

Supportive therapy in haematology

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1985 **MARTINUS NIJHOFF PUBLISHING**
a member of the KLUWER ACADEMIC PUBLISHERS GROUP
BOSTON / DORDRECHT / LANCASTER



Distributors

for the United States and Canada: Kluwer Academic Publishers, 190 Old Derby Street, Hingham, MA 02043, USA

for the UK and Ireland: Kluwer Academic Publishers, MTP Press Limited, Falcon House, Queen Square, Lancaster LA1 1RN, UK

for all other countries: Kluwer Academic Publishers Group, Distribution Center, P.O. Box 322, 3300 AH Dordrecht, The Netherlands

Library of Congress Cataloging in Publication Data

Main entry under title:

Supportive therapy in haematology.

1. Blood--Diseases--Treatment. 2. Blood--Transfusion. 3. Hematology. I. Das, P. C. II. Smit Sibinga, C. Th. III. Halie, Martin Rudolf. [DNLM: 1. Blood Transfusion. 2. Hematologic Diseases--therapy. WH 100 S959] RC636.S86 1985 616.1'506 85-4977 ISBN-13:978-1-4612-9617-1 e-ISBN-13:978-1-4613-2577-2 DOI:10.1007/978-1-4613-2577-2

ISBN-13:978-1-4612-9617-1

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Softcover reprint of the hardcover 1st edition 1985

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Martinus Nijhoff Publishing, 190 Old Derby Street, Hingham, MA 02043, USA.

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Foreword

As appropriately outlined in the first chapter in part II in this book, the history of contemporary blood transfusion is only three-quarters of a century old. On the surface, there is not much left in common between an arm to arm blood transfer carried out as an heroic measure in the twenties, when patient or donor had to be weighed in order for the physician to decide when to stop, and blood component therapy of today, when several patients can benefit from appropriately measured and standardized amounts of various purified blood fractions. Yet, the basic principles of blood transfusion have remained the same, and much is owed to the efforts of pioneers, in this as in so many other fields of curative medicine. How technical advances in blood transfusion have always developed hand-in-hand with its scientific foundations is probably clear to every potential reader of this book. But an additional message that will come home forcefully from reading it is the remarkable way in which blood transfusion has cut across so many of the traditionally defined areas, both in terms of basic biology and of clinical applications. These interactions between blood transfusion therapy and various established disciplines have been notably and mutually beneficial. For example, blood groups have been for a long time almost the only system in which Mendelian inheritance could be analyzed easily in the human species, and blood group frequencies were in practice all there was to human population genetics until the sixties. Red cell serology, because of its simplicity, served as a model system to investigate antigens from other cells. Extension of the concepts and techniques to white

cells was pioneered in Holland by Van Loghem and Van Rood, and it led eventually to the discovery of the HLA system and its subsequent explosive development. In biochemistry, the work on the ABO and MN blood group substances has provided pointers to general features of the biosynthesis and role of glycolipids and glycoproteins in the cell membrane, and the identification of serological specificities associated with specific oligosaccharides has proven for the first time how gene products that are not proteins can exhibit Mendelian inheritance. With the discovery of the role of Duffy blood groups in the infection of red cells by *Plasmodium vivax*, it was again the study of red cell antigens that has identified a specific mode of parasite-host cell interaction which may be a model for other systems in parasitology. In the last three years, with the cloning of factor IX and factor VIII, coagulation proteins have successfully graduated from blood component therapy to molecular biology.

If interactions with basic biology have been fruitful, one of the most gratifying aspects of work in blood transfusion is the interface with clinical activities. The blood banker, perhaps more than anyone else in a hospital, comes in day to day contact with colleagues of all specialties. Obstetric catastrophes are perceived no less urgently in the blood bank than in the labour ward. The prevention and management of haemolytic disease of the newborn relies on a high standard of blood transfusion no less than on a good neonatology unit. Good liaison between the blood bank, surgeons and anaesthetists has made it possible for the massive

transfusions required by open heart surgery to become now part of the hospital routine. In medicine and its subspecialties the problem list is long and still growing in length. Plasmapheresis brings the blood bank in contact with rheumatologists and nephrologists dealing with autoimmune disease, or with the treatment of congenital hyperlipidaemias; albumen replacement therapy is relevant to nutritional problems and gastrointestinal disorders; and, coming nearer home, platelets and/or granulocyte transfusions are essential in the supportive treatment of cytopenic or immunocompromised patients. It is quite remarkable that chapters in this book cover most of the details of each of these widely different topics. In addition, it deals with the use of specialized blood derivatives such as immunoglobulins and interferon.

No therapy is free of side effects, and we have always been aware that we should weigh risks and benefits carefully. 'Homologous serum jaundice' was the scourge of the fifties and sixties, but safeguards were then developed. AIDS has now hit, and probably by the time this volume is in the libraries several countries may have already adopted regular serological testing of blood donors for HTLV-III.

If the branches of blood transfusion are intertwined with all specialties, its roots are in haematology. In this respect, the subject has been traditionally closely associated with the investigation and management of acquired haemolytic anaemias, and immunohaematology in general. In order to emphasize that blood transfusion is more of a biological than a pharmacological therapeutic procedure, we have for years impressed on the students that it is, so to speak, a temporary graft. This concept has been vindicated now that bone marrow transplantation has become established as the major advance in the treatment of blood disorders in the last decade. Blood transfusion is deeply involved with bone marrow transplantation both on conceptual and on practical grounds. Conceptually, the 'matching' between donor and recipient that has been our prime concern in blood transfusion is proving even more critical in bone marrow transplantation. If blood group incompatibility leads to transfusion reactions, bone mar-

row incompatibility leads to graft failure and to graft *versus* host disease. From the practical point of view, many blood banks are taking responsibility for HLA typing and related matching procedures, and all of them are deeply involved in the enormous commitment to supportive care that the immediate post-transplantation period demands.

Because of the common roots of blood transfusion and haematology, it seems appropriate that a number of chapters are devoted particularly to the role of blood transfusion and blood components in the management of haematological disorders, such as leukaemia, sickle cell anaemia, thrombocytopenias and haemophilia. This leads one naturally to a more general and important question. Blood transfusion has come a long way from ABO blood grouping and cross-matching: whither is it now going? On one hand, this will depend on developments in medicine and particularly in haematology as a whole. For instance, the high level transfusion therapy of thalassaemia major has frequently shifted the pathophysiology of these patients from that of chronic anaemia with ineffective erythropoiesis to that of organ damage consequent on iron overload. Thus, iron-chelation becomes as important a task in management as blood transfusion itself. At the same time, thalassaemia major has become largely preventable through genetic counselling and prenatal diagnosis, while some hope it may become amenable within the foreseeable future to genetic manipulation as well. Blood component therapy has become more and more tailored towards the needs of the individual patient. Ideally, a patient with haemophilia A should receive pure factor VIII, but the formidable task of complete biochemical purification is likely to be overtaken before too long by a product synthesized through recombinant DNA technology. Opinions are much more divided on the future of so-called artificial blood. As explained in this volume, oxygen-binding chemicals have very different properties from haemoglobin-containing red cells and, even if problems of short-term and long-term toxicity are overcome, they are unlikely to be a viable substitute for red blood cells except in exceptional circumstances.

The message from this volume is that techniques

must be updated, needs and indications and preparations change, and concomitantly we can see a healthy evolution in the role of specialists in the health profession. We should not be afraid of changes, but responsive to them. Developments can vary depending on organizational differences in different countries: authors from seven countries in three continents have contributed to this volume. Even more important, new developments have often come from the drive of individual leaders in the field. With this proviso, I feel it is likely that in general blood transfusion will continue to fulfil its essential function perched, as it is, on the interface between the laboratory and its clinical commitment. On the laboratory side conservation,

purification and safety of blood components are ever improving and yet they pose ever increasing challenges. On the clinical side, immunohaematology seems to be evolving gradually towards the management of a widening range of immune disorders of haemopoiesis and of bone marrow transplantation. We are only beginning to understand the physiology of immune reconstitution after such procedures. This book reviews what is established and at the same time it sets the stage for the future at a time of change. I wish it the success that the hard work by the contributors and by the editors truly deserves.

London, July 1985

Professor L. Luzzatto

Preface

In the last few decades rapid developments in basic knowledge and in sophisticated technology have had considerable effects on the therapeutic use of blood and its components, which has accordingly become complex. It has also gained widespread application in depth and breadth in almost all the specialities of medicine. There are, however, other factors that are directly applicable to supportive therapy; many of these have been studied and are well understood but their importance in the clinics has seldom been emphasized. By documenting both the fundamental aspects and clinical experience our contributors have attempted to inform clinicians of the various forms of supportive therapy available in haematology including those that are proven value and those that are potentially applicable. The conception of the various topics included in the book grew as we progressed from hospital residents through specialists and then as members of multi-disciplinary teams in University Hospitals.

Clinicians faced with a problem in supportive care ought to be able to find the appropriate information in one book rather than in half a dozen specialised journals. Our hope is that this book provides the answers for all those engaged in primary care, especially those in the field of acute clinical medicine; more importantly to junior hospital doctors and residents anxious to perform optimally in exacting situations, and to the staff of blood banks, transfusion and haematology departments who wish to update their overall knowledge of supportive therapy.

We are grateful to our expert contributors – clinicians and research workers – who have gladly revised and updated chapters following independent referees' comments. We are also grateful to Dr. S.H. Davies, Royal Infirmary of Edinburgh for constant encouragement while the topics of the book crystallised and matured; he willingly provided much time in discussion and constructive criticism; to Professor H.O. Nieweg, Department of Medicine, University of Groningen, for his most helpful comments. We are indebted to our reviewers in Australia, Europe, the United Kingdom, North America and New Zealand for their invaluable suggestions. We are also indebted to Mr. Jeffrey K. Smith of the publishers Martinus Nijhoff for his considerable help. Our thanks also to Mrs. Anje Doedens-Luchtenberg, our secretary, who shared something of the turmoil of preparation of the text.

Groningen, July 1985

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Part One

Pathophysiology

1. Role of cell culture in the management of haematological diseases

D.C. LINCH

Introduction

Morphological examination of the bone marrow allows analysis of cells at the level of the terminal five or six divisions in the differentiation pathway. A greater number of divisions have already occurred, however, between the haematopoietic stem cells and the earliest recognizable precursors, and it is at this level that many haematological disorders arise. These cells involved in the early stages of differentiation are clearly too few to be examined microscopically and the enumeration of these cells and the study of their regulation is dependent upon *in vitro* clonal assays.

In 1961 Till and McCulloch described an assay for haemopoietic cells capable of forming colonies in the spleens of irradiated syngeneic mice [1]. These colonies (CFU-S) are derived from single cells and contain red cells, granulocytes, megakaryocytes, and further colony forming cells [2]. The cell giving rise to the CFU-S is thus multipotential and capable of self-replication and is by definition a stem cell. Lymphocytes are not found in the spleen colonies but studies using radiation-induced chromosome markers suggest that some of the cells forming CFU-S are closely related, if not identical, to the T lymphocyte and B lymphocyte stem cells [3, 4]. This CFU-S assay is obviously inapplicable to the study of human haemopoiesis.

In 1965 and 1966 Pluznik and Sachs and Bradley and Metcalf independently reported *in vitro* methods for culturing murine granulocyte/monocytic colonies in agar [5, 6] and this system was soon modified for growth of human progenitor cells [7].

The progenitor cells are immobilized in a nutrient semi-solid matrix to support cell growth and keep the progeny of a single cell together as a colony or cluster. After 5–7 days in bone marrow cultures, small cell aggregates predominate and are known as clusters (4–40 cells). By 10–14 days lesser numbers of larger cell aggregates are seen (between 40 and several thousand cells) and are called colonies (CFU-GM). Cluster forming cells are partially separable from colony forming cells (GM-CFC) by physical means [8] and it is probable that cluster forming cells are the immediate progeny of colony forming cells. The length of culture after which cluster forming or colony forming cells are counted is somewhat arbitrary and 7 and 14 days respectively are usually used (Table 1). The number of cell clusters decreases after one week in culture due to cell death and dispersion in the semi-solid medium. Some aggregates scored as clusters at 7 days will also be scored as colonies at 14 days, and there is clearly a spectrum of progenitor cells with proliferative potentials between 2 and approximately 12 divisions. Although GM-CFC may have large proliferative potential, they do not self-replicate *in vitro* and must be considered as progenitor cells and not stem cells. GM-CFC but not cluster forming cells are also present in small numbers in the peripheral blood and these blood derived progenitor cells may be more primitive than the majority of marrow derived progenitor cells [9]. GM-CFC have a peak buoyant density of approximately 1.069 and less than 10% of normal GM-CFC have a density of <1.062 [10].

Individual CFU-GM consist of neutrophils,

monocytes or eosinophils. Occasionally mixed colonies of neutrophils and monocytes are seen, and micromanipulation studies have shown that these mixed colonies are derived from single cells [11]. Mixed colonies containing eosinophils are not seen, indicating that monocytes and neutrophils are more closely related than is either cell type to the eosinophil lineage (Fig. 1). The eosinophil colonies exhibit an initial growth lag with virtually no eosinophil colonies present at 7 days and colony numbers not maximal until nearly 3 weeks. The number of eosinophil colonies in human blood and marrow is surprisingly high, forming 20%–30% of all 14 day colonies [12, 13, 14]. Although, in general, more mature cells are seen in culture as time proceeds, there is marked asynchrony of maturation between different colonies and also within individual colonies. The *in vitro* granulopoiesis is usually dysplastic, and well granulated multilobed neutrophils are few.

In the culture system developed by Pike and Robinson a double nutrient agar layer was used [7]. The overlay contained the haemopoietic cells to be cultured and the underlay contained blood leukocytes acting as 'feeder cells'. These cells produce soluble factors which stimulate growth and are called colony stimulating activity (CSA). The main source of CSA in leucocyte conditioned medium is the monocytic cells [15] and as CSA stimulates monocyte growth there is an apparent positive feedback loop. However, in the presence of high concentrations of monocytes, there is inhibition of CFU-GM growth due to the production of Pro-

Table 1. Incidence of hemopoietic progenitor cells in blood and marrow.*

	Blood		Marrow	
	(Progenitors/10 ⁵ ficolli hypaque separated cells)			
	Mean	Range	Mean	Range
Myeloid clusters	<1	—	172	65–450
GM-CFC	4	<1–15	72	20–200
<i>CFU-E</i>	2	<1–7	280	90–700
<i>BFU-E</i>	25	5–120	85	30–195

* Normal ranges vary between laboratories.

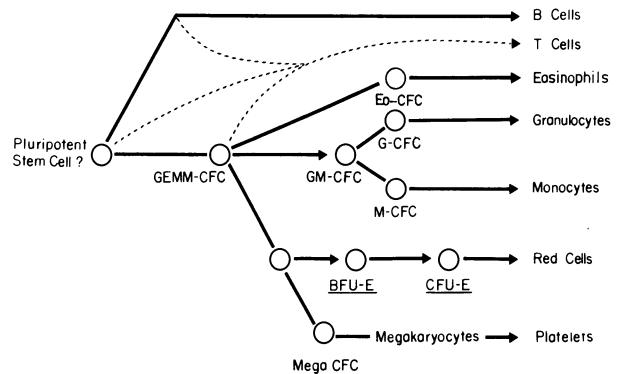


Fig. 1. Schematic representation of haematopoietic differentiation.

taglandin E [16] and acidic isoferritins [17]. More recently a complex network of cellular interactions has been proposed that regulates the production of CSA and the acidic isoferritins. This is illustrated in Fig. 2. It is apparent that CSA is also produced by T cells especially when stimulated by mitogens or in mixed lymphocyte reactions [18, 19], and different subpopulations of T cells can modulate T cell CSA and monocyte acidic isoferitin production [20].

Cell separation studies of blood leukocytes also revealed that granulocytes and their products are capable of decreasing the production and release of CSA by monocytes [21]. The colony inhibiting activity (CIA) has been highly purified, and it has been suggested by Broxmeyer and co-workers that this substance is lactoferrin, which is contained in the specific granules of neutrophils [22]. Other studies on the inhibitory activity of CSA by Bagby *et al.* have suggested that lactoferrin does not decrease monocyte CSA production but causes the monocytes to produce an inhibitory monokine that reduces T cell CSA production [23]. More recently, Broxmeyer and co-workers have implicated a third iron binding protein, transferrin, as the mediator by which the T8⁺T cells suppress T cell CSA production [20].

Although it is widely assumed that CSA is central to the control of granulopoiesis, it has proved extremely difficult to demonstrate clearly its *in vivo* significance (reviewed by Moore [24]. This may be due to the fact that circulating CSA levels have little influence on granulopoiesis and that it is local levels of CSA produced in intimate cell relation-

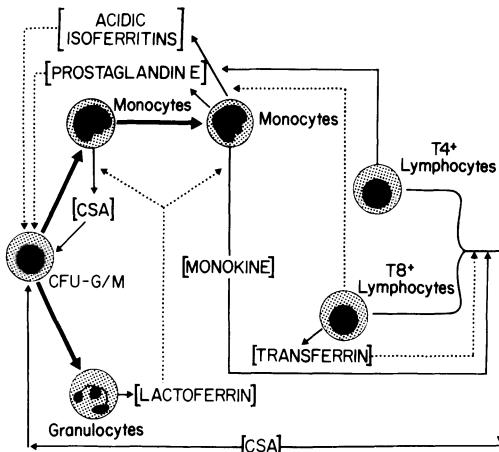


Fig. 2. Putative cellular interactions in the regulation of granulopoiesis.

ships within the bone marrow which are important. The physiological relevance of the proposed cellular interactions and the role of the iron binding proteins in granulopoiesis are especially difficult to prove. Broxmeyer showed that injection of human lactoferrin into mice after cyclophosphamide therapy dampened the marrow rebound granulopoiesis that usually occurs [25] but Winton *et al.* were unable to demonstrate that iron saturated murine lactoferrin had an inhibitory effect on *in vitro* production of granulopoietic stimulators by mouse macrophages and bone marrow cells [26]. Furthermore, the level of lactoferrin which produces suppression of CSA production *in vitro* (10^{-14} M of 8% iron saturated lactoferrin) is several orders of magnitude less than the *in vivo* steady state plasma levels of greater than 10^{-8} M. Bagby and Bennet [27] have shown that lactoferrin polymerizes under physiological conditions and that polymerized lactoferrin is not inhibitory. This may account for the apparent discrepancies between the *in vitro* concentrations required for CSA inhibition and the *in vivo* levels, but it remains uncertain.

CSA is also produced by many other tissues, and for routine laboratory use placental conditioned medium [28] or medium conditioned by the monocyte-like cell line GCT [29] are frequently used in place of feeder layers. CSA from placental conditioned medium and the GCT cell line both stimulate neutrophil macrophage and eosinophil colony formation, but there is evidence that stimulators

for specific cell lines can be separated from one another [30].

Committed human erythroid progenitor cells can also be grown *in vitro* using plasma clot [31] or methylcellulose [32] as the semi-solid matrix and erythropoietin as the growth stimulator. After 7 days in culture of adult bone marrow, haemoglobinized aggregates of cells appear and are known as CFU-E. CFU-E were originally said to have between 8 and 64 cells, but under optimal conditions day 7 CFU-E may contain in excess of 1000 cells. The colonies degenerate over the ensuing week. The progenitor cells giving rise to CFU-E are referred to here by the nomenclature *CFU-E*. Their growth *in vitro* is dependent on relatively low levels of erythropoietin and in the mouse the size of the *CFU-E* compartment *in vivo* varies with the erythropoietin levels [33, 34, 35].

After 2–3 weeks in culture, at higher erythropoietin levels, blood and marrow give rise to larger (approximately 1,000–100,000 cells) haemoglobinized colonies known as erythroid bursts (BFU-E). They are called bursts because they are frequently not discrete colonies but consist of several subcolonies in close proximity to one another. The clonal origin of these bursts was proven by Prchal *et al.* who grew bursts from a female heterozygous for glucose-6-phosphate dehydrogenase isoenzymes, and showed that only one type of isoenzyme was found in each BFU-E, although there was heterogeneity between bursts [36].

Red cell maturation within BFU-E is asynchronous and usually dyserythropoietic [37]. Megakaryocytes are frequently found in BFU-E, suggesting that the erythroid and megakaryocytic lineages are closely related (see Fig. 1).

There is heterogeneity among the BFU-E cell population, and Gregory and Eaves [38] showed that the larger bursts with more subcolonies appeared later in culture and required higher levels of erythropoietin for growth *in vitro*. It is generally accepted that the macroscopic BFU-E arise from cells preceding those which give rise to the smaller BFU-E, which in turn precede those giving rise to the CFU-E. Approximately 25 BFU-E are found per 10^5 peripheral blood mononuclear cells, which is about 30% of the prevalence found in the mar-

row, although the total nucleated cell concentration is considerably higher in the marrow. Although erythropoietin is required for achievement of full differentiation and haemoglobinization of the progeny of a *BFU-E*, there is controversy as to whether erythropoietin has any effect on the early progenitor cells *in vitro* [39, 40].

Marked fluctuations in the level of endogenous erythropoietin in the mouse induced by acute bleeding have little or no effect on the *BFU-E* compartment, suggesting that *BFU-E* are also not regulated by erythropoietin *in vivo* [34]. Iscove has postulated that a specific factor known as burst promoting activity (BPA) regulates the proliferation of primitive *BFU-E* [39]. Human active BPA has been highly purified [41].

BPA will induce the development of *BFU-E* from *CFU-S* in murine long-term cultures [43], and Niskanen *et al.* have shown that a crude BPA preparation, when injected into mice, will increase the number of human *BFU-E* implanted in diffusion chambers [44].

The cellular origins of BPA have stimulated much interest and controversy. Nathan *et al.* demonstrated that T cells were capable of BPA production [45], and this has been widely confirmed [46, 47]. Other workers showed that adherent cells or monocytes could produce BPA [48–52] and Zuckerman [52] and Reid *et al.* [53] have demonstrated that peripheral blood T cells and monocytes have a synergistic role in the production of BPA. Monocytes produce more BPA than T cells per cell, but both moncytoid and T cell lines produce BPA which is directly active on isolated *BFU-E* [54]. Most of these studies used peripheral blood as the source of *BFU-E*. When bone marrow was used, Lipton *et al.* could show no T cell dependence for *BFU-E* growth [55], and Linch *et al.* could not clearly demonstrate an obligatory role for either T cells or monocytes [56]. It was suggested by Lipton *et al.* that most marrow *BFU-E* were more mature than blood *BFU-E* and that they therefore required less BPA, and were thus less dependent on T cells. These results could also be explained, they pointed out, if an alternative cell type in the marrow also produced BPA. Meytes *et al.* have shown that a non-adherent non-T cell in the blood can

produce BPA [57], and a bone marrow radioresistant non-myeloid non-T cell has been identified as a potent BPA producer [54]. Furthermore, in children with severe combined immunodeficiency and virtual absence of either mature or immature T cells, erythropoiesis is remarkably normal even in the face of recurrent infections, suggesting that T cells have no obligatory role in steady state erythropoiesis.

In 1975, Metcalf *et al.* developed a colony forming assay for murine megakaryocyte progenitors (Mega CFC) [58], and assays are now available for the growth of human Mega CFC in methylcellulose [59] or in plasma clot [60]. These assays have used crude erythropoietin [59] or human serum from patients with aplastic anaemia [60] as a source of megakaryocyte colony stimulating activity, but with these impure sources of Mega CSA CFU-GM and *BFU-E* also grow. As the cells in CFU-Mega may be no larger than monocytes grown *in vitro*, recognition of CFU-Mega can be very difficult. This problem can be overcome, however, by immunofluorescent staining of the colonies with either polyclonal or monoclonal anti-platelet antibodies [60, 61].

Animal studies have suggested that there are two trophic hormones regulating platelet production [62], and preliminary data suggests a similar dual control in man [63]. Thrombopoietin is produced in response to thrombocytopenia and appears to both increase the ploidy in megakaryocytes and platelet production. Thrombopoietin does not cause proliferation of Mega-CFC which are regulated by Mega CSA(s). Mega CSA is not raised in the serum following acute thrombocytopenia but is present in high levels in aplastic anaemia serum. This two level regulation of platelet production is analogous to the control of erythropoiesis by BPA and erythropoietin. The origins of thrombopoietin and Mega CSA and the factors regulating their production and release are presently obscure.

Mega CFC, like *BFU-E* and GM-CFC, are committed progenitor cells and do not self-replicate. Amplification can occur between the stem cell and the progenitor cell, and for many studies enumeration of stem cell numbers would be desirable. Gordon [64] and Jacobson [65] developed a system for

growing progenitor cells implanted in diffusion chambers into the peritoneal cavity of sublethally irradiated mice or rabbits, and murine *CFU-S* proliferate under these conditions [66]. Hoelzer *et al.* showed that human GM-CFC similarly increase in numbers in diffusion chambers, partly perhaps by self-renewal and partly by input from an early precursor cell pool [67]. When a semi-solid matrix is used in the diffusion chamber, colonies are formed (*CFU-d*) which may contain either neutrophils, eosinophils, megakaryocytes or erythrocytes [65, 68], and Jacobson *et al.* have shown that at least some of these colonies grow from cells more primitive than the GM-CFC grown in agar cultures [69]. This technique has not proved to be useful for counting human stem cells as opposed to progenitor cells and the latter are more readily assayed *in vitro*. Culture in diffusion chambers may, however, be of considerable value in the determination of the *in vivo* significance of proposed haemopoietic regulators [70] and the study of the kinetics of haemopoiesis [71].

In an attempt to study stem cell proliferation, Dexter and co-workers developed a system in the mouse whereby stem cell proliferation and differentiation could be maintained for many months in culture [72]. These cultures depend on the prior establishment of a suitable population of marrow-derived adherent cells, supporting the concept of a haemopoietic inductive micro environment [73]. The adherent cells in these cultures consist of a complex multilayer of large fat containing cells, flattened endothelial cells, macrophages and some fibroblasts [74]. *CFU-S* proliferate in this system for many months, and differentiation to GM-CFC and *BFU-E* occurs without added stimulators. The GM-CFC mature to granulocytes and monocytes without added CSA, though the *BFU-E* do not mature to red cells. This system has been adapted with more limited success to human material [75], and probably the best results have been obtained by Gartner and Kaplan, who managed to maintain GM-CFC for at least 20 weeks [76]. There has been little success to date in maintaining human *BFU-E*. The major problem with this system is that it is not clonal, so that it is impossible to enumerate the human stem cells at any stage. Moore *et al.* have

used continuous cultures to infer that human stem cells do not bear the HLA-Dr antigen as do their immediate progeny [77], though this must still be considered a tentative suggestion.

If marrow is incubated in suspension culture with appropriate conditioned medium, there is an increase in GM-CFC as detected in agar subcultures. This has also been used as a measure of the 'pre-*CFU-C*' compartment [69, 78]. Such assays are again limited by their lack of clonality.

In 1977 Johnson and Metcalf reported the growth of murine multipotential haemopoietic stem cells in agar cultures [79]; these colonies are clonally derived and frequently contain *CFU-S* (reviewed by Johnson) [80]. The following year Fausser and Messner described an assay system for human pluripotent progenitor cells forming mixed colonies containing granulocytes, erythroblasts, megakaryocytes and macrophages (CFU-GEMM) in individual colonies [81]. More recently Messner *et al.* have provided preliminary evidence that these CFU-GEMM also contain T cells [82]. Approximately 25% of CFU-GEMM give rise to secondary colonies, but only a small proportion of these colonies are mixed; most GEMM-CFC are not therefore true stem cells [83]. In the Fausser and Messner system marrow or blood is cultured in methylcellulose in the presence of leukocyte conditioned medium or Mo cell conditioned medium and after 4 days erythropoietin is added. Because the frequency of GEMM-CFC is relatively low, $1-2 \times 10^5$ cells/dish are often plated per dish and, as *BFU-E* and *CFU-GM* also grow under these conditions, there can be considerable crowding and even coalescence of colonies, frequently making the enumeration of CFU-GEMM rather precarious. It must be noted that Fausser and Messner did demonstrate the clonal origin of mixed colonies in their system by cocultures of male and female cells and then showing that cells from individual mixed colonies either all had or did not have a Y chromatin body [81]. In general, to ensure the clonality of mixed colonies, cells should be plated at a maximum concentration of 5×10^4 /ml.

Assay systems have also been developed for growing human T cell and B cell colonies [84, 85], but unlike other haemopoietic cells, mature T and

B cells are capable of extensive proliferation. Blood-derived T cell colonies growing in response to phytohaemagglutinin stimulation should clearly not be considered as progenitor cell assays. T cell colonies can also be grown from human bone marrow [86], and although some of these colonies may be derived from progenitor cells this is not proven.

Acute myeloid leukemia

Under standard conditions for CFU-GM growth, few normal appearing colonies develop. This is because of the replacement of normal progenitors by leukaemic blasts and because the leukaemic blasts release factors that inhibit normal haemopoiesis. Work from Broxmeyer and coworkers has suggested that the leukaemia-associated factors are the family of acidic isoferritins which may be normal haemopoietic regulators [17, 87, 88]. The leukaemic blasts are themselves insensitive to acidic isoferritins and frequently proliferate in standard cultures, giving rise to cell clusters and occasional colonies. These cell aggregates are largely composed of blast cells, and mature polymorphonuclear leucocytes with well-formed granules are rarely seen. These leukaemic colonies and clusters are not autonomous but are still dependent on a source of CSA. The leukaemic progenitor cells are less dense than normal GM-CFC with approximately 30% of leukaemic progenitors having a density <1.062 g/dl [10]. In 1974 Moore *et al.* reported on the results of agar cell cultures in 108 cases of untreated AML in which they correlated the growth characteristics with the response to chemotherapy. In this study a high plating efficiency was a poor prognostic factor, and the pattern of colony cluster growth was also related to the response to chemotherapy [89]. In patients in whom there was no growth, a preponderance of large clusters, or a high cluster to colony ratio, only 18% obtained a complete remission. In patients with a preponderance of small clusters of colonies with a normal colony to cluster ratio, the complete remission rate was 52%. The latter pattern is least common and may represent residual normal haemopoiesis. Since this original study many centers have

confirmed that the growth characteristics in acute myeloid leukaemia are helpful in predicting which patients will enter remission [90, 91, 92]. This remains true with the use of more effective modern induction regimes [93, 94]. Direct comparison of the results from various centers is not possible because the different centers use different sources of CSA, score the cultures at different times, and use different criteria for classifying the colonies and clusters. The same trends apply, however, and the growth of large leukaemic clones is generally a poor prognostic indicator. Greenberg *et al.* have also assessed CSA production from leukaemic marrow and found that those with low CSA production have significantly lower complete remission rates [95]. Culture studies are thus capable of predicting the response to induction therapy. They do not appear, however, to predict the length of remission [94]. It must therefore be concluded that at present such studies have little role in the routine management of AML.

Francis has shown that the larger leukaemic clones require more CSA for growth, and by analogy with normal progenitor cells, she has suggested that the larger clones originate from leukaemic cells at an earlier stage of differentiation than those giving rise to smaller clusters [93]. An improved culture system for the growth of myeloid leukaemic blast progenitor cells has also been described [96]. The blood or marrow is depleted of E rosetting cells and is then stimulated with PHA leukocyte conditioned medium. A similar technique using irradiated feeder cells with PHA has been used by Lowenberg and colleagues [97]. The cells giving rise to colonies in PHA-leukocyte feeder cultures have a different density from those that give rise to colonies in conventional culture system [97]. Many of the colonies in the PHA cultures are larger than those in the standard cultures and in some cases these colonies contain further leukaemic colony-forming cells. The ability to form secondary colonies is an indicator of self-renewal and the primitiveness of that cell. It thus appears that among the leukaemic blast cell population there are stem cells which can self-replicate or differentiate to 'more mature blasts', blasts capable of colony formation and not self-renewal; and blast cells which at least

in culture are inert. Buick *et al.* have shown that a high capacity for self-renewal is a poor prognostic factor with regard to remission induction [98]. This is in agreement with the views of Francis, that the large leukaemic clusters grown under standard CFU-GM conditions are derived from a more primitive leukaemic progenitor and are a bad prognostic feature. The overlap of the self-renewal in leukaemic colonies between those who do remit and those who do not is so large that this type of assay is of little prognostic value in individual cases.

This model of leukaemia in which there is great functional heterogeneity within the blast cell population has major implications for the understanding and treatment of leukaemia, especially by serotherapy. The leukaemic colony-forming cells will not necessarily have the same phenotype as the majority of blasts, and it is these highly proliferative stem cells at which therapy must be directed. In M4 leukaemias for instance, the monocytic blast cells express Mo2 and MY4 antigens, but cell sorting experiments have shown that the colony-forming cells are invariably MY4 negative [99]. Discrepancies between the phenotype of the majority of blast cells and the majority of colony-forming cells have also been reported in other forms of AML [100, 101]. Wouters and Lowenberg have documented a case in which the leukaemic colony-forming cells and leukaemic cluster-forming cells had different phenotypes [102].

Monoclonal antibodies to leukaemia specific antigens have not, and may well not be, produced. To contemplate *in vivo* immunotherapy with monoclonal antibodies or *in vitro* purging of marrow with serotherapy during an autologous bone marrow transplant, it would be necessary to know the phenotype of the most primitive leukaemic cells. Analysis of patients with AML, heterozygous for G6PD, have shown that in some patients the 'leukaemic hit' goes as far back as the multipotential stem cell [103]. Colony-forming assays for leukaemic cells at this primitive stage of differentiation are not available, and as few patients are G6PD heterozygotes, a rational approach to immunotherapy in AML seems very distant. The use of anti-HLA-DR antibodies has been suggested for *in vitro* purging of AML marrows. Whereas normal

progenitor cells express HLA-DR, true stem cells responsible for regeneration may not [104]. Most leukaemic colony-forming cells do express DR [100] but it is quite possible that the early leukaemic stem cells will not do so. Similar reservations must apply to the use of drugs for marrow purging, which kill both normal and leukaemic colony-forming cells and yet spare those normal cells that give rise to GM-CFC in Dexter type cultures [105].

In vitro leukaemic colony growth may be of value to determine cases in which progenitor cells are resistant to certain cytotoxic drugs [98], but such studies are not yet at a stage where they can be used to individualize treatment protocols.

BFU-E growth from AML marrow (M1–M5) is usually greatly reduced or absent. It is not clear whether the few remaining erythroid colonies are derived from the leukaemic clone or represent residual normal erythropoiesis, but it is likely that both mechanisms apply.

In erythroleukaemia (M6) spontaneous erythroid growth without added erythropoietin may be seen, and whereas in some cases this may be due to extreme hypersensitivity of the progenitors to erythropoietin in the serum used in the cultures, studies with anti-erythropoietin antiserum have suggested that in some cases the erythroid growth is autonomous of erythropoietin control [106]. Growth of autonomous erythroid colonies in acute leukemia is not pathognomonic of erythroleukaemia, however, as it has also been observed in non erythroid AML and in refractory anaemia with excess blasts [106].

Acute lymphoblastic leukaemia

The prevalence of GM-CFC and BFU-E is usually decreased in ALL at presentation [10]. Methods have been developed for the growth of leukaemic colonies in common (Non B non T) ALL [107, 108], and as in AML the colonies consist predominantly of leukaemia blasts. Secondary colonies have been grown from these blast colonies, indicating self-renewal by some progenitors [108]. These culture systems presently have no role in the management of individual patients.

Haemopoietic dysplasia

Many investigations of patients with haemopoietic dysplasia or preleukaemia have shown that culture studies can be useful prognostic indicators. Some patients have normal CFU-GM growth, and these patients have a better prognosis than those who do not [109–113]. A low incidence of CFU-GM or a frankly leukaemic growth pattern with a marked increase in the cluster to colony ratio is correlated with a malignant course with progressive cytopenia and lethal infection or transformation to acute leukaemia. In many cases the cluster and colony-forming cells also have an abnormally light buoyant density as in acute myeloid leukaemia [10, 110]. The cells in colonies from preleukaemic patients with abnormal colony growth frequently show maturation defects. This also generally indicates a malignant course though not necessarily transformation to acute leukaemia [114]. These maturation defects can also be demonstrated in liquid cultures [115, 116].

Several studies of erythroid progenitor cell growth have shown that erythroid progenitor cell numbers may be high in the presence of refractory anemia and haemopoietic dysplasia [117, 118], and this probably reflects the marked ineffective erythropoiesis that is occurring.

Recently it has been shown that leukaemic blast cell colonies can be grown from the blood of many preleukaemic patients although the clinical significance of this is not yet clear [119].

Thus it appears that culture studies may give some prognostic information in preleukemia, although the clinical parameters of pancytopenia and splenomegaly, and the presence of chromosomal abnormalities, are probably more predictive of a malignant course. Furthermore, the patients with haemopoietic dysplasia respond poorly to therapy either in the chronic phase or after transformation, and knowledge of which patients will have a poor prognosis does not modify treatment in individual cases.

Chronic granulocytic leukemia

In the chronic stable phase of chronic granulocytic leukaemia (CGL) the prevalence of GM-CFC in the bone marrow is moderately increased (2–5 fold) whereas the level in the blood is markedly increased (absolute increase of approximately 9000 fold) [10, 120, 121]. Approximately 70% of GM-CFC in CGL have a buoyant density of <1.062 [10]. The GM-CFC numbers in the blood are linearly related to the peripheral leucocyte count when both are expressed on log/log axes. The colony to cluster ratio in the marrow is essentially normal during the chronic phase, and clusters can also be grown from the blood in similar proportions. With treatment the number of circulating GM-CFC falls towards the normal range. Cytogenetic studies of colonies from patients with Ph' + CGL have shown that nearly all the colonies are derived from the malignant clone and very few normal residual progenitor cells are detectable [10, 122]. In long term cultures, however, the Ph' + GM-CFC are soon replaced by Ph'-progenitor cells, indicating that some Ph'-stem cells do remain [123].

Erythroid progenitors and GEMM-CFC are also increased in the marrow of CGL and increased to a greater extent in the blood [121, 124, 125]. This increase in the blood *BFU-E* is related to the white blood cell count in a manner similar to the GM-CFC increase, although the increase of circulating *BFU-E* is less than for GM-CFC (180 vs 9000 fold increase) [121, 126]. *CFU-E* are present in large numbers in the blood in CGL whereas *CFU-E* are few in normal blood [121]. In one study many of the circulating erythroid progenitor cells appeared to be erythropoietin independent [121] but in another study relatively normal erythropoietin dose response curves were reported [126]. Although there are inevitably differences in culture conditions from laboratory to laboratory, this large discrepancy is surprising.

As CGL begins to transform, the white cell count tends to rise despite treatment, and the circulating progenitor cell levels rise towards the pre-treatment levels. With onset of blast crisis the GM-CFC level may fall [10, 127, 128]. This change

to an acute leukaemic growth pattern may herald blast crisis by several months [10, 128], and in patients with a suitable donor this might be considered as an urgent indication for bone marrow transplantation (BMT) as BMT during blast crisis is far less successful than during stable or accelerated phase.

Polycythaemia rubra vera

Polycythaemia rubra vera (PRV) is a myeloproliferative disorder characterized by an increase in the red cell mass and, frequently, an increase in other blood elements. Culture studies have revealed that the prevalence of erythroid progenitors cells is only modestly increased in both marrow and blood [121, 129]. The PRV erythroid progenitors have similar physical characteristics to normal progenitors. GM-CFC were found to have a normal buoyant density in one study [130] but were found to be larger and less dense than normal in another [131]. The regulation of erythroid progenitor cell maturation is grossly abnormal. The erythropoietin progenitor cell growth dose response curve is markedly shifted to the left, and many erythroid progenitors grow in the absence of added erythropoietin [132, 121]. Some erythropoietin is, however, present in the serum of the cultures, and the growth of CFU-E without added erythropoietin may be largely due to increased erythropoietin sensitivity rather than erythropoietin independence. This explanation was suggested by Zanjani *et al.*, who found they could inhibit this erythropoietin independent growth with an anti-erythropoietin antibody [133]. Casadevaal *et al.* further showed that there was no erythropoietin independent CFU-E or BFU-E growth in a serum free culture system [134]. It is also possible that the erythroid progenitors in PRV are hypersensitive to other factors such as BPA as well as erythropoietin. Recent studies have shown that GEMM-CFC are also abnormal in PRV confirming experiments in G6PD female heterozygotes that PRV is a stem cell disorder [135]. In PRV the GEMM-CFC are hypersensitive to erythropoietin [136, 137] and the peripheral blood GEMM CFC are cycling in contrast

to the quiescent behavior of normal circulating GEMM-CFC [127]. Prchal *et al.* cultured haemopoietic colonies from G6PD female heterozygotes and analysed the isoenzyme pattern of individual colonies; these studies revealed that normal progenitor cells were still present in the marrow, though quiescent *in vivo* at the prevailing low erythropoietin levels [138]. Recent studies have suggested that there is a decline in the prevalence of these normal progenitor cells over time with disease progression [139].

Cell culture studies demonstrating increased erythropoietin sensitivity may thus have a diagnostic role in PRV in those few cases without splenomegaly, leucocytosis, or thrombocytosis, in whom a diagnosis of secondary polycythaemia is entertained.

Other chronic myeloproliferative disorders

At the progenitor cell level the myeloproliferative disorders are characterized by their similarities rather than differences, underlying the fact that these are all stem cell diseases. In primary myelofibrosis the marrow progenitor cell numbers are reduced [140], but the GM-CFC levels and BFU-E levels are markedly increased in the peripheral blood [141, 142]. This may occasionally be of diagnostic value, as in other conditions in which there may be a neutrophil leukocytosis, there is usually only a two-to three-fold increase in circulating GM-CFC [143]. As with other myeloproliferative disorders, the colony forming cells have an abnormally low buoyant density [10]. Circulating erythroid progenitor cells from patients with myelofibrosis and myeloid metaplasia may grow in the absence of added erythropoietin [144], as is the case in PRV and at least some cases of CGL [121, 145].

In essential thrombocythaemia the incidence of GM-CFC in the marrow may be normal or raised [130] and the number of circulating progenitor cells is frequently high. The GM-CFC in essential thrombocythaemia are of abnormally low density [130]. Circulating erythroid progenitors may again grow in the absence of added erythropoietin [145] and Reid *et al.* have used this phenomenon to

diagnoses forms frustes of essential thrombocythaemia or similar myeloproliferative diseases in patients with arterial disease and only moderately raised platelet counts, not diagnostic in themselves of a myeloproliferative disease [146].

Aplastic anemia

In most cases of aplastic anaemia, culture studies show a low prevalence of GM-CFC, *BFU-E* and GEMM-CFC [147, 148], which is in accord with the view that most cases of aplastic anaemia are due to an intrinsic stem cell defect. This concept is supported by the reported successes of identical twin bone marrow grafts without preparative immunosuppression [149, 150]. A second possible mechanism of aplastic anaemia is a failure of the supporting microenvironment to provide the appropriate environment for the stem cells. In such circumstances normal *in vitro* progenitor cell growth might occur in the presence of exogenous stimulators but this has only rarely been reported [151]. It should be noted, however, that if accessory cell dysfunction caused a maturation block between the stem cells and the assayable progenitor cells, then such a lesion would not be detectable by present methods. It has been shown by Gordon and Gordon-Smith that the marrow derived fibroblasts from some cases of aplastic anaemia were unable to augment the effect of CSA on normal progenitor cells, as do normal marrow derived fibroblasts, but the significance of this finding is presently not clear [152]. A third possibility is an immune mediated suppression of haematopoiesis. Antibodies reactive with normal GM-CFC are found in the serum of about 25% of patients with aplastic anaemia [153], but it appears that these antibodies are usually alloantibodies induced by transfusion and that true autoantibodies are rarely implicated as the cause of aplastic anaemia. Occasionally drug-induced pancytopenia can be shown by culture studies to be antibody mediated [154].

The possibility of cell mediated suppression of haemopoiesis has attracted much attention. Various groups have shown that the marrow from some cases of aplastic anaemia will grow CFU-GM *in*

vitro after treatment with antithymocyte globulin (ATG) and complement [155-157], and that this may be matched by a clinical response to ATG. Studies from other centres with other batches of ATG have not confirmed these findings [158, 159], which is perhaps not surprising in view of the variable specificity and toxicity of ATG, and the fact that some batches of ATG may stimulate CFU-GM growth independent of any effect on T cells [157]. Experiments in co-culturing blood lymphocytes from patients with aplastic anaemia with normal allogeneic marrow suggested the presence of suppressor T cells in aplastic anaemia [151, 155, 160], but it was later shown that this suppression of allogeneic marrow was frequently due to sensitization to major HLA antigens following blood transfusion [161] or to minor HLA antigens when apparently matched HLA target marrows were used as targets [162]. Furthermore, there is recent evidence to suggest that T cells which can suppress erythropoiesis are genetically restricted, such that suppressor lymphocytes might not be detected in random co-culture experiments [163, 164]. T cell depletion experiments, such as rosetting with sheep red blood cells on the patient's own marrow, overcome these problems, but aspirates from aplastic marrows may yield too few cells for such manipulations. To convincingly demonstrate T cell mediated suppression there should be little growth of haematopoietic colonies from unfractionated patient marrow or blood, there should be a marked increment in colony growth after removing the T cells, and readdition of the T cells should suppress this growth. By this means T cell suppression has been demonstrated from several centres [159, 162].

Bacigalupo *et al.* have provided evidence that the suppression is mediated by lymphocytes with receptors for sheep red blood cells and the Fc portion of IgG [165]. It has also been shown that normal $E^+\gamma Fc^+$ lymphocytes will suppress CFU-GM *in vitro* if first stimulated by mitogens [166, 167], suggesting that the level of T cell activation is crucial to suppression. The data from Torok-Storb demonstrated suppressor T cells in approximately 20% of cases, whereas Bacigalupo *et al.* found suppressor cells in 50%. The reason for this difference is not clear, but whereas Bacigalupo *et al.* studied mar-

row CFU-GM, Torok-Storb *et al.* studied peripheral blood BFU-E, and it is possible that progenitor cell circulation may be grossly disturbed in aplastic anaemia.

In a large study from Bagby and colleagues, cultures were performed on unfractionated and T cell depleted marrow from 234 patients with neutropenia and granulocytic hypoplasia of diverse aetiologies [168]. Inhibitory T cells were found in 16.5% of patients, more commonly those with selective acquired, granulocytic hypoplasia of unknown aetiology or with associated collagen vascular disease. Ninety-five patients had disease severe enough to merit immunosuppressive therapy, 27 of these patients had inhibitory T cells *in vitro* and in 22 of these there was a significant increase in CFU-GM growth when glucocorticoids were added to the cultures. By contrast, glucocorticoids increased colony growth in only 3 of the 68 patients without demonstrable inhibitory T cells. Most interestingly, 21 of 22 patients with steroid sensitive inhibitory T cells responded clinically to high dose prednisone therapy compared to none of those patients without demonstrable *in vitro* steroid enhancement of CFU-GM growth. It must be noted that the demonstration of suppressor cells *in vitro* does not necessarily indicate a primary pathogenetic mechanism; as it has been shown in chickens that bursectomy leads not only to hypogammaglobulinaemia but also to the generation of T cells capable of suppressing immunoglobulin synthesis in syngeneic birds [169]. Nonetheless, even if the suppressor T cells in aplastic anaemia are a secondary phenomena, they might still be involved in the maintenance of the hypoplastic state. In 141 of Bagby's patients, the primary disease was a low grade haemopoietic malignancy (e.g. pre-leukaemia) and the appearance of inhibitory T cells in 18 of these was almost certainly secondary.

It has recently been suggested that the effect of suppressor T cells is mediated via γ interferon [170] which has potent antiproliferative effects *in vitro* [171] and *in vivo* [172]. This may partially explain the phenomenon of post viral aplasia.

In addition to T cell mediated suppression in aplastic anaemia it is possible that natural killer (NK) cells are also involved in some cases, as it has

been shown that such cells can inhibit progenitor cell growth *in vitro* [173]. NK cells are stimulated by interferon and it is possible that NK cells are involved in cases of post viral aplasia. NK cells are not classical T cells as defined by monoclonal antibodies and response to mitogens, but they do express the receptor for sheep red blood cells [174]. It is possible therefore that some of the cases of E $^{+}$ Fc $^{+}$ suppressor cells described by Bacigalupo *et al.* were of the NK lineage.

Pure red cell aplasia

In congenital pure red cell aplasia (Diamond-Blackfan syndrome) erythroid progenitor cells are present in near normal numbers in the marrow at diagnosis, although their response to erythropoietin is blunted [175]. With disease progression progenitor cell numbers fall and this may in part be due to iron overload from repeated transfusions.

Acquired pure red cell aplasia (PRCA) is frequently thought to be immune mediated and many cases are associated with thymomas. Antibody mediated PRCA has been well described. In one case the antibody was apparently directed against erythropoietin [176], but more common is an antibody against erythroid progenitor or precursor cells [177-180]. Antibodies to erythroid progenitors are apparently common in transient erythroblastopenia of childhood [181], though such antibodies may in some cases be alloantibodies in transfused patients. Patients with antibody mediated red cell failure may respond to plasmapheresis [181] or immunosuppressive therapy [179, 182]. Pure red cell aplasia may also be associated with a proliferation of T lymphocytes [164, 183-185]. These patients tend to have near normal progenitor cell numbers which are probably suppressed *in vivo* by their own T cells. To demonstrate suppression by these T lymphocytes, *in vitro* close cell contact is required and plasma clot assays with cells plated at 10 6 cells/ml should be used rather than methylcellulose assays with cells plated at 10 5 /ml. These T cells have receptors for the Fc portion of IgG, stain with OKT3 and OKT8 like antibodies, but not with OKT1 like antibodies. They are frequently HLA-

Dr positive [186]. The nature of these T cell proliferations is uncertain, but it is of interest that many patients with such proliferations are unresponsive to steroids but may respond to therapy with cyclophosphamide or chlorambucil.

It must be concluded that culture studies in red cell aplasia with T cell proliferations do not aid management, but culture studies should be performed wherever antibody mediated PRCA is suspected, prior to embarking upon a course of plasmapheresis.

Neutropenia

Neutropenia is a heterogeneous condition and the culture patterns are understandably varied. In cases with peripheral neutrophil destruction the incidence of GM-CFC tends to be raised, as is the proportion in DNA synthesis [187]. Even in cases in which decreased granulopoiesis is thought to be a factor, normal progenitor cell levels are not infrequently found.

In the congenital neutropenia known as Kostman's syndrome, GM-CFC numbers are usually normal or raised and in other congenital neutropenias progenitor cell numbers may be raised, reduced, or absent [188].

The majority of patients with chronic acquired idiopathic neutropenia have adequate marrow GM-CFC and the mechanism of neutropenia is obscure [185].

Occasional patients with acquired leukopenia have anti-GM-CFC antibodies [153] and there is good evidence, in occasional cases, that these antibodies are responsible for the disease [190]. 'Suppressor T cell' proliferations are found in some other cases of neutropenia, as described under PRCA, and most of these cases have normal progenitor cell growth when the marrow is cultured directly at low plating numbers [191]. The exact role of the T cells is obscure, as direct proof of their suppressor activity is lacking. Some of these patients have rheumatoid arthritis, and other patients with rheumatoid arthritis and neutropenia may also have 'suppressor T cells' without overt T cell proliferations [192, 193]. In some cases at least

these CFC suppressor T cells are also of the OKT8 lineage [194].

In drug-induced neutropenia the GM-CFC numbers are usually reduced if there is granulocytic hypoplasia, rather than the granulocytic hyperplasia that occurs with peripheral granulocyte destruction. In some cases it has been shown that the drug plus patient's acute phase serum will inhibit colony formation whereas drug plus recovery serum will not, presumably implying that a drug antibody complex was involved, at the progenitor cell level, in the generation of the neutropenia [195, 154]. Such studies may be useful in determining which drug is responsible for neutropenia or pancytopenia when the cause is in doubt, and several drugs have been used.

Cryopreservation of bone marrow

Cryopreservation of bone marrow and autologous bone marrow rescue following ablative chemotherapy or irradiation are now practiced in a variety of clinical settings. It is generally accepted that progenitor cell assays must be available at such centres for the continual evaluation of the quality of cryopreservation and thawing procedures. With care, progenitor cell recoveries of 80% can be obtained [196]. However, if the viable cell recovery is good then the progenitor cell yield is invariably excellent, and progenitor cell assays are probably not necessary.

Manipulation of bone marrow prior to transplantation

Bone marrow can be treated to remove T cells in allogeneic marrow transplantation in an attempt to reduce graft versus host disease [197-199] or to remove residual malignant cells in autologous transplants [200]. The reagents used should clearly be screened for reactivity against all haemopoietic progenitor cells prior to clinical trials, and each procedure should be monitored by appropriate culture studies.

The number of cells required for transplantation

has not been defined. In an analysis of 73 cases of aplastic anaemia treated by allogeneic bone marrow transplantation, Storb and colleagues showed that there was a significantly increased risk of graft failure/rejection when less than 3×10^8 nucleated cells/kg were infused [201]. In a smaller study Faille *et al.* found no such relationship between total nucleated cell numbers infused and neutrophil regeneration, but they did find a significant correlation between the latter and the number of GM-CFC/kg injected [202]. It is widely accepted that fewer GM-CFC are required for transplantation in acute leukaemia than in aplastic anaemia, although the precise reasons for this are not clear. Spitzer and colleagues have found a similar correlation between GM-CFC infused and time to haemopoietic regeneration in autologous bone marrow transplants [203], and this has been confirmed by some other groups [204]. We have not observed such a relationship, but this is perhaps not surprising. It is likely that a delay in regeneration would only be seen below a critical number of progenitor cells, and if all patients received greater than this number, no correlation would be expected. Data from animal experiments indicate that the number of progenitor cells required for autologous transplantation is several fold less than for allogeneic transplantation [205]. It is not possible to give absolute progenitor requirements, however, because the colony seeding efficiency varies greatly between different laboratories. Furthermore, it must always be remembered that the GM-CFC number is only an approximate index of the number of stem cells present. This may be quite reliable in normal marrow, but GM-CFC numbers may not reflect stem cell numbers in some cases of heavily treated marrows, as used in autologous marrow transplants. In such cases, CFU-GEMM assays may provide a more accurate assessment of regenerating potential. Conversely, marrow treated *in vitro* with cytotoxic drugs can be rendered devoid of GM-CFC, and yet near normal regeneration can occur [206].

Conclusions

In vitro haematopoietic culture studies have greatly increased our understanding of the regulation of normal haemopoiesis and the biology of many haematological diseases, but they have had little impact on the management of individual patients. Culture studies may provide prognostic information in AML and the 'preleukaemic' states, but this information cannot yet be utilized to plan therapy. In CGL culture studies may predict oncoming blast crisis and this could be an urgent indication for bone marrow transplantation if a donor and the appropriate facilities were immediately available. Growth of myeloid and erythroid progenitor cells may be of diagnostic value in the chronic myeloproliferative diseases, but in the vast majority of cases the diagnosis is apparent from other clinical and laboratory parameters. In aplastic anaemia culture studies have shown that some cases are immune mediated, and the reports that such studies can predict which patients will respond to immunosuppressive therapy clearly generate great interest. In the selective cytopenias culture studies may help to explain the pathogenesis in a given case, but only when an anti-progenitor cell antibody is suspected, and plasmapheresis contemplated, are such investigations essential. It must be concluded that investigation of haemopoietic progenitor cells largely remains a research tool, and only in centres manipulating bone marrow prior to transplantation are culture facilities mandatory.

References

1. Till JE, McCulloch EA: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation Research*, 1961; 14: 213-22.
2. Siminovitch L, McCulloch EA, Till JE: The distribution of colony forming cells among spleen colonies. *J Cell Compar Physiol*, 1963; 62: 327-36.
3. Wu AM, Till JE, Siminovitch L, McCulloch EA: Cytological evidence for a relationship between normal haematopoietic colony forming cells and cells of the lymphoid system. *J Exp Med*, 1963; 127: 455-63.
4. Abramson S, Miller RG, Phillips RA: The identification in adult bone marrow of pluripotent and restricted stem

- cells of the myeloid and lymphoid systems. *J Exp Med*, 1977; 145: 1567-79.
5. Pluznik DH, Sachs L: The cloning of normal mast cells in cell culture. *J Cell Comp Physiol*, 1965; 66: 319-24.
 6. Bradley TR, Metcalf D: The growth of mouse bone marrow cells in vitro. *Aust J Exp Biol Med Sci*, 1966; 44: 287-300.
 7. Pike BL, Robinson WA: Human bone marrow colony growth in agar gel. *J Gell Physiol*, 1970; 76: 77-84.
 8. Johnson GR, Dresch C, Metcalf D: Heterogeneity in human neutrophil macrophage and eosinophil progenitor cells demonstrated by velocity sedimentation. *Blood*, 1977; 50: 823-31.
 9. Tebbi K, Rubin S, Cowan DH, McCulloch EA: A comparison of granulopoiesis in culture from blood and marrow cells of nonleukaemic individuals and patients with acute leukaemia. *Blood*, 1976; 48: 235-43.
 10. Moore MAS, Williams N, Metcalf D: In vitro colony formation by normal and leukaemic human haematopoietic cells: characterization of the colony forming cells. *J Natl Cancer Inst*, 1973; 50: 603-23.
 11. Dao C, Metcalf D, Bilski Pasquier G: Eosinophil and neutrophil colony forming cells in culture. *Blood*, 1977; 50: 833-39.
 12. Chervenick PA, Boggs DR: In vitro growth of granulocytic and mononuclear cell colonies from blood of normal individuals. *Blood*, 1971; 37: 131-35.
 13. Dao C, Metcalf D, Zultoun R, Bilski Pasquier G: Normal human bone marrow cultures in vitro: cellular composition and maturation of granulocytic colonies. *Br J Haematol*, 1977; 37: 127-36.
 14. Dresch C, Johnson GR, Metcalf D: Eosinophil colony formation in semi-solid cultures of human bone marrow cells. *Blood*, 1977; 49: 835-44.
 15. Moore MAS, Williams N: Physical separation of colony stimulating cells from in vitro colony forming cells in haematopoietic tissue. *J Cell Physiol*, 1972; 80: 195-206.
 16. Kurland JI, Broxmeyer HE, Pelus LM, Bockman RS, Moore MAS: Role of monocyte-macrophage derived colony stimulating factor and prostaglandin E in the positive and negative feedback control of myeloid stem cell proliferation. *Blood*, 1978; 52: 388-97.
 17. Broxmeyer HE, Bognacki J, Domer MH, Lu L, Castro-Malaspina H: Monocyte macrophage derived acidic iso-ferritins: normal feedback regulators of granulocyte-macrophage progenitor cells in vitro. *Blood*, 1982; 60: 595-607.
 18. Cline MJ, Golde DW: Production of colony stimulating activity by human lymphocytes. *Nature*, 1974; 248: 703-4.
 19. Parker JW, Metcalf D: Production of colony stimulating factor in mitogen stimulated lymphocyte cultures. *J Immunol*, 1974; 112: 502-10.
 20. Broxmeyer HE, Juliano L, Lu L, Dupont B: Release of monocyte derived acidic iso-ferritin inhibitory against myeloid haematopoietic progenitor cells is controlled by HLA-Dr restricted lymphocyte-monocyte interactions. *J Exp Med* (in press).
 21. Broxmeyer HE, Moore MAS, Ralph P: Cell free granulocyte colony inhibiting activity derived from human polymorphonutrophils. *Exp Haematol*, 1977; 5: 87-94.
 22. Broxmeyer HE, Smithyman A, Eger RR, Meyers A, De-Sousa M: Identification of lactoferrin as the granulocyte derived inhibitor of colony stimulating activity production. *J Exp Med*, 1978; 148: 1052-65.
 23. Bagby GC, Vasliki D, Rigas VD, Bennett RM, Vandembark AA, Garewal HS: Interaction of lactoferrin monocytes and T lymphocyte subsets in the regulation of steady state granulopoiesis in vitro. *J Clin Invest*, 1981; 63: 56-63.
 24. Moore MAS: Humoral regulation of granulopoiesis. *Clinics Haematol*, 1979; 8: 287-309.
 25. Broxmeyer HE: Inhibition in vivo of mouse granulopoiesis by cell free activity derived from human polymorphonuclear neutrophils. *Blood*, 1978; 51: 889-898.
 26. Winton EF, Kincade JM, Vegter WP, Parker MB, Barnes KC: In vitro studies of lactoferrin and murine granulopoiesis. *Blood*, 1981; 57: 574-78.
 27. Bagby GC, Bennett RM: Feedback regulation of granulopoiesis: polymerization of lactoferrin abrogates its ability to inhibit CSA production. *Blood*, 1982; 60: 108-12.
 28. Burgess AW, Wilson WMA, Metcalf D: Stimulation by human placental conditioned medium of haemopoietic colony formation by human marrow cells. *Blood*, 1977; 49: 573.
 29. Persio JF, Brennan JK, Lichtman MA, Speiser BL: Human cell lines that elaborate colony stimulating activity for the marrow cells of man and other species. *Blood*, 1973; 51: 507-19.
 30. Nicola NA, Metcalf D, Johnson Gr, Burgess AW: Separation of functionally distinct human granulocyte-macrophage colony stimulating factors. *Blood*, 1979; 54: 614-27.
 31. Tepperman AD, Curtis JE, McCulloch EA: Erythropoietic colonies in cultures of human marrow. *Blood*, 1974; 44: 659-69.
 32. Iscove NN, Sieber F, Winterhalter KH: Erythroid colony formation in cultures of mouse and human bone marrow: analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on agarose concanavalin A. *J Cell Physiol*, 1974; 83: 309-20.
 33. Axelrad A, McLeod DL, Shreeve MM, Heath DA: In: Robinson WA (ed), *Haemopoiesis in Culture*. DHEW Publication NIH 74-205, Washington, U.S. Government Printing Office, 1974, pp 226-34.
 34. Iscove NN: The role of erythropoietin in regulation of population size and cell cycling of early and late erythroid precursors in mouse bone marrow. *Cell Tiss Kin*, 1977; 10: 323-34.
 35. Udupa KG, Reissman KR: In vivo erythropoietin requirements of regenerating erythroid progenitors (BFU-E and CFU-E) in bone marrow of mice. *Blood*, 1979; 53: 1164-69.
 36. Prchal JF, Adamson JW, Steinman L, Fialkow PJ: Human erythroid colony formation *in vitro*: evidence for clonal origin. *J Cell Physiol*, 1976; 89: 489-92.

37. Reid CDL, Baptista LC, Deacon R, Chanarin I: Megaloblastic change is a feature of colonies derived from an early erythroid progenitor (BFU-E) stimulated by monocytes in culture. *Br J Haematol*, 1981; 49: 551–61.
38. Gregory CJ, Eaves AC: Human marrow cells capable of erythropoietic differentiation in vitro: Definition of three erythroid colony responses. *Blood*, 1977; 49: 855–64.
39. Iscove NN: Erythropoietin independent stimulation of early erythropoiesis in adult marrow cultures by conditioned media from lectin stimulated mouse spleen cells. ICN-UCLA Symposium on Haemopoietic Cell Differentiation. In: Golde DW, Cline MJ, Metcalf D, Fox CF (eds), *Molecular and Cell Biology*. Academic Press, New York, 1978, pp. 37–82.
40. Dessimis EN, Drantz SB: Effect of pure erythropoietin on DNA synthesis by human marrow day 15 erythroid burst forming units in short term liquid culture. *Br J Haematol*, 1984; 56: 295–306.
41. Porter PN, Ogawa M: Characterization of human erythroid burst promoting activity derived from bone marrow conditioned media. *Blood*, 1982; 59: 1207–12.
42. Westbrook C, Gason J, Selsted M, Golde DW: Purification of a human T lymphocyte derived burst promoting activity. *Blood*, 1983; 62: 417 (suppl. 1, abstract).
43. Eliason JF, Dexter TM, Testa NG: The regulation of haemopoiesis in long term bone marrow cultures. III The role of burst forming activity. *Exp Haematol*, 1982; 10: 444–50.
44. Niskanen E, Oki A, Cline MJ, Golde DW: Human T lymphocytes products stimulate human hematopoietic progenitor cell proliferation in diffusion chambers *in vivo*. *Blood*, 1982; 60: 368–72.
45. Nathan DG, Chess L, Hillman DG, Clarke B, Breard J, Merler E, Housman D: Human erythroid burst forming unit: T cell requirement for proliferation in vitro. *J Exp Med*, 1973; 147: 324–29.
46. Mangan KF, Desforges JF: The role of T lymphocytes and monocytes in the regulation of human erythropoietic peripheral blood burst forming units. *Exp Haematol*, 1980; 8: 717–27.
47. Torok-Storb BJ, Martin PJ, Hansen JA: Regulation of in vitro erythropoiesis by normal T cells: Evidence for two T cell subsets with opposing function. *Blood* 58: 171–74.
48. Eaves CJ, Eaves AC: Erythropoietin dose response curves for three classes of erythroid progenitors in normal human marrow and in patients with polycythaemia vera. *Blood*, 1978; 52: 1196–1210.
49. Murphy MJ, Urabe A: Modulatory effect of macrophages on erythropoiesis. In: Murphy MJ (ed), *In vitro Aspects of Erythropoiesis*. Springer, Berlin, 1978: pp. 189–95.
50. Gordon LI, Miller WJ, Branda RF, Zanjani ED, Jacob HS: Regulation of erythroid colony formation by bone marrow macrophages. *Blood*, 1980; 55: 1047–50.
51. Kurland JI, Meyers PA, Moore MAS: Synthesis and release of erythroid colony burst-potentiating activities by purified populations of murine peritoneal macrophages. *J Exp Med*, 1980; 151: 839–52.
52. Zuckerman KS: Human erythroid burst forming units. Growth in vitro is dependent on monocytes but not T lymphocytes. *J Clin Invest*, 1981; 67: 702–9.
53. Reid CDL, Baptista LC, Chanarin I: Erythroid colony growth in vitro from human peripheral blood null cells: Evidence for regulation by T lymphocytes and monocytes. *Br J Haematol*, 1981; 48: 155–64.
54. Linch DC, Nathan DG: T cell and monocyte-derived burst promoting activity directly act on erythroid progenitor cells. *Nature*, 1984; 312: 775–777.
55. Lipton JM, Reinherz EL, Kudisch M, Jackson PL, Schlossman SF, Nathan DG: Mature bone marrow erythroid burst-forming units do not require T cells for induction of erythropoietin dependent differentiation. *J Exp Med*, 1980; 152: 350–60.
56. Linch DC, Boyle D, Beverley PCL: T cell and monocyte requirements for erythropoiesis. *Acta Haematol*, 1982, 67: 324–28.
57. Meytes D, Ma A, Ortega JA, Shore NA, Dukes PP: Human erythroid burst promoting activity produced by PHA stimulated radioresistant peripheral blood mononuclear cells. *Blood*, 1979; 54: 1050–57.
58. Metcalf D, McDonald HR, Odartchanko N, Sardat B: Growth of mouse megakaryocyte colonies in vitro. *Proc Natl Acad Sci*, 1975; 72: 1744–48.
59. Vainchenker W, Bouget J, Guichard J, Breton Goriou J: Megakaryocyte colony formation from human bone marrow precursors. *Blood*, 1979; 54: 940–45.
60. Mazur EM, Hoffman R, Chasis J, Marchesi S, Bruno E: Immunofluorescent identification of human megakaryocyte colonies using an antiplatelet glycoprotein anti-serum. *Blood*, 1981; 57: 277–86.
61. Vainchenker W, Reschamps JF, Bastin JM, Guichard J, Titeux M, Breton-Goriou J, McMichael AJ: Two monoclonal antiplatelet antibodies as markers of human megakaryocyte maturation: Immunofluorescent staining and platelet peroxidase detection in megakaryocyte colonies and in vivo cells from normal and leukaemic patients. *Blood*, 1982; 59: 514–21.
62. Williams N, Levine RF: The origin development and regulation of megakaryocytes. *Br J Haematol*, 1982; 52: 173–80.
63. Hoffman R, Mazur E, Bruno E, Floyd V: Assay of an activity in the serum of patients with disorders of thrombopoiesis that stimulates formation of megakaryocyte colonies. *N Engl J Med*, 1981; 305: 533–38.
64. Gordon MY: Quantitation of haemopoietic cells from normal and leukaemic RFM mice using an in vivo colony assay. *Br J Cancer*, 1974; 30: 421–27.
65. Jacobson N: Chamber centrifugation: A harvesting technique for estimation of the growth of human haemopoietic cells in diffusion chambers. *Br J Haematol*, 1975; 20: 171–78.
66. Shulman LN, Robinson SH: Maintenance of haemopoietic stem cells in diffusion chamber cultures. *J Lab Clin Med*, 1977; 90: 581–88.

67. Hoelzer D, Harriss EP, Slack M, Kurble E: Growth of in vitro colony forming cells from normal human peripheral blood leukocytes cultured in diffusion chambers. *J Cell Physiol*, 1976; 89: 89–100.
68. Steinberg HN, Handler ES, Handler EE: Assessment of erythrocyte colony formation in an in vivo plasma clot diffusion chamber system. *Blood*, 1976; 47: 1041–48.
69. Jacobsen N, Broxmeyer HE, Grossbard E, Moore MAS: Colony forming units in diffusion chamber (CFU-d) and colony forming units in agar culture (CFU-C) obtained from normal human bone marrow: a possible parent progeny relationship. *Cell Tiss Kinet*, 1979; 12: 213–26.
70. MacVittie T, McCarthy KF: The influence of a granulocyte inhibitor on haematopoiesis in an in vivo culture system. *Cell Tiss Kinet*, 1975; 8: 553–59.
71. Gerard E, Carsten AL, Cronkite EP: The proliferative potential of plasma clot erythroid colony forming cells in diffusion chambers. *Blood Cells*, 1978; 4: 105–28.
72. Dexter TM, Allen TD, Lajtha LG: Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J Cell Physiol*, 1977; 91: 335–44.
73. Trentin JJ: Haemopoietic microenvironments. *Transplant Proc*, 1976; 10: 77–82.
74. Dexter TM: Cell interactions in vitro. *Clinic haematol*, 1979; 8: 453–68.
75. Moore MAS, Sheridan AP: Pluripotential stem cell replication in continuous human, prosimian and murine bone marrow culture. *Blood Cells*, 1979; 5: 297–311.
76. Gartner S, Kaplan HS: Long term culture of human bone marrow cells. *Proc Natl Acad Sci USA*, 1980; 77: 4756–59.
77. Moore MAS, Broxmeyer HE, Sheridan APC, Meyers PA, Jacobsen N, Winchester RJ: Continuous human bone marrow culture: Ia antigen characterization of probable pluripotent stem cells. *Blood*, 1980; 55: 682–90.
78. Iscove NN, Messner H, Till JE, McCulloch EA: Human marrow cells forming colonies in culture: analysis by velocity sedimentation and suspension culture. *Ser Haematol*, 1972; 5: 37–45.
79. Johnson GR, Metcalf D: Pure and mixed erythroid colony formation in vitro stimulated by spleen conditioned medium with no detectable erythropoietin. *Proc Natl Acad Sci USA*, 1977; 74: 3879–82.
80. Johnson GR: Haemoglobin stem cells in vitro. In: Stamatoyannopoulos G, Nienhuis AW (eds), *Haemoglobins in Development and Differentiation*. Alan Liss Inc., New York, 1981: pp. 23–34.
81. Fausser AA, Messner HA: Granuloerythropoietic colonies in human bone marrow, peripheral blood and cord blood. *Blood*, 1978; 52: 1243–46.
82. Messner HA, Izquierde CA, Jamal N: Identification of T lymphocytes in human mixed haematopoietic colonies. *Blood*, 1981; 58: 402–5.
83. Messner HA, Fausser AA, Buick R, Chang LJ-A, Lepine J, Curtis JC, Senn J, McCulloch EA: Assessment of human pluripotent haemopoietic progenitors and leukaemic blast forming cells in culture. *Haematol Blood Transfus*, 1981; 26: 246–47.
84. Rozenszjan LA, Shohan D, Kalechman I: Clonal proliferation of PHA stimulated human lymphocytes. *Immunology*, 1975; 29: 1041–45.
85. Bobak D, Whisler R: Human B lymphocyte colony responses. General characteristics and modulation by monocytes. *J Immunol*, 1980; 125: 2764–69.
86. Triebel F, Robinson WA, Hayward AR, Goube de Laforest P: Existence of a pool of T lymphocyte colony forming cells (T-CFC) in human bone marrow and their place in the differentiation of the T lymphocyte lineage. *Blood*, 1981, 58: 911–15.
87. Broxmeyer HE, Jacobsen N, Kurland J, Mendelsohn N, Moore MAS: In vitro suppression of normal granulocyte stem cells by inhibitory activity derived from leukaemic cells. *J Natl Cancer Ins*, 1978; 60: 497–502.
88. Broxmeyer HE, Bognacki J, Dorner MH, De Sousa M: Identification of leukaemia associated inhibitory activity as acidic isoferritins. A regulatory role for acidic isoferritins in the production of granulocytes and monocytes. *J Exp Med*, 1981; 153: 1426–31.
89. Moore MAS, Spitzer G, Williams N, Metcalf D, Buckley J: Agar culture studies in 127 cases of untreated acute leukaemia: the prognostic value of reclassification of leukaemia according to in vitro growth characteristics. *Blood*, 1974; 44: 1–18.
90. Spitzer G, Dicke KA, Gehan EA, Smith T, McCredie K, Barlogie B, Freireich E: A simplified in vitro classification for prognosis in acute leukaemia. *Blood*, 1976; 48: 795–807.
91. Vincet PC, Sutherland R, Bradley M, Lind D, Gunz F: Marrow culture studies in acute leukaemia at presentation and during remission. *Blood*, 1977; 49: 903–12.
92. Beran M, Reizenstein P, Uden A: Response to treatment in acute non lymphocytic leukaemia: Prognostic value of colony forming and colony stimulating capacities of bone marrow and blood cells compared to other parameters. *Br J Haematol*, 1980; 44: 39–50.
93. Francis GE, Tuma GA, Berney JJ, Hoffbrand AV: Sensitivity of acute myeloid leukaemia cells to colony stimulating activity: relation of response to chemotherapy. *Br J Haematol*, 1981; 49: 259–67.
94. Kirschner JJ, Goldberg J, Nelson DA, Gottlieb AJ: Predictive value of the CFU-C assay in acute nonlymphocytic leukaemia. *Am J Med*, 1982; 72: 615–19.
95. Greenberg PL, Mara B, Heller P: Marrow adherent cell colony stimulating activity production in acute myeloid leukaemia. *Blood*, 1978; 52: 362–78.
96. Buick RN, Till JE, McCulloch EA: Colony assay for proliferative blast cells circulating in myeloblastic leukaemia. *Lancet*, 1977; i: 862–63.
97. Swart K, Hagemeijer A, Lowenberg B: Acute myeloid leukaemia colony growth in vitro: differences of colony forming cells in PHA supplemented and standard leukocyte feeder cultures. *Blood*, 1982; 59: 816–21.
98. Buick RN, Chang LJ-A, Messner HA, Curtis JE, McCulloch EA: Self renewal capacity of leukaemic blast

- progenitor cells. *Cancer Res*, 1981; 41: 4849-52.
99. Griffin JD, Mayer RJ, Weinstein HJ, Rosenthal DS, Coral FS, Beveridge RP, Schlossman SF: Surface marker analysis of acute myeloblastic leukaemia: identification of differentiation associated phenotypes. *Blood*, 1983; 62: 557-63.
100. Griffin JD, Linch DC, Schlossman SF: A monoclonal antibody reactive with normal and leukaemic human myeloid progenitor cells. *Leukaemia Research* (in press).
101. Lange B, Ferrero D, Pessano S, Hubbell H, Palumbo A, Lai SK, Rovera G: Discrimination between normal haemopoietic stem cells and myeloid leukaemia cells using monoclonal antibodies. In: Lowenberg B, Hagenbeck A (eds), *Minimal Residual Disease in Acute Leukaemia*. Martinus Nijhoff Publishers, Boston, 1984: pp. 55-66.
102. Wouters R, Lowenberg B: On the maturation order of AML cells: a distinction on the basis of self renewal properties and immunological phenotypes. *Blood*, 1984; 63: 634-89.
103. Fialkow PJ, Singer JW, Adamson JW: Acute non-lymphocytic leukaemia: heterogeneity of stem cell origin. *Blood*, 1981; 57: 1068-73.
104. Moore MAS, Broxmeyer HE, Sheridan APC, Meyers PA, Jacobsen N, Winchester RJ: Continuous human bone marrow culture: Ia characterization of probably pluripotential stem cells. *Blood*, 1980; 55: 682-90.
105. Douay L, Gorin NC, Laporte JP, Najman A, Duhamel G: Study of ASTA Z 7557 effects on probable human pluripotential stem cells in long term marrow cultures: application to autologous bone marrow transplantation (Abstract). *Exp Haematol* (in press).
106. Anderson WF, Beckman B, Beltran G, Fisher JW, Shicke WJ: Erythropoietin independent erythroid colony formation in patients with erythroleukaemia and related disorders. *Br J Haematol*, 1982; 52: 311-17.
107. Smith SD, Vyeki EM, Lowman JT: Colony formation in vitro by leukaemic cells in acute lymphoblastic leukaemia. *Blood*, 1978; 52: 712-19.
108. Izaguirre CA, Curtis J, Messner H, McCulloch EA: A colony assay for blast cell progenitors in non-B non-T (common) acute lymphoblastic leukaemia. *Blood*, 1981; 57: 823-29.
109. Senn JS, Pinkerton PH: Defective in vitro colony formation by human bone marrow preceding overt leukaemia. *Br J Haematol*, 1972; 23: 277-82.
110. Greenberg PL, Mara B: The preleukaemic syndrome: Correlation of in vitro parameters of granulopoiesis with clinical features. *Am J Med*, 1979; 66: 951-58.
111. Berthier R, Douady F, Metral J, Newton I, Schweitzer A, Hollard D: In vitro granulopoiesis in oligoblastic leukaemia: Prognostic value characterization and serial cloning of bone marrow colony and cluster forming cells in agar culture. *Biomedicine*, 1979; 30: 931-37.
112. Vermer D, Spitzer G, Dicke K, McCredie K: In vitro agar culture patterns in preleukaemia and their clinical significance. *Leuk Res*, 1979; 3: 42-49.
113. Lidbeck J: In vitro colony and cluster growth in haematopoietic dysplasia (the preleukaemic syndrome): Clinical correlations. *Scand J Haematol*, 1980; 24: 412-20.
114. Lidbeck J: In vitro colony and cluster growth in haematopoietic dysplasia (the Preleukemic syndrome); Identification of a maturation defect in agar cultures. *Scand J Haematol*, 1980; 25: 113-23.
115. Elias L, Greenberg P: Divergent patterns of marrow cell suspension culture growth in the myeloid leukaemias: correlation of in vitro findings with clinical features. *Blood*, 1977; 50: 263-74.
116. Koeffler HP, Golde DW: Cellular maturation in human preleukaemia. *Blood*, 1978; 52: 355-61.
117. Milner RG, Testa NG, Geary GC, Dexter TM, Muldal S, Mac Iver JE, Lajtha LG: Bone marrow culture studies in refractory cytopenia and smouldering leukaemia. *Br J Haematol*, 1977; 35: 251-61.
118. Koeffler HP, Cline MJ, Golde DW: Erythropoiesis in preleukaemia. *Blood*, 1978; 51: 1013-14.
119. Senn JS, Messner HA, Pinkerton PH, Chang L, Nitsch B, McCulloch EA: Peripheral blood blast cell progenitors in human preleukaemia. *Blood*, 1982; 59: 106-9.
120. Goldman JM, Th'ng KH, Lowenthal RM: In vitro colony forming cells and colony stimulating factor in chronic granulocytic leukaemia. *Br J Cancer*, 1974; 30: 1-12.
121. Eaves AC, Henkelman DH, Eaves CJ: Abnormal erythropoiesis in the myeloproliferative disorders: an analysis of underlying cellular and humoral mechanisms. *Exp Haematol*, 1980; 8: 235-47 (suppl. 8).
122. Dube ID, Eaves AC, Eaves CJ: A new technique for the cytogenetic analysis of cells from single haemopoietic colonies of bone marrow or peripheral blood origin. *Am Soc Human Gen 31st annual Meeting (Abs)*, 1979.
123. Coulombel L, Kalouset DK, Eaves CJ, Gupta CM, Eaves AC: Long term marrow culture reveals chromosomally normal hematopoietic progenitor cells in patients with Ph⁺ chronic myelogenous leukaemia. *N Engl J Med* 1983, 1493-1497.
124. Moriyama Y: Circulating erythropoietic precursors in patients with aplastic anaemia and chronic granulocytic leukaemia. *Acta Haematologic Japonica*, 1978; 41: 1381-83.
125. Hara H, Kai S, Fushimi M, Taniwaki S, Ifuku H, Okamoto T, Ohe Y, Fujita S, Noguchi K, Kanamara A, Nagai K, Inada E: Pluripotent erythrocytic and granulocytic haemopoietic precursor cells in chronic granulocytic leukaemia. *Exp Haematol*, 1981; 9: 871-77.
126. Goldman JM, Shiota F, Th'ng KH, Orchard KH: Circulating granulocytic and erythroid progenitor cells in chronic granulocytic leukaemia. *Br J Haematol*, 1980; 46: 7-13.
127. Rhodes CA, Robinson WA, Entringer MA: Granulocyte colony formation in chronic granulocytic leukaemia during stable accelerated and blastic disease. *Proc Soc Exp Biol Med*, 1978; 157: 337-41.
128. Goldberg J, Tice DG, Gottlieb AJ, Nelson DA: Blood colony and cluster forming activity during various stages of chronic granulocytic leukaemia. *Am J Clin Path*, 1980; 74: 771-76.

129. Mladenovic J, Adamson JW: Characteristics of circulating erythroid colony forming cells in normal and polycythaemic man. *Br J Haematol*, 1982; 51: 377-84.
130. Greenberg P, Mara B, Bax I, Brossel R, Schrier S: The myeloproliferative disorders. Correlation between clinical evolution and alterations of granulopoiesis. *Am J Med*, 1976; 61: 878-91.
131. Singer JW, Adamson JW, Ernst C, Lin N, Steinmann L, Murphy S, Fialkow PJ: Polycythaemia vera: physical separation of normal and neoplastic committed granulocyte-macrophage progenitors. *J Clin Invest*, 1980; 66: 730-35.
132. Prchal JF, Axelrad AA: Bone marrow responses in polycythaemia vera. *N Engl J Med*, 1974; 290: 1382-85.
133. Zanjani ED, Lutton JD, Hoffman R, Wasserman LR: Erythroid colony formation: dependence on erythropoietin. *J Clin Invest*, 1977; 59: 841-46.
134. Casadevaal N, Vainchenker W, Lacombe C, Vina G, Chapman J, Breton Gorijs J, Varet B: Erythroid progenitors in polycythaemia vera: demonstration of their hypersensitivity to erythropoietin using serum free cultures. *Blood*, 1982; 59: 447-51.
135. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L: Polycythaemia vera: stem cell and probable clonal origin of the disease. *N Engl J Med*, 1976; 295: 913-16.
136. Fauser AA, Messner HA: Pluripotent hemopoietic progenitors (CFU-GEMM) in polycythaemia vera: analysis of erythropoietin requirement and proliferative activity. *Blood*, 1981; 58: 1224-27.
137. Ash RC, Derrick RA, Zanjani ED: In vitro studies of human pluripotential haematopoietic progenitors in polycythaemia vera. *J Clin Invest*, 1982; 69: 1112-18.
138. Prchal JF, Adamson JW, Murphy S, Steinmann L, Fialkow PJ: Polycythaemia vera. The in vitro response of normal and abnormal stem cell lines to erythropoietin. *J Clin Invest*, 1978; 61: 1044-47.
139. Adamson JW, Singer JW, Catalano P, Murphy S, Lin N, Steinmann L, Connie E, Fialkow PJ: Polycythaemia Vera. Further in vitro studies of haemopoietic regulation. *J Clin Invest*, 1980; 66: 1363-68.
140. Rickard KA, Brown RD, Wilkinson T, Kronenberg H: The colony forming cell in the myeloproliferative disorders and aplastic anaemia. *Scand J Haematol*, 1979; 22: 121-28.
141. Chervenick P: Increase in circulating stem cells in patients with myelofibrosis. *Blood*, 1973; 41: 67-71.
142. Ohl S, Carsten AL, Chanana AD, Chikkappa G, Cronkite EP: Increased erythrocytic and neutrophilic progenitors in myelofibrosis with myeloid metaplasia. *Eur J Cancer*, 1976; 12: 131-35.
143. Morra L, Ponassi A, Gigli G, Vercelli M, Sacchetti C: Blood colony forming cells and leucocyte colony stimulating activity in patients with neutrophilic leucocytosis. *Scand J Haematol*, 1979; 22: 311-19.
144. Lutton JD, Levere RD: Endogenous erythroid colony formation by peripheral blood mononuclear cells from patients with myelofibrosis and polycythaemia vera. *Acta Haematol*, 1979; 62: 94-99.
145. Prchal JF, Axelrad AA, Crookston JH: Erythroid colony formation in plasma culture from cells of peripheral blood in myeloproliferative disorders. *Blood*, 1974; 44: 912-19.
146. Reid CD, Chanarin I, Lewis J: Formes frustes in myeloproliferative disorders. Identification by the growth of an endogenous erythroid clone in vitro in patients with arterial vascular disease. *Lancet*, 1982; i: 14-15.
147. Kurnick JE, Robinson WA, Dickey CA: In vitro granulocytic colony forming potential of bone marrow from patients with granulocytopenia and aplastic anaemia. *Proc Soc Exp Biol Med*, 1971; 137: 917-20.
148. Hara H, Kai S, Fushimi M, Taniwaki S, Okamoto T, Ohe Y, Fujita S, Noguchi K, Senba M, Hamano T, Kanamaru A, Nagai K: Pluripotent hemopoietic precursors in vitro (CFU-M) in aplastic anaemia. *Exp Haematol*, 1980; 8: 1165-71.
149. Robins MM, Noyes WD: Aplastic anaemia treated with bone marrow transfusion from an identical twin. *N Engl J Med*, 1961; 265: 974-76.
150. Pillow RP, Epstein RB, Buckner CD, Giblett ER, Thomas ED: Treatment of bone marrow failure by isogenic marrow infusion. *N Engl J Med*, 1966; 275: 94-97.
151. Kagan WA, Ascensao JL, Fialk MA, Coleman M, Valera EB, Good RA: Studies on the pathogenesis of aplastic anaemia. *Am J Med*, 1979; 66: 444-49.
152. Gordon MY, Gordon Smith EC: Bone marrow fibroblast function in relation to granulopoiesis in aplastic anaemia. *Br J Haematol*, 1983; 53: 483-90.
153. Fitchen JH, Cline MJ: Serum inhibitors of myelopoiesis. *Br J Haematol*, 1980; 44: 7-16.
154. Kelton JG, Huang AT, Mold N, Logue G, Rosse WF: The use of in vitro techniques to study drug induced pan-cytopenia. *N Engl J Med*, 1979; 301: 621-24.
155. Ascensao JA, Pahwa R, Kagan W, Hansen J, Moore M, Good R: Aplastic anaemia: evidence for an immunological mechanism. *Lancet*, 1976; 1: 669-71.
156. Amare M, Abdou NL, Robinson MG, Abdou NI: Aplastic anaemia associated with bone marrow suppressor T cell hyperactivity: successful treatment with anti-thymocyte globulin. *Am J Haematol*, 1978; 5: 25-32.
157. Faille A, Barrett AJ, Balirand N, Ketels F, Gluckman E, Najaen Y: Effect of anti-lymphocyte globulin on granulocyte precursors in aplastic anaemia. *Br J Haematol*, 1979; 42: 371-80.
158. Sullivan R, Queensberry PJ, Parkman R, Zuckerman KS, Levey RH, Rappaport J, Ryan M: Aplastic anaemia: lack of inhibitory effect of bone marrow lymphocytes on in vitro granulopoiesis. *Blood*, 1980; 56: 625-32.
159. Bacigalupo A, Podesta M, van Lint MT, Vumercati R, Carri R, Rossi E, Rosso M, Carella A, Santini G, Damasio E, Giordano D, Marmont AM: Severe aplastic anaemia: correlation of in vitro tests with clinical response to immunosuppression in 20 cases. *Br J Haematol*, 1981; 47: 423-33.
160. Hoffman R, Zanjani ED, Lutton JD, Zalusky R, Wasserman LR: Suppression of erythroid colony formation by

- lymphocytes from patients with aplastic anaemia. *N Engl J Med*, 1977; 296: 10-13.
161. Singer JW, Brown JE, James MC, Doney K, Warren RP, Storb R, Thomas ED: Effect of peripheral blood lymphocytes from patients with aplastic anaemia on granulocyte colony growth from HLA matched and mismatched marrows: effect of transfusion sensitization. *Blood*, 1978; 52: 37-46.
 162. Torok-Storb BJ, Sieff C, Storb R, Adamson J, Thomas ED: In vitro tests for distinguishing possible immune mediated aplastic anaemia from transfusion induced sensitization. *Blood*, 1980; 55: 211-15.
 163. Torok-Storb BJ, Hansen JA: Modulation of in vitro BFU-E growth by normal Ia-positive T cells is restricted by HLA-Dr. *Nature*, 1982; 298: 473-74.
 164. Lipton JM, Nadler LM, Canellos GP, Kudisch M, Reiss C, Nathan DG: Evidence for genetic restriction in the suppression of erythropoiesis by a unique subset of T lymphocytes in man. *J Clin Invest*, 1983; 72: 694-706.
 165. Bacigalupo A, Podesta M, Mingari MC, Moretta L, van Lint MT, Marmont A: Immune suppression of haemopoiesis in aplastic anaemia: activity of T-gamma lymphocytes. *J Immunol*, 1980; 125: 1449-53.
 166. Bacigalupo A, Podesta M, Mingari MC, Moretta L, Piaggio G, van Lint MT, Durando A, Marmont AM: Generation of CFU-C suppressor T cells in vitro: an experimental model for immune mediated marrow failure. *Blood*, 1981; 57: 491-96.
 167. Ascenso JL, Kay NE, Banisadre M, Zanjani ED: Cell-cell interaction in human granulopoiesis: role of T lymphocytes. *Exp Haematol*, 1981; 9: 473-78.
 168. Bagby GC, Lawrence HJ, Neerhout RC: T-lymphocyte mediated granulopoietic failure. *N Engl J Med*, 1983; 309: 1073-78.
 169. Blaese RM, Weiden PL, Koski I, Dooley N: Infections agammaglobulinaemia: transmission of immunodeficiency with grafts of agammaglobulinaemic cells. *J Exp Med*, 1974; 140: 1097-1101.
 170. Zoumbos NC, Gascon P, Djeu JY, Young NS: Interferon is a mediator of haematopoietic suppression in aplastic anaemia in vitro and possibly in vivo. *Proc. Natl. Acad. Sci., USA*, 1985; 82: 188-192.
 171. Verma DS, Spitzer G, Guterman JU, McCredie KB, Diebe KA: Human leukocyte interferon preparation blocks granulopoietic differentiation. *Blood*, 1979; 54: 1423-1427.
 172. Guterman JD, Fines S, Quesda J, Horning SJ, Levine JF, Alexanian R, Barnhardt L, Kramer M, Spiegel H, Colburn W, Trown P, Merigan T, Dziewanowski Z: Recombinant leucocyte A interferon: pharmacokinetics, single dose tolerance, and biological effects in cancer patients. *Ann. Int. Med.*, 1982; 96: 549-556.
 173. Hansson M, Beran M, Andersson B, Kiessling R: Inhibition of in vitro granulopoiesis by autologous allogeneic human NK cells. *J Immunol*, 1982; 129: 126-32.
 174. Beverly PCL, Callard RE: Distinctive functional characteristics of human T lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. *Eur J Immunol*, 1981; 11: 329-34.
 175. Lipton JM, Nathan DG, Kudisch M: Diamond-Blackfan anaemia: heterogeneity of the block in erythroid progenitor maturation (Abst) *Ped Res*, 1982; 16: 780 (suppl.)
 176. Peschle C, Marmont AM, Marone G, Genovese A, Sasso G, Condorelli M: Pure red cell aplasia: studies on an IgG serum inhibitor neutralizing erythropoietin. *Br J Haematol*, 1975; 30: 411-17.
 177. Krantz SB, Mooe WH, Zaentz SD: Studies on red cell aplasia. V Presence of erythroblast activity in YG globulin fraction of plasma. *J Clin Invest*, 1973; 52: 324-26.
 178. Browman GP, Freedman MH, Blajchman MA, McBride JA: A complement independent erythropoietic inhibitor acting on the progenitor cell in refractory anaemia. *Am J Med*, 1976; 61: 572-78.
 179. Marmont A, Peschle C, Sanguineti M, Condorelli M: Pure red cell aplasia: response of three patients to cyclophosphamide and/or anti-lymphocyte globulin and demonstration of two types of serum IgG inhibitors to erythropoiesis. *Blood*, 1975; 45: 247-61.
 180. Koenig HM, Lightsey AL, Nelson DP, Diamond LK: Immune suppression of erythropoiesis in transient erythroblastopenia of childhood. *Blood*, 1979; 54: 742-46.
 181. Messner HA, Fauser AA, Curtis JE, Dolten D: Control of antibody mediated pure red cell aplasia by plasmapheresis. *N Engl J Med*, 1981; 304: 1334-38.
 182. Krantz SP, Kao V: Studies on red cell aplasia II. Report of a second patient with an antibody to erythroblast nuclei and a remission after immunosuppressive therapy. *Blood*, 1969; 34: 1-13.
 183. Hoffman R, Kopel S, Hsu SD, Daniak N, Zanjani ED: T cell chronic lymphocytic leukaemia: Presence in bone marrow and peripheral blood of cells that suppress erythropoiesis in vitro. *Blood*, 1978; 52: 255-60.
 184. Nagasawa T, Abe T, Nakagawa T: Pure red cell aplasia and hypogammaglobulinaemia associated with T-cell chronic lymphocytic leukaemia. *Blood*, 1981; 57: 1025-31.
 185. Linch DC, Cawley JC, McDonald SM, Masters G, Roberts BE, Antonis AH, Waters AK, Sieff C, Lydyard PM: Acquired pure red cell aplasia associated with an increase of T cells bearing receptors for the Fc of IgG. *Acta Haematol*, 1981; 65: 270-74.
 186. Callard RE, Smith CM, Worman C, Linch DC, Cawley JC, Beverley PCL: Unusual phenotype and function of an expanded population of T cells in patients with haemopoietic disorders. *Clin Exp Immunol*, 1981; 43: 497-505.
 187. Greenberg PL, Shrier SL: Granulopoiesis in neutropenic disorders. *Blood*, 1973; 41: 753-69.
 188. Falk PM, Rich K, Feig S, Stiehm ER, Golde DW, Cline MJ: Evaluation of congenital neutropenic disorders by in vitro marrow culture. *Ped*, 1977; 59: 739-48.
 189. Greenberg PL, Mara B, Steed S, Boxer L: The chronic idiopathic neutropenic syndrome: correlation of clinical features with in vitro parameters of granulocytopoiesis. *Blood*, 1980; 55: 915-21.

190. Cline MJ, Opelz G, Saxon A, Fahey JL, Golde DW: Autoimmune panleukopenia. *N Engl J Med*, 1976; 295: 1489-93.
191. Linch DC, Cawley JC, Worman CP, Galvin MC, Roberts BE, Callard RE, Beverley PCL: Abnormalities of T cell subsets in patients with neutropenia and an excess of lymphocytes in the bone marrow. *Br J Haematol*, 1981; 48: 137-45.
192. Abdou NI, NaPombejara C, Balentine L, Abdou NL: Suppressor cell mediated neutropenia in Felty's syndrome. *J Clin Invest*, 1978; 61: 738-43.
193. Starkebaum GA, Arend WP, Singer JW, Nardella FA, Gavin SE: Felty's syndrome: humoral and cellular immune aspects. (Abstr) *Arthritis Rheumatism*, 1979; 22: 662.
194. Bagby GC: T lymphocytes involved in inhibition of granulopoiesis in two neutropenic patients are of the cytotoxic/suppressor (T3⁺ T8⁺) subset. *J Clin Invest*, 1981; 68: 1597-1600.
195. Barrett AJ, Weller L, Rozengurt N, Longhurst P, Humble JG: Amidopyrine agranulocytosis: drug inhibition of granulocyte colonies in the presence of patients serum. *Br Med J*, 1976; 2: 850-51.
196. Linch DC, Knott L, Patterson KG, Coven DA, Harper PG: A comparison of three methods of bone marrow progenitor cell concentration prior to cryopreservation. *J Clin Path*, 1982; 35: 186-90.
197. Rodt H, Kolb HZ, Netzel B, Haas RJ, Wilms K, Gotze Ch B, Link H, Thierfelder S: Effect of anti-T cell globulin on GVHD in leukaemic patients treated with BMT. *Transplant Proc*, 1981; 13: 257-61.
198. Reisner Y, Kapoor N, O'Reilly RJ, Good RA: Allogeneic bone marrow transplantation using stem cells fractionated by lectins. *Lancet*, 1980; ii: 1320-23.
199. Prentice HG, Blacklock HA, Janossy G, Gilmore MJML, Price-Jones L, Tidman N *et al.*: Depletion of T lymphocytes in donor marrow prevents significant graft versus host disease in matched allogeneic leukaemic marrow transplant recipients. *Lancet*, 1984; i: 472-76.
200. Ritz J, Sallan SE, Bast RC, Lipton JM, Clavell LA, Feeney M, Hercend T, Nathan DG, Schlossman SF: Autologous bone marrow transplantation in CALLA positive acute lymphoblastic leukemia after in vitro treatment with J5 monoclonal antibody and complement. *Lancet*, 1982; ii: 60-63.
201. Storb R, Prentice RL, Thomas ED: Marrow transplantation for aplastic anaemia. An analysis of factors associated with graft rejection. *N Engl J Med*, 1977; 296: 61-64.
202. Faillé A, Maraninch E, Gluckman A, Devergie N, Balitrand N, Ketels F, Dresch C: Granulocyte progenitor compartments after allogeneic bone marrow grafts. *Scand J Haematol*, 1981; 26: 202-14.
203. Spitzer G, Verma DS, Fisher R, Zander A, Vellekoop L, Litam J, McCredie KB, Dicke KA: The myeloid progenitor cell - its value in predicting haematopoietic recovery after autologous bone marrow transplantation. *Blood*, 1980; 55: 317-23.
204. Gorin NC, Najman A, Douay L, Salmon Ch, Duhamel G: autologous bone marrow transplantation in the treatment of non-Hodgkin's lymphoma of high grade malignancy. (Abstract). *Exp Haematol*, 1983; 11: 153 (suppl. 13).
205. Gorin NC, Herzig G, Bull MI, Graw RG: Long-term preservation of bone marrow and stem cell pools in dogs. *Blood*, 1978; 51: 257-65.
206. Kaiser H, Stuart RK, Brookmayer R, Colvin M, Santos GW: Autologous bone marrow transplantation in acute leukaemia: a phase 1 study of in vitro treatment of marrow with 4 hydroperoxycyclophosphamide to purge tumour cells. (Abstract). *Blood*, 1983; 62: 799 (suppl. 1).

2. Oxygen transport to the tissue and blood viscosity

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I. Introduction

For a long time it was assumed that essential to shock was a decrease in blood pressure. As a result, treatment consisted of restoring the blood pressure with vasoconstrictors. Blood pressure is still given a relatively important role as a criterion of the state of the circulation, but this is due more to the fact that the blood pressure is easier to measure than any other pathophysiological parameters.

During the last two decades more information has become available about the microcirculation, and circulatory disturbances began to be approached in terms of disturbances in flow. During the last few years we have gone one step further still and it is the inadequate O_2 -transport to the tissue, rather than insufficient flow, which is looked upon as the most essential pathological criterion for a threatened or disturbed circulation.

A disturbance in O_2 -transport is usually the result of a maldistribution in the flow, so that the total flow or cardiac output can be either reduced, normal or even elevated, and yet accompanied by decreased oxygen consumption.

The disturbance in the equilibrium between neurohumoral vasoconstriction and metabolic vasodilatation is one of the factors that lead to a maldistribution of flow at the level of the microcirculation. In tissues where the flow is high, more oxygen is transported through the dilated arteriolar-capillary network, but it is quite possible that less oxygen is extracted from the blood because the cells bordering directly on the dilated capillaries only take up that amount of O_2 which they can

utilize themselves while the O_2 -supply to the cells which are located some distance away from the dilated capillaries is limited by the oxygen diffusion through the tissues [1].

In *in vitro* studies using glass capillaries as well as the arterioles and capillaries of the rat mesentery in *in vivo* experiments, Pries *et al.* [2] have demonstrated that the 'erythrocyte redistribution' or 'red cell flow fractionation' at blood vessel branching points depends on the following factors: (1) flow rate; (2) hematocrit; and (3) capillary diameter.

Flow rate

At the point where a capillary branches off from a feeding channel, the hematocrit in the capillary will decrease as the flow decreases until, in the case of a very low rate of flow in the feeding channel, the flow in the capillary will be practically cell-free.

Hematocrit

Red cell fractionation will be more pronounced at a high hematocrit than at a low hematocrit, so that the variation in hematocrit in the branching vessels will be more marked at a high hematocrit. Maldistribution of the erythrocytes occurs less often during hemodilution than during hemoconcentration.

Capillary diameter

Vasoconstriction or capillary stenosis predisposes to an unequal flow of the erythrocytes. In the case of a capillary bifurcation, if one of the daughter branches is partly occluded, then the hematocrit in the occluded branch will decrease (e.g. from 0.19 to

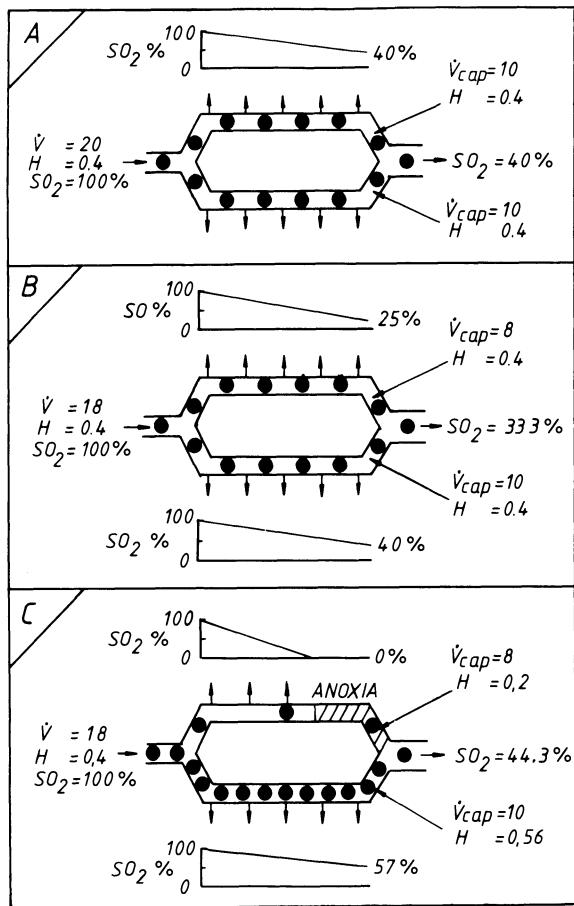


Fig. 1. Effects of change in flow distribution and hematocrit distribution on O_2 -supply in a simplified capillary network [2]. (\dot{V} = flow rate in $\mu l \cdot sec^{-1}$, H = hematocrit, SO_2 = O_2 -saturation, \dot{V}_{cap} = capillary flow rate).

0.12) while that in the non-occluded branch it will rise (e.g. from 0.19 to 0.24) with no significant change in flow volume.

Maldistribution can be the result of inadequate flow distribution, inadequate hematocrit distribution or both. Small changes in flow generally result in an appreciably greater change in O_2 -delivery over the normal range of flow rates. At a very low flow rate it can even happen that some tissues receive no oxygen at all because the capillaries are only filled with plasma. Figure 1 shows the three possible situations with their consequences for the O_2 -transport and O_2 -delivery to the tissues: Situation A with an uniform flow and hematocrit distribution, situation B with an unequal flow and uniform hematocrit distribution, and situation C

with an unequal flow and unequal hematocrit distribution [2].

Both an increased and a decreased arteriovenous O_2 -difference can be a sign of maldistribution, diminishing the real value of the clinical parameters, O_2 -consumption ($\dot{V}O_2$) and O_2 -extraction (O_2 EX), which are derived, among other things, from the arterial-mixed venous O_2 -gradient. The objection to the O_2 -availability (O_2 AV) as a clinical parameter is that it reflects only the O_2 -supply and not the O_2 -distribution.

Shoemaker [1] has attempted to provide a number of parameters for determining the prognosis of survival of intensive care patients in various states of shock. He distinguished 35 parameters with a correct prediction score of between 19 and 82% (showing the appendix), but a strikingly high predictive value was achieved when the erythrocyte volume was combined with O_2 -transport (Table 1).

Red Cell Mass (RCM) introduces not only a volume parameter but also the entire complex of rheological factors related to erythrocyte aggregation and blood viscosity; this could be an explanation for the high prediction score achieved by the first three variables (Table 1).

This is in agreement with the way in which Chien [3] defined the O_2 -transport capacity of the blood as being determined by the ratio between the number of erythrocytes (Ht in %) and the blood viscosity (η in cP) at a particular shear rate (γ in s^{-1}); the maximal O_2 -transport capacity ($\approx Ht/\eta = %/cP$) can be presented in fig. 2.

Table 1. Percentage of correct predictions of survival for each cardiorespiratory variable (adapted from Shoemaker).¹

Variable	Measurements or derived calculations	% correct predictions
Efficiency of tissue O_2 -extraction	Arterial mixed venous O_2 gradient \div red cell mass	82
Red cell mass	Blood volume $-$ plasma volume	69
O_2 -transport/Red cell mass ratio	O_2 consumption \div red cell mass	64

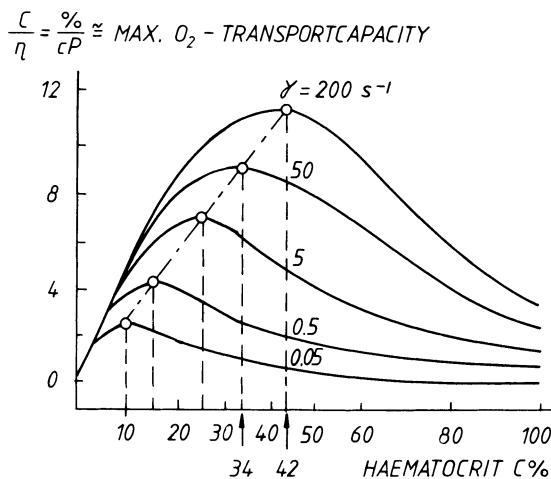


Fig. 2. The relationship between a maximal O_2 -transport capacity; this being a compromise represented by the highest possible ratio between hematocrit (% cells) and the viscosity in centipoise at five different shear rates. It is clear that every shear rate is characterized by a single optimal value for the O_2 -transport at one specific value for the hematocrit. The lower the shear rate, the lower the hematocrit must be to achieve optimal O_2 -transport (Ht/η). At $\gamma = 200 \text{ s}^{-1}$ a Ht of 42% is optimal, but at $\gamma = 0.05 \text{ s}^{-1}$ the optimal Ht is 10% (Chien) [3].

In the case of shock, trauma or low-flow states (i.e. in a range of relatively low shear rates) the maximal O_2 -transport capacity of the blood is attained at a moderate (normovolemic) hemodilution. This is the optimal compromise between maximizing the number of erythrocytes, as the transport medium, and minimizing the blood viscosity as a factor which limits O_2 -transport due to erythrocyte aggregation. At a Ht between 30 and 35%, the behavior of blood is practically Newtonian and erythrocyte aggregation plays a less important role.

Under conditions of normal myocardial function the cardiac output is determined by the peripheral factors: vascular diameter, circulatory filling pressure and viscosity. The vascular factor is difficult to measure and is, furthermore, fairly reliably represented by the circulatory filling pressure. As the circulatory filling pressure cannot be measured under clinical circumstances, for practical use it is more common to substitute the pulmonary capillary wedge pressure (PCWP).

It has become common clinical practice to present the heart's ventricular function curves graph-

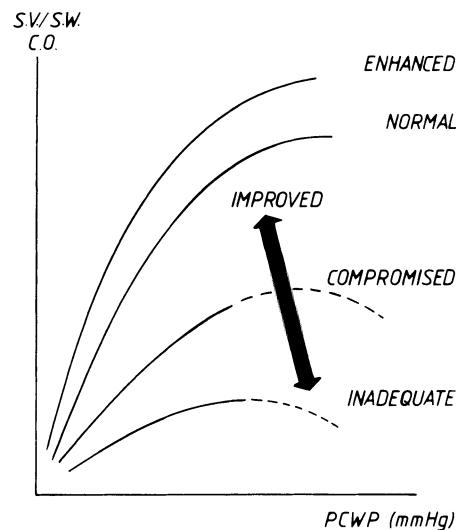


Fig. 3. Graphic classification of the ventricular function curves according to Starling.

ically in such a way that the PCWP and cardiac output (CO) are recorded on the two axes. The various Starling curves are created in this way (Fig. 3). Although this representation is valid and valuable, it gives a one-sided picture since it takes into account only the cardiac output and the wedge pressure (PCWP) while ignoring, to a certain extent, the peripheral factors, viscosity (η) and vascular diameter (r^4).

II. Experimental work and clinical implications

Experimental work has shown that low viscosity and a high-normal PCWP increase the chance of survival after shock provocation, while high viscosity and a low PCWP are unfavorable [6]. According to Guyton, the cardiac output in the presence of a non-pathological cardiac function is determined by the venous return, which is determined in turn by three factors: the vascular diameter, the circulatory filling pressure and the blood viscosity [4, 5]. Clinically, these three factors are represented reasonably well by PCWP and viscosity.

To construct a complete representation of the most important factors which determine flow, i.e. the central and peripheral factors (contractility of

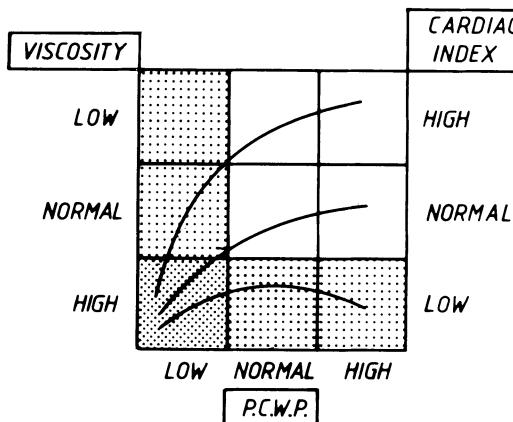


Fig. 4. Diagrammatic representation in which the ventricular function curves are plotted together with the peripheral function factors. The combination of central and peripheral factors can give a more complete picture.

the cardiac ventricles, vascular diameter, viscosity and wedge pressure), it would be reasonable to make a combination of the two (Fig. 4).

Assuming that the vascular diameter is represented rather well by the wedge pressure – vasodilatation generally produces a decrease in PCWP and vasoconstriction mostly an increase – then the most important factors in the circulation are taken into consideration.

If we assume that the low-shear viscosity (shear rate = 0.04 s^{-1}) is a very sensitive rheological parameter and is generally representative of the whole blood viscosity, and if we assume that the normal values (for men) of the low-shear viscosity (η_{LS}), the pulmonary capillary wedge pressure (PCWP) and the cardiac index (CI) are $50-100 \text{ mPa} \cdot \text{s}$, $5-15 \text{ mmHg}$ and $2-3 \text{ L} \cdot \text{min}^{-1} \text{ m}^{-2}$ respectively, then the circulation diagram shown in Fig. 5 can be constructed.

For instance, if we have a patient with a low-shear viscosity (η_{LS}) of $120 \text{ mPa} \cdot \text{s}$ and a PCWP of 3 mmHg (which is a common situation), it is very likely that the cardiac index will be low ($\text{CI} < 2 \text{ L} \cdot \text{min}^{-1} \text{ m}^{-2}$). To improve this situation it is not reasonable to stimulate the heart and try to improve the contractility; instead it would be much more effective to improve the viscosity and wedge pressure. If this is done by lowering the viscosity (e.g. to $50 \text{ mPa} \cdot \text{s}$) and increasing the wedge pres-

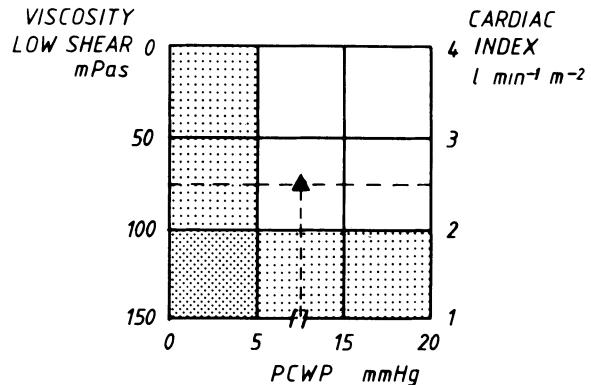


Fig. 5. Circulation diagram with the clinical values which can determine the high, normal and low regions.

sure (e.g. to 10 mmHg) by hemodilution, the cardiac index will improve (to $3 \text{ L} \cdot \text{min}^{-1} \text{ m}^{-2}$) without an increase in oxygen-demand of the heart. It is generally impossible to bring the three values to one point, without the help of a 3-dimensional scale. In a 2-dimensional scale, however, it is quite possible to let the three values be represented by a line determined by two values (η_{LS} and CI), while the third value (PCWP) always remains on that line (see Fig. 6). The subject in Fig. 6 is a patient with a normal PCWP; the high viscosity was compensated by an increased cardiac function, e.g. by a positive inotropic drug, resulting in a normal cardiac index. A more acceptable situation and a reasonable starting point for therapy would be to decrease viscosity. This diagram with the WVC values (PCWP, viscosity and CI) permits a better clinical analysis of the circulation and a differentiation of the underlying factors. If there is no equipment for viscosity measurement available, one can make a simplification by taking hematocrit instead of viscosity, with the optimal range between 30 and 40%.

In the clinical situation, reduction in viscosity, whether by hemodilution or by defibrillation or other agents, is still a form of treatment to which unduly little attention is paid [6, 9, 10, 11, 12, 13]. As the suggested therapy for a low cardiac output syndrome, for example, Kaplan [7, 8] lists: (1) fluid challenge, (2) inotropic drug, (3) vasodilatory drug, (4) fluid load, and (5) cardiac assist device. This scheme underestimates the fact that a low cardiac output is frequently seen in cases with a normal PCWP and a normal cardiac contractility,

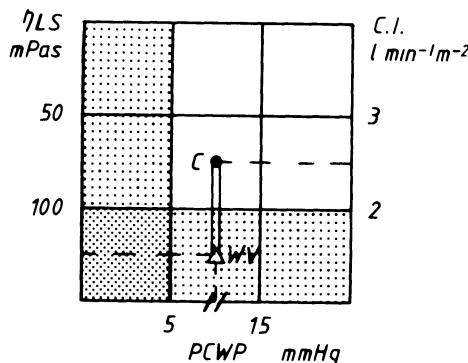


Fig. 6. Patient status defined by three values: cardiac index, low-shear viscosity and PCWP. The so-called WVC line (PCWP, viscosity and CI) expresses the most important cardiac and peripheral factors which are clinically measurable.

due for example, to a normovolemic polycythemia. It is therefore advisable, in the case of a low cardiac output syndrome, to measure not only the cardiac output and the PCWP, but also the viscosity, and to take immediate steps to normalize the viscosity (together with the PCWP) if it is too high. The advantage of this procedure is that an improvement in venous return and cardiac output can be achieved without any strain on the heart – in fact, the load on the heart is reduced since the O_2 -demand drops.

When the cardiac contractility is increased by means of a positive inotropic drug, then the O_2 -demand of the heart is definitely increased. Therefore, the suggested course of treatment for the low cardiac output syndrome should begin with:

- (1) Normalization of (a) the viscosity, (b) the wedge pressure (PCWP). In many cases points (a) and (b) can be combined by hemodilution techniques, with or without venesection, and with or without defibrillation.
- (2) Vasodilatory drug plus fluid load; this usually amounts to reducing the viscosity. If everything has been done to achieve an efficient decrease in afterload with a normal preload, then one can resort to increasing the cardiac contractility with the aid of:
- (3) an inotropic drug;
- (4) or as a last resort, a cardiac assist device.

The effects of this therapeutic schedule can be diagrammatically shown in Fig. 7. The hemo-

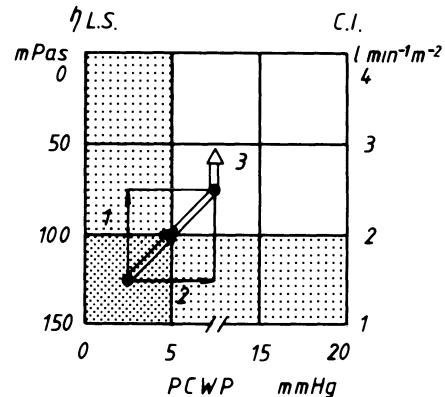


Fig. 7. Treatment of a low cardiac output syndrome: steps 1 and 2 should be to normalize the low-shear viscosity and the wedge pressure (PCWP); only in the third place should one resort to positive inotropic drugs.

dynamic situation described below (Table 2) is produced if, during surgery, a sympathetic blockade is realised (by epidural and/or neuroleptanesthesia) and the resultant vasodilatation as well as possible blood loss are compensated by infusion of dextran, albumin and a salt solution until a hematocrit of 35% is reached (isovolemically).

In comparison to phase 1, phase 2 (Fig. 8 left and middle panel) is characterized by a cardiac output which is almost twice as high with no change in wedge pressure (PCWP), cardiac O_2 -demand, as expressed by rate pressure product (RPP = Haematocrit \times Systolic BP). If, in phase 3 (Fig. 8 right panel), after the infused fluid has been excreted with the urine, dopamine was administered inadvisedly on the basis of a single observation of an increased systemic pulmonary artery pressure, then the situation shown in the last column is produced. This illustrates clearly how unfavorable it is for the heart (RPP) when dopamine is administered instead of treating the hypovolemia.

III. Conclusions

It should be remembered that reperfusion and the restoration of an adequate O_2 -transport should be the most important goal of treatment in circulatory failure [1]; this is generally true for hemorrhagic, traumatic, septic or cardiogenic shock. In order to

Table 2.

		Before operation	End of the operation	Recovery after Dopamine (administered inadvisedly)
Haemoglobin	(mmol · l)	9.6	7.6	7.8
Haematocrit	(liter/liter or %)	47	35	37
Heart rate	(beats/min)	70	70	115
Systolic blood pressure	(mmHg)	120	120	115
Diastolic blood pressure	(mmHg)	60	60	55
Rate pressure products	(mmHg/min)	8400	8400	12 225
Right arterial pressure	(mmHg)	10	10	4
Syst. pulm. arterial pres.	(mmHg)	20	20	34
Diast. pulm. arterial pres.	(mmHg)	14	13	7
Pulm. capillary wedge pres.	(mmHg)	11	11	6
Cardiac output	(l · min ⁻¹)	3.4	6.2	6.0
Cardiac index	(l · min ⁻¹ m ⁻²)	1.8	3.7	3.6
Total peripheral resistance	(dyn · s · cm ⁻⁵)	1330	900	945
Pulmonary vascular resistance	(dyn · s · cm ⁻⁵)	117	56	135
Viscosity LS	(mPa · s)	112	68	75

accomplish this, measures should be taken to guarantee the following:

- (1) an adequate circulating volume (clinically represented in the PCWP);
- (2) an adequate flow (clinically represented in the CI); and
- (3) a good quantitative flow, with the elimination of maldistribution.

One of the key principles of resuscitation from hemorrhage is that the first goal of the administration of fluid should be the restoration of perfusion

since this is initially much more important than the restoration of oxygen delivering capacity. The restoration of perfusion in fact restores the delivery of oxygen to the periphery in the most effective and immediate manner, and there is abundant reserve in the oxygen delivering system as it is normally found. Experimentally, the maintenance of perfusion even in the absence of oxygenation delays deterioration and death of cells, probably by the removal of noxious materials and the delivery of necessary substrates [14]. It is very clear that the

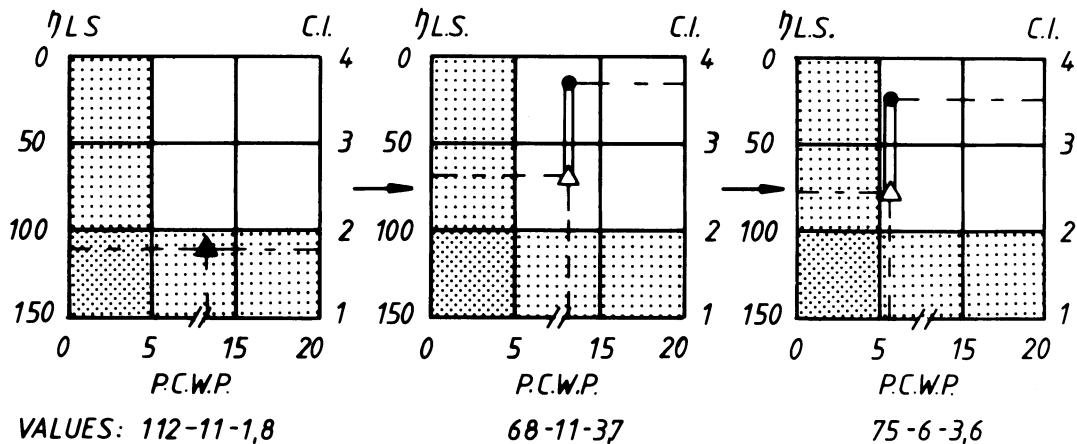


Fig. 8. The course of Mr A. (bifurcation prosthesis) during and after operation.

primary aim of resuscitation is to restore blood volume. In addition to maintenance of the circulating volume and cardiac output, a normalization or reduction of blood viscosity would therefore seem to be of essential importance for restoration of the microcirculation and for an adequate flow distribution. For this purpose the viscosity is the most suitable approach, since in general a reduction of viscosity: (a) improves the flow; (b) decreases the haematocrit; and (c) brings about a kind of vasodilatation [6].

The therapeutic approach described above seems highly sensible since it takes both central and peripheral factors into consideration and since it analyzes the essential factors in the circulation, i.e. the circulating volume, the flow and an optimal rheological state which is determined primarily by the hematocrit and viscosity. These last two factors are essential for an adequate O_2 -transport at the level of the capillaries in order to prevent shunting and maldistribution. Even a circulation with an ideal PCWP and CI can be qualitatively poor if, for example, there is insufficient O_2 -extraction and O_2 -consumption. On the basis of the above arguments, the ideal prognostic parameter for the circulation should be formulated as follows:

$$\frac{\text{PCWP} \times \text{CI}}{\text{viscosity or hematocrit}}.$$

Assuming that the viscosity cannot yet be measured clinically on a routine basis, then an attempt should be made to achieve the following values:

PCWP >10 (Normal 0–10 mmHg)

CI > 3.6 (Normal 2.6–3.6 l/min \cdot m $^{-2}$)

Ht <35 (Normal 35–45%)

This parameter could be called the WCHR or WCVR (wedge pressure, cardiac index, hematocrit or viscosity ratio) and could be supported by the following arguments:

- (1) it is based theoretically on a combination of accepted pathophysiological principles in which both peripheral and central factors have been incorporated [4, 5];
- (2) it is based empirically on experimental and clinical findings [1, 6];
- (3) the three components can be measured directly and reliably [6].

It may be more important and sensible, however, to display this parameter graphically instead of as a mathematical formula.

Appendix A

Viscosity terminology

- Viscosity is a measure of the internal friction of a flowing liquid, i.e. of the ease with which a liquid flows
- Viscosity = $\frac{\text{shear stress}}{\text{shear rate}} = \frac{\text{dyn} \cdot \text{cm}^{-2}}{\text{s}^{-1}} = \text{dyn} \cdot \text{cm}^{-2} \cdot \text{s}$
or $\eta = \frac{\tau}{\gamma}$ (eta = tau/gamma)
- Unit of viscosity in the CGS system: $\text{dyn} \cdot \text{s} \cdot \text{cm}^{-2}$ or poise Unit of viscosity in the SI system: $\text{N} \cdot \text{s} \cdot \text{m}^{-2}$ or $\text{Pa} \cdot \text{s}$ (Pascal-second)
- The viscosity of water at 20°C = $10^{-3} \text{ Pa} \cdot \text{s} = 1 \text{ mPa} \cdot \text{s} = 1 \text{ centipoise} = 10 \text{ poise}$
- The shear stress is the force per unit area which is necessary to overcome the friction between two layers of a liquid which are moving in relation to one another; it is measured in dyn per cm 2 .
- The shear rate is the velocity per unit distance at which two layers of a liquid are moving in relation to one another and is dependent upon both the velocity (v) and the distance (y) between the two moving layers: the shear rate is thus v/y. The unit of measurement is s $^{-1}$ (the velocity v in cm per sec., the distance y in cm, hence v/y is cm \cdot s $^{-1}$ /cm = s $^{-1}$)

Appendix B.

Percentage of correct predictions of survival for each cardiorespiratory variable (adapted from ref. (1)).

Rank	Variable	Measurements of derived calculations	% correct predictions
1. ETOE	Efficiency of tissue O ₂ -extraction	C(a- \bar{v})O ₂ ÷ RCM	82
2. RCM	Red cell mass	BV – PV	69
3. OTR	O ₂ -transport / Red cell mass ratio	$\dot{V}O_2$ ÷ RCM	64
4. PVR	Pulmonary vascular resistance	79.92 (MPAP – PCWP) ÷ CI	53
5. LCW	Left cardiac work	CI × MAP × .0144	52
6. BV	Blood volume	PV ÷ (l-Ht) × Surface area	52
7. MAP	Mean arterial pressure	Direct measurement	52
8. O ₂ AV	O ₂ -availability or O ₂ -delivery	CaO ₂ × CI × 10	51
9. BFVR	Blood flow/Volume ratio	CI ÷ BV	50
10. TOE	Tissue O ₂ -extraction ratio	C(a- \bar{v})O ₂ ÷ RCFR	50
11. pH	Arterial pH	Direct measurement	47
12. LWSW	left ventricular stroke work	SI × MAP × .0144	47
13. TTI	Time tension index	MAP × HR × Duration of systole	46
14. RCFR	Red cell flow rate	CI × Ht	43
15. MTT	Mean transit time	Direct measurement	42
16. OTRF	O ₂ transport/Red cell flow ratio	$\dot{V}O_2$ ÷ RCFR	42
17. CI	Cardiac index	Direct measurement	40
18. RVSW	Right ventricular stroke work	SI × MPAP × 0.144	40
19. PCWP (PW)	Pulmonary capillary wedge Pressure	Direct measurement	39
20. $\dot{V}O_2$	O ₂ -consumption	C(a- \bar{v})O ₂ × CI × 10	38
21. PaCO ₂	Arterial CO ₂ -tension	Direct measurement	38
22. O ₂ EXT	O ₂ -extraction rate	(CaO ₂ -CvO ₂) ÷ CaO ₂	37
23. RCW	Right cardiac work	CI × MPAP × .0144	37
24. C(a- \bar{v})O ₂	Arterial mixed venous O ₂ -content difference	CaO ₂ – CvO ₂	36
25. PvO ₂	Mixed venous O ₂ tension	Direct measurement	35
26. MPAP	Mean pulmonary arterial pressure	Direct measurement	35
27. SaO ₂	Arterial O ₂ saturation	Direct measurement	34
28. SI	Stroke index	CI ÷ HR	34
29. MSER	Mean systolic ejection rate	SI ÷ Duration of systole	31
30. Hb	Hemoglobin	Direct measurement	31
31. Temp.	Rectal temperature	Direct measurement	27
32. CVP	Central venous pressure	Direct measurement	24
33. SVR	Systemic vascular resistance	79.92 (MAP-CVP) ÷ CI	24
34. CBV	Central blood volume	MTT × CI × 16.7	22
35. HR	Heart rate	Direct measurement	19

References

- Shoemaker WC: Pathophysiology and therapy of shock states. In: *Handbook of Critical Care*, Berk JL, Sampliner JE (eds). Little, Brown and Company, Boston, 1982: 253–85.
- Pries AR, Gaethgens P, Kanzow G: Microvascular distribution of blood volume flow and hematocrit as related to oxygen delivery. In: *Advances in Physiological Sciences*. Vol. 25. Oxygen Transport to Tissue, Rovach AGB, Bora E, Kessler N, Silver IA (eds). Pergamon Press, Akademiai Kiado, Budapest. Proceedings of the 28th International Congress of Physiological Sciences.
- Chien S: Present state of blood rheology. In: *Hemodilution. Theoretical Basis and Clinical Application*. Proceedings of an international symposium held in Rottach-Egern. Tegernsee, 1971. Messmer K, Schmid-Schönbein H (eds). S. Karger, Basel, 1972: 1–45.
- Guyton AC: *Textbook of Medical Physiology*. Saunders, Philadelphia, 1976.

5. Cowley AW: Basic principles of cardiac output and arterial pressure regulation. In: *Hemodynamic Changes in Anaesthesia*. Vth European Congress of Anaesthesiology Paris. September, 1978: 121-51.
6. Gosling H: Blood viscosity and shock, the role of hemodilution, hemoconcentration and defibrillation. *Anesthesiology and Intensive Care Medicine*, Springer Verlag, Berlin, 1984, 160.
7. Kaplan JA (ed): In: *Cardiac Anesthesia*. Grune & Stratton, New York, 1979: 99-100.
8. Kaplan JA: Vasodilators or inotropic agents in the management of intraoperative cardiac failure. 7th World Congress of Anaesthesiologists, Hamburg, 1980.
9. Messmer K: Hemodilution. *Surg Clin N Amer*, 1975; 55: 659-78.
10. Wood JH, Fleischer AS: Observations during hypervolemic hemodilution of patients with acute focal cerebral ischemia. *Preliminary Communications*. *JAMA*, 1982; 248: 2999-3005.
11. Ehrly AM: Dosierungen und Langzeitwirkungen von Arwin. *Folia Angiologica*, 1975; XXIII: 377-81.
12. Dormandy JA, Reit HL, Goyle KB: Treatment of severe intermittent claudication by controlled defibrillation. *Lancet*, 1977; i: 625-26.
13. Lowe GDO, Meek DR, Prentice CRM, Campbell AE, Forbes CD: Subcutaneous anrod in prevention of deep vein thrombosis after operation for fractured neck of femur. *Lancet*, 1978; ii: 698-700.
14. Collins JA: The pathophysiology of haemorrhagic shock. In: *Massive Transfusion in Surgery and Trauma*. Collins JA, Murawski K, Shafer WA (eds). Alan Liss, NY, 1982: pp. 5-28.

3. Infection prevention in neutropenic patients

D. VAN DER WAAIJ and H.G. DE VRIES-HOSPERS

Introduction

Infection remains the principal cause of morbidity and mortality in patients with severe granulocytopenia. The interrelationships between fever, neutropenia, infection and disease status are complex. Neutropenia can be associated with bone marrow malignancies, at various stages or in relation to remission induction therapy, and even with infection. Patients with acute leukaemia often present with neutropenia due to bone marrow failure as a result of leukaemic cell infiltration. It is not clear whether the marrow suppression in acute nonlymphocytic leukaemia is due to suppression of stem cell activity or whether it is purely the result of occupancy of space in the marrow compartment by leukaemic cells. Many chemotherapeutic drugs used to treat leukaemia depress the marrow and impair granulocytopoiesis; this reduces cellular immunity and finally may influence the humoral response in an unpredictable way. In addition to their effects on the bone marrow, many chemotherapeutic drugs cause mucosal damage and ulceration, which may promote the spread of potentially pathogenic microorganisms in the oropharynx and the intestines.

Suppression of marrow activity, suppression of immunocompetence and the mucosal damage associated with aggressive chemotherapy appear to constitute the major cause of morbidity and mortality in patients with malignancies of the bone marrow, particularly in cases of acute nonlymphocytic leukaemia. A quantitative relationship has been established by several authors be-

tween the degree of granulocytopenia and the risk of major infections [1-3]. This is understandable since the circulating granulocytes constitute a major defence against invading bacteria and may enhance the effectiveness of many antibacterial drugs. A logical consequence has been to compensate for the lack of cellular defence by prophylactic antibiotic treatment. Many authors advocate aggressive therapeutic and preventive measures that may include broadspectrum therapy with two [4-8], or three or more [9, 10] antibiotics as soon as fever or other clinical signs of infection develop. Other workers hold that antibiotic prophylaxis requires supplementary treatment with granulocyte transfusions either therapeutically [11, 12] or prophylactically [13]. Fever alone is a quite non-specific sign of infection and may result from several other causes. The EORTC International Antimicrobial Therapy Group [7] reports that in the large patient population ($n = 365$) involved in their study, 17% of the febrile episodes were probably not due to infections and that another 20% were only possibly due to infection. Some centres therefore advocate no systemic antibiotics until bacterial infection is microbiologically or otherwise more exactly diagnosed to permit better-adjusted antibiotic therapy. Others, however, state that pyrexial episodes are not uncommon in cancer patients, especially those with nonlymphocytic leukaemia and neutropenia. Overwhelming infection is a frequent cause of death and, with a restrictive antibiotic regimen, patients may often die before the infecting organism has been indentified.

The infecting pathogens isolated from neu-

tropenic patients are in general Gram-negative bacilli such as *Esch. coli*, species of the *Klebsiella*-, *Enterobacter*-, *Serratia* group, *Proteus* or *Pseudomonadaceae* [4–6]. Less frequently, Gram-positive organisms such as staphylococci, streptococci, diphtheroids or *Clostridium* species are associated with life-threatening infections in these patients [14, 15, 16]. Many potential pathogens may invade from the oropharynx [44]. Kurkle and co-authors [46] have found a significantly increased risk of infection in neutropenic patients when their oropharynx was colonized by Gram-negative bacilli. When *Klebsiella*, *Enterobacter*, *Proteus* or *Pseudomonas spp.* were isolated, the patients were found to have significantly more days with infection at levels of neutropenia at or below 500 cells/ μ l than patients without Gram-negative bacilli. Patients who had no Gram-negative colonization of their oropharynx during treatment had 78 infections in 951 neutropenic patient days while those who acquired Gram-negative colonization had 194 infections in 545 neutropenic patient days (36%).

Multi-antibiotic systemic prophylaxis

Information about the portal of entry and the most probable group of microorganisms that cause infections in granulocytopenic patients is essential in every approach to prophylactic treatment. Also, a rational approach to the design of appropriate therapeutic regimens depends upon clear knowledge of the various types of infection that occur in these patients and on the composition of the so-called hospital flora. Particularly the latter may vary from place to place. To meet these prerequisites Tattersall and co-authors [9] have attempted to reduce mortality by prompt empirical treatment with a combination of five antibiotics. They report success in 53% of a total of 16 bacteriologically confirmed infections. A more comprehensive study by the EORTC International Antimicrobial Therapy Project Group [7] on three different empirical antibiotic combinations shows that prompt empirical multi-antibiotic treatment may be successful in 59% of the cases with bacteraemia and in 76% of the patients with a clinically documented infection but without bacteraemia.

There was, however, little reduction in the rate of improvement when the infecting pathogen was susceptible to only one or two antibiotics given. Resistance of the pathogen to both of the prescribed antibiotics, not surprisingly, proved to be associated with a high rate of infection progression, i.e. with the duration of antibiotic treatment. Earlier and later studies report essentially similar results [14, 15, 24–27]; all mention the adverse influence of prolonged neutropenia, and of bacterial resistance and bacterial overgrowth in the oropharynx and intestines.

Reverse isolation and bowel decontamination for infection prevention

Consideration of the factors associated with morbidity and mortality in granulocytopenic patients leads to the conclusion that a decrease in the load of potential pathogens carried by leukaemic patients may permit alteration of host defences without an increase in infection rate. Approaches to the control of infection in the granulocytopenic patient have included attempts to decrease exposure to exogenous and endogenous potential pathogens [17–23]. This involves the use of 'protective environments', topical antiseptic and antibiotic agents, sterile diets, or decontaminated food in combination with bowel decontamination. Different studies report different levels of success with this approach to infection prevention (EORTC Gnotobiotic Project Group, 22). Levine and co-authors [18] reported a marked reduction in the number of severe infections whereas Schimpff and co-workers [20] and the EORTC Gnotobiotic Project Group [22] found a higher incidence of positive blood cultures in isolated and decontaminated patients than in those treated under normal ward conditions. Isolation alone appeared to have only limited value in reducing the incidence of infections. Typing of bacteria indicated that, although most infections in patients who were only treated in a protected environment for prophylaxis were of endogenous origin, a significant number appeared to be exogenous.

Co-trimoxazole prophylaxis

Since the application of reverse isolation (protected environment) with or without bowel decontamination is limited by the great cost involved and the patient compliance, oral prophylaxis with trimethoprim-sulphamethoxazole (co-trimoxazole) has been tried in granulocytopenic patients [28–32]. Without isolation precautions, this appeared to be equally protective against infections as bowel sterilisation in a protected environment [32]. This may explain why it has led to rather widespread adoption of this regimen [28–34].

Although two patients who were receiving co-trimoxazole prophylactically were reported to have died due to a bacteraemia with a co-trim resistant *Enterobacteriaceae* species [35], a number of other studies have shown that prophylactic co-trimoxazole, often given in combination with an antifungal agent, reduces acquired infections in patients with profound neutropenia. Urinary-tract infections and bacteraemia due to Gram-negative bacilli in particular, were reported to be significantly reduced by co-trimoxazole prophylaxis with a consequent reduction in the need for additional antibiotic therapy. Failure of prophylaxis by oral co-trim treatment seems likely to be associated with broad-spectrum parenteral antibiotic therapy. As will be discussed later in this chapter, parenteral antibiotic therapy may considerably lower the threshold for colonization with potential pathogens. When co-trim prophylaxis is continued only co-trim resistant strains may be selected to cause 'overgrowth' and possibly infection. To investigate the influence of co-trim on the flora of the digestive tract, two groups have studied the effect of this drug on the faecal flora. Knothe [36] applied three different daily dose levels in groups of four volunteer subjects. Four volunteers received one tablet a day for seven days and had negative stool cultures for *Enterobacteriaceae* species after an average interval of ten days. Other bacterial species were not influenced by the drug. The four volunteers who received two regular co-trim tablets a day had *Enterobacteriaceae* negative stool cultures after about five days of treatment, while those receiving four tablets of co-trimoxazole appeared to behave al-

most similarly. Näff's [37] study with human volunteers ($n = 12$) was less comprehensive than Knothe's. Näff found that the faeces of subjects given four tablets of co-trim per day in his study were completely free of *Enterobacteriaceae* species in three to four days. Enterococci, however, appeared to increase in incidence from around 10% to 100% of the stools during treatment. Other bacteria did not change. Development of resistance was not seen in this study. Similar results were reported by Moorhouse and Farrel [38].

In this chapter we will not further elaborate on the development and the incidence of co-trim resistance because this is still low, between 5 and 15%, in most hospitals, and secondly because this subject was recently comprehensively reviewed by Lacey [39]. The results of the studies by Näff and by Knothe may explain the beneficial effect of co-trim prophylaxis in patients with acute leukaemia. It may provide a continuous systemic level of active drug, particularly in the urinary tract, and secondly, it may suppress the potentially pathogenic Gram-negative bacilli in the digestive tract that are otherwise most frequently involved in these infections. Co-trim apparently differs in more than its chemistry from other antibiotics. If the drug is given on a long-term daily basis without additional antibiotic treatment, leukaemic patients appear to do well in general and are not subject to the rapid overgrowth by resistant strains that occurs during long-term (prophylactic) treatment with many other antibiotics [38, 39]. This is perhaps due to the fact that co-trim does not affect the endogenous microflora, at least not when applied in daily doses of up to six tablets per day [40] (i.e. 2880 mg per day).

Toxicity of co-trimoxazole

In some studies a delay in marrow recovery has been reported [29, 31, 40], though this was not seen by others [28, 38]. In addition to possible adverse effects on the marrow, hypersensitivity reactions to co-trim may occur in leukaemic patients and perhaps slightly more frequently than in a normal population [43]. These two considerations call for caution in co-trim prophylaxis. A delay in marrow

recovery of about a week [31, 40] could favour growth of malignant cells and has financial consequences in relation to longer hospitalization. The skin hypersensitivity complication is an absolute contraindication to the use of co-trim in hypersensitive patients. However, skin hypersensitivity appears to be largely attributable to the sulpha compound in the combination, so that prophylactic use of trimethoprim alone may reduce the group in which this kind of treatment is contraindicated. Further studies on the prophylactic use of trimethoprim alone are necessary to answer this question.

Colonization resistance of the digestive tract

Colonization resistance (CR) is the resistance that potential pathogens encounter when they try to colonize one of the three tracts with open communication with the outside world. The colonization pattern of potential pathogens, i.e. number of strains and species and their concentration, and their site may be of key importance to both the urinary- and the respiratory tract since most endogenous infections find their source in the digestive tract [24, 14, 43–46]. This is not confined to patients with normal marrow function and is certainly also the case in leukaemic patients. The CR of the digestive tract is the result of cooperation between the host and his autochthonous microflora [47–50].

Host factors in the CR

For a better understanding of the contribution of the host organism to the CR, a brief outline of the various mechanisms that control colonization in the urinary bladder may be helpful. If a potential pathogen tries to colonize a normal urinary bladder, it will meet with three major opposing forces. The first is mechanical, i.e. complete bladder emptying. For colonization – prolonged stay and multiplication – a pathogen must adhere to the mucosa of the bladder where there are two more defensive mechanisms: the first is the mucin layer on the mucosa [51] and, if present as a result of previous immune experiences, specific IgA anti-bodies [52].

Both may interfere with adherence either by blocking of receptor sites on the mucosal cells or by covering adherence pili of the bacteria [53–56]. Cell desquamation, a continuous process due to cell renewal, is the fourth defensive mechanism.

Essentially the same four colonization restricting forces are active in the respiratory and in the digestive tract. In the mouth, the saliva, whether or not mixed with specific IgA, may have a similar function as the mucin in the intestines [57]. Swallowing of the saliva is the mechanical cleansing factor and continuous cell renewal also leads to frequent extrusion of cells into the contents of the mouth and oropharynx. In the intestines, peristalsis, mucin secretion (IgA) and cell desquamation constitute the host factors active in the control of digestive tract colonization. Obviously these host factors involved in the CR are influenced by the clinical condition of the patient and certainly by remission induction therapy.

Flora factors in the CR

If germ-free animals are mono-associated with a potential pathogen such as *Escherichia coli*, the four host factors which adequately control colonization of the respiratory and urinary tracts, fail to do so in the digestive tract. The pathogen in question will grow to abnormally high concentrations comparable with 'overgrowth' in the entire alimentary canal of the mono-associated individual. This condition will continue as long as the animals are maintained mono-associated. In addition, depending on the invasiveness of the pathogen, penetration of the mucosa with subsequent migration to lymph nodes, spleen and liver [58, 59] will occur. Enhanced translocation of bacteria from the digestive tract into the lymphatic tissues as a result of bacterial overgrowth, will not necessarily cause an inflammatory response or lead to other clinical symptoms. However, if an individual with this type of colonization (overgrowth) has neutropenia, a life-threatening condition is likely to evolve.

In individuals with a conventional microflora largely consisting of (many) anaerobic bacterial species, potential pathogens can only colonize the digestive tract if they are orally administered in

high numbers [47, 60–62]. For colonization with Gram-positive as well as Gram-negative pathogens, rather high oral doses of 10^6 bacteria are required [59–62]. This figure is in the order of 10^5 fold lower in animals without an (anaerobic) intestinal microflora, i.e. germ-free animals or in animals with an antibiotic decontaminated gut [47, 59]. The same appears to apply to man, i.e. if an individual is treated with antibiotics with activity on Gram-positive bacteria and if these antibiotics reach the intestinal lumen, the threshold for colonization following oral contamination with resistant potential pathogens may decrease to a fraction of what it was. Antibiotics may reach the alimentary canal either by incomplete absorption following oral administration or because of excretion into that tract. If along one or both of these lines, the antibiotic concentration in the digestive tract rises to or exceeds the minimum inhibitory level of the CR-associated (anaerobic)microflora, the CR of the digestive tract begins to decrease. The degree of the CR decrease and the rapidity of this decrease depend on the level and rate at which a steady state concentration of an antibiotic active to anaerobic Gram-positive bacteria is reached in the intestines.

Antibiotic treatment and colonization resistance

In the foregoing paragraph, the importance of the maintenance of a normal autochthonous anaerobic intestinal flora in immunocompromised patients has been discussed. When this was recognized in the early seventies, a series of experiments was set up to screen antibiotics for their effect on the CR-constituting intestinal flora in mice [63–68]. In these experiments groups of mice were treated with different doses of a particular antimicrobial drug for several weeks. During this treatment period the concentration of potential pathogens in faeces and parameters for measuring the CR were investigated at regular intervals. The results of these studies show that antimicrobial drugs can roughly be subdivided into three major classes [69]:

- (1) Antibiotics that affect the CR-constituting microflora at any clinical dose level.
- (2) Antibiotics that decrease the CR only when

high clinically applicable doses are used.

- (3) Antibiotics that do not decrease the CR even when applied in unusually high doses.

By analogy to traffic lights, these three groups could be referred to respectively as 'red' (CR-decreasing), 'orange' (only CR-affecting after higher doses) and 'green' (no danger for the CR-flora). At a certain dose level potential pathogens that were susceptible to the antibiotic being screened, disappeared from the faeces. If at this dose level – the minimum pathogen eradicating dose – the CR was still unaffected, the drug was considered worthy of further study for application to infection prophylaxis in immunocompromised individuals. Only if this minimal CR-decreasing dose was fourfold or more higher than the minimum pathogen eradicating dose was the drug regarded as 'safe' for application to what soon became known as selective decontamination of the digestive tract (SD). Subsequent studies in man have indicated that the CR-constituting flora of mice and man respond similarly to antibiotics. Antibiotics labelled 'red' after screening in mice, are the same that generally are known as the drugs associated with 'overgrowth' in man. 'Green' antimicrobials, on the other hand, have also been found to be non-toxic to the CR-constituting flora in man, even when applied in doses high enough to eliminate the potential pathogens that are susceptible to the drug used. One of these drugs is obviously co-trimoxazole. However, in terms of safety, to the patient as opposed to the patient's CR, co-trimoxazole may have its limitations as briefly mentioned above, and could therefore be classified as green-orange.

Four tablets a day can apparently be regarded as a minimal effective dose for eradication of *Enterobacteriaceae* species, whereas double that daily dose enhances *Candida* colonization [70]. Overgrowth by *Candida* species or other resistant microorganisms can be regarded as a sign of a CR-decrease.

Clinical monitoring

In the last five years, a biochemical determination of CR status has been developed. (The micro-

biological assessment of CR status is difficult and time-consuming.) Welling and co-workers found that certain enzymes in faeces are specific for CR-constituting anaerobic bacteria. One of these enzymes is a peptidase and degrades a dipeptide beta-aspartylglycine (beta-aspgly) to aspartic acid and glycine. Beta-aspgly is a normal end-product of protein metabolism in man and animals and is excreted into the intestines and urine. This implies that this substrate is always present in the intestines, even in starving subjects. In the presence of a normal flora, beta-aspgly is, however, readily metabolized by bacterial enzymes as mentioned above. A study in mice has shown that the concentration of beta-aspgly correlates perhaps linearly with the degree of interference with the CR. In the presence of a normal flora, faeces is always free of beta-aspgly, whereas in individuals with an antibiotic decontaminated gut the concentration is as high as in germ-free animals [71]. The determination of the concentration of beta-aspgly in faeces is simple and cheap.

Clinical application of selective decontamination (SD)

A prospectively controlled clinical trial performed in 105 patients in the Haematology Department of the University of Groningen, from January 1977 until October 1978, has shown the value of selective decontamination [40]. Adult granulocytopenic ($<1 \times 10^9/l$ blood) patients with acute leukaemia or aplastic anaemia were allocated at random to receive either SD (53 patients) or to serve as a control group (52 patients) not receiving any prophylactic treatment.

SD was accomplished by daily oral administration of nalidixic acid (8 g) or co-trimoxazole (2880 mg) or polymyxin (800 mg) to suppress growth of *Enterobacteriaceae*- and *Pseudomonadaceae* species in the digestive tract. Growth of yeasts was inhibited by oral administration of amphotericin B (2 g) or nystatin (6×10^6 I.U.). With the exception of co-trimoxazole, which was administered three times a day, the daily dose of all the other drugs was divided into four aliquots. Antimycotic drugs were preferably given as a sus-

pension; antibacterial agents were given as tablets. All patients received the normal non-sterilized hospital food and were treated in normal hospital bedrooms (4–6 beds) under open ward conditions. Both groups were comparable, in relation to the duration and the degree of granulocytopenia. Granulocyte counts were done three times a week. Bacteriological monitoring was restricted to cultures of throat swabs and faeces. In the SD-group as well as in the control group these cultures were performed only aerobically thrice weekly, starting at the moment of randomization, usually coinciding with admission. Cultures of other samples such as blood, urine, sputum or pus from lesions were cultured in addition when an infection was suspected or when the patient had an axillary temperature above 38.5°C . Blood cultures were incubated aerobically and anaerobically.

The clinical results of this randomized study were very promising: infections caused by aerobic Gram-negative rods or yeasts occurred in the control group 18 times in 12 patients: in the selectively decontaminated group only two patients developed one infection each ($p<0.01$). One of these two patients became infected with an *Acinetobacter calcoaceticus* via a skin lesion, the other patient developed a *Candida* septicaemia preceded by intestinal *Candida* colonization after he had refused his oral amphotericin B for a couple of days. Clinically documented infections occurred 15 times in 12 control patients and four times in three selectively decontaminated patients ($0.01< p<0.05$). In patients with granulocyte counts exceeding $0.5 \times 10^9/l$ the differences were no longer significant. Nine patients in the control group died as a result of acquired infections, but none of the patients in the SD-group died ($p<0.01$). At the moment of randomization the patients in both groups were equally colonized by aerobic Gram-negative potential pathogens. After starting SD with either nalidixic acid or co-trimoxazole, it took about one week of SD treatment before the faecal cultures became free of susceptible Gram-negative rods. The non-absorbable polymyxin appeared to have a much more rapid effect on the endogenous *Enterobacteriaceae* as the faecal cultures became negative after oral treatment for 1–3 days. Once negative,

most of the cultures of the throat swabs and the faeces remained free of Gram-negative potential pathogens. The difference with the control group was significant ($p<0.0005$). This reduction in positive cultures paralleled with and may explain the clinical efficacy of selective decontamination. A certain fraction of the faecal samples however, was occasionally positive. In 25.6% of the faecal samples taken during SD, Gram-negative rods were cultured. This was probably due to the fact that the patients received non-sterile normal hospital food and were in no way managed in isolation. Of these Gram-negative bacteria 72% were susceptible to the drugs used for SD in the corresponding patient, and in general they disappeared in a few days without changing the (antimicrobial) SD regimen. Even one-third of the Gram-negative rods that were resistant to the existing SD-regimen disappeared without readjustment. This observation in itself gives further evidence that the CR is not greatly disturbed by SD.

Resistance of Gram-negative rods

During the trial period, surveillance cultures of the throat swabs and the faeces were performed three times per week. As mentioned above, in about one quarter of the faecal samples Gram-negative rods were cultured, usually in low concentrations (less than 10^4 /g of faeces). Two-thirds of these bacteria disappeared without changing the antibiotic regimen for selective decontamination. For the other Gram-negatives it was necessary to switch treatment to another SD-drug, and this promptly resulted in negative cultures.

To discriminate between the induction of resistance and the 'pickup' of resistant environmental bacteria, we compared the biochemical profile (API) of the resistant and susceptible Gram-negative rods. This indicated that the induction of resistance occurred rarely [41]; however, when it occurred, it was almost always during the first week of treatment and only in those patients who were selectively decontaminated with either nalidixic acid or co-trimoxazole as a single drug.

Monitoring of the CR during SD

Supernatants of faecal specimens of both SD- and control patients were analyzed for the presence of the dipeptide beta-aspartylglycine. In patients receiving no antibiotics or only SD-drugs this dipeptide was not present. However, during treatment with systemic antibiotics such as aminoglycosides and (ureido-)penicillins which are suppressive to the anaerobic colon flora, beta-aspartylglycine [71] appeared in about 50% of the faecal samples. More indirect evidence that the CR was not disturbed during SD comes from the observation that the concentration of the enterococci in the faeces – which are naturally resistant to the SD-drugs – did not increase, even after weeks of treatment.

Infection prevention by selective decontamination

The results of this randomized study prompted us to continue with selective decontamination as a method of infection prevention in granulocytopenic patients. As our study indicated that the 'infection preventing capacity' became significant below a granulocyte count of $0.5 \times 10^9/l$, the criterion for starting SD was reduced to this level of granulocytes. Moreover, *Staphylococcus aureus* was included in the series of pathogens that should and could be eliminated selectively. The drug of choice for this purpose appeared to be cefradine in a daily dose of 6 grams.

As soon as neutropenic patients are admitted to the hospital, a complete bacteriological inventory must be made. This includes cultures of throat swabs, faeces (in case of need an anal swab can be used), nose, urine, vagina, prepuce, and pus from lesions. Apart from this bacteriological investigation a complete physical examination is performed. Later on, the surveillance cultures are confined to those of the throat swab and the faeces with a frequency of two to three per week. However, when potential pathogens are cultured from one or more of the other sites, those cultures must be repeated until they have become negative as a result of treatment. After the bacteriological inventory, selective decontamination can be started with

drugs directed at three groups of microorganisms:

- (1) *Enterobacteriaceae-* and *Pseudomonadaceae* species
- (2) Yeasts, such as *Candida* and *Torulopsis* species
- (3) *Staphylococcus aureus*.

1) *Gram-negative bacilli*. These enteric bacteria can be eliminated by means of one or more of the antimicrobial drugs listed in Table 1. A choice of the SD-drugs should be made on the basis of the susceptibility pattern of the potential pathogens found in the inventory-flora. When there is no time to wait for the results of these cultures – which may take up to five days – treatment could be started with a combination of polymyxin plus one of the absorbable drugs: nalidixic acid or co-trimoxazole.

If the isolated Gram-negative rods are known to be susceptible to polymyxin, it is advisable to start treatment with that drug, as it may have a rapid effect on the Gram-negative aerobic faecal flora. As mentioned above, faecal cultures are usually free from Gram-negative rods within one to three days after the onset of treatment with this drug. However, *Proteus* species are naturally resistant to polymyxin. In such cases polymyxin should be given in combination with one of the absorbable drugs. These drugs however, require longer – about one week – before the faecal cultures become negative for Gram-negatives [72]. Another

future possibility might be to use a combination of polymyxin with tobramycin for SD: Polymyxin in a daily dose of 400 mg and tobramycin in 320 mg (not more) orally. Negative faecal cultures can than be expected in about three to four days. Our clinical experience with this combination is limited as it is confined to only 25 patients. In one of these patients oral tobramycin appeared to be ineffective in suppressing a colonizing *Proteus* species. From studies performed in eight volunteers, there is evidence that daily oral doses of 320 mg of tobramycin may already slightly decrease the CR; higher doses of 500 mg per day had a more marked influence on the CR but were not more effective in suppressing endogenous Gram-negative bacilli. A CR-decrease was assumed on the basis of the appearance of beta-aspartylglycine [73] in the faecal samples of some of these volunteers. The combination of polymyxin and tobramycin may be advantageous because of the relatively rapid elimination of Gram-negative rods from the digestive tract. This can be particularly important in view of the fact that the longer the Gram-negatives are present, the greater the chance that they become resistant.

Elimination of Gram-negative bacteria from the gut is in general relatively easily achieved. In the oropharynx this is often more difficult. However, in patients with a normal CR the oropharynx is the part of the digestive tract which is not, or only sparsely, colonized by these bacteria. If Gram-negative bacilli remain during SD in the mouth of a patient, it is sometimes necessary to add absorbable drugs to the SD-regimen. Especially co-trimoxazole may successfully help to eliminate the colonizing strain(s). Possible infectious foci in such as teeth and sinuses should be eliminated. Another possible approach for the elimination of colonizing Gram-negative bacilli from the mouth, is the application of Orabase® premixed with 2% of the SD-drugs. However, Orabase® is a sticky substance with a poor compliance and most patients are not willing to apply this paste to their gums for a long period. If necessary, one could start with the application during the hours of sleep. In serious cases, where these measures are insufficient, Orabase® can also be applied during daytime.

Table 1. Drugs for selective decontamination, their target organisms and the dose at which they should be applied.

Drugs for SDD	Directed against	Daily dose for adults
Nalidixic acid	Enterobacteriaceae-species	8 g
Co-trimoxazole	Enterobacteriaceae-species	2880 mg
	Staph. aureus	2880 mg
Polymyxin B or E	Enterobacteriaceae-species (except <i>Proteus</i>)	800 mg
	<i>Pseudomonadaceae</i> -species	800 mg
Amphotericin B	yeasts	2 g
Nystatin	yeasts	6×10^6 I.U.
Cefradin	Staph. aureus	6 g

(2) *Yeasts*. In contrast to the Gram-negative rods, the primary source of yeasts is the oropharynx. Without antimycotic treatment more than 50% of the granulocytopenic patients become colonized by *Candida*- or *Torulopsis*- species. From this site the rest of the digestive tract can become contaminated or colonized by the yeast, which will result in positive faecal cultures. For elimination of yeasts either amphotericin B or nystatin could be used. Because of the lower MICs of yeasts for amphotericin B we prefer this drug. To optimize drug activity in the oropharynx this drug is preferably given as a suspension. However, in spite of this, it is very difficult to free the oropharynx of yeasts, i.e. to obtain negative throat swab cultures. For improvement, lozenges containing these polyene antibiotics can be applied. The addition of lozenges is particularly advocated when the patient has mucosal lesions, clinically suspect for *Candida* infection or when foreign bodies such as a nasogastric tube are present.

Dentures must not be worn, except during meals and at visiting time.

(3) *Staphylococcus aureus*. This micro-organism can best be eliminated with cephadrine. However, only a minority (about 10%) of our patients has so far been found colonized by *Staphylococcus aureus*. Only in such patients is cephadrine administered. Cephadrine treatment can generally be stopped after a three week course, provided the cultures are negative. A second-choice drug for elimination of *Staph. aureus* is co-trimoxazole (6 tablets/day).

All drugs for selective decontamination are continued as long as the granulocyte count is below $0.5 \times 10^9/l$ blood.

Food

Usually, selectively decontaminated patients are not isolated, and they can eat the normal non-sterile hospital food. However, food which can often be heavily contaminated, such as croquettes, raw meat and salads, should be avoided. Patients with insufficient oral intake can be fed parenterally or via a nasogastric tube. In the case of enteral

hyperalimentation we prefer the use of sterile food suspensions which are commercially available in most countries now.

Adverse effects

Adverse effects due to selective decontamination have occurred, but they are infrequent. They mainly consisted of allergic reactions, especially related to co-trimoxazole and nalidixic acid, and nausea due to amphotericin B suspension. In other cases it was difficult to detect the cause and distinguish between one of the antibiotics or the cytostatic drugs. Prolonged duration of granulocytopenia due to the use of co-trimoxazole was not found in our study although others have encountered the phenomenon.

Systemic treatment

Successfully selectively decontaminated patients should be free of potential pathogens in their digestive tract as indicated and have negative cultures of their faeces and their throat swabs.

Although the cultures become generally negative by SD-treatment, this does not absolutely guarantee that all potential pathogens are really absent. When such a patient develops fever, careful examination of the patient is necessary to try to obtain an explanation for the rise of the temperature. When allergic reactions due to drugs or transfusions can be excluded and only possible or suspected infectious causes remain, prompt institution of a combination of cidal antibiotics is indicated. The choice of antibiotics for that purpose must be guided by the most recent findings from the surveillance cultures, including the data from the inventory cultures. If possible, the combination of antibiotics should be CR-indifferent, for example tobramycin plus cephadrine or moderate doses of cefuroxime. If a *Pseudomonas* infection is considered possible, addition of a ureidopenicillin such as piperacillin is necessary. However, this drug is excreted via the liver and is active against many anaerobes and is therefore likely to decrease the colonization resistance of the digestive tract. In the near future, cephalosporins that are less CR-de-

creasing and with anti-pseudomonas activity, such as ceftazidime, may become more generally available. This drug does not appear to affect the CR, at least not in mice.

Viridans streptococci and enterococci must be taken into account as possible causes of infection, especially when the (oropharyngeal) mucosal barrier has been obviously damaged by chemotherapy. Even (oral) anaerobes may have a portal of entry in such cases. If oral streptococci are isolated from the blood, systemic therapy must be adjusted according to the susceptibility pattern of the microorganisms isolated.

Systemic treatment of suspected *Candida* infections can best be done by i.v. administration of a combination of amphotericin B (0.3 mg/kg/day) and 5-fluorocytosine (150 mg/kg/day) [74].

References

1. Bodey GP, Buckley M, Sathe YS, Freireich EJ: Quantitative relations between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med, 1966; 64: 328-40.
2. Gaya H: The treatment of infection in acute leukemia. Br J Hosp Med, 1975; 13: 124-29.
3. Gurwith MJ, Brunton JL, Lank BA, Ronald AR, Harding GKM: Granulocytopenia in hospitalized patients. I. Prognostic factors and etiology of fever. Am J Med, 1978; 64: 121-32.
4. Klastersky J, Henri A, Hensgens C, Daneau D: Gram-negative infections in cancer. Study of empiric therapy comparing carbenicillin-cephalotin with and without gentamicin. JAMA, 1974; 227: 45-48.
5. Schimpff SC, Landesman S, Hahn DM, Stanford HC, Fortner CL, Young VM, Wiernik PH: Ticarcillin in combination with cephalotin or gentamicin as empiric antibiotic therapy in granulocytopenic cancer patients. Antimicrob Ag Chemother, 1976; 10: 837-44.
6. Schimpff SC: Therapy of infection in patients with granulocytopenia. Med Clin North Amer, 1977; 61: 1101-18.
7. EORTC International antimicrobial therapy project group. Three antibiotic regimens in the treatment of infection in febrile granulocytopenic patients with cancer, 1978; 137: 14-29.
8. Issell BF, Keating MJ, Valdivieso M, Bodey GP: Continuous infusion tobramycin combined with carbenicillin for infections in cancer patients. Am J Med Sci, 1979; 227: 311-18.
9. Tattersall MHN, Spiers ASD, Darrell JH: Initial therapy with combination of five antibiotics in febrile patients with leukemia and neutropenia. Lancet, 1972; i: 162-65.
10. Tattersall MHN, Hutchinson RM, Gaya H, Spiers ASD: Empirical antibiotic therapy in febrile patients with neutropenia and malignant disease. Eur J Cancer, 1973; 9: 417-23.
11. Graw RG, Herzig GP, Perry S, Henderson ES: Normal granulocyte transfusion therapy. Treatment of septicemia due to gram-negative bacteria. N Engl J Med, 1972; 287: 367-71.
12. Herzig RH, Herzig GP, Graw RG, Bull MI, Ray KK: Successful granulocyte transfusion therapy for gram-negative septicemia. A prospective randomized controlled study. N Engl J Med, 1977; 296: 701-5.
13. Ford JM, Cullen MH: Prophylactic granulocyte transfusions. Exp Hematol, 1977; 5: 65-72.
14. Schimpff SC, Young VM, Greene WH, Vermeulen GD, Moody MR, Wiernik PH: Origin of infection in acute non-lymphocytic leukemia. Significance of hospital acquisition of potential pathogens. Ann Intern Med, 1972; 77: 707-14.
15. Gaya H, Tattersall MHN, Hutchinson RM, Spiers ASD: Changing patterns of infection in cancer patients. Eur J Cancer, 1973; 9: 401-6.
16. Mortensen N, Mortensen BT, Nissen NI: Bacteraemia in patients with leukaemia and allied neoplastic diseases. Scand J Inf Dis, 1976; 8: 145-49.
17. Bodey GP, Gehan EA, Freireich EJ: Protected environment-prophylactic antibiotic programm in chemotherapy of acute leukemia. Am J Med Sci, 1971; 262: 138-59.
18. Levine AS, Siegel SE, Schreiber AD, Hauser H, Goldstein JM, Seidler F, Perrey SR, Bennett J, Henderson ES: Protected environment and prophylactic antibiotics. N Engl J Med, 1973; 288: 477-83.
19. Dietrich M: Gnotobiotics in hematology: improvement of treatment of acute leukemia. Eur J Cancer, 1975; 11: 49-55.
20. Schimpff SC, Greene WH, Young VM, Fortner CL, Jepsen L, Cusack H, Block JB, Wiernik PH: Infection prevention in acute nonlymphocytic leukemia. Ann Intern Med, 1975; 82: 351-58.
21. Pizzo PA, Levine AS: The utility of protected environment regimens for the compromised host: a critical assessment. Progr Hematol, 1977; 10: 311-32.
22. EORTC. Gnotobiotic Project Group: Protective isolation and antimicrobial decontamination in patients with high susceptibility to infection. A prospective cooperative study of gnotobiotic care in acute leukemia patients III: The quality of isolation and decontamination. Infection, 1978; 6: 175-91.
23. Kurrle E, Bhaduri S, Heimpel H, Hoelzer D, Krieger D, Vanek E, Kubanek B: The efficiency of strict reverse isolation and antimicrobial decontamination in remission induction therapy of acute leukemia. Blut, 1980; 40: 187-95.
24. Kurrle E, Bhaduri S, Krieger D, Gaus W, Heimpel H, Pflieger H, Arnold R, Vanek E: Risk factors for infections of the oropharynx and the respiratory tract in patients with acute leukemia. J Inf Dis, 1981; 144: 128-36.
25. Schimpff SC, Greene W, Young VM, Wiernik PH: Signifi-

- cance of *Pseudomonas aeruginosa* in the patient with leukemia or lymphoma. *J Inf Dis*, 1974; 130 (suppl): 24–31.
26. Pollack M, Nieman RE, Reinhardt JA, Charachie P, Jett MP, Hardy PH: Factors influencing colonization and antibiotic-resistance patterns of gram-negative bacteria in hospital patients. *Lancet*, 1972; ii: 668–71.
27. Myerowitz RL, Medeiros AA, O'Brien TF: Recent experience with bactillemia due to gram-negative organisms. *J Inf Dis*, 1971; 124: 239–46.
28. Enno A, Darrell J, Hows J, Catovsky D, Goldman JM, Galton DAG: Co-trimoxazole for prevention of infection in acute leukaemia. *Lancet*, 1978; ii: 395–97.
29. Gurwith MJ, Brunton JL, Lank BA, Harding GKM, Ronald AR: A prospective controlled investigation of prophylactic trimethoprim/sulfamethoxazole in hospitalized granulocytopenic patients. *Am J Med*, 1979; 66: 248–56.
30. Dekker AW, Rozenburg-Arsk M, Sixma JJ, Verhoef J: Prevention of infection by trimethoprim-sulfamethoxazole plus amphotericin B in patients with acute nonlymphocytic leukemia. *N Engl J Med*, 1981; 95: 555–59.
31. Kurle E, Bhaduri S, Kreiger D, Pflieger H, Heimpel: Antimicrobial prophylaxis in acute leukemia; prospective randomized study comparing two methods for selective decontamination. *Klin Wochenschr*, 1983; 61: 691–98.
32. Kurle E: Infektprophylaxe bei der Induktionstherapie akuter Leukämien. *Klin Wochenschr*, 1981; 59: 1075–79.
33. Watson JG, Powels RL, Lawson DN, Morgenstern GR, Jameson B, McElwain TJ, Judson I, Lumley H, Kay HEM: Co-trimoxazole versus nonadsorbable antibiotics in acute leukaemia. *Lancet*, 1982; i: 6–9.
34. Wade JC, de Jongh CA, Newman KA, Crowley J, Wiernik PH, Schimpff SC: A comparison of trimethoprim/sulfamethoxazole to nalidixic acid: Selective decontamination as infection prophylaxis during granulocytopenia. *J Inf Dis*, 1983; 147: 624–31.
35. Wilson JM, Guiney DG: Failure of oral trimethoprim-sulfamethoxazole prophylaxis in acute leukemia. *N Engl J Med*, 1982; 306: 16–20.
36. Knothe H: The effect of a combined preparation of trimethoprim and sulfamethoxazole following short-term and long-term administration on the flora of the human gut. *Cancer Chemotherapy*, 1973; 18: 285–96.
37. Näff H: Über die Veränderungen der normale Darmflora des Menschen durch bactrim. *Pathol Microbiol*, 1971; 37: 1–22.
38. Moorhouse EC, Farrell W: Effect of co-trimoxazole on faecal enterobacteria: no emergence of resistant strains. *J Med Microbiol*, 1973; 6: 249–52.
39. Lacey RW: Do sulphonamid-trimethoprim combinations select less resistance to trimethoprim than the use of trimethoprim alone? *J Med Microbiol*, 1982; 15: 403–27.
40. Sleijfer DT, Mulder NH, De Vries-Hospers HG, Fidler V, Nieweg HO, Van der Waaij D, van Saene HKF: Infection prevention in granulocytopenic patients by selective decontamination of the digestive tract. *Eur J Cancer*, 1980; 16: 859–69.
41. De Vries-Hospers HG, Van der Waaij D, Sleijfer DT, Mulder NH, Nieweg HO, Van Saene HKF: Selective decontamination of the digestive tract in granulocytopenic patients: the occurrence of resistance. In: *New Criteria for Antimicrobial Therapy: Maintenance of Digestive Tract Colonization Resistance*, Van der Waaij D, Verhoef J (eds). Excerpta Medica, Amsterdam-Oxford, 1979: 117–29.
42. De Vries-Hospers HG, Sleijfer DT, Mulder NH, Van der Waaij D, Nieweg HO, van Saene HKF: Bacteriological aspects of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients. *Antimicrob Agents Chemother*, 1981; 19: 813–20.
43. Bernstein LS: Adverse reactions to trimethoprim-sulfamethoxazole, with particular inference to long-term therapy. *Med J Aust*, 1975; 2: 5–7.
44. Van der Waaij D, Tielemans-Speltie TM, de Roeck-Houben AMJ: Infection by and distribution of biotypes of Enterobacteriaceae species in leukaemic patients treated under ward conditions and in units for protective isolation in seven hospitals in Europe. *Infection*, 1977; 5: 188–94.
45. Linzenmeier G, Haralambie E: Zur gegenwärtige Kenntnis der Stuhlflora mit hinweisen auf praktische Diagnostik von Eubiose und Dysbiose. *Ärtl Lab*, 1980; 26: 89–92.
46. Kurle E, Bhaduri S, Gaus W, Heimpel H, Fliedner JM: Pathogenicity of microorganisms for oropharyngeal and respiratory tract infections in acute leukaemia patients under total and selective decontamination. In: *Recent Advances in Germfree Research*, Sasaki S, Ozawa A, Hashimoto K (eds). University Press, Tokyo, 1981: 693–96.
47. Van der Waaij D, Berghuis-de Vries JM, Lekkerkerk-van der Wees JEC: Colonization resistance of the digestive tract in conventional and antibiotic treated mice. *J Hyg*, 1971; 69: 405–11.
48. Van der Waaij D, Vossen JM, Korthals Altes C, Hartgrink C: Reconvetionalization following antibiotic decontamination in man and animals. *Am J Clin Nutr*, 1977; 30: 1887–95.
49. Costerton JW: Effects of antibiotics on adherent bacteria. In: *Action of Antibiotics in Patients*. Sabath LD (ed). Hans Huber Publishers, Bern, Stuttgart, Vienna, 1982: 160–76.
50. Van der Waaij D: The digestive tract in immunocompromised patients: importance of maintaining its resistance to colonization, especially in hospital inpatients and those taking antibiotics. In: *Action of Antibiotics in Patients*, Sabath LD (ed). Hans Huber Publishers, Bern, Stuttgart, Vienna, 1982: 104–18.
51. Parson CL, Shrom SH, Hanno PM, Mulholland SG: Bladder surface mucin: Examination of possible mechanisms for its antibacterial effect. *Invest Urol*, 1978; 16: 104–18.
52. Williams RC, Gibbons RJ: Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science*, 1972; 177: 697–99.
53. Laforce FM, Hopkins J, Trow R, Wang WLL: Human oral defenses against gram-negative rods. *Am Rev Resp Dis*, 1976; 114: 929–35.
54. Ofek I, Mirelman D, Sharon N: Adherence of *Escherichia*

- coli* to human buccal cells mediated by mannose receptors. *Nature*, 1977; 265: 623–25.
55. Johanson WG, Woods DE, Chandhuri T: Association of respiratory tract colonization with adherence of gram-negative bacilli to epithelial cells. *J Inf Dis*, 1979; 139: 667–73.
 56. Woods DE, Straus DC, Johanson WG, Berry WK, Bass JA: Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. *Inf Immun*, 1980; 29: 1146–51.
 57. Gahnberg L, Olson J, Krasse B, Carlén A: Interference of salivary immunoglobulin A antibodies and other salivary fractions with adherence of *Streptococcus mutans* to hydroxyapatite. *Inf Immun*, 1982; 37: 401–6.
 58. Van der Waaij D, Berghuis-de Vries JM, Lekkerkerk-van der Wees JEC: Colonization resistance of the digestive tract and the spread of bacteria to the lymphatic organs in mice. *J Hyg*, 1972; 70: 335–42.
 59. Berg R: Inhibition of translocation from the gastrointestinal tract by normal cecal flora in gnotobiotic and antibiotic-decontaminated mice. *Infect Immun*, 1980; 29: 1073–81.
 60. Buck AC, Cooke EM: The fate of ingested *Pseudomonas aeruginosa* in normal persons. *J Med Microbiol*, 1969; 2: 521–25.
 61. Cooke EM, Hettiaratchy GT, Buck AC: Fate of ingested *Escherichia coli* in normal persons. *J Med Microbiol*, 1972; 5: 361–69.
 62. Williams Smith H: Survival of orally administered *E. coli* in alimentary tract of man. *Nature*, 1975; 255: 500–2.
 63. Van der Waaij D, Berghuis JM: Determination of the colonization resistance of the digestive tract of individual mice. *J Hyg*, 1974; 72: 379–87.
 64. Van der Waaij D, Berghuis-de Vries JM: Selective elimination of *Enterobacteriaceae* species from the digestive tract in mice and monkeys. *J Hyg*, 1974; 72: 205–11.
 65. Thijm HA, Van der Waaij D: The effect of three frequently applied antibiotics on the colonization resistance of the digestive tract of mice. *J Hyg*, 1979; 82: 397–405.
 66. Van der Waaij D, Aberson J, Thijm HA, Welling GW: The screening of four aminoglycosides in the selective decontamination of the digestive tract in mice. *Infection*, 1982; 10: 35–40.
 67. Wiegersma N, Jansen G, Van der Waaij D: The effect of twelve antimicrobial drugs on the colonization resistance of the digestive tract of mice. *J Hyg*, 1982; 88: 221–30.
 68. Van der Waaij D, Hofstra W, Wiegersma N: Effect of betalactam antibiotics on the resistance of the digestive tract to colonization. *J Inf Dis*, 1982; 146: 417–22.
 69. Van der Waaij D: Colonization resistance of the digestive tract as a major lead in the selection of antibiotics for therapy. In: *New Criteria for Antimicrobial Therapy: Maintenance of Colonization Resistance*, Van der Waaij D, Verhoef J (eds). *Excerpta Medica*, Amsterdam-Oxford, 1979: 271–80.
 70. Hughes WT, Kuhn S, Chandhary S, Feldman S, Verzosa M, Aur RJA, Pratt Ch, George SL: Successful chemoprophylaxis for *Pneumocystis carinii* pneumonitis. *N Engl J Med*, 1977; 297: 1419–26.
 71. Welling GW: Comparison of methods for the detection of beta-aspartylglycine in fecal supernatants of leukemic patients treated with antimicrobial agents. *J Chron*, 1982; 232: 55–62.
 72. De Vries-Hospers HG, Sleijfer DT, Mulder NH, Van der Waaij D, Nieweg HO, Van Saene HKF: Bacteriological aspects of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients. *Antimicrob Ag Chemother*, 1981; 19: 813–20.
 73. Medoff G, Kobayashi GS: Strategies in the treatment of systemic fungal infections. *N Engl J Med*, 1980; 302: 145–55.
 74. Mulder JG, Wiersma WE, Welling GW, van der Waaij D: Low dose oral tobramycin for selective decontamination of the digestive tract: a study in human volunteers. *J Antimicrob Chemother*, 1984; 13: 495–504.

4. Apheresis units: designs and future applications

J.A. STAHL and A.A. PINEDA

Apheresis units have been established to separate whole blood from a donor to obtain a specific component for transfusion into a patient, collection of a specific blood component for further processing to obtain a specific product, or the removal of a portion of a patient's blood as an adjunct to the therapy of a disease state.

Apheresis blood components are needed to support a number of patients with both non-malignant and malignant disease states as more aggressive therapeutic regimens have been developed. The technology of apheresis has had a major impact upon the practice of blood transfusion over the past 15 years. In therapeutic practice, the removal of whole blood was one of the therapies used by physicians in history to remove the 'bad' elements, but apheresis technology has added the sophistication of the removal of plasma or cells suspected to be the 'bad' blood element contributing to particular disease states rather than removal of whole blood [1].

The first introduction of equipment to perform apheresis procedures was into blood transfusion centers as they held responsibility for supplying blood products for transfusion to patients, and the development of apheresis equipment was primarily to obtain blood components for transfusion. The collection of blood components for transfusion has also led to development of apheresis units outside the blood transfusion center to meet demands for blood components. One department frequently having independent apheresis capabilities of the blood transfusion service is the oncology department within the hospital [2].

For component collection from unrelated donors the blood transfusion center is a logical location for the apheresis unit, as an apheresis donor panel can be established from existing blood donors by exposure of the donors to the apheresis procedures during their regular blood donations. The blood transfusion center also has established equipment and staff to process blood components to assure safe transfusion products.

Component collection in a department such as oncology lends itself to convenience for the related donor, friend or associate who may be visiting the patient and donating a needed blood component during the same trip to the hospital. For the unrelated donor, the hospital-based department apheresis unit may give the donor a stronger feeling of being involved in the patient's therapy, but an experienced blood transfusion center can convey this involvement as a form of donor encouragement without having the donor visit the hospital, which may in some donors cause hesitation as the hospital generally represents illness to the non-medical individual.

When an apheresis unit exists in a hospital which has a blood transfusion center, to avoid duplication of skilled personnel and equipment, it is best that a good working relationship be established so that processing of the collected components may be carried out by the existing blood transfusion staff [2]. The unit in a hospital not associated with a blood transfusion service must be capable of performing all the tests necessary to assure safe transfusion policies.

Apheresis as a therapeutic modality poses spe-

cial considerations as it involves direct patient care responsibilities. The blood bank/transfusion service was instrumental in the beginning of therapeutic apheresis as they had available trained apheresis personnel and instruments to perform the procedures. Availability of the machinery and personnel has in certain facilities caused the blood bank/transfusion service director to become the director of a therapeutic apheresis facility.

One aspect of therapeutic apheresis that seems to meet the agreement of the majority of those involved in this field is that the procedure must be performed where hospital care backup is available if required [4].

The approaches to therapeutic apheresis are:

- (1) to transport the patient to the blood bank/transfusion center for therapy;
- (2) to transport the apheresis equipment and personnel to the bedside of the patient within the hospital; or
- (3) to have independent therapeutic apheresis units responsible for patient therapy.

Each of the preceding approaches is outlined in terms of advantages and disadvantages in Table 1.

Physical layout

The location and access to the apheresis unit should be based upon the aim of the unit. As previously described, the unit may handle donors for component collection, combined donor and patient responsibilities or patient care responsibilities only.

For component collection, an area where either blood donations or other apheresis procedures are being carried out will be the most comfortable for the donor as the procedure is lengthy and the activity of the center will help pass the time. Also, when done where whole blood donations are carried out, visibility invites questions and interest on the part of the regular whole blood donor, which is a form of recruitment [3]. It is essential that privacy be easily established if the donor suffers an untoward reaction; privacy will decrease donor anxiety and prevent other blood donors from being frightened away from volunteering for apheresis.

For patient therapy in the blood bank/transfu-

Table 1. Location: advantages/disadvantages.

I. Patient therapy in blood bank/transfusion service

A. ADVANTAGES

1. Cost effective, as machines and personnel are available.
2. Skilled apheresis personnel are available and able to work in familiar surroundings.
3. Availability and understanding of blood products to meet transfusion requirements.
4. Surroundings for an outpatient may prove more pleasant than a return visit to the hospital environment.

B. DISADVANTAGES

1. For the hospitalized, hospital transport may be uncomfortable, time consuming and may pose risk to patient's condition.
2. Need to develop a patient treatment unit separated from donor area.
3. Staff may be unskilled/unexperienced in the care of a seriously ill patient or may not have full understanding of current medical care.

II. Blood bank/transfusion service to patient bedside

A. ADVANTAGES

1. Cost effective as machine and personnel are available.
2. Shared patient care responsibility between apheresis personnel and patient care unit personnel.
3. Availability of rapid emergency help.
4. Familiar surroundings to the patient, which may alleviate some anxiety related to the procedure.

B. DISADVANTAGES

1. Time consuming for apheresis personnel.
2. Transport of apheresis equipment may be difficult unless a lightweight mobile instrument is selected or a machine may be 'stationed' in the treatment facility.
3. Apheresis personnel may have to work in unfamiliar surroundings, which may be uncomfortable.
4. Products for blood transfusion may not be available, requiring a 'runner' between blood bank/transfusion center and patient's bedside.

III. Dedicated therapeutic apheresis unit

A. ADVANTAGES

1. Apheresis personnel trained in care of specific disease process.
2. Avoidance of patient/machine transport.
3. Centralization of all items to perform apheresis procedure and provide patient care.
4. May be combined simultaneously with other therapeutic procedures, such as hemodialysis.

B. DISADVANTAGES

1. May be duplication within institution of apheresis instrumentation, thus costly.
 2. May be too dedicated, thus restricting therapy to a limited number of patients.
 3. Establishes a need for apheresis medical specialists in a wide variety of disease processes.
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sion service, it is best to establish patient privacy unless the patient's physical condition appears normal. The presence of a patient on an apheresis instrument, whose physical appearance betrays his disease, may be unpleasant and frightening to the normal, healthy donor.

The therapy of the patient at his bedside requires privacy. If the patient is in a general care unit where he may be exposed to the other patients, privacy should be easily established in case of reactions.

In general the area where apheresis is carried out should be well lighted, have adequate ventilation, and temperature should be comfortable [5].

Supportive apheresis

Apheresis is used to collect large quantities of blood component from donors for transfusion. The most commonly collected components for transfusion are platelets and granulocytes. Lymphocytes can be collected for use in production of transfer factor and interferon. More recently there has been collection of platelet-rich plasma to supply platelets and fresh frozen plasma or plasma for fractionation into a plasma derivative or a fraction of young red cells (neocytes).

It must be remembered that the apheresis donor is a special individual who is willing to endure special inconveniences to donate. The apheresis procedure leads to a sacrifice of personal time, possibly loss of work time and income, and the donor must accept that his blood will pass through an extracorporeal circuit and then be returned to his body [3]. Though rare, the apheresis donor is exposed to hazards from exposure of his blood to extracorporeal circuits, machinery and anti-coagulant solutions [6].

The apheresis donor must meet all the qualifications for whole blood donors set up by local and national governing boards of the area. The donor may be recruited from a patient's family members, friends, associates or regular blood donors who have a special desire to help their fellow man; frequently police and firemen fit into this unique category of donors. A large hospital apheresis de-

partment may find it advantageous to have a collaborative arrangement with the community blood bank to fill non-related donor requirements [3].

Prior to the procedure, the donor should be informed of what the procedure will consist of, such as 1 or 2 venipunctures dependent on apheresis instrument used, the administration of anti-coagulant and sodium chloride solution, length of time and possible side effects, such as citrate reactions.

It is essential that the donor be made comfortable during the apheresis procedure as the procedure is lengthy and discomfort will discourage the donor from further donations. The donor should loosen any tight clothing, necktie or belt, and be positioned so he can see what is happening around him.

Platelets may be collected from the donor for transfusion into a patient with active bleeding or prophylactically when peripheral platelets counts are below $10,000\text{--}20,000$ per mm^3 . To increase the platelet count to $30,000\text{--}50,000/\text{mm}^3$, it will take a transfusion of 1 unit of platelets per 10 kg patient's body weight. One unit is equal to approximately 5.5×10^{10} platelets; thus a man of 70 kg would require a transfusion of 3.85×10^{11} platelets. The advantage of collecting these platelets by apheresis technique is that it limits the patient's exposure to a number of donors, thus decreasing chances of alloimmunization, transfusion reactions, and spread of blood borne disease such as hepatitis.

Granulocytes may also be collected from a donor to support the neutropenic patient during infectious episodes which are resistant to antibiotic therapy or in special neutropenic patients prophylactically to prevent infection. To collect a therapeutic quantity of granulocytes from a donor, the use of a sedimenting agent, such as hydroxyethyl starch, may be employed and steroid medication to mobilize the peripheral granulocyte pools is recommended [8, 9, 10, 11]. As both these agents have been associated with reactions in the donor, their use should be discussed with the donor, and the donor should be carefully observed and questioned during the procedure.

Complications

The potential hazards to the donor are:

(1) Citrate reactions related to hypocalcemia and manifested as paraesthesia, especially in the oral area, cooling sensations, quivering sensation in the diaphragm. Severe citrate toxicity can lead to severe chest pain, tetany, convulsions and cardiac arrhythmias. The treatment for citrate toxicity is reducing the return rate of citrated blood until symptoms subside or the administration of a calcium preparation [6].

(2) Hypothermia exhibited in the form of mild coolness to shaking chills and attributed to cooling of the blood in the extracorporeal circuit [4]. This reaction is avoidable by doing the apheresis in a warm environment and having blankets available to cover patient at first sign of coolness.

(3) Hypotension is sometimes noted and related to extracorporeal volume. This is easily controlled by selection of an apheresis circuit which will limit the extracorporeal volume to 15% or less and keeping the donor supine during the procedure [6].

(4) Hemolysis has been reported and associated with a mechanical obstruction to flow. Careful observation of the procedure should eliminate any adverse effects related to reinfusion of hemolysed blood to the donor [4, 6].

(5) Air embolism is a potential risk with any intravenous administration or extracorporeal circuit, but once again with careful observation of the apheresis procedure this should be avoided [4].

(6) Allergic or hypersensitivity reactions to hydroxyethyl starch, steroids and other solutions have been reported and appropriate therapy would be dependent on the symptoms exhibited [6].

In general, the hazards related to apheresis are rare when the procedure is performed by trained, experienced apheresis personnel. The procedure can be a positive experience for the donor, proven by the increasing number of regular donors in most established apheresis programs.

Therapeutic apheresis

The utilization of plasma exchange and/or cyta-

pheresis to treat or modify a disease has increased dramatically since the advent of plasma/cell separator technology. Therapeutic apheresis has been reported in a wide variety of medical specialities including, but not limited to, oncology [12, 13, 14], dermatology [15, 16, 17], neurology [20, 21, 22], nephrology [26, 27, 28], cardiology [29, 30, 31], hematology [32] and immunology [33, 34].

Cytapheresis or cytoreduction has been used in patients with leukemias to reduce the number of peripheral white cells [35]. In certain circumstances, the cytoreduction of counts in the patient with chronic granulocytic leukemia is performed to coincide with the need for granulocyte support in a neutropenic patient [36]; this results in a two-fold effectcell burden reduction for the leukemic patient and a large dose of granulocytes for the neutropenic patient.

Cytapheresis, in the form of red cell apheresis, has been reported effective in treating certain complications of sickle cell anemia crisis [37, 38]. Lymphocytapheresis, alone or in combination with plasma exchange, is currently being evaluated as to its therapeutic effects in multiple sclerosis and rheumatoid arthritis [39].

By far the most accepted and practiced form of therapeutic apheresis today is plasma exchange [44]. Plasma exchange is the removal of large quantities of a patient's plasma and replacement with a substitute fluid. Therapeutic plasma exchange was attempted on rabbits as early as 1902 and used on humans as early as 1909 [40]. Early trials of plasma exchange were done by manual methods, which was time consuming, work intensive, and difficult for the patient. Also, results were difficult to evaluate as exchanges were of very small volumes, sometimes insignificant in reducing unwanted substances. The cell separators have allowed plasma exchange to be accomplished rapidly, with minimal morbidity and mortality.

Plasma exchange is a refinement both on the early blood letting days and whole blood exchanges, but still holds many mysteries. In certain diseases, a reduction in a specific element of the plasma can be given credit for improvement in the symptoms of the disease, but in others there is the question of what exactly is being removed that

affected the disease or is it the replacement fluid and concomitant therapy that had the effect. Another problem with therapeutic plasma exchange is that it is an extremely expensive procedure; thus it ought not be undertaken without careful consideration. Currently efforts are being made to set standards for therapeutic apheresis [3, 4, 5] procedures and the following are suggestions which are under consideration:

(1) Patients should be accepted for apheresis only when referred by a specialist in that patient's disease. The physician should establish that the patient has not responded to conventional therapy for his disease and that a rationale exists for applying the procedure.

(2) Consultation between the patient's physician and the apheresis physician must consider:

- (a) patient's existing clinical status, including complications, likely prognosis, patient's ability to withstand the procedure and a therapeutic goal.
- (b) adequacy of vascular access. Will peripheral venipunctures be possible or are the risks of alternate methods of access outweighed by the potential therapeutic gain?
- (c) cost justification. Can a reasonable result be expected that is cost effective?
- (d) drug therapy. What is the current regimen and what effect will the plasma exchange have as plasma bound drugs will be partially eliminated and dosages and administration schedules may have to be adjusted accordingly.

(3) Consultation between the patient and/or patient's family, the patient's physician, and apheresis physician must consider:

- (a) potential risks of the apheresis procedure.
- (b) cost of the procedure.

The potential side effects of therapeutic apheresis summarized by Dr. Joshua Levy, are shown in Table 2.

The patient should be evaluated prior to each therapeutic apheresis procedure to determine effects that the particular procedure may have on him. Careful, complete records from previous treatments may help to eliminate a repeat of a previously experienced side effect, e.g. a patient

Table 2. Potential side effects of plasma exchange.

Hypovolemia-shock: lightheadedness, hypotension, tachycardia, diaphoresis, nausea, tinnitus, syncope.
Congestive heart failure – pulmonary edema: tachycardia, hypertension, tachypnea, orthopnea, distended neck veins, peripheral edema.
Citrate reactions: circumoral paresthesias, tremor, muscle twitching, chills, nausea, vomiting, tetany.
Hypothermia (mild)
Hemorrhage: platelet reduction
Thrombosis: reduction in antithrombin-III
Embolism: platelet, air
Hemolysis
Blood loss (transfusion side effects)
Hematoma and tenderness at venipuncture site
Cardiac arrhythmias (K, Ca)
Infection

who became symptomatic of hypovolemia may benefit from a volume bolus prior to blood removal.

Vascular access

In therapeutic apheresis, vascular access is frequently one of the most difficult aspects of the procedure confronting the apheresis staff. In single donor apheresis, a firm median antecubital vein is usually chosen for the procedure, as is in patients. In the latter, however, due to the frequency of therapeutic apheresis and the fact that the underlying disease may have effects on the vascular system of the patient, this is not always possible. Alternative sites of peripheral access may be chosen, central cannulae may be employed, or in extreme conditions or ones where simultaneous hemodialysis therapy may be used, artificial vascular access may be indicated.

Peripheral access for apheresis must be in a vein where there is good muscular support. Veins in the hand and forearm do not have this support; thus they collapse, preventing blood to flow effectively into the plasma/cell separator. In a double access procedure, return of the unremoved blood components and replacement fluids may be introduced via a hand or forearm vein, reserving the antecubitals for bleeding into the apheresis systems, alternating

extremities as much as possible.

Also important is the size and length of the needle or catheter selected. Most procedures require a 16 gauge needle or catheter to ensure adequate flow rates. If a catheter is used, the shortest practical length should be chosen as resistance to flow varies directly with the length of the catheter [41].

Central cannulation may be considered in the patient where no peripheral access is possible. The insertion of subclavian or femoral catheters should only be undertaken by experienced personnel and adherence to strict aseptic technique is mandatory as this is an easy site to develop infection. If the catheter will be indwelling, the entry site must be protected and any exposure of the area should result in cleansing with an antiseptic, covering with a sterile dressing and when available, protection with a waterproof, sterile, adhesive cover such as used in surgery. To keep the catheter patent during non-use, either a keep open drip or regular flushing of the catheter with heparinized saline will be necessary. If the subclavian catheter is used, flow is best established with the patient-bed elevated, withdrawing with a syringe any pre-apheresis samples, attaching the apheresis harness and initiating flow into the plasma/cell separator as the patient exhales. During inhalation, a negative pressure exists within the thoracic cavity and any priming solution or anticoagulant in the apheresis circuit may be sucked through the catheter into the patient.

When subclavian catheters are used for returning, care must be taken to avoid rapid infusion of cold, citrated blood through this catheter which may be in close proximity to the heart. The warming of the reinfusion blood and the use of heparin anticoagulation are two possible considerations to overcome this danger.

Another alternative is an external arterio-venous shunt, but this method of access requires a surgical procedure. The external shunts are implanted cannulae, usually with Teflon tips attached to a silicone body. The tubes exit the body through two skin wounds and when not in use, the two ends of the shunt are joined together by a Teflon connector allowing blood to flow from artery to vein. An external shunt is an inconvenience to the patient and poses risks such as hemorrhage from sep-

aration and infection at entry and exit sites of the skin [41].

Internal arteriovenous fistulas are the most common route of access for hemodialysis and may also be used for apheresis procedures. The internal fistula is a surgical side by side anastomosis of vein and artery and is a permanent situation which does not restrict the patient from normal daily routines. This route of access should be considered only in patients who will also be receiving hemodialysis therapy or for patients with no alternative means of access for apheresis therapy in a disease where the procedure is of proven benefit.

Another question of great importance in therapeutic apheresis is anticoagulation. In single donor apheresis, citrate anticoagulation is routine, with the possible side effect of hypocalcemia due to reinfusion of citrated blood. Patients may have already low serum calcium levels or central cannulae which may make reinfusion of citrated blood a potential danger; in this case the use of heparin may be indicated for anticoagulation rather than citrate solutions. Adding heparin to a reduced citrate dose is a viable alternative.

Many other points must be addressed in therapeutic apheresis, such as replacement fluids, type and amount, frequency of apheresis procedures, laboratory settings and number of apheresis procedures to be done prior to evaluating the effects of the procedure on the disease and the patient's overall condition.

Apheresis personnel

A physician must be responsible for both donor and patient apheresis procedures. The physician must have a knowledge of the apheresis procedure and possible side effects and how to handle untoward reactions. The Technical Manual of the American Association of Blood Banks advises that there be provisions for emergency medical care for donor apheresis and that a physician who is familiar with the apheresis procedure and the patient's condition be present during a therapeutic apheresis procedure. It has also been suggested that the responsible physician be available to the patient on a

24 hour basis if a problem arises thought to be related to the apheresis therapy [4].

In regard to the apheresis operator qualifications, opinions vary. Some feel that medical technologists, nurses can be qualified and competent machine operators, but most agree that licensed, registered nurse are better equipped to handle therapeutic apheresis as they are more comfortable with drugs, intravenous fluids, cardio-pulmonary resuscitation procedures and general patient care procedures [3, 4, 5].

In selecting personnel it is important to investigate local legislation which may dictate qualifications for apheresis technologists and then select a staff who meets the qualifications and is trained and experienced in apheresis.

Future apheresis trends

In donor apheresis, the future of apheresis appears to be directed at automation with increased donor safety, reduction in donation time, and improved component yields. In addition there is a need for producing purer cell fractions and separation of subpopulations of cells. Automation reduces work load on apheresis staff and may allow an increased number of donations to be accomplished without additional staff. Automation also brings with it more safety monitors built into the system, which may decrease need for close monitoring.

These systems are usually faster than work intensive systems and thus reduce donation times. As operator variability is eliminated, more consistent and possibly improved component yields may be achieved.

The area of therapeutic apheresis will probably see more change in the future than donor apheresis. As information is compiled, more defined indications and apheresis protocols will be established. Also, as the actual role the apheresis procedure plays in modifying the disease is defined, greater sophistication of the procedures will be achieved. In plasma exchange, the procedure appears to be heading in the direction of selective removal, processing of the plasma to remove a specific plasma component contributing to the dis-

ease, and return of the depleted plasma to the patient, thus eliminating the cost and potential risks of replacement fluids. The selective plasma component removal may possibly be accomplished by 1) extracorporeal perfusion of plasma over affinity columns, 2) extracorporeal perfusion of plasma over sorbents embedded in membrane filtration systems, 3) on-line separation of membrane filtration and cryoprecipitation, 4) chemical precipitation, or 5) cryoprecipitation [43].

The removal of specific plasma components could result in a complete understanding of the pathophysiology of the disease disorder under treatment. Certainly, the next few years will bring new variations in available systems, as well as totally new concepts.

Cost considerations

The maintenance of an apheresis unit is costly and the establishment of a new unit deserves careful investigation. An apheresis unit requires special instrumentation, personnel and supplies and not every hospital can justify its own apheresis unit. The community or regional blood bank may be the logical location for component collection and distribution to a number of hospitals each with demands too low to justify their own unit; the same blood bank may offer a therapeutic apheresis service, either transporting the apheresis equipment to the hospital or the patient to the blood bank [3].

The cost of donor apheresis is covered by charges to the recipient for the product. Therapeutic apheresis is much more costly due to possible need for replacement fluids, number of procedures required and time involved. The payment for the procedure should be considered prior to initiation of the procedure as third party reimbursement is restricted to limited number of diseases at this time [4].

Conclusion

Apheresis is a relatively young medical specialty which has exciting possibilities in the future, especially the branch of therapeutic apheresis. The

need for apheresis availability to service demands is great and a facility considering establishing a unit should investigate the literature, discuss plans with experienced colleagues and visit established units before setting out on a venture of its own.

References

1. Schmidt PJ: Therapeutic plasma exchange. *Arch Intern Med*, 1981; 141: 1661-62.
2. Wright SK: The organization of a hospital-based hemapheresis unit. In: *Fundamentals of a Pheresis Program*, Nusbacher J, Berkman EM (eds). Washington D.C., American Association of Blood Banks, 1979; 1-22.
3. Huestis DW, Bove JR, Busch S: Hemapheresis. In: *Practical Blood Transfusion*, 3rd edn, Huestis DW, Bove JR, Busch S (eds). Boston, Little, Brown and Company, 1981: 315-372.
4. Levy J: Safety and standards in therapeutic apheresis. *Plas Ther*, 1982; 3: 195-216.
5. Cona JA: Establishing a therapeutic pheresis program. *Artif Organs*, 1981; 5: 229-33.
6. Kotwas L: Pheresis donor reactions and complications: Prevention, recognition, and management. In: *Fundamentals of a Pheresis Program*, Neubacher J, Berkman EM (eds). Washington DC, American Association of Blood Banks, 1979; 66-77.
7. Penner JA: Blood Component therapy for patients with coagulation disorders. In: *Clinical Practice of Blood Transfusion*, Petz LD, Swisher SN (eds). New York, Churchill Livingston, 1981: 501-526.
8. Höcker P, Mann M, Pittman E: The collection and transfusion of granulocytes. *Plas Ther*, 1982; 3: 183-94.
9. Glasser L, Huestis DW, Jones JF: Functional capabilities of steroid-recruited neutrophils harvested for clinical transfusion. *N Engl J Med*, 1977; 297: 1033-36.
10. Mc Credie K, Friereich EJ, Hester JP, Valejos C: Increased granulocyte collection with the blood cell separator and the addition of etiocholanolone and hydroxethyl starch. *Transfusion*, 1974; 14: 357-64.
11. Mishler JM: Donor conditioning agents: Usage and effects on in vitro and in vivo neutrophil function. In: *Cell Separation and Cryobiology*, Rainer H, Schattauer FK (eds). Stuttgart, New York, Verlag, 1978.
12. Graf J, Carpenter N, Medenica R, Miescher PA: Plasmapheresis in cancer patients. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 229-233.
13. Israel L, Edelstein R, Samak R: Repeated plasma exchange in patients with metastatic cancer. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 238-243.
14. Beyer JH, Schuff-Werner P, Kaboth U, Klee M, Kostering H, Nagel GA: Plasmapheresis: First clinical results in malignant tumors. *Schweiz Med Wochenschr*, 1981; 111: 1522-24.
15. Andersen E, Andresen R, Clenmensen OJ: Treatment of psoriasis with plasmapheresis (letter). *Arch Dermatol*, 1982; 118: 74.
16. Hunziker T, Schwarzenbach HR, Krebs A, Nydegger UE, Camponovo F, Hess M: Plasma exchange in pemphigus vulgaris. *Schweiz Med Wochenschr*, 1981; 111: 1637-42.
17. Rebora A, Verino G, Aratari E, Scala D, Adani R, Strada P: Plasma exchange in sporadic erythroderma (letter). *Br J Dermatol*, 1982; 106: 119-20.
18. Brehm G: Plasma exchange in thyrotoxicosis. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 121-125.
19. Kallee E: Artificial reflux of thyroid hormones from tissue into blood stream mediated by hormone-binding human serum proteins. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 126-129.
20. de Jager AE, The TH, Smit Sibinga CTh, Das PC: Plasma exchange in five patients with acute Guillain Barre syndrome. *Int J Artif Organs*, 1981; 4: 230-33.
21. Miller RG: Plasmapheresis in myasthenia gravis (letter). *Ann Neurol*, 1981; 10: 369-97.
22. Newsom-Davis J, Hawkey C, Vincent A: Plasma exchange in the treatment of myasthenia gravis. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 180-185.
23. Gordon GV: Rheumatoid vasculitis. *Int J Dermatol*, 1981; 20: 546-47.
24. Dequeker J, Naesens M, Martens J, Pieters R: The effect of plasma exchange on synovitis in rheumatoid arthritis. *Scand J Rheumatol*, 1981; 10: 273-279.
25. Wallace DJ, Goldfinger D, Gatti R, Lowe C, Ian P, Bluestone R, Klinenberg R: Plasmapheresis and lymphocytapheresis in the management of rheumatoid arthritis. *Arthritis and Rheumatism*, 1979; 22: 703-10.
26. Bonomini V, Vangelista A, Stefoni S, Frasca G, Nanni Costa A: Effect of plasma exchange and thoracic duct drainage on immunological status in glomerulonephritis. *Proc Eur Dial Transplant Assoc*, 1981; 18: 736-42.
27. Sommerlad KH, Leber HW, Rawer P, Scholz R, Graubner M, Schütterle G: Plasma exchange and immunosuppression in glomerulonephritis. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 161-167.
28. Cardella CJ: Does plasma exchange have a role in renal transplant rejection? *Plas Ther Trans Tech*, 1982; 3: 153-56.
29. Schriewer H, Assmann G: Aspects of familiar hypercholesterolemia with regard to plasma exchange. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 104-108.
30. Stoffel W, Borberg H, Greve V: Application of specific extracorporeal removal of low density lipoprotein in familial hypercholesterolemia. *Lancet*, 1981; ii: 1005-7.

31. Strauer BE, Fateh-Moghadam A, Kment A, Samtleben W, Volger E: Use of plasmapheresis and immunosuppressive therapy in coronary microangiopathies. *Bibl Haematologica*, 1981; 47: 213-27.
32. Bloom AL: Current status and trends in the treatment of hemophiliac patients with inhibitors. *Prog Clin Biol Res*, 1981; 72: 123-38.
33. Valbonesi M, Garelli S, Mosconi L, Manca F, Cantarella S, Celada F: Patients with high titers of circulating immune complexes are most likely to benefit from plasmapheresis treatment. *Int J Artif Organs*, 1981; 4: 234-37.
34. Rother U: Diagnostic methods in autoimmune and immune complex disease. In: *Plasma Exchange Therapy*. Borberg H, Reuther P (eds). Stuttgart, New York, Georg Thieme Verlag, 1981: 141-148.
35. Huestis DW, Price MJ, White RF, Inman M: Leukapheresis of patients with chronic granulocytic leukemia (CGL), using the Haemonetics blood processor. *Transfusion*, 1976; 16: 255-60.
36. Höcker P, Mann M, Pitterman E: The collection and transfusion of granulocytes. *Plas Ther Trans Tech*, 1982; 3: 183-94.
37. Kleinman S, Thompson-Breton R, Rifkind S, Goldfinger D: Exchange red blood cellpheresis in the management of complications of sickle cell anemia. *Proceedings of the Haemonetics Research Institute Advanced Component Seminar*. Boston, 1979.
38. Davis K, Thorp D, Taylor A, Dart C, Taylor A: Red cell exchange in sickle cell disease. *Plas Ther*, 1980; 1: 27-32.
39. Gilcher RO, Jone JV, Rock GA: Meetings in perspective: *Haemonetics Research Institute Advanced Component Seminar*, Boston. *Plas Ther Trans Tech*, 1982; 3: 331-32.
40. Fleig C: Autotransfusion of washed corpuscles as a blood washing procedure in toxemia: Heterotransfusion of washed corpuscles in anemia. *Bull Med Acad Sci of Montpellier*, 1909; 1 (4).
41. Gutch CF, Stoner MH: Review of hemodialysis for nurses and technicians. St. Louis, CV Mosby, 1979: 98-111.
42. American Association of Blood Banks: Technical manual. Washington D.C.: American Association of Blood Banks, 1981; 7 & 34.
43. Pineda A, Taswell HF: Selective plasma component removal: Alternatives to plasma exchange. *Artif Organs*, 1981; 5: 234-39.
44. Kanneth H, Rock GA: Therapeutic plasma exchange. *N Engl J Med*, 1984; 310: 762-71.

5. Platelets for transfusion: collection, storage and clinical efficacy

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Introduction

Platelet replacement therapy has assumed an important role in transfusion therapy in recent years in the treatment of patients with bone marrow failure. However, it is clear that optimal preparation and storage of these components is necessary to produce a product of benefit to the recipient. This section will review current standards of platelet collection and storage as well as the evaluation of the results of platelet transfusion.

Platelet collection and storage

Platelets may be collected as units of platelet concentrate produced by differential centrifugation from units of whole blood obtained from random blood donors. This is the standard or traditional method of platelet collection and remains, quantitatively, the most important source of platelets for transfusion. Platelets may also be obtained by apheresis of single donors by manual technique or by the use of continuous or discontinuous flow blood cell separator machines. Any of the above techniques requires close attention to standard procedures if the best possible product is to be produced.

Standard collection

Platelets are only one of the products of whole blood collection. Separation of platelets from blood requires consideration of the best conditions

for production of red cells and plasma for which there is heavy demand in all transfusion services. In addition, the efficient use of personnel time may partially dictate centrifugation speeds and times that are selected. Much information is available on the optimal separation of platelets from whole blood. The blood may be collected into CPD, CPDA-1 or ACD anticoagulant and recent information would indicate CPDA-2 is also adequate for platelet collection [1]. ACD is no longer commonly used because it is not the ideal anticoagulant for red cell storage. U.S. Federal regulations require that platelets be separated from whole blood within 4 hours of blood collection. However, the American Association of Blood Banks (AABB) [2] accepts separation within 6 hours of collection, and there is good evidence that the subsequent platelet product does not deteriorate if separation is delayed for 8 hours [1]. The blood should be maintained at 20–24°C prior to platelet separation. Centrifugation should also be carried out at room temperature.

Centrifugation speeds and times should be selected to allow maximum separation of platelets without loss of platelet viability and function. U.S. Federal regulation requires that 75% of the units of platelet concentrate produced contain at least 5.5×10^{10} platelets with pH greater than 6.0 after three days of storage [2]. AABB requires that testing be done once a month on 4 units of platelet concentrate at the end of their storage period to ensure that these standards are being met. Because of day to day variability in machines and operators, this surveillance may be insufficient for quality con-

trol. These standards should be regarded only as the minimum controls necessary.

The initial centrifuge spin to produce platelet rich plasma (PRP) has not been shown to alter platelet function or viability in any way, so attention should be directed toward achieving the maximum efficiency of platelet separation. Using the Sorval RC-3 centrifuge with horizontal head (HG-4L), the AABB recommends speeds of 2500 rpm for 3 min (onset of centrifugation to onset of braking) for the preparation of PRP [2]. There is an inverse relationship between time of centrifugation and centrifuge speed in the separation of PRP [3]. However, Murphy [4] as well as Kahn *et al.* [3] have found that at speeds above 3000 rpm minor defects in the tachometer or timer in the RC3 may result in reduced platelet recovery. For this reason Murphy recommends spins of 2500 to 3000 rpm for 4 to 6 min as a compromise between optimization of PRP separation and efficient use of blood banking time. Slichter and Harker [5] were able to improve the platelet separation somewhat by using a slower speed (1000 g) for 9 min. However, the increased time for this procedure may be prohibitive in most bloodbanks.

There is concern that any of the above techniques results in PRP that is depleted of the largest and most metabolically active platelets, as these are sedimented into the red cell layer [6]. However, to date there is no practical method to recover these platelets. In contrast to the earlier work by Corash *et al.* [7] and others [6], Mezzano *et al.* [8] were unable to demonstrate difference in density or volume between platelets in PRP and those lost in the red cells. There is also concern that contamination of the PRP with white blood cells, predominantly lymphocytes, results in decreased platelet viability with storage, as the white cells are more metabolically active than platelets and may cause more dramatic falls in pH within the concentrate [9]. Removal of the white cells is favored by the longer, slower spins described above, e.g. 2500 rpm \times 6 min. Other authors [10] have suggested that the white count is not important in determining post storage pH of platelet concentrate. However, in this study the platelet count of the tested units is not stated to be constant and any

change in pH due to changing white cell count may have been obscured by the change induced by variable platelet counts in the units. Because of these conflicting results, the importance of reducing white cell contamination in platelet concentrates in order to keep post storage changes in pH to a minimum is not certain.

PRP must be centrifuged at room temperature to produce platelet concentrate. This procedure can result in significant platelet damage and difficulty in resuspension if the spin is too vigorous [5]. The AABB recommends 5000 g (4000 rpm) for 5 min [2]. Slichter and Harker found that platelet viability and survival were significantly reduced at forces greater than 4000 g and recommend spins at 3000 g for 20 min as optimal. The difference demonstrated is small, however, compared to the additional time required for the longer spin. Ninety-five percent of the platelets in PRP should be recovered with this spin. When the platelet poor plasma is expressed from the bag, at least 30 ml of plasma must remain with the platelets to allow maintenance of viable pH with storage. However, for many units this volume will be insufficient and volumes of 50 to 70 ml are optimal for storage beyond 24 h [4]. Immediately after collection there will be significant platelet aggregate formation in many of the units of concentrate. The most practical way to promote disaggregation of the platelets is to allow them to rest undisturbed at room temperature for 60 to 90 min [11]. Gentle manipulation will then usually allow easy resuspension. This phenomenon of aggregation appears to occur because preparation of platelet concentrate results in a degree of platelet activation associated with ADP and platelet factor 4 release. However, this is a reversible phenomenon that will subside with time after platelet collection.

Any of the above centrifugation methods should result in recovery of at least 70% of the platelets in the initial unit of whole blood. This should be confirmed in individual laboratories by careful quality control.

Platelet storage

Living, healthy platelets have a smooth, disc-like

shape. They function metabolically, consuming both oxygen and glucose and producing CO_2 and lactate, the latter under more anaerobic conditions. They aggregate in the presence of agents such as collagen, thrombin, ADP and epinephrine and are hemostatically active when transfused. As platelets become damaged they show progressive changes in morphology, transforming from discs to spheres and finally to dendritic and balloon-like cells as a consequence of loss of microtubular structures. These progressive abnormalities in platelet morphology have been shown to predict decreases in platelet recovery and survival following transfusion [12]. Severe abnormalities are irreversible and associated with loss of cell viability and function [13].

A large number of variables have been investigated and found to be important in maintaining stored platelets in the optimum condition for transfusion.

Temperature

After controversy for a number of years it is now accepted that platelet storage at 20°C to 22°C is superior to storage at 4°C . Storage of platelets at 4°C for even 24 hours causes them to change shape from discs to spheres and to suffer other physical and metabolic changes. Following transfusion they function only briefly and survive for less than 2 days. After 72 hours' storage at 4°C , they fail to function effectively even shortly after transfusion and survive in the circulation less than 24 hours [13, 14]. In contrast, platelets stored at 20°C to 22°C retain their disc-like shape and metabolic activity. Although their *in vitro* function as measured, for example, by platelet aggregation, is reduced after as short a time as 24 hours' storage [15], when platelets stored at 22°C are transfused they circulate with a normal survival time and will correct the abnormal bleeding time associated with thrombocytopenia. While storage at 22°C has been shown to be superior to that at 4°C , it has also been demonstrated that storage at temperatures above 27°C causes adverse changes in the morphology and pH of the stored concentrates [12] and so should be avoided. Standard platelet incubators are available and useful in maintaining storage temperatures within this critical range.

pH

The post storage pH of platelet concentrates has been shown to be directly related to changes in platelet morphology [12]. As pH in the platelet concentrates drops toward 6.0, the platelets gradually change shape from discs to spheres. Below pH 6.0 these changes are severe and irreversible [13].

There is an inverse relationship between the platelet concentration in units of concentrate and the post storage pH. Several studies have shown that at platelet counts greater than approximately $1.7 \times 10^6/\mu\text{l}$ a large proportion of concentrates will have pH's less than 6.0 after 72 hours' storage [13] with associated severe changes in platelet morphology which indicate nonviability [12]. If units of platelet concentrate are stored in 50 to 70 ml of plasma the majority will have platelet counts less than $1.7 \times 10^6/\mu\text{l}$ and maintain a pH greater than 6.0 over 72 hours [13].

The change in pH within platelet concentrates is due to production of lactic acid and CO_2 by platelet metabolism [18]. If oxygen supply within the concentrate is adequate, glucose will be metabolized to CO_2 , whereas if oxygen is scarce, lactic acid will be produced. The ability to allow O_2 and CO_2 to diffuse into and out of the platelet concentrate is one of the most important characteristics of the plastic from which platelet storage bags are made. Polyvinyl chloride containers such as the Fenwall PL-146 bag have allowed platelet storage for 72 hours without serious loss of platelet viability or function [13, 15]. Recent studies with new containers made from plastic designed to increase permeability to O_2 and CO_2 have shown that it is possible to store platelets at 22°C for 5 days with maintenance of pH and post transfusion recovery and survival [16, 17]. Further refinements in these plastics may soon allow storage of platelets for as long as a week. In designing the material for these new containers, it has been important to optimize O_2 transport without facilitating excess CO_2 diffusion out of the bag which causes the pH to rise. Severely elevated pH will cause spontaneous platelet aggregation and abnormal platelet morphology [19].

Agitation

When platelets are stored at room temperature, some form of gentle agitation is necessary to maintain optimal viability [13]. Agitation brings plasma and platelets into frequent contact with the storage bag walls, where diffusion of O_2 and CO_2 takes place. The type of agitation used will also affect the success of platelet storage. Holme *et al.* [20] have shown that horizontal agitation at 70 cycles/min is superior to the ferris wheel form (5 cycles/min) as measured by post transfusion recovery, survival and maximal aggregation following 72 hours' storage. Platelet morphology is also better maintained with the horizontal agitation. Newer methods of agitation such as the Helmer tumbler agitation may give similar results to the horizontal shakers [17].

Bacterial contamination

One of the concerns about storage of platelets at 22°C rather than 4°C, especially for periods of 3 days or longer, is the potential for the proliferation of small numbers of bacteria inadvertently inoculated into the concentrate during blood collection. Although Slichter and Harker found no bacterial growth in over 300 units of concentrate [13] stored for 72 hours, and other investigators [21] have found no contamination in smaller numbers of tested units, Buchholz *et al.* [22] demonstrated bacteria in 1.4% of units of platelet concentrate. The frequency of recovery of bacteria increased with the duration of storage up to 3 days. Most of the contaminating bacteria were not those generally considered pathogenic, e.g. *staphylococcus epidermidis* or *diphtheroids*. However, many patients receiving platelets are severely immunosuppressed or frankly granulocytopenic and so may be susceptible to less virulent organisms. In the study by Buchholz *et al.* six septic episodes were described in patients, which could be attributed to bacterial contamination of transfused platelets. Although there is some evidence that platelets can inhibit bacterial growth when the contamination consists of certain organisms at low concentration [21], there is no doubt that inoculation of microorganisms into platelet concentrates is a major concern with room temperature storage and particularly as storage is extended for longer periods of time.

Rigid adherence to aseptic technique in phlebotomy is necessary to prevent this serious side effect in the recipient.

With the numerous practical problems of optimizing all the variables important in platelet storage discussed above, it is likely that the results of platelet storage in a routine blood bank are less successful than those reported in the literature. It is therefore important that blood bankers be aware of the major issues and attempt to deal with them if a useful platelet product is to be given to the recipients. Developments such as the new platelet storage bags may make some of these variables less critical, especially for storage of 3 days duration or less.

Cryopreservation of platelets

Even with the newer plastic containers platelet storage at room temperature is limited to a few days. Techniques are available which will allow storage of platelets suspended in plasma and 5% DMSO and frozen at -120°C in liquid nitrogen for longer than 3 years [23]. In Baltimore we have developed a clinical program of platelet cryopreservation over the past 9 years which has gradually increased in size and which now represents a major part of the platelet transfusion support for leukemia patients who are heavily alloimmunized against random donor platelets [24]. Many of these patients have rare HLA types, making location of suitably matched donors difficult if family members are not compatible. However, the patients can be platelepheresed while in remission with normal platelet counts and their platelets frozen for subsequent transfusion during future courses of chemotherapy or leukemic relapse. Frozen HLA-matched transfusions can also be stored for these difficult patients, and random donor platelets can be stored for use during emergencies or periods of platelet shortage for nonimmunized recipients.

The results of our autologous frozen platelet transfusion program have been very consistent over recent years. The average overall freeze-thaw loss is 15 to 20%. The mean one hour post transfusion recovery with autologous frozen platelets is 55 to 60% of that obtained with fresh platelets. The post transfusion survival of platelets which survive

initial destruction appears normal, with 70% of the recovered platelets circulating 24 hours after transfusion [25]. The clinical effectiveness of the transfusions as measured by cessation of spontaneous bleeding and ability to withstand minor surgical procedures has been satisfactory. Other investigators [26] have achieved similar results with platelets frozen in DMSO but have been able to store them successfully at -80°C , which overcomes the necessity for an expensive liquid nitrogen freezer. However, it is not known if the higher temperature will allow storage for longer than 6 months.

The use of glycerol-glucose as the cryopreservative agent for platelet freezing has had success in initial reports from one group of investigators [27]. However, in our experience, glycerol-glucose is not as effective as DMSO in preventing platelet damage and loss during freezing [28]. We continue to recommend DMSO for platelet cryopreservation. More than 90% of the DMSO is removed in a single wash step before transfusion, and we have seen no significant side effects from infusion of the small amount that remains.

Single donor platelets

There are a number of real and theoretical concerns that make the transfusion of large numbers of platelets from one donor preferable to the use of pooled random donor platelets in some patients. Patients who have become alloimmunized following multiple transfusions or pregnancy will have a better response to HLA matched platelets than to the random donor units [29]. Treating a patient who will require long-term platelet support with single donor platelets from the beginning of therapy has the theoretical potential benefit of preventing or delaying immunization. However, this hypothesis requires scientific proof before such practice becomes widespread because of the increased cost of single donor platelets [30]. At present, conclusive published evidence to support the routine use of single donor platelets for nonimmunized recipients is lacking.

Before the increased availability of blood cell separator machines, single donor platelets were prepared using standard blood bags arranged in a

quadruple plateletpheresis pack. This allowed 4 units of blood to be collected in sequence from one donor and each one separated into red cells, plasma and platelet concentrate by centrifugation [31]. The red cells and plasma would be returned to the donor before the next unit of blood was withdrawn. Two to three hours were required to process 4 units of blood. The 4 units of platelet concentrate could then be pooled and transfused to the recipient. In 1968, Tullis *et al.* [32] described plateletpheresis with a discontinuous blood flow system which utilized a disposable plastic centrifuge bowl. This method has been incorporated into the Haemonetics Blood Cell Separator which is now widely used for platelet collection.

Briefly, plateletpheresis with the Haemonetics machine is accomplished as follows. Blood from the donor enters the centrifuge bowl at 60 to 80 ml per minute. When the buffy coat approaches the exit port of the bowl, blood collection is slowed and platelet collection begun. If collection is stopped when red cells begin to be collected with the platelets only 40% of the platelets in the blood are harvested. The efficiency can be increased to greater than 80% if collection is continued 15 ml into the red cell mass. This increase in platelet harvest is accompanied by collection of 1×10^9 lymphocytes and 3.3 ml of red blood cells with each filling of the centrifuge bowl [33]. Recent modifications of this machine allow collection of a platelet product which is free of red cells and greatly depleted of white blood cells. This is accomplished by use of a 'surge pump' device which directs a stream of previously collected donor plasma back into the centrifuge bowl as the buffy coat approaches the exit port. The plasma stream elutriates the platelets from the top of the red and white cell mass. The product collected has less than 10% of the white cells collected by the previous method and is essentially red cell free. This eliminates the need for red cell crossmatches with the donor and for subsequent centrifugation steps to remove red cells. Such an additional spin to remove red cells will result in a loss of approximately 20% of the platelets. The number of platelets collected per minute of procedure time has not changed with use of the surge pump. During a 90 minute procedure, 7 fill-

ings of the centrifuge bowl can be accomplished with collection of 4×10^{11} platelets [34].

Other blood cell separators are also widely used to harvest platelets from single donors. Both the IBM 2997 and the Fenwall CS 3000 are continuous flow machines. The IBM machine uses rotating, hollow plastic belts as its blood separation device. The 'single stage' belt allows collection of a large number of platelets, but these are contaminated with white cells (mean = 3 to 6×10^9 white cells per collection) and red cells (mean collection hematocrit 2%) [35]. The 'dual stage' belt is a modification for the IBM machine in which a thin spiral channel is introduced to connect the inner and outer portion of the belt. Within this spiral channel, platelets are concentrated along the outer wall and extracted through the collection line. Using this procedure a mean of 3.7×10^{11} platelets can be harvested from a donor in 90 minutes with a mean of 2×10^8 white cells in a virtually red cell free product [36, 37].

The Fenwall machine also utilizes continuous blood flow from the donor, but a different separation principle is employed in the centrifuge. In a 90 minute procedure, similar platelet yields to those achieved with the IBM are obtained with similar low red and white cell contamination [38]. The Fenwall and Haemonetics machines have the advantage of reduced time for software assembly compared to the IBM.

The choice of blood cell separator depends on many considerations including the other uses, such as plasma and white cell collection, to which the machine will be put, the availability of maintenance support and software supplies and individual preferences of the operators.

The platelets produced by any of the above methods, using blood cell separators or manual collection, have been shown to function hemostatically in the recipient by raising the platelet count and shortening the bleeding time following transfusion [39, 40, 41, 42]. However, the cell separators use an open system which introduces the possibility of bacterial contamination during collection and makes storage beyond 24 hours impossible.

Donor considerations

Many investigators have shown that the platelet yield from any collection procedure is directly influenced by the donor's prepheresis platelet count [31, 43]. The frequency with which plateletpheresis can be safely and efficiently performed has been investigated. Although it is possible to perform daily plateletpheresis on a single donor, this is rarely necessary and there is a gradual fall in the donor's prepheresis platelet count to less than 70% of the original prepheresis count after 6 to 8 donations by either manual bag or intermittent flow plateletpheresis [43]. Subsequent prepheresis platelet counts may not decline any further, implying bone marrow compensation for the continued platelet loss. However, the initial decline in prepheresis counts may result in a donor platelet count well below 150,000 per μl , with considerably reduced platelet yields. It is therefore advisable to do prepheresis donor platelet counts when the same donor is used at very frequent intervals, i.e. more than once per week.

There are other concerns with frequent platelet donation. These include the associated lymphocyte loss with apheresis and its implications for the donor and recipient. The use of the standard or red cell rich method of plateletpheresis with the Haemonetics blood cell separator or platelet collection with the single stage IBM channel results in a mean loss of 6×10^9 lymphocytes with each procedure. Weekly plateletpheresis can result in a significant decrease in circulating lymphocyte count with a disproportionate loss of B cells [44, 45]. The lowered lymphocyte count may persist for many months after pheresis is discontinued [45]. Immunoglobulin levels show much less alteration unless weekly pheresis is continued for many months [44]. No well-documented ill effects have occurred in donors that could be related to these changes. The advent of newer modifications of the blood cell separator machines such as the 'surge pump' for the Haemonetics and the dual stage channel for the IBM have largely eliminated the problem of lymphocyte loss to the donor, so that this issue should be of less concern in the future.

There is an additional concern about the pre-

sence of lymphocytes in platelet preparations. There is both experimental [46] and clinical [47] evidence that lymphocytes are more immunogenic than platelets and that their presence in platelet transfusions increases the incidence of transfusion reactions and perhaps decreases post transfusion platelet count increments in immunized recipients. However, in a prospective, randomized trial we found that removal of 80% of the lymphocytes from random donor platelets did not reduce the rate of alloimmunization in leukemia patients receiving repeated platelet transfusion [48]. These results are in contrast to the results of a study from The Netherlands [49] where the rate of alloimmunization was decreased with the use of a lymphocyte poor platelet product. These conflicting data can perhaps be explained by the very different way in which the platelet concentrates were prepared in the Dutch study, which allowed only ca. 5×10^6 leukocytes to be administered with each transfusion. This is compared to ca. 7×10^8 white cells with each transfusion in our study. However, the Dutch method of platelet concentrate preparation is not compatible with North American blood banking practices, as it included such procedures as storage of the platelets at 4°C prior to transfusion. We were unable to improve upon the leukocyte depletion in our platelet product using standard centrifugation procedures and so cannot recommend it as a method of reducing alloimmunization. The spin to remove the white cells also results in the loss of at least 20% of the platelets. In addition, to achieve consistency, white cell free red cells, supplied as either frozen or washed cells must be used, adding to the expense and inconvenience of both forms of transfusion.

Aspirin used by the donors within 36 hours of platelet donation will inhibit the function of the transfused platelets in the recipient and so should be avoided. Although the aspirin effect on platelets is a permanent effect and the lifespan of platelets is 8 to 10 days, it is only necessary for 20% of the donor's platelets to be recently released from the bone marrow and unaffected by aspirin for the hemostatic effect of the harvested platelets to be normal [4].

Clinical evaluation of platelet transfusion

As with all forms of medical therapy it is important to be able to assess the effectiveness of platelet transfusion. It is necessary to determine when additional transfusions are necessary and to assess when a patient has become immunized against random donor platelets and requires specially matched platelets.

In the bleeding thrombocytopenic patient the qualitative or hemostatic effect can be determined by observing that the bleeding has stopped after platelet transfusion. In patients who are not actively bleeding and receiving prophylactic transfusion, the qualitative effect can still be measured by determining the bleeding time before and after transfusion. The bleeding time becomes progressively abnormal as the platelet count drops below $100,000/\mu\text{l}$ [13]. A transfusion which raises the platelet count above $100,000/\mu\text{l}$ should normalize the bleeding time if the platelets function normally [14]. Although the bleeding time gives useful information, it requires the creation of small skin wounds that may serve as a nidus for infection in patients with marrow hypofunction who are often granulocytopenic, and so cannot be recommended for routine evaluation of platelet transfusion.

Isotopic labelling of platelets with ^{51}Cr or ^{111}In allows the performance of platelet survival studies. These techniques have been used to demonstrate normal platelet survival following transfusion in nonthrombocytopenic recipients receiving stored autologous platelets [13]. In thrombocytopenic recipients the platelet survival time is reduced to about 4 days [13]. Platelet survival can also be calculated using post-transfusion platelet count increments, and there is excellent agreement between the survivals calculated by that method and by ^{51}Cr survival [13]. Although representing a useful tool for clinical investigation, isotopic labelling of platelets is too time consuming and inconvenient to be used as a standard method of following the response to platelet transfusion. Serial counts performed on the recipient at 1 h and 24 h after transfusion should demonstrate a prompt and sustained rise in the platelet count. One unit (0.7×10^{11} platelets) of concentrate should raise the count 9,000 to

10,000 per μl in the average-sized adult. There is considerable variation in the number of platelets in any single unit of concentrate and an even greater variability in the size of the recipient. In order to make different transfusions to the same individual as well as transfusions given to different recipients comparable we have used the following formula. It corrects the post transfusion platelet count increment for these two factors, i.e. the size of the transfusion and the size of the recipient. The value obtained is called the corrected count increment (CCI) [50].

$$\text{CCI} = \frac{(\text{Post-transfusion count} - \text{Pre-transfusion count})/\mu\text{l} \times \text{BSA (m}^2\text{)}}{\text{No. of platelets transfused} \times 10^{11}}$$

For example: A recipient with a post-transfusion peripheral blood platelet count increment of 40,000/ μl (increment = platelet count post-transfusion – platelet count pre-transfusion) and a body surface area of 2 m^2 would have a CCI after receiving a transfusion of 4×10^{11} platelets determined as follows:

$$\text{CCI} = \frac{40,000 \text{ (plt}/\mu\text{l)} \times 2(\text{m}^2)}{4 \text{ (plt} \times 10^{11}\text{)}} = 20,000$$

We accept a CCI of greater than 10,000 at 1 h and greater than 7,500 at 24 h post-transfusion as a good response [50, 51]. Anything less is a poor response which merits investigation.

Many clinical factors will reduce the response to platelet transfusion. The survival of transfused platelets in a febrile patient is significantly reduced at temperatures greater than 38.5°C [52]. Serious infection, significant hemorrhage or disseminated intravascular coagulation will consume platelets and reduce their survival [53]. Platelet sequestration can be a major factor reducing platelet count increments in a patient with hepatomegaly or splenomegaly. Alloimmunization is the final and major clinical factor affecting the results of platelet transfusion. It must be differentiated from all the other clinical variables and this is often difficult in a severely ill thrombocytopenic patient. We have found the one hour post-transfusion count increment to be helpful in this situation [50]. In the presence of fever, infection, hemorrhage and even D.I.C. the one hour CCI is usually adequate, i.e.,

greater than 10,000, even though platelet survival as measured by a suboptimal 24 hour CCI is short. A low one hour CCI is almost always due to alloimmunization. Exceptions occur in the patients who are seriously ill or with hepatosplenomegaly which may reduce the one hour post transfusion response. It is important, when using the CCI to make decisions about the presence or absence of alloimmunization, to give transfusions consisting of healthy viable platelets (ideally less than 24 hours post collection) because defects that arise with storage can affect platelet recovery and survival after transfusion.

Platelets are known to possess HLA antigens on their surface [54]. Immunization against these antigens accounts for the majority of cases of refractoriness to random donor platelet transfusion [55, 56]. These HLA antibodies can be detected in the serum of immunized patients using the micro lymphocytotoxicity assay [57]. Lymphocytotoxic antibody (LCTAb) levels can be determined as the percent cytotoxicity of patient serum against a panel of different lymphocytes selected to represent the majority of the HLA antigens. For many years we have used the level of lymphocytotoxic antibody in patient serum as an indicator of alloimmunization. The presence of these antibodies serves as serological confirmation of the clinical impression of alloimmunization gained by measuring post transfusion platelet count increments. The LCTAb measurement is particularly helpful in seriously ill patients with multiple medical problems or hepatosplenomegaly where the count increments are less useful. A clear diagnosis of alloimmunization is important because of the expense and inconvenience of obtaining HLA matched platelets for patients who are clearly refractory to random donor platelets. In a study of the transfusion records of 189 leukemic patients [56] we found that the presence in a patient's serum of antibodies cytotoxic against more than 20% of the cells on a lymphocyte panel virtually precluded a good response to random donor platelets as measured by corrected count increments after transfusions. Conversely, in the absence of lymphocytotoxic antibody in the serum, 80% of the patients responded well to random donor platelets. Those who lack

antibody but yet fail to respond to random platelets will achieve no better increments with HLA matched transfusion and their expense can be avoided.

A number of platelet antibody tests have been evaluated for use in the diagnosis of alloimmunization and as platelet crossmatches. Many of the older tests such as platelet aggregometry appear to lack the sensitivity to detect all cases of platelet/recipient incompatibility [58]. More recently developed assays which measure platelet-associated immunoglobulin such as platelet immunofluorescence or platelet ELISA may offer more promise. Kickler *et al.* [59] have been very successful in using a radiolabelled antiglobulin test for platelet crossmatching. A negative crossmatch with their technique was highly predictive of a good response to the platelet transfusion as measured by corrected count increments at 1 and 20 hours following transfusion. When the crossmatch was positive the 1 hour increment was adequate in only 9 of 89 transfusions. However, in none of these was the platelet survival adequate with very low 20 hour increments in all. We have developed a micro-ELISA test for use in detecting antibodies against platelets [60]. It appears to be sensitive enough to detect most of the HLA antibodies that arise in the serum of our multi-transfused patients and has the advantage that frozen-dessicated platelets or platelets stored in 1% sodium azide may be used as the test cells. Kickler [59] was also able to store platelets in sodium azide for up to 8 weeks before testing. This offers the possibility that a semi-permanent panel of different platelets from potential donors may be developed to be available for crossmatch with alloimmunized patient serum. In our experience no more than 5% of patients who are refractory to random donor platelets fail to demonstrate HLA antibodies in their serum, suggesting platelet specific antibodies. However, it is possible that tests such as the platelet ELISA or radiolabelled antiglobulin test may make it possible to successfully select from among a number of potential donors for patients with particularly uncommon HLA types or who fail to respond well to closely HLA matched platelets. In addition, in most series up to 30% of perfectly HLA matched platelet transfusions fail to show a

satisfactory response as measured by post-transfusion count increments. If a significant proportion of these failures is due to platelet specific antibody a platelet crossmatch should detect the incompatibility and an unsuccessful transfusion could be avoided.

In conclusion, the evaluation of the clinical efficacy of platelet transfusions requires the careful monitoring of post-transfusion platelet counts. It is important to make a clear diagnosis of alloimmunization in multitransfused patients in order to be able to administer the optimal form of platelet therapy to thrombocytopenic patients. This can be done by measuring HLA or lymphocytotoxic antibodies in patient serum or by the use of some of the newer platelet antibody tests. There are recent data to suggest that these latter tests will have further usefulness in platelet crossmatching. However, it should be remembered that all of the platelet crossmatch studies have been conducted in one laboratory to date with relatively small numbers of patients. Confirmation by others is required.

References

1. Bolin RB, Cheney BA, Smith DJ, Gildengorin V, Shigekawa R: An *in vivo* comparison of CPD and CPDA-2 preserved platelet concentrates after an 8-hour preprocess hold of whole blood. *Transfusion*, 1982; 22: 491-95.
2. American Assoc. of Blood Banks, Technical Manual, Widmann FK (ed). J.B. Lippincott Co., 1981: 48.
3. Kahn RA, Cossette I, Friedman LI: Optimum centrifugation conditions for the preparation of platelet and plasma products. *Transfusion*, 1976; 16: 162-65.
4. Murphy S: Harvesting of platelets for transfusion and problems of storage. In: *The Blood Platelet in Transfusion Therapy*, Greenwalt TJ, Jamieson GA (eds.). Alan R. Liss, Inc., New York, 1978: 101.
5. Slichter SJ, Harker LA: Preparation and storage of platelet concentrates. I. Factors influencing the harvest of viable platelets from whole blood. *Br J Haematol*, 1976; 34: 395-402.
6. Karpatkin S, Khan Q, Freedman M: Heterogeneity of platelet function. Correlation with platelet volume. *Am J Med*, 1978; 64: 542-46.
7. Corash L, Tan H, Gralnick HR: Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Blood*, 1977; 49: 71-87.
8. Mezzano D, Hwang K, Aster RH: Characteristics of total

- platelet populations and of platelets isolated in platelet-rich plasma. *Transfusion*, 1982; 22: 197–202.
9. Beutler E, Kuhl W: Platelet glycolysis in platelet storage. IV. The effect of supplemental glucose and adenine. *Transfusion*, 1980; 20: 97–100.
 10. Moroff G, Friedman A, Robkin-Kline L: Factors influencing changes in pH during storage of platelet concentrates at 20–24°C. *Vox Sanguinis*, 1982; 42: 33–45.
 11. Mourad N: A simple method for obtaining platelet concentrates free of aggregates. *Transfusion*, 1968; 8: 48.
 12. Kunicki TJ, Tuccelli M, Becker GA, Aster RH: A study of variables affecting the quality of platelets stored at 'room temperature'. *Transfusion*, 1975; 15: 414–21.
 13. Slichter SJ, Harker LA: Preparation and storage of platelet concentrates. II. Storage variables influencing platelet viability and function. *Br J Haematol*, 1976; 34: 403–17.
 14. Filip J, Aster RH: Relative hemostatic effectiveness of human platelets stored at 4° and 22°C. *J Lab Clin Med*, 1978; 91: 618–24.
 15. Murphy S, Gardner FH: Platelet storage at 22°C; metabolic, morphologic, and functional studies. *J Clin Invest*, 1971; 50: 370–77.
 16. Murphy S, Holme S: Storage of platelets for five days in two containers – value of paired studies. *Transfusion*, 1981; 21: 637–38.
 17. Murphy S, Kahn RA, Holme S, Phillips GL, Sherwood W, Davisson W, Buchholz DH: Improved storage of platelets for transfusion in a new container. *Blood*, 1982; 60: 194–200.
 18. Murphy S, Gardner F: Platelet storage at 22°C: role of gas transport across plastic containers in maintenance of viability. *Blood*, 1975; 46: 209–18.
 19. Rogers AB: The effect of pH on human platelet aggregation induced by epinephrine and ADP. *Proc Soc Exp Biol Med*, 1972; 139: 1100–02.
 20. Holme S, Vaidja K, Murphy S: Platelet storage at 22°C: Effect of type of agitation on morphology, viability and function *in vitro*. *Blood*, 1978; 52: 425–35.
 21. Myhre BA, Walker LJ, White ML: Bacteriocidal properties of platelet concentrates. *Transfusion*, 1974; 14: 116–23.
 22. Buchholz DH, Young VM, Friedman NR, Reilly JA, Mardiney MR, Jr: Detection and quantitation of bacteria in platelet products stored at ambient temperature. *Transfusion* 13; 268–75.
 23. Daly PA, Schiffer CA, Aisner J, Wiernik PH: Successful transfusion of platelets cryopreserved for more than 3 years. *Blood*, 1979; 54: 1023–27.
 24. Schiffer CA, Aisner J, Dutcher JP, Daly PA, Wiernik PH: A clinical program of platelet cryopreservation. In: *Cytapheresis and Plasma Exchange: Clinical Indications*, Vogler WR (ed). Alan R. Liss, Inc., New York, 1982: 165–80.
 25. Schiffer CA, Aisner J, Wiernik PH: Frozen autologous platelet transfusion for patients with leukemia. *N Engl J Med*, 1978; 299: 7–12.
 26. Vecchione JJ, Mellaragno AJ, Hollander A, Defina S, Emerson CP, Valeri CR: Circulation and function of human platelets isolated from units of CPDA-1, CPDA-2, and CPDA-3 anticoagulated blood and frozen with DMSO. *Transfusion*, 1982; 22: 206–9.
 27. Dayian G, Pert JH: A simplified method for freezing human blood platelets in glycerol-glucose using a statically controlled cooling rate device. *Transfusion*, 1979; 19: 255–60.
 28. Kotelba-Witkowska B, Schiffer CA: Cryopreservation of platelet concentrates using glycerol-glucose. *Transfusion*, 1982; 22: 121–24.
 29. Yankee RA, Grumet FC, Rogentine GN: Platelet transfusion therapy. The selection of compatible platelet donors for refractory patients by lymphocyte HLA typing. *N Engl J Med*, 1969; 281: 1208–12.
 30. Schiffer CA, Slichter SJ: Platelet transfusions from single donors. *N Engl J Med*, 1982; 307: 245–48.
 31. Schiffer CA, Buchholz DH, Wiernik PH: Intensive multi-unit plateletpheresis of normal donors. *Transfusion*, 1974; 14: 388–94.
 32. Tullis JL, Tinch RJ, Baudanza P, Gibson JG, DiForte S, Conneely G, Murthy K: Plateletpheresis in a disposable system. *Transfusion*, 1971; 11: 368–77.
 33. Aisner J, Schiffer CA, Wolff JH, Wiernik PH: A standardized technique for efficient platelet and leukocyte collection using the model 30 blood processor. *Transfusion*, 1976; 16: 437–45.
 34. Hogge DE, Schiffer CA: Collection of platelets depleted of red and white blood cells with the 'surge pump' adaptation of the Haemonetics blood cell separator. *Transfusion*, 1983; 23: 177–81.
 35. Kalmin ND, Grindon AJ: Pheresis with the IBM 2997. *Transfusion*, 1981; 21: 325–29.
 36. Hester JP, McCredie KB, Freireich EJ: The use of disposable plastic channels in an IBM modified separator for granulocyte and platelet collection. *Transfusion*, 1978; 18: 380.
 37. IBM Corporation. IBM 2997 blood cell separator. Customer thrombocytapheresis data. 1981.
 38. Katz AJ, Genco PV, Blumberg N, Snyder EL, Camp B, Morse EE: Platelet collection and transfusion using the Fenwal CS-3000 cell separator. *Transfusion*, 1981; 21: 560–63.
 39. Slichter SJ: Efficacy of platelets collected by semi-continuous flow centrifugation (Haemonetics Model 30). *Br J Haematol*, 1978; 38: 131–40.
 40. Daly PA, Schiffer CA, Aisner J, Wiernik PH: A comparison of platelets prepared by the Haemonetics Model 30 and multiunit bag plateletpheresis. *Transfusion*, 1979; 19: 778–81.
 41. Patel IP, Ambinder E, Holland JF, Aledort LM: *In vitro* and *in vivo* comparison of single-donor platelets and multiple-donor pooled platelets: transfusion in leukemic patients. *Transfusion*, 1978; 18: 116–19.
 42. Chao FC, Tullis JL, Tinch RJ, Conneely GS, Baudanza P: Plateletpheresis by discontinuous centrifugation: Effect of collecting methods on the *in vitro* function of platelets. *Br J*

- Haematol, 1979; 39: 177-87.
43. Glowitz RJ, Slichter SJ: Frequent multiunit plateletpheresis from single donors: Effects on donors' blood and the platelet yield. *Transfusion*, 1980; 20: 199-205.
 44. Koepke JA, Parks WM, Goekin JA, Klee GG, Strauss RG: The safety of weekly plateletpheresis: Effect of the donors' lymphocyte population. *Transfusion*, 1981; 21: 59-63.
 45. Senhauser DA, Westphal RG, Bohman JE, Neff JC: Immune system changes in cytapheresis donors. *Transfusion*, 1982; 22: 302-304.
 46. Claas FHJ, Smeenk RJT, Schmidt R, Van Steenbrugge GJ, Eernisse JG: Alloimmunization against the MHC antigens after platelet transfusions is due to contaminating leukocytes in the platelet suspension. *Exp Hematol*, 1981; 9: 84-89.
 47. Herzig RH, Herzig GP, Bull MI, Dechter JA, Lohrmann HP, Stout FG, Yankee RA, Graw RG: Correction of poor platelet transfusion responses with leukocyte-poor HLA matched platelet concentrates. *Blood*, 1975; 46: 743-50.
 48. Schiffer CA, Dutcher JP, Aisner J, Hogge D, Wiernik PH, Reilly JP: A randomized trial of leukocyte depleted platelet transfusion to modify alloimmunization in patients with leukemia. *Blood*, 1982; 60: 182a.
 49. Eernisse JG, Brand A: Prevention of platelet refractoriness due to HLA antibodies by administration of leukocyte-poor blood components. *Exp Hematol*, 1981; 9: 77-83.
 50. Daly PA, Schiffer CA, Aisner J, Wiernik PH: Platelet transfusion therapy. One-hour postransfusion increments are valuable in predicting the need for HLA-matched preparations. *JAMA*, 1980; 243: 435-38.
 51. Tosato G, Appelbaum FR, Trapani RJ, Dowling R, Deisseroth AB: Use of *in vitro* assays in selection of compatible platelet donors. *Transfusion*, 1980; 20: 47-54.
 52. Freireich EJ, Kliman A, Gaydos LA, Mantel N, Frei E: Response to repeated platelet transfusion from the same donor. *Ann Inter Med*, 1963; 59: 277-87.
 53. Brand A, Van Leeuwen A, Niterink A, Langerak J, Eernisse JG, Van Rood JJ: Platelet supportive care: Immunological and clinical aspects. *Neth J Med*, 1979; 22: 133-37.
 54. Colombani J: Blood platelets in HL-A serology. *Transplant Proc*, 1971; 3: 1078-87.
 55. Mueller-Eckhardt G, Breidenbach M, Mahn I, Mueller-Eckhardt C: The role of alloimmunization in platelet survival studies. *Blut*, 1980; 40: 93-99.
 56. Hogge DE, Dutcher JP, Aisner J, Schiffer CA: Lymphocytotoxic antibody is a predictor of response to random donor platelet transfusion. *Am J Hematol*, 1983; 14: 363-69.
 57. Mittal KK, Mickey MR, Singal DP, Terasaki PL: Serotyping for homotransplantation XVIII; refinement of the microdroplet lymphocyte cytotoxicity test. *Transplantation*, 1968; 6: 913.
 58. Filip DJ, Duquesnoy RJ, Aster RH: Predictive value of cross-matching for transfusion of platelet concentrates to alloimmunized recipients. *Am J Hematol*, 1976; 1: 471-79.
 59. Kickler TS, Braine HG, Ness PM, Koester A, Bias W: A radiolabeled antiglobulin test for crossmatching platelet transfusions. *Blood*, 1983; 61: 238-42.
 60. Schiffer CA, Young V: Detection of platelet antibodies using a micro-enzyme-linked immunosorbent assay (ELISA). *Blood*, 1983; 61: 311-17.

6. Leukapheresis: clinical experience and post-transfusion control

J. McCULLOUGH

Introduction

The availability of platelet transfusion support to patients with bone marrow failure (such as aplastic anemia, or malignancy undergoing chemotherapy), has reduced the incidence of hemorrhage. However, infection then became the major cause of death in these patients [1]. There is an inverse relationship between the granulocyte count and the percent of days patients spend with infections [2]. Early studies showed that patients were at an increased risk of infection when the granulocyte count fell below $1000/\mu\text{l}$ [2]; although more recent data indicates that serious infection is not a problem until the granulocyte count falls below $500/\mu\text{l}$ [3]. One approach to the management of infection in patients with bone marrow failure is to replace depleted granulocyte stores by giving granulocyte transfusions. Because of the relatively small number of granulocytes in the circulation of normal people, early experience with granulocyte transfusions involved the use of patients with chronic myelogenous leukemia (CML) as donors [4]. Using large doses of granulocytes, increments in peripheral blood granulocyte count were observed and clinical improvement in the patients occurred a few hours following transfusion. Usually only one or two transfusions were necessary. During the early 1970's there were many reports describing patients who improved following granulocyte transfusion. The reports concluded that granulocyte transfusions were useful, as evidenced by: a) clinical improvement following transfusions, often in patients previously unresponsive to anti-

biotics, and b) appearance of granulocytes in sites of infection which did not have an inflammatory response prior to transfusion. Because of this, there was great interest in making granulocyte transfusions widely available for use in treating granulocytopenic patients. This chapter will describe the methods presently available for granulocyte collection, the function of the cells collected and approaches to evaluating the effectiveness of the transfusions. The latter is especially important because as blood cell separators became available during the 1970's, the source of granulocytes shifted from CML patients to normal donors. As a result, the dose of granulocytes transfused fell to approximately 10% of that used in earlier studies, and it became increasingly difficult to achieve obvious clinical benefits from a single transfusion [5]. Before considering granulocyte collection, it is appropriate to briefly review granulocyte production.

Granulocyte production and kinetics

The average daily production of granulocytes is approximately 1.6×10^9 cells/kg [6], which is equivalent to approximately 1.1×10^{11} granulocytes/day in the average-sized adult. The granulocyte has a life span of 10–14 days. During the 8–11 days the granulocyte remains in the bone marrow, it undergoes about 4–7 days of proliferation from the promyelocyte to the myelocyte stage and about 4 days of differentiation from a myelocyte to a mature segmented granulocyte. The granulocyte is then re-

leased into the peripheral blood where it circulates with a half life of about six hours. The total blood granulocyte pool in the average-sized adult is estimated at 70×10^7 cells/kg or a total of approximately 5×10^{10} granulocytes [6]. Approximately half of the granulocytes in the blood are in a circulating pool and half in a marginal pool. The marginal granulocyte pool is composed of cells loosely adherent to the walls of small vessels and in equilibrium with the circulating pool but not in the axial stream of blood. Leukapheresis techniques presently in use remove approximately $1-2 \times 10^{10}$ granulocytes. By comparison, 2.5×10^{10} granulocytes are available in the circulating granulocyte pool, and an additional 2.5×10^{10} are immediately available in equilibrium in the marginal granulocyte pool and 4×10^{11} are available in the bone marrow storage pool [6]. The combination of the circulating and marginal granulocyte pools represents only approximately 1/2 of the average daily production of granulocytes or 1/200 of the total body stores of granulocytes. Thus, present collection techniques remove only a small portion of the total body granulocytes. There is not a substantial risk of granulocyte depletion in the donor; however, the dose of granulocytes being provided to an infected patient is quite small.

Collection of granulocytes for transfusion

Granulocytes can be collected by: (1) continuous flow centrifuge leukapheresis using the IBM Blood Cell Separator, Aminco Celltrifuge, the IBM 2997 Blood Cell Separator or the Fenwal CS-3000 Blood Cell Separator; (2) intermittent flow centrifuge leukapheresis using the Haemonetics Model-30; (3) filtration leukapheresis (FL) using the Fenwal Leukapheresis pump or the Leukopherator; (4) gravity leukapheresis; or (5) harvesting of buffy coats from ordinary units of whole blood. A detailed discussion of granulocyte collection techniques is available elsewhere [7], however, some consideration of collection techniques will be included here to emphasize the effects of different collection techniques on cell function, the dose of cells obtained by different techniques, and risks of leukapheresis to the donor.

Continuous flow centrifuge leukapheresis (CFCL)

The first instrument designed for granulocyte collection was the IBM Blood Cell Separator, in which there is a continuous flow of blood out of the donor's vein through the Blood Cell Separator and back to the donor. Another similar instrument (the Celltrifuge) was subsequently manufactured by the American Instrument Company. In these instruments, whole blood continuously flows through a centrifuge bowl where it is separated into red blood cells, buffy coat and plasma. The buffy coat is collected for transfusion. Usually $8-10\text{ l}$ of donor blood (twice the donor's blood volume) are processed during a procedure which requires approximately $3\frac{1}{2}\text{ h}$. The granulocyte yield using these techniques is unsatisfactory unless some modifications are used (Table 1).

A more recent IBM Blood Cell Separator (Model 2997) uses a disposable belt-like chamber instead of a centrifuge bowl for separation of whole blood into components. The instrument involves a continuous flow centrifuge process similar to other continuous flow procedures except for the design of the separation chamber. It appears that this instrument produces a granulocyte yield much larger than that obtained with the original IBM Blood Cell Separator or the Aminco Celltrifuge (Table 1).

The Fenwal CS-3000 Blood Cell Separator is the

Table 1. Granulocyte yield $\times 10^9$ using different instruments and leukapheresis techniques*

	Procedure modifications			
	None	HES**	Steroids	HES + ster- oids**
IBM blood cell separator	4	10	10	22
Aminco celltrifuge	-	11	-	35
IBM-2997		12	-	25
Fenwal CS-3000	3	10	-	20
Haemonetics-30				

* Values are summarized from ref. 7.

** HydroxyEthyl Starch.

newest continuous flow centrifuge. Whole blood is separated into components in a disposable plastic bag, the configuration of which is determined by placing the bag inside a mold. Granulocyte production using this instrument is equivalent to that obtained with the IBM 2997 (Table 1) [8].

Intermittent flow centrifuge leukapheresis (IFCL)

In the Haemonetics Model-30, blood flows from the donor into the disposable centrifuge bowl where red cells, plasma, platelets and granulocytes are separated and removed from the bowl sequentially rather than simultaneously as in CFCL. When the granulocytes have been obtained, the blood flow is reversed and the plasma, red cells and some or most of the platelets are returned to the donor. This cycle of filling the centrifuge bowl, removing granulocytes and returning the remainder of the blood to the donor is repeated as many times as necessary to obtain the desired granulocyte yield.

Use of corticosteroids and hydroxyethyl starch to improve granulocyte yields

The yield of granulocytes obtained by CFCL or IFCL from normal donors can be greatly increased using two general approaches: (1) increasing the efficiency of granulocyte extraction from whole blood by adding a red cell sedimenting agent, with or without (2) elevation of the donor's granulocyte count by administration of corticosteroids.

Hydroxyethyl starch (HES) is the sedimenting agent in most widespread use. It is a branched chain polymer of glucose developed for use as a blood volume expander. When a 6% suspension of HES is added to donor blood as it passes through the separation chamber, the granulocyte yield is approximately doubled (Table 1). HES has been considered safe for use in normal donors [9], although it is difficult to determine the exact incidence of adverse reactions during leukapheresis which are due to HES. Recently there have been reports of anaphylaxis due to HES [10] and lichen planus-like lesions in a donor who received HES [11]. Small, but measurable, blood levels of HES

have been found up to 250 days after a single infusion [12]. Thus, although HES is presently in widespread use during leukapheresis, additional studies defining its long-term effects are desirable.

Corticosteroids induce a granulocytosis, probably by slowing the egress of granulocytes from the circulation and increasing the release of granulocytes from the bone marrow [6]. Since the granulocyte yield from leukapheresis is directly related to the donor's granulocyte count, steroid treatment of donors is an effective method of improving the yield for transfusion. Steroid treatment of the donor approximately doubles the granulocyte yield (Table 1), although it is not customary to use steroid treatment of the donor without addition of hydroxyethyl starch to donor blood. Thus, using steroids and HES, the granulocyte yields obtained by CFCL and IFCL are approximately $20-30 \times 10^9$ (Table 1). Prednisone, dexamethasone, hydrocortisone and etiocholanolone are the steroids which have been used in leukapheresis [7]. Etioclanolone causes unpleasant side effects and is no longer available in the United States. Prednisone or dexamethasone is given orally the evening before leukapheresis. Intravenous injection of dexamethasone or hydrocortisone immediately before leukapheresis does not significantly improve the granulocyte yield because of the lag time in elevation of the donor's granulocyte count. It appears that administration of steroids in several divided doses 12–18 h prior to leukapheresis causes a greater increase in granulocyte count and granulocyte yield than the equivalent amount of steroids given as one dose [8, 13, 14].

Filtration leukapheresis (FL)

During filtration leukapheresis, granulocytes are isolated from whole blood by their selective adherence to nylon fibers. After a predetermined time or volume of blood has been processed, the disposable system is flushed to elute granulocytes from the fibers. Since a high proportion of granulocytes adhere to the fibers, the yield of granulocytes from FL is much greater than from systems involving granulocyte separation by centrifugation. Red cell sedimenting agents are of no

value in FL since granulocyte isolation is not based on centrifugation. Steroids have been used to elevate the donor's granulocyte count in hopes of improving granulocyte yields. However, the data are not clear as to whether this is effective. The use of FL for granulocyte collection has declined drastically in the last several years following a report of priapism in normal donors [15], severe perineal pain in women [16] and complement activation [17, 18]. It appears that the risks of this procedure to normal donors are unacceptably high.

Gravity leukapheresis

In this procedure HES is used to sediment erythrocytes and allow isolation of granulocytes from whole blood in ordinary plastic bags [19]. The procedure can yield a large number of granulocytes and is relatively simple because it requires only the equipment and skills used for plasmapheresis. Granulocytes obtained by gravity leukapheresis are as effective clinically as those obtained by filtration leukapheresis [20]. However, it is cumbersome and time consuming [21] and has not been used extensively other than in the laboratory where it was developed.

Function of granulocytes collected by leukapheresis

Granulocytes collected by CFCL have normal bactericidal activity, phagocytosis, chemotaxis and intracellular metabolic activity [7]. Granulocytes collected by IFCL also apparently function normally *in vitro* [7, 22, 23]. It is generally agreed that granulocytes collected by FL have reduced phagocytic capacity, bacterial killing and chemotactic response [7]. Some investigators have reported that granulocytes collected by FL function normally *in vitro*. However, it appears that the process of collection of granulocytes by reversible adherence to nylon fibers is damaging to the cell. Many subtle but important variations in collection technique affect the extent of cell damage and it is very difficult to obtain granulocytes in this manner which function normally *in vitro*.

In vivo function of granulocytes collected by leu-

kapheresis is more difficult to study. CFCL granulocytes have a normal intravascular recovery and half life following transfusion [24] and migrate into a localized skin abrasion normally [25]. Granulocytes collected by FL provide a lower increase in granulocyte count after transfusion [24]; however, they are clinically effective and migrate to skin windows and exudates following transfusion [26]. It has been estimated that eight times more granulocytes collected by FL than CFCL would be required to obtain an equivalent number of transfused cells at a skin abrasion site [25].

Adverse reactions to leukapheresis in normal donors

The incidence of reactions in leukapheresis donors ranges from 4% to 37% [7]. The reaction most commonly reported is anxiety with or without other vasovagal symptoms [27]. Symptoms related to the citrate anticoagulant are also common. These include parasthesias, chills, and muscle tremors. These symptoms are easily reversed by slowing the rate of blood flow. The risk of cardiac arrhythmia due to hypocalcemia seems remote because donors experience severe symptoms before the ionized calcium falls to dangerous levels [28].

Leukapheresis donors may experience most of the reactions associated with ordinary blood donations such as nausea, vomiting, syncope, seizures, etc. In addition, reactions which are unique to leukapheresis include hypervolemia (due to HES infusion), stiffness of the arms (because of prolonged extension), a feeling of fatigue after the procedure and problems such as hemolysis due to mechanical equipment failure. Reactions to hydroxyethyl starch used in leukapheresis are rare and have been discussed earlier. Also, as previously mentioned, filtration leukapheresis is no longer widely used because of the occurrence of priapism in men, perineal pain in women and complement activation.

One other risk unique to leukapheresis is the potential of cellular depletion. As previously mentioned, the number of granulocytes removed is a small portion of the total body reserve and gran-

ulocyte depletion has not been a practical problem. The potential for, and risks of lymphocyte depletion, are less well understood. The total body lymphocyte pool is approximately 1×10^{12} and one leukapheresis procedure will remove about 0.1% of this ($2-4 \times 10^9$). Some subsets of lymphocytes are long lived with a slow rate of reproduction, and thus may be more susceptible to depletion. In addition, alterations in the helper suppressor T cell ratio have been implicated in several immunologic diseases. Thus, the issue of possible lymphocyte depletion is of major concern.

Lymphocytapheresis of rheumatoid arthritis patients caused a decrease in the level of circulating lymphocytes after only 3-4 procedures in one study [29]. The lymphocyte count remained less than 50% of preapheresis levels in some patients for at least one year. In a separate study, ten weekly plateletapheresis donations caused an average decrease of 20% in the lymphocyte count, which was primarily due to a loss of B-lymphocytes [30]. Additional data to define the changes in lymphocytes resulting from leukapheresis and an analysis of the clinical significance of such changes would be helpful.

Evaluation of the success of granulocyte transfusion

Because the initial experience with granulocyte transfusion involved use of cells obtained from patients with CML, very large doses of granulocytes (approximately 6×10^{10}) were used [4]. Increases in circulating granulocyte count of approximately $1000/\mu\text{l}$ were observed and patients often improved within a few hours following transfusion. There was a direct relationship between the dose of granulocytes transfused and the increase in granulocyte count. Doses $<1 \times 10^{10}$ were rarely clinically effective [4]. Additional evidence of the *in vivo* effectiveness of these early granulocyte transfusions was the appearance of granulocytes in a skin window after transfusion; persistence of the Ph^1 chromosome for many days [4, 31]; and *in vivo* evidence that transfused CML granulocytes phagocytized bacteria *in vivo* [31]. Although these methods were valuable experimentally, the change in granulocyte

count was used as the routine practical method of evaluating the effectiveness of granulocyte transfusion. When CML donors were used, increases of 700-1,000/ μl in the granulocyte count were common [4]. However, it was soon learned that the incidence and severity of transfusion reactions were related to the speed of transfusion, and it became customary to transfuse granulocytes over 2-4 h. Because of their short intravascular half life, evaluation of the success of a transfusion by measuring the increase in granulocyte count became less meaningful. In addition, as the source of donors shifted from patients with CML to normal individuals, the dose of granulocytes transfused fell to approximately 10% of that used in early studies. The actual increase in granulocyte count provided by this smaller dose of cells was approximately $300/\mu\text{l}$ or less. The error in the granulocyte count and the patient variables is such that changes of this magnitude are difficult to evaluate statistically. Thus, evaluation of the *in vivo* effect of granulocyte transfusion by measuring the change in granulocyte count is not satisfactory.

Evaluation of the *in vivo* effectiveness of granulocyte transfusion could also be done as described in the previous section on function of granulocytes collected by leukapheresis. Under experimental conditions it was shown that granulocytes had a normal intravascular recovery and survival [24, 32] and migrated to a skin abrasion [25]. In careful studies of two patients, ^{51}Cr labeled granulocytes collected by CFCL had a normal intravascular survival and localized at a known site of infection [33]. Arnold *et al.* [34] used the unique approach of determining the number of granulocytes in the oral cavity as a method of evaluating granulocyte transfusions. The number of orogranulocytes was reduced in granulocytopenic patients and increased following transfusion in direct proportion to the number of cells transfused. Although this approach has not been widely used, it seems reasonably simple and practical. However, none of these approaches provided a practical approach to monitoring the success of granulocyte transfusions.

Previously, studies of granulocyte intravascular kinetics were done using DF^{32}p or ^{51}Cr labels. DF^{32}p is a rather specific label for granulocytes but

it is no longer available for human use in the United States. ^{51}Cr labels red blood cells and platelets as well as leukocytes and thus gives variable results due to technical problems. Dose limitations make ^{51}Cr unsatisfactory for external body imaging, and DF^{32}p is a beta emitter, also unsuitable for imaging.

$^{111}\text{Indium}$ is an efficient granulocyte label with a half life of 67 hours. It emits photons in high abundance which are ideal for external body imaging. The label appears to be firmly bound to the cell since no elution has been demonstrated [35]. When a pure suspension of granulocytes is labeled with $^{111}\text{Indium}$, and injected into the original normal donor (autologous injection), approximately 30% of the granulocytes are recovered and they have a half life of approximately five hours [36]. Although this 30% recovery is somewhat lower than the 45% established as normal using DF^{32}p , the indium technique does allow comparison of variables which may affect the fate of granulocyte *in vivo* [37]. The normal function *in vivo* of $^{111}\text{Indium}$ granulocytes is supported by localization of these cells at known sites of infection in patients with intra-peritoneal sepsis [38], kidney transplant rejection [39] and other localized infections [40]. $^{111}\text{Indium}$ granulocytes can be used to study the effect of different variables which may be important in the success of granulocyte transfusion. Two such examples are the effect of histocompatibility testing and the effect of storage.

Histocompatibility testing for granulocyte transfusion

Most studies of histocompatibility testing for granulocyte transfusion have involved HLA typing and transfusion of cells from donors with chronic myelogenous leukemia. When lymphocytotoxic (HLA) or leukoagglutinating antibodies are present directed against donor cells, there is no increase in granulocyte count following transfusion [5]. In detailed studies of four patients, chromium-51 labeled granulocytes did not localize at sites of infection but were sequestered in the spleen when the cells were incompatible in a leukoagglutination crossmatch [33]. Two studies [41, 42] showed that

there was a direct relationship between the number of HLA antigens matched between donor and recipient and the increase in granulocyte count following transfusion. Thus, these early studies of histocompatibility testing using CML cells indicated that incompatibility in the lymphocytotoxic and/or leukoagglutination test was associated with lack of ability of granulocytes to circulate and to localize at sites of infection. However, subsequent studies have not confirmed a relationship between post-transfusion granulocyte increment and HLA matching [43]. In addition, clinical improvement following granulocyte transfusion occurred regardless of whether HLA matched or mismatched granulocytes were used [44, 45].

This is further supported by Ungerleider *et al.* [46] who found no relationship between the increment in granulocyte count following transfusion or transfusion reactions and the presence of leukocyte antibodies detected by granulocytotoxicity, lymphocytotoxicity, leukoagglutination or capillary agglutination. The major shortcoming of this study, however, was the use of post-transfusion granulocyte count as the index of success. The results were standardized based on 10^{11} granulocytes transfused; but since transfusions usually contain 10^{10} granulocytes, the observed changes in granulocyte count must have been 100–300/ μl . Because of the effect of many patient variables on granulocyte counts in this range, this is probably not a satisfactory method of evaluating a single transfusion of granulocytes.

Two studies in dogs have established that prior exposure to blood from the granulocyte donor causes a decreased post-transfusion increment and migration into a skin chamber [47, 48]. In one study [47], testing by lymphocytotoxicity (HLA) revealed antibodies in only 30% of the immunized dogs, while in the second study [48] most immunized dogs had lymphocytotoxins, granulocytotoxins and leukoagglutinins. Thus, the optimum serologic tests to detect immunization were not revealed from these dog studies. Subsequently, however, Chow and Epstein [49], using a neutropenic dog model, showed that compatibility by granulocyte immunofluorescence results in significantly greater increases in peripheral granulocyte

count than compatibility by lymphocytotoxicity or leukoagglutination.

Studies in humans also stress the value of granulocyte immunofluorescence (GIF) [50]. In 25 patients with gram negative sepsis, the presence of GIF antibodies was associated with death in five of six and their absence associated with recovery in 12 of 13. This relationship did not occur with lymphocytotoxicity (HLA) testing and leukoagglutination was not studied.

Dutcher *et al.* [51] reported that indium labeled granulocytes localized at sites of infection in 20/20 non-immunized patients, but in only 3/14 immunized patients who had lymphocytotoxic (HLA) antibody. In later studies [52], these investigators showed that alloimmunization significantly increased pulmonary retention of indium labeled granulocytes during the 30 minutes after injection. However, in both studies, most of the patients had both lymphocytotoxic and leukoagglutinating antibodies so the role of antibodies detected in different assays could not be established.

We have shown that granulocyte agglutinating antibodies alone and in combination with other leukocyte antibodies were associated with decreased intravascular recovery of incompatible granulocytes [37, 53]. No other antibody technique studied including granulocytotoxicity, lymphocytotoxicity, granulocyte immunofluorescence or antibody-dependent lymphocyte-mediated granulocytotoxicity had this effect. One patient whose serum reacted in granulocyte immunofluorescence and other assays, but not granulocyte agglutination, had an intravascular recovery value much lower than control patients ($p = .056$), also suggesting that immunofluorescence may have clinical significance.

Probably more important than survival in the intravascular space is the granulocyte's ability to migrate to a site of inflammation. In our study [53], five patients had either a false negative or false positive indium scan or a combination false positive-false negative. These patients all had antibodies detected by both agglutination and immunofluorescence assays. No other antibody combination was present in these five patients. Neither granulocytotoxic, nor lymphocytotoxic, nor anti-

bodies causing lymphocyte-mediated granulocytotoxicity alone or in any combination were associated with reduced intravascular recovery, failure of granulocytes to localize at a site of infection or abnormal pulmonary sequestration. This supports previous observations that granulocyte agglutinating antibodies cause a reduced intravascular recovery and failure of cells to localize at a known site of infection.

Our studies and those summarized here do not establish which antigen system(s) is (are) involved in interference with the *in vivo* fate of granulocytes. Granulocyte agglutination detects granulocyte specific and some HLA antigens, immunofluorescence identifies both granulocyte specific and HLA antigens, while lymphocytotoxicity identifies HLA antigens. Our data indicate that granulocytes incompatible in a lymphocytotoxicity (HLA) cross-match circulate normally, migrate to a site of infection normally and are not abnormally sequestered in the lungs. Although the studies of Dutcher *et al.* [51, 52] implicate lymphocytotoxic antibodies, they do not distinguish the effects of different antibody methods. Chow *et al.* [49] reported that in dogs immunofluorescence was more predictive of the fate of granulocytes *in vivo* than agglutination or lymphocytotoxicity although data were not shown. The clinical studies of Dahlke *et al.* [50] also support the role of immunofluorescence, although agglutination testing was not done. Our data, which seem consistent with previous studies, indicate that the combination of antibodies detected by agglutination and immunofluorescence are the best *in vitro* predictor of the *in vivo* fate of granulocytes.

In most highly immunized patients both agglutinating and lymphocytotoxic antibodies are present; however, the routine use of the agglutination test appears preferable. Immunofluorescence might also be used to detect immunization, but it requires expensive equipment and more highly skilled personnel.

Preservation of granulocytes for transfusion

As granulocytes have become a clinically available blood component, it is of increasing importance to define the changes which occur in these cells during

preservation. This allows definition of proper conditions for handling the granulocyte concentrates in the interval between collection and transfusion. Granulocytes maintain their bactericidal capacity and important metabolic activity such as the hexose monophosphate shunt for up to three days of storage in plastic bags at 1–6° [54]. However, during the first 24 hours there is a 30–50% loss of chemotactic response [55, 56]. A major factor in this loss of chemotactic response is exposure to cold. This causes a loss of microtubules, microfilaments and ability to adhere to a foreign surface [57]. These changes are reduced by storage of granulocytes at room temperature and this also results in a better chemotactic response [56, 57].

Since the interval between collection and transfusion may be several hours or more, it would be very helpful if granulocytes could be stored. This would also facilitate donor scheduling if granulocytes could be preserved for transfusion. Studies *in vitro* suggest that storage of granulocytes may be possible; however, there is very little information available regarding the effectiveness *in vivo* of stored granulocytes. Price and Dale [58] reported that rabbit granulocytes stored at 4°C had a higher intravascular recovery and migration into subcutaneous sponges than cells stored at room temperature. In subsequent studies of human granulocytes by these investigators, cells were stored only at 4°C. After 24 h of storage, the intravascular recovery was reduced by approximately 33% and migration into a skin window by 75% [59].

We have used the ¹¹¹indium labelled granulocyte technique described earlier to study the fate *in vivo* of stored human granulocytes [60]. This was combined with a skin chamber technique which allowed measurement of intravascular recovery, survival and extravascular migration of cells. Granulocytes stored for eight hours at room temperature had no reduction of intravascular recovery or survival and approximately a 50% reduction in migration into the skin chambers. Storage of granulocytes for eight hours at 1–6°C reduced the intravascular recovery by 66% and the extravascular migration by 86%. Storage for longer than eight hours caused more severe reductions in the *in vivo* effectiveness of the transfused granulocytes. Thus,

¹¹¹indium labelling appears to also provide helpful information regarding the *in vivo* effectiveness of granulocytes preserved for short intervals *in vitro*.

Summary

Granulocyte transfusions are used in the management of infection in granulocytopenic patients. Several instruments are available to obtain granulocytes for transfusion. There is ample evidence that these cells function normally *in vitro* and can be clinically effective *in vivo*. During the last several years, new antibiotics have become available and clinicians are much better prepared to deal with granulocytopenic patients. At the same time, it has become apparent that the dose of granulocytes usually given is quite small, that many patients become immunized to granulocytes and that methods of preservation of granulocytes for transfusion are not optimal. Thus, the use of granulocyte transfusion has stabilized or declined. As work with granulocyte transfusions continues, it is essential that the effects of variables on the results of transfusion be carefully documented. One method for determining the *in vivo* effect of transfused granulocytes is the use of ¹¹¹indium-labelled granulocytes.

Acknowledgement

Supported in part by grant R01-HL-26568-02 from the National Heart, Lung and Blood Institute and research funds from the American Red Cross.

References

1. Hersh EM, Bodey GP, Nies BA, Freireich EJ: Causes of death in acute leukemia. A ten-year study of 414 patients from 1954 to 1963. *JAMA*, 1965; 193: 105–9.
2. Bodey GP, Buckley M, Sathe YS, Freireich EJ: Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med*, 1966; 64: 328–40.
3. Three antibiotic regimens in the treatment of infection in febrile granulocytopenic patients with cancer. The EORTC

- International Antimicrobial Therapy Project Group. *J Infect Dis*, 1978; 137: 14-29.
4. Freireich EJ, Levin RH, Whang J, Carbone PP, Bronson W, Morse EE: The function and fate of transfused leukocytes from donors with chronic myelocytic leukemia in leukopenic recipients. *Ann NY Acad Sci*, 1965; 113: 1081-89.
 5. Graw RG, Jr, Herzig G, Perry S, Henderson ES: Normal granulocyte transfusion therapy. Treatment of septicemia due to gram-negative bacteria. *N Engl J Med*, 1972; 287: 367-71.
 6. Cline MJ, *The White Cell*. Harvard University Press, Cambridge, Massachusetts and London, 1975.
 7. McCullough J: Leukapheresis and granulocyte transfusion. *Clinical Reviews in Clinical Science*, CRC Press, Inc., April, 1979: p. 275.
 8. Lin AT, Smith J, Haux-Porten J, Porten R, Cullis H, Buchholz D, McCullough J: Granulocyte collection for transfusion using the Fenwal CS-3000 Blood Cell Separator. *Transfusion* (in review).
 9. Mishler JM: Hydroxyethyl starch as an experimental adjunct to leukocyte separation by centrifugal means: review of safety and efficacy. *Transfusion*, 1975; 15: 449-60.
 10. Ring J, Seifert J, Messmer K, Brendel W: Anaphylactoid reactions due to hydroxyethyl starch infusion. *Eur Surg Res*, 1976; 8: 389-99.
 11. Bode U, Deisseroth AB: Donor toxicity in granulocyte collections: Association of lichen planus with the use of hydroxyethyl starch leukapheresis. *Transfusion*, 1981; 21: 83-85.
 12. Maguire LC, Strauss RG, Koepke JA, Bowman RJ, Zelenski KR, Lambert RM, Hulse JD, Atnip AK: The elimination of hydroxyethyl starch from the blood of donors experiencing single or multiple intermittent-flow centrifugation leukapheresis. *Transfusion*, 1981; 21: 347-53.
 13. Hinckley ME, Huestis DW: Premedication for optimal granulocyte collection. *Plasma Ther*, 1981; 2: 149-52.
 14. Blumberg N, Shah I, Katz AJ, Morse EE: Granulocyte yields using the Haemonetics 30: Effects of variations in corticosteroid regimen and donor selection. *Vox Sang*, 1982; 44: 225-32.
 15. Dahlke MB, Shah SL, Sherwood WC, Shafer AW, Brownstein PK: Priapism during filtration leukapheresis. *Transfusion*, 1979; 19: 482-86.
 16. Wiltbank TB, Nusbacher J, Higby J, MacPherson JL: Abdominal pain in donors during filtration leukapheresis. *Transfusion*, 1977; 17: 159-62.
 17. Hammerschmidt DE, Croddock PR, McCullough J, Kronenberg RS, Dalmasso AP, Jacob HS: Complement activation and pulmonary dysfunction during nylon-fiber filtration leukapheresis. *Blood*, 1978; 51: 721-30.
 18. Strauss RG, Spitzer RE, Stitzel AE, Urmson JR, Maguire LC, Koepke JA, Thompson JS: Complement changes during leukapheresis. *Transfusion*, 1980; 20: 32-38.
 19. Djerassi I: Gravity leukapheresis - a new method for collection of transfusible granulocytes. *Exp Hematol*, 1977; 5: 139-42.
 20. Ambinder EP, Button GR, Cheung T, Goldberg JD, Holland JF: Filtration versus gravity leukapheresis in febrile granulocytopenic patients: A randomized prospective trial. *Blood*, 1981; 57: 836-41.
 21. Aisner J, Schiffer CA, Daly PA, Buchholz DH: Evaluation of gravity leukapheresis and comparison with intermittent centrifugation leukapheresis. *Transfusion*, 1981; 21: 100-5.
 22. Glasser L: Functional considerations of granulocyte concentrates used for clinical transfusions. *Transfusion*, 1979; 19: 1-6.
 23. Strauss RG, Maguire LC, Koepke JA, Thompson JS: Properties of neutrophils collected by discontinuous-flow centrifugation leukapheresis employing hydroxyethyl starch. *Transfusion*, 1979; 19: 192-96.
 24. McCullough J, Weible BJ, Deinard AS, Boen J, Fortuny IE, Quie PG: In vitro function and post-transfusion survival of granulocytes collected by continuous-flow centrifugation and by filtration leukapheresis. *Blood*, 1976; 48: 315-326.
 25. Appelbaum FR, Norton L, Graw RG, Jr: Migration of transfused granulocytes in leukopenic dogs. *Blood*, 1977; 49: 483-88.
 26. Alavi JB, Root RK, Djerassi I, et al.: A randomized clinical trial of granulocyte transfusions for infection in acute leukemia. *N Engl J Med*, 1977; 296: 706-11.
 27. Sandler SG, Nusbacher J: Health risks of leukapheresis donors. *Haematologia*, 1982; 15: 57-69.
 28. Olson PR, Cox C, McCullough J: Laboratory and Clinical effects of the infusion of ACD solution during plateletpheresis. *Vox Sang*, 1977; 33: 79-87.
 29. Wright DG, Karsh J, Fauci AS, Klippel JH, Deisseroth AB, Decker JL: Lymphocytapheresis. In: *The Lymphocyte*, Sell KW, Miller WV (eds). Alan R. Liss, Inc., New York, NY, 1981: 217-24.
 30. Koepke JA, Parks WM, Goeken JA, Klee GG, Strauss RG: The safety of weekly plateletpheresis: Effect on the donors' lymphocyte population. *Transfusion*, 1981; 21: 59-63.
 31. Shohet SB: Morphologic evidence for the in vivo activity of transfused chronic myelogenous leukemia cells in a case of massive staphylococcal septicemia. *Blood*, 1968; 32: 111-18.
 32. Price TH, Dale DC: Blood kinetics and in vivo chemotaxis of transfused neutrophils: Effect of collection method, donor corticosteroid treatment, and short-term storage. *Blood*, 1979; 54: 977-86.
 33. Eyre HJ, Goldstein IM, Perry S, Graw RG, Jr: Leukocyte transfusions: function of transfused granulocytes from donors with chronic myelocytic leukemia. *Blood*, 1970; 36: 432-44.
 34. Arnold R, Pflieger H, Dietrich M, Heimpel H: The clinical efficacy of granulocyte transfusions: studies on the oral cavity. *Blut*, 1977; 35: 405-14.
 35. Thakur ML, Coleman RE, Welch MJ: Indium-III-labeled leukocytes for the localization of abscesses: preparation, analysis, tissue distribution, and comparison with gallium-67 citrate in dogs. *J Lab Clin Med*, 1977; 89: 217-28.
 36. Weible BJ, Forstrom L, McCullough J: Studies of the

- kinetics of indium-III-labeled granulocytes. *J Lab Clin Med*, 1979; 94: 246-55.
37. McCullough J, Weiblen BJ, Clay ME, Forstrom L: Effect of leukocyte antibodies on the fate in vivo of indium-III-labeled granulocytes. *Blood*, 1981; 58: 164-70.
 38. Ascher NL, Ahrenholz DH, Simmons RL, Weiblen B, Gomez L, Forstrom LA, Frick MP, Henke C, McCullough J: Indium III autologous tagged leukocytes in the diagnosis of intraperitoneal sepsis. *Arch Surg*, 1979; 114: 386-92.
 39. Frick MP, Henke CE, Forstrom LA, Simmons RA, McCullough J, Loken MK: Use of 111-in-labeled leukocytes in evaluation of renal transplant rejections: A preliminary report. *Clin Nucl Med*, 1979; 4: 24-25.
 40. Forstrom LA, Weiblen BJ, Gomez L, Ascher NL, Hoogland DR, Loken MK, McCullough J: Indium-111-oxine labelled leukocytes in the diagnosis of occult inflammatory disease. In: *Indium-111 Labeled Neutrophils, Platelets, and Lymphocytes*. Thakur ML, Gottschalk A (eds), 1979: 123-30.
 41. Graw RG, Jr, Eyre HJ, Goldstein IM, Terasaki PI: Histocompatibility testing for leukocyte transfusion. *Lancet*, 1970; ii: 77-78.
 42. Goldstein IM, Eyre HJ, Terasaki PI, Henderson ES, Graw RG, Jr: Leukocyte transfusions: role of leukocyte alloantibodies in determining transfusion response. *Transfusion*, 1971; 11: 19-24.
 43. Herzig RH, Herzig GP, Graw RG, Jr, Bull MI, Ray KK: Successful granulocyte transfusion therapy for gram-negative septicemia. A prospectively randomized controlled study. *N Engl J Med*, 1977; 296: 701-5.
 44. McCredie KB, Freireich EJ, Hester JP, Vallejos C: Leukocyte transfusion therapy for patients with host-defense failure. *Transplant Proc*, 1973; 5: 1285-89.
 45. McCredie KB, Hester JP, Freireich EJ, Brittin GM, Vallejos C: Platelet and leukocyte transfusions in acute leukemia. *Hum Pathol*, 1974; 5: 699-708.
 46. Underleider RS, Appelbaum FR, Trapani RJ, Deisseroth AB: Lack of predictive value of antileukocyte antibody screening in granulocyte transfusion therapy. *Transfusion*, 1979; 19: 90-94.
 47. Westrick MA, Debelak-Fehir KM, Epstein RB: The effect of prior whole blood transfusion on subsequent granulocyte support in leukopenic dogs. *Transfusion*, 1977; 17: 611-14.
 48. Appelbaum FR, Trapani RJ, Graw RG: Consequences of prior alloimmunization during granulocyte transfusion. *Transfusion*, 1977; 17: 460-64.
 49. Chow HS, Alexander DL, Epstein RB: Detection and significance of granulocyte alloimmunization in leukocyte transfusion therapy on neutropenic dogs. *Transfusion* 1983; 23: 45-48.
 50. Dahlke MB, Keashen M, Alavi JB, Koch PA, Eisenstaedt R: Granulocyte transfusions and outcome of alloimmunized patients with gram-negative sepsis. *Transfusion*, 1982; 22: 374-78.
 51. Dutcher JP, Schiffer CA, Johnston GS, Papenberg D, Daly PA, Aisner J, Wiernik PH: The effect of histocompatibility factors on the migration of transfused ¹¹¹indium-labeled granulocytes. *Blood*, 1982; 58: 181a (abstract).
 52. Dutcher JP, Fox JJ, Riggs C, Johnston GS, Schiffer CA: Pulmonary retention of indium-III-labeled granulocytes in alloimmunized patients. *Blood*, 1982; 58: 171a (abstract).
 53. McCullough J, Clay M, Richards K, Forstrom L, Loken M: Leukocyte antibodies: Their effect on the fate in vivo of indium-111-labeled granulocytes. *Blood*, 1982; 60: 80a (abstract).
 54. McCullough J: Liquid preservation of granulocytes. *Transfusion*, 1980; 20: 129-37.
 55. Glasser L: Effect of storage on normal neutrophils collected by discontinuous-flow centrifugation leukapheresis. *Blood*, 1977; 50: 1145-50.
 56. McCullough J, Weiblen BJ, Peterson PK, Quie PG: Effects of temperature on granulocyte preservation. *Blood*, 1978; 52: 301-19.
 57. Palm SL, Furcht LT, McCullough J: Effects of temperature and duration of storage on granulocyte adhesion, spreading, and ultrastructure. *Lab Invest*, 1981; 45: 82-88.
 58. Price TH, Dale DC: Neutrophil preservation. The effect of short-term storage on in vivo kinetics. *J Clin Invest*, 1977; 59: 475-80.
 59. Price TH, Dale DC: Blood kinetics and in vivo chemotaxis of transfused neutrophils: effect of collection method, donor corticosteroid treatment, and short-term storage. *Blood*, 1979; 54: 977-86.
 60. McCullough J, Weiblen BJ, Fine D: Effects of storage of granulocytes on their fate in vivo. *Transfusion*, 1983; 23: 20-24.

7. Plasma proteins for therapeutic use: past, present and future

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1. Prologue: promise and fulfillment

The development and clinical use of plasma proteins has a history of sporadic quantum jumps coupled with years of stagnation. The quantum jumps occur with the development of new technology, whereas the stagnation occurs when there is unimaginative management of the plasma resource and lack of funding for new product development.

Critics invariably point out the worldwide commitment to the Cohn cold-ethanol procedure [1] and deplore the lack of new fractionation technology and the lack of new therapeutic products. The durability of the Cohn system stems from the firm physical-chemical foundation built into the original fractionation scheme as well as the adaptability of this scheme to large-scale operation. On the other hand, the limited range of fractionation technology used and plasma products available compared with the potential for this industry merits serious investigation. The development of new plasma products depends on an ordered progression from biochemical investigations to physiologic and pathologic models to manufacturing and clinical trials. This review is intended to predict what could be an exciting future but, at the same time, to address historical pitfalls.

The current roster of therapeutic products composed of human plasma proteins is readily divisible into three categories: (a) coagulants, (b) immune globulins, and (c) plasma volume expanders. The individual members of these categories are listed in Tables 1 and 2, and they are discussed below. A parallel cataloging of animal proteins would in-

clude animal antihemophilic factor (Factor VIII) preparations, various antitoxins and antivenins, and the antilymphocytic globulins used in tissue and organ transplantation. A detailed discussion of such products, however, is beyond the scope of this chapter.

The birth of plasma fractionation coincided with the development of blood banking and the consequent availability of plasma from the outdated blood. The perceived need for colloid to treat the battle casualties of World War II gave great impetus to the development of albumin as a therapeutic agent. The result was the cold-ethanol fractionation scheme [1], which is almost uniformly used for large-scale manufacture of therapeutic immunoglobulin preparations and albumin-rich products. Other products such as fibrin and fibrinogen [2, 3], though once thought to be useful, have fallen into disuse. As early as 1948 the possibility of using ethanol fractions for the treatment of hemophilia was studied, and Fraction I was found to be active *in vivo* [4]. However, since many of the congenital deficiencies of plasma proteins were as yet uncharacterized, such proteins as Factor IX and C1 inactivator were not investigated.

Another quantum advance was the development in the early 1960's of the hardware for collecting fresh plasma by plasmapheresis. This has allowed the production of fractions rich in the more labile plasma components, e.g., Factor VIII, as well as specific immune globulins such as high titer anti-D.

Despite the development of many powerful protein separation techniques over the last 30 years, these have had little impact on the large-scale pro-

duction of plasma proteins. Ion exchange chromatography has been shown to be useful for the production not only of Factor IX concentrates [5] but also for albumin and globulin [6]. Affinity chromatography has yet to be developed on a commercial level.

Two major basic technologies are likely to revolutionize the production of plasma proteins for therapeutic use in the next decade. The power of recombinant DNA for the production of trace components of plasma seems to be unmatched. The ability to produce monoclonal antibodies offers a new powerful tool for the development of immunotherapy not only for infectious diseases but also for cancer, autoimmune disease and transplantation.

Recombinant DNA technology has already been used to clone cells that produce a variety of plasma proteins [7-14]. If these advances can be exploited to provide such products at a cost significantly below current prices, the ultimate impact could be a profound alteration in methods of treatment. For example, treatment of hemophilia A is primarily 'demand' therapy, i.e., treatment is begun in response to hemorrhage. A significant reduction in the price of Antihemophilic Factor could lead not only to worldwide availability but possibly to universal prophylactic therapy. At the same time one would anticipate a safer product since it would be free from extraneous human viruses and allogeneic proteins.

The clinical investigation of monoclonal antibodies has already begun. It includes trials of anti-lymphocyte antibodies to prevent graft versus host reactions and for treatment of infectious diseases and malignancy [15]. It is difficult to predict the clinical future of products made by hybridoma technology (see below); however, one can be sure that monoclonal antibodies will be tried in virtually every type of disease.

1.1. Use and utility of plasma products: Congenital and acquired diseases

The philosophies expressed in using blood products differ for congenital and acquired deficiencies. For congenital deficiencies, there often is a clear

association between an absent or defective protein and the development of a defined pathology: hemophilia and hemorrhage, α_1 -antitrypsin deficiency and emphysema, etc. (However, a protein deficiency need not always be associated with pathology, e.g., congenital analbuminemia.) In these situations, replacement therapy can have a dramatic normalizing effect, as demonstrated by the congenital coagulation defects and agammaglobulinemias. Similarly, for congenital deficiencies of C1 inactivator [16] and α_1 -antitrypsin [17] there is some clinical evidence supporting the efficacy of replacement therapy.

The usefulness of blood products for acquired deficiencies is far less defined. Some treatments are based on sound physiologic principles, such as resuscitation with albumin after blood loss. Massive volume loss is unarguably an indication for rapid volume replacement, but even here there is considerable controversy as to whether crystalloid solutions serve as well as albumin [18]. The limiting factor in the development of blood products has been the lack of proof of efficacy. This is particularly true for acquired diseases in which, as pointed out above, there is often no direct relationship between the decreased concentration of a protein and the morbidity and/or mortality. All too often an investigation begins with an assumption of effectiveness rather than a critical experimental design.

An historical example is provided by fibrinogen. Although many patients who bleed have low plasma fibrinogen levels, there have never been any experiments to demonstrate that fibrinogen replacement is beneficial. When fibrinogen was first introduced on the market, it was greeted with great enthusiasm and correspondingly enthusiastic use. Over the years, the use of fibrinogen in the United States dwindled until ultimately it was removed from the market [3]. There is still no experimental data to indicate what conditions (if any) might be benefited by fibrinogen replacement therapy.

By contrast, two examples illustrating the ordered development of efficacious blood products involve Rh immunoglobulin and the 'J5' serum. The Rh immunoglobulin story began with

the observation that antibody formation was inhibited by the concomitant administration of antibody and antigen [19]. Finn *et al.* [20] pursued these observations in search of a method to block Rh immunization. Animal models of erythroblastosis are not available; however, a rabbit model indicated that antibody administered 3 days after the red cell antigen was infused could still prevent antibody formation [21]. Trials in male volunteers established that Rh sensitization of humans could be blocked. Subsequently, the administration of the antibody to women immediately postpartum was shown to prevent erythroblastosis fetalis [22].

The recent development of a successful therapeutic approach to severe gram-negative infection followed a similar ordered course. The first step was the concept of a 'class' antigen for gram-negative endotoxemia [23]. This was followed sequentially by the development of such an antigen [24], development of an animal model [25, 26], and finally a carefully designed clinical trial which showed a reduction in mortality of 50% in extremely sick patients [27].

2. Therapeutic plasma proteins: Present and future

2.1. Coagulant protein preparations

Antihemophilic Factor and Factor IX Complex are proven therapeutic preparations. The availability of these fractions and the development of treatment programs have revolutionized the lives of hemophilic patients. Successful as these products are, however, they could be better. The major problem is the transmission of viral disease, particularly hepatitis.

Although screening has had a major impact on decreasing the contamination of pooled plasma products with hepatitis B virus, it has revealed that the vast majority of such products are contaminated with non-A, non-B hepatitis viruses [28, 29].

For many years the albumin products have been heated at 60°C during manufacture to prevent hepatitis transmission [30, 31]. New data show that under certain conditions more labile proteins such as Factor VIII and Factor IX can also be heated to

reduce, if not prevent, transmissible disease in recipients [32-34]. Other potential processing methods including the addition of specific antibody, treatment with detergent, and the combined use of β -propiolactone and ultraviolet radiation are also being investigated [35-38]. It is likely that all coagulant protein products will be subjected to some such treatment in the future, unless (or until) virus-free coagulants can be produced by recombinant DNA technology.

2.1.1. Factor VIII

Factor VIII concentrates currently available for the treatment of hemophilia A are 10- to 100-fold purified as compared to plasma and are concentrated 10- to 50-fold over plasma. Almost uniformly the first step in production is the harvesting of Factor VIII-rich cold insoluble 'cryoprecipitate'. This is followed by other purification procedures, often including Al(OH)_3 adsorption and, for the higher purity products, polyethylene glycol fractionation [39]. The major protein constituents in the final product are fibrinogen and fibronectin with lesser amounts of immunoglobulins and other plasma proteins. With increasing purity, there is a decreasing content of the higher polymers of von Willebrand factor [40]. The overall yield of Factor VIII coagulant in purified Antihemophilic Factor is probably about 20%, as compared to about 50% in single donor cryoprecipitate.

In addition to the treatment of Factor VIII concentrates to reduce the content of infectious agents, as noted above, further progress can also be made to increase the yield from the starting plasma and to increase the purity. The former has obvious economic advantages to the manufacturer and the user. The advantages of further purification might be more convenient dosage forms and the possibility of less antigenic stimulation. However, in view of the rapid developments in recombinant DNA technology, producers may not invest in refinement of current techniques.

Cryoprecipitate, which is currently used for the treatment of hemophilia A, von Willebrand's disease, and on occasion, hypofibrinogenemia, may be effective in other acquired bleeding disorders. The recent report of Janson *et al.* [41] indicates that

cryoprecipitate contains material capable of correcting the prolonged bleeding time in patients with kidney failure. This effect may be related to the higher molecular weight polymers of von-Willebrand factor found in cryoprecipitate and may be similar (or identical) to the component effective in the treatment of bleeding in von-Willebrand's disease. Cryoprecipitate is also being tried for clinical use as a source of fibronectin.

2.1.2. Factor V

There is a clinical need for a therapeutic concentrate of Factor V for those few patients deficient in this factor. Although laboratory scale purification is possible, it is unlikely that there will be commercial development of such a product.

2.1.3. Preparations of vitamin K dependent coagulants

For the last 20 years, Factor IX Complex preparations containing all of the vitamin K dependent factors, Factors II, VII, IX and X (and presumably Protein C), have been widely used clinically for both congenital and acquired disorders. These concentrates are produced either by adsorption and elution from inorganic precipitates such as Al(OH)_3 and $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ or by use of anion exchange resins such as DEAE-Sephadex. The content of Factors II, VII, IX and X is comparable for the Al(OH)_3 and $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ preparations, whereas the ion exchange preparations are relatively depleted of Factor VII.* As in the case of Factor VIII concentrates, the major adverse effect of Factor IX Complex has been the transmission of viral hepatitis. Similarly, one can anticipate that, in the near future, either physical or chemical treatments or production by recombinant DNA technology will yield product with low viral content.

A second serious adverse effect of the current Factor IX concentrates is the induction of thrombohemorrhagic disorders [42]. These complications may be due to active coagulant enzymes [43], active enzyme along with phospholipids [44], or

high loads of infused zymogens [45]. Presumably, preparations with higher purity would be less likely to evoke this type of reaction. Such preparations, enriched in either Factor VII [46] or Factor IX, are under development [47]. On the other hand, the standard Factor IX Complex has not been reported to produce thromboembolic complications when used for purposes such as reversing the effects of vitamin K antagonists. Thus, since purified products enriched in a single factor will be useful only for treating the congenital deficiency of that factor, there will still be a role for the combined preparation containing all the vitamin K dependent coagulants.

Two commercial preparations of 'activated' Factor IX Complex, FEIBA® (Immuno) and Auto-plex® (Hyland), are currently available for the treatment of hemorrhage in patients with inhibitors to Factor VIII. These products contain increased quantities of activated Factors VII, IX and X [48, 49]. Clinical experience indicates that they offer, at best, a marginal increment in effectiveness compared to non-activated Factor IX Complex [50, 51]. One might anticipate that the current 'activated' products will disappear in light of this marginal effectiveness, their extreme expense, lack of knowledge concerning the hemostatically active ingredient(s), and consequent inability to standardize effectiveness. One can expect better characterized products such as activated Factor VII, activated Factor IX, or animal Factor VIII to be investigated and, if proven successful, to become the dominant therapy for patients with Factor VIII inhibitors.

The newly described vitamin K dependent protein, Protein C, has many interesting properties, including its modulating effect on Factor V and Factor VIII activity [52, 53]. A great deal of physiological and biochemical investigation is underway, but it is unlikely that it will find clinical application in the near future.

2.2. Plasma proteinase inhibitors

The plasma proteinase inhibitors form an interesting class of proteins with varying potential for therapeutic use. They were described nearly 80

* In the absence of a standard preparation for defining the unit of coagulant activity, a unit is defined by the activity in 1 mL of normal plasma.

years ago by Opie [54] as inhibitors of leukocyte proteinases. In some cases pathology is clearly associated with congenital deficiency of the inhibitor, e.g., C1 inactivator [16], α_2 -plasmin inhibitor [55], α_1 -antitrypsin [56, 57]. In the case of other proteinase inhibitors, e.g., antithrombin III, the association of deficiency with pathology is likely, even though formally unproven.

2.2.1. C1 inactivator

The physiology and pathology of C1 inactivator have been well described. All of the pathology associated with the congenital deficiency state is compatible with excessive and prolonged complement activation. Vogelaar *et al.* reported the infusion of C1 inactivator into congenitally deficient patients [58]; further investigations have been described by Gadek *et al.* [16]. These trials indicate that infusion during the acute hyperenzymic state shortens and ameliorates the acute crises. No studies of antiproteinase maintenance therapy for this disorder have been reported, and it is unlikely that it will be attempted since it is well established that androgens (danazol) increase the endogenous production of C1 inactivator and diminish or prevent most acute attacks. C1 inactivator therapy has also been used to prevent hypotension associated with certain surgical procedures [59].

The data available support the use of C1 inactivator replacement therapy only during acute crises in congenitally deficient patients.

2.2.2. Antithrombin III

With the advent of heparin chromatography for purification, the search for a therapeutic use for antithrombin III began. Despite the many elegant biochemical studies of antithrombin III, the physiologic and pathologic roles of this inhibitor are not well understood. There are clusters of thromboses in families with congenitally low antithrombin III; however, there are no population studies formally proving that decreased antithrombin III is associated with an increased incidence of thrombosis. On the other hand, clinical reports [60, 61] of individuals with low levels of antithrombin III indicate that once thrombosis begins it is progressive and sometimes fatal, even in young patients, despite con-

ventional anticoagulant therapy.

No formal clinical trial of antithrombin III replacement for congenital deficiency has been reported, nor have there been sufficient anecdotal cases to provide convincing data of efficacy. In the reported congenitally deficient patients treated with antithrombin III, there has been no progression of the thrombotic process [62, 63]. Such therapy will probably be used only during acute thrombotic episodes since thrombosis in these patients is infrequent and maintenance therapy would be expensive and inconvenient. An alternative to treatment with antithrombin III concentrates would be replacement therapy with plasma.

Many acquired conditions cause significant decreases in plasma antithrombin III levels. Acquired deficiencies in which patients have been infused with antithrombin III include toxic shock syndrome, pre-eclampsia, 'shock', hemolytic-uremic syndrome, cirrhosis, undifferentiated mild disseminated intravascular coagulation (DIC), and DIC in acute hepatic failure [64-68]. The accrued data are insufficient to permit any conclusion as to the efficacy of the treatment, though the studies in cirrhosis indicate normalization of fibrinogen catabolism. Several small clinical trials [69] have investigated thrombosis in patients undergoing total hip replacement following infusion of antithrombin III. The results do not indicate a benefit to the patient.

If the use of antithrombin III is to be recommended in the future, the data must be more convincing than that currently available. Animal models may aid in identifying patients who could benefit from replenishment of antithrombin III.

2.2.3. α_1 -antitrypsin

The presence of altered, dysfunctional α_1 -antitrypsin is clearly associated with pathologic processes resulting from proteolysis of structural proteins by enzymes such as elastase [56, 57]. Patients with abnormal alleles of α_1 -antitrypsin are prone to neonatal non-viral hepatitis and chronic, debilitating emphysema. An animal model has clearly demonstrated the capability of excess pulmonary elastolytic activity to produce emphysema.

In the face of such evidence, it is surprising that

the clinical therapy data are limited to a single trial demonstrating decreased elastolytic activity in bronchial secretions after treatment with α_1 -antitrypsin [17]. Although longitudinal studies would be needed to formally prove the efficacy of this approach, this single trial gives very strong support to the usefulness of replacement therapy in α_1 -antitrypsin deficiency. The technology exists to produce a therapeutic product by recombinant DNA [8] or by conventional plasma fractionation. The estimate of the patient numbers (20,000–80,000 ZZ genotypes in the United States) suggests that it should be a commercially attractive product.

2.2.4. α_2 -antiplasmin (α_2 -plasmin inhibitor)

This recently characterized plasma protein is the primary inhibitor of plasmin *in vivo*. Patients with a homozygous deficiency have a severe hemorrhagic state due to the rapid removal of fibrin by plasmin [55]. It seems unlikely that a therapeutic preparation of α_2 -plasmin inhibitor will be needed for the congenitally deficient patients since the administration of synthetic fibrinolytic inhibitors such as ϵ -aminocaproic acid gives satisfactory inhibition of fibrinolysis *in vivo*.

2.3. Albumin products

Plasma volume expanders containing human albumin are usually prepared at two different concentrations. In the United States, the more concentrated solution is 25% Normal Serum Albumin; in some other countries, 20% albumin is preferred. A high proportion ($\geq 96\%$) of the total protein in these solutions is albumin. The pH and sodium concentration are in the physiological range. In an effort to inactivate any hepatitis viruses that may be present in these products, manufacturers subject them to heating for 10 h at 60°C. Despite the use of one or more stabilizers, such as sodium caprylate, some protein-protein interaction occurs during this heating step. Thus, in addition to the principal component, monomeric albumin (molecular weight 66 500), these solutions contain some albumin dimer, trace amounts of higher oligomers, and a small quantity of high molecular weight aggregate (polymer).

The less concentrated solutions usually contain 3.5–5.0% protein. Unlike the concentrated albumin products, their protein composition is variable. For example, in the United States, two such products coexist. In one of these, 5% Normal Serum Albumin, at least 96% of the total protein is albumin. In the other, Plasma Protein Fraction (which is also a 5% protein solution), as little as 83% of the total protein may be albumin. Because of the increased opportunity for protein–protein interactions and the inherently lower thermal stability of the globulins, the latter product may differ from Normal Serum Albumin in molecular size distribution as well as electrophoretic pattern. That is, after the heating for 10 hours at 60°C, these low-purity (i.e., low albumin percentage) products contain three to four times as much high molecular weight aggregate as does 5% Normal Serum Albumin (Table 1).

An additional consequence of its lower purity is the greater tendency – unless special manufacturing precautions are taken – of Plasma Protein Fraction to contain prekallikrein activator. Just as the aggregate content of Plasma Protein Fraction may

Table 1. Currently available human plasma protein products

Product ¹	Active protein component(s)	Other major protein components
Antihemophilic Factor	Factor VIII	Fibrinogen; fibronectin
Factor IX Complex	Factor IX; Factors II, X, (VII)	–
Anti-Inhibitor Coagulant Complex	Factor IXa?	–
Immune Globulins ²	Factor Xa?	–
Immune Globulin Intravenous ³	IgG antibodies	–
Normal Serum Albumin	IgG or Modified IgG	–
Plasma Protein Fraction	Albumin	Aggregated protein; β -globulin

¹ The products are those licensed in the United States, and the names reflect the present official U.S. designations.

² For intramuscular administration; see Table 2.

³ See Table 3.

raise legitimate concern about its safety in the treatment of shock, the potent hypotensive effect of prekallikrein activator [59, 70–72] may severely limit the usefulness of Plasma Protein Fraction in emergency situations.

The clinical use of human albumin products is discussed in Part two, Chapter 2. Additional details of their manufacture and properties are given in a recent review [73]. Certain properties of the albumin molecule itself, especially its ability to circulate without prior glycosylation, make it a prime candidate for synthesis by recombinant DNA technology. Indeed, cloning, synthesis, and other technical steps have been accomplished, and commercialization has been considered. In spite of the potential superiority (stemming, for example, from its freedom from hepatitis viruses and the need for stabilization and heating) of albumin prepared in this way, however, its economic advantage over conventionally manufactured albumin remains to be demonstrated.

2.4. *Immunoglobulin products*

2.4.1. *Immune globulins for intramuscular use*

Like the therapeutic preparations of human albumin, which are administered by intravenous infusion, the human immunoglobulin products intended for intramuscular injection have been available since the 1940's [74]. They are usually manufactured as concentrated IgG solutions (10–18% protein) that contain small quantities of IgA and IgM. The list of current products (Table 2) includes a number of specific immune globulins directed against particular antigens (hepatitis B surface antigen, blood group antigen D, tetanus toxin) or viruses (rabies, varicella-zoster), as well as the 'broad-spectrum' product, Immune Serum Globulin. The long availability of the latter permits the tracing of its clinical history. As in the case of many new drugs, initial enthusiasm led to use for a wide range of conditions. That range has now shrunk to a modest list of three indications – prophylaxis of hepatitis A, prophylaxis of measles when active immunization is inappropriate (for example, in infants younger than one year), and maintenance of immunodeficient patients. In parallel with the find-

ing that Immune Serum Globulin was ineffective for various applications, a number of specific immune globulins whose effectiveness was marginal or nonexistent faded into disuse (Table 2). These include Mumps Immune Globulin, Pertussis Immune Globulin, and Poliomyelitis Immune Globulin. Two other immunoglobulin products have also disappeared, even though they were effective. Measles Immune Globulin is no longer made because its function is fulfilled by Immune Serum Globulin, and Vaccinia Immune Globulin became a product without a disease when worldwide eradication of smallpox was accomplished.

By contrast, several of the specific immune globulins are firmly established, at least for certain indications. Rh_o(D) Immune Globulin has been shown unequivocally to prevent immunization of previously unsensitized D-negative women if they receive it soon after the delivery of a D-positive infant [22]. Related uses include administration after miscarriage, abortion, or aminiocentesis or to D-negative recipients of mismatched (i.e., D-positive) blood or blood cells [74]. Still greater success in preventing postpartum immunization of D-negative mothers has been reported if Rh_o(D) Immune Globulin is given antenatally [75]. In some parts of the world this has become routine practice, although there is not universal agreement on its merit. Hepatitis B Immune Globulin was found to be prophylactically effective for neonates born to women who are hepatitis B carriers [76]. It is also recommended for post-exposure prophylaxis fol-

Table 2. Immune globulins* prepared from human plasma

Effectiveness established or highly probable

Immune Serum Globulin
Hepatitis B Immune Globulin
Rabies Immune Globulin
Rh_o(D) Immune Globulin
Tetanus Immune Globulin
Varicella-Zoster Immune Globulin

Effectiveness not established or use not recommended

Measles Immune Globulin
Mumps Immune Globulin
Pertussis Immune Globulin
Poliomyelitis Immune Globulin

* For intramuscular administration; see footnotes to Table 1.

lowing direct exposure to hepatitis B-positive material [74]. Similarly, Varicella-Zoster Immune Globulin is used for prophylaxis in susceptible, immunocompromised children exposed to varicella and in neonates whose mothers develop varicella near the time of delivery [77].

Truly satisfactory evidence for the prophylactic effectiveness of Rabies Immune Globulin and Tetanus Immune Globulin is not available. Each is used in conjunction with the corresponding agent for active immunization (rabies vaccine and tetanus toxoid, respectively). The apparent efficacy of the combined prophylactic regimens, however, makes it likely that the use of these immune globulins will continue.

If new immune globulins for intramuscular administration are developed, they will probably resemble the current products in composition and differ from them primarily in specificity. New specificities such as diphtheria and rubella have been suggested for potentially useful products. However, their ultimate effectiveness, the appropriate route of administration, and the proper role (if any) of passive immunization in these conditions are uncertain.

2.4.2. *Immune globulins for intravenous use*

When immunoglobulins were first isolated from venous plasma, it seemed logical to re-introduce them therapeutically by the same route, i.e., intravenously. The severe adverse reactions that resulted, however, relegated these products to intramuscular administration. Nonetheless, intravenous infusion offered many potential advantages (larger doses, less discomfort, more rapid achievement of maximum levels in the circulation), especially for patients with small or damaged muscle mass. These advantages stimulated the search for immunoglobulin preparations that could be given safely by the intravenous route. The initial pathway in their development involved isolation of a globulin fraction by conventional methods such as cold-ethanol precipitation, followed by modification through treatment with proteolytic enzymes or other agents. The first enzyme used was pepsin [78] and the modified product consisted primarily of $F(ab')_2$. Other treatments employed

include incubation at pH 4 or with plasmin, cleavage of interchain disulfide bridges by reduction or sulfhydryl treatment, adsorption with fumed silica, addition of stabilizing materials, e.g., disaccharides or albumin, or combinations of these. More recently, the treatments have tended to become gentler and to produce less structural alteration. Some methods now involve no deliberate modification but, instead, are directed toward isolating 'native' immunoglobulins. As a result of these diverse approaches there exist, worldwide, a variety of different intravenous immunoglobulin preparations with a wide range of characteristics (Table 3).

Most of these preparations (indicated by the single designation, Immune Globulin Intravenous, in Table 2) are of the 'broad-spectrum' type. When studied in prospective clinical trials, they have been found suitable for maintaining immunodeficient patients [81-83]. In addition, individual preparations have shown promise for preventing or modifying infections in surgical [84], leukemic [85], and transplant [86, 87] patients and for raising the circulating platelet level in patients with idiopathic thrombocytopenic purpura [88, 89]. Several major questions such as the optimal dosage, the precise indications, the mechanism of action, and the means for identifying appropriate target populations still remain. Nonetheless, studies in progress, if sufficiently well controlled, may provide definitive answers.

Beyond the intravenous immunoglobulins for general use lies a potentially vast array of specific immune globulins, and a recent report [27] should provide additional stimulus for their development. Ziegler *et al.* [27] found that unfractionated human antiserum to the common core region of bacterial lipopolysaccharide ('J5 antiserum') significantly decreased mortality in patients with gram-negative bacteremia. Thus, despite the formidable problems encountered in demonstrating the efficacy of treatments for bacterial infections [90], intravenous administration of specific, high-titer immune globulins may offer clinical success where antibiotic therapy has failed. Moreover, in addition to immunoglobulins directed against organisms such as *Pseudomonas*, *Pneumococcus*, *Meningococcus*, and *Streptococcus*, one can also envisage

intravenous products for use in toxin-mediated diseases like botulism or tetanus in which rapid achievement of high plasma antibody concentration is essential. Finally, specific intravenous immune globulins for prevention or modification of viral infection (e.g., cytomegalovirus) have been prepared and are undergoing clinical trials.

2.4.3. Monoclonal antibodies and other hybridoma products

If the spectrum of potential intravenous immune globulin products manufactured from human plasma appears to be vast, that of comparable products prepared by hybridoma technology must be considered limitless. After its inception in 1975

[91], this technology began providing experimental immunologists with a continuously expanding arsenal of highly specific research reagents. Soon, however, monoclonal antibody reagents were developed for laboratory tests used in diagnosing diseases, grouping and typing blood cells, and identifying adventitious agents. The next logical (and important) step was the development of monoclonal antibodies for diagnoses *in vivo*. Use of such antibody products for prophylaxis or treatment, however, offers the greatest clinical challenge and, probably, the highest rewards.

The advent of human hybridomas [92, 93] introduced the possibility of manufacturing large quantities of specific immune globulins directed against

Table 3. Representative preparations of immune globulin intravenous¹

Type of product	Type of treatment	Method of preparation	Producer
Deliberately fragmented	Enzymic	Pepsin	Behringwerke
		Plasmin	Green Cross
		Plasmin	Travenol (Hyland)
		Plasmin	Mérieux
		Plasmin	Serotherapeutisches Institut
Disulfide-cleaved	Chemical	S-sulfonation	Behringwerke
		Reduction, alkylation	Miles (Cutter)
		S-sulfonation	Teijin
'Intact'	Chemical	β -Propiolactone	Biotest
		pH 4	Commonwealth Serum Laboratories
		pH 4	Netherlands Red Cross
		pH 4	Swiss Red Cross
'Intact'	Fractionation	Ethanol, PEG, HES ²	Armour Pharma
		Ethanol, PEG	Continental
		Ethanol, PEG	Green Cross
		Ethanol, DEAE-Sephadex	Travenol (Hyland)
		Ethanol, salt, PEG	Immuno
		DEAE-Sephadex	Irish BSB ³
		Ethanol, DEAE-Sephadex	KabiVitrum
		Ethanol, PEG	Mérieux
		SiO ₂ , QAE-Sephadex	Minnesota University
		Ethanol	Scottish NBTS
		DEAE-Sephadex	Winnipeg Rh Institute ³
		DEAE-Sephadex	Zentralinstitut für das Bluttransfusionswesen ³

¹ Compiled from information presented in references 77, 79, and 80 or supplied by manufacturers. The list of products is not comprehensive; their commercial availability and approval by national authorities vary from country to country.

² Abbreviations: BSB, Blood Transfusion Service Board; DEAE, diethylaminoethyl; HES, hydroxyethyl starch; NBTS, National Blood Transfusion Service; PEG, polyethylene glycol; QAE, diethyl(2-hydroxypropyl)aminoethyl.

³ Specific immune globulins only.

a variety of infectious agents without reliance on blood or plasma donations or on continual immunogenic stimulation. Nonetheless, this exciting possibility represents only one area of application. Monoclonal antibodies can be (and, in some cases, have already been) designed and employed for immunosuppression (see above) or for neutralization of bacterial toxins, snake venoms, spider venoms, or even drugs [cf. ref. 94]. For some of these applications, human immunoglobulins may not be required and murine products may suffice. Reports of varying degrees of success in the treatment of cancer with monoclonal antibodies [15, 95] have evoked a wave of enthusiasm and spurred the development of a variety of serotherapeutic agents. These include products consisting of monoclonal antibodies *per se*, as well as those in which the tumor-directed antibody serves as the carrier for a radionuclide or a cytotoxic substance such as ricin or diphtheria toxin.

Although monoclonal antibodies have received most of the attention, lymphocyte products other than immunoglobulins should not be overlooked. Many of these 'lymphokines', each with its own biologic activity, have been described [96]. Their specific interactions with cells may underlie still another generation of clinically useful proteins.

3. Epilogue: Brave new world

In the next decade, the major change in the preparation of therapeutic blood products will be the introduction of recombinant DNA technology. This will result not only in a different spectrum of available products but also in new producers and different price structures. Of the classic products now obtained by fractionation (Tables 1 and 2), only albumin and the general immune globulin for treatment of agammaglobulinemia and hypogammaglobulinemia may continue to be made from plasma. Specific immunoglobulin products will probably be produced only in hybridomas.

There are many syndromes for which progress in therapy must await better understanding of the pathogenesis. The multiple traumatized patient is a particular target for treatment with a bewildering

array of blood products including, but not limited to, albumin products for shock, coagulant proteins as well as coagulant inhibitors to treat disseminated intravascular coagulation, and more recently fibronectin as a tonic to improve opsonization. Until the multiple pathologies are understood, there will be no improvement in the *status quo*.

References

1. Cohn EJ, Strong LE, Hughes WL Jr, Mulford DJ, Ashworth JN, Melin M, Taylor HL: Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J Am Chem Soc*, 1946; 68: 459-75.
2. Bering EA Jr: Chemical, clinical, and immunological studies on the products of human plasma fractionation. XX. The development of fibrin foam as a hemostatic agent and for use in conjunction with human thrombin. *J Clin Invest*, 1944; 23: 586-90.
3. Bove JR: Fibrinogen - is the benefit worth the risk? *Transfusion*, 1978; 18: 129-36.
4. Alexander B, Landwehr G: Studies of hemophilia. II. The assay of the antihemophilic clot-promoting principle in normal human plasma with some observations on the relative potency of certain plasma fractions. *J Clin Invest*, 1948; 27: 98-105.
5. Melin M, Jansky JW, Leonardos G, Di Francesco A, Pennell RB, Tullis JL: The preparation of a prothrombin-containing fraction from human plasma by chromatography. *Vox Sang*, 1964; 9: 227 (abstract).
6. Curling JM (ed): *Methods of Plasma Protein Fractionation*. Academic Press, New York, 1980.
7. Choo KH, Gould KG, Rees DJG, Brownlee GG: Molecular cloning of the gene for human anti-haemophilic factor IX. *Nature*, 1982; 299: 178-80.
8. Chandra T, Kurachi K, Davie EW, Woo SLC: Induction of α_1 -antitrypsin mRNA and cloning of its cDNA. *Biochem Biophys Res Commun*, 1981; 103: 751-58.
9. Suggs SV, Wallace RB, Hirose T, Kawashima EH, Itakura K: Use of synthetic oligonucleotides as hybridization probes: isolation of cloned cDNA sequences for human β_2 -microglobulin. *Proc Natl Acad Sci USA*, 1981; 78: 6613-17.
10. Lawn RM, Adelman J, Bock SC, Franke AE, Houck CM, Najarian RC, Seeburg PH, Wion KL: The sequence of human serum albumin cDNA and its expression in *E. coli*. *Nucleic Acids Res*, 1981; 9: 6103-14.
11. Ricca GA, Hamilton RW, McLean JW, Conn A, Kalinyak JE, Taylor JM: Rat α_1 -acid glycoprotein mRNA. Cloning of double-stranded cDNA and kinetics of induction of mRNA levels following acute inflammation. *J Biol Chem*, 1981; 256: 10362-68.

12. Crabtree GR, Kant JA: Molecular cloning of cDNA for the α , β , and γ chains of rat fibrinogen. A family of coordinately regulated genes. *J Biol Chem*, 1981; 256: 9718–23.
13. Odink KG, Fey G, Wiebauer K, Diggelmann H: Mouse complement components C3 and C4. Characterization of their messenger RNA and molecular cloning of complementary DNA for C3. *J Biol Chem*, 1981; 256: 1453–58.
14. Prochownik EV, Orkin SH: Isolation of the human anti-thrombin III gene. *Blood*, 1982; 60 (suppl. 1): 806 (abstract).
15. Sinkovics JG, Dreesman GR: Monoclonal antibodies of hybridomas. *Rev Infect Dis*, 1983; 5: 9–34.
16. Gadek JE, Hosea SW, Gelfand JA, Santaella M, Wickerhauser M, Triantaphyllopoulos DC, Frank MM: Replacement therapy in hereditary angioedema. Successful treatment of acute episodes of angioedema with partly purified C1 inhibitor. *N Engl J Med*, 1980; 302: 542–46.
17. Gadek JE, Klein HG, Holland PV, Crystal RG: Replacement therapy of alpha 1-antitrypsin deficiency. Reversal of protease-antiprotease imbalance within the alveolar structures of PiZ subjects. *J Clin Invest*, 1981; 68: 1158–65.
18. Skillman JJ: Use of colloid in massive transfusions. In: *Massive Transfusion in Surgery and Trauma*, Collins JA, Murawski K, Shafer AW (eds). Alan R. Liss, New York, 1982: 65–68.
19. Smith T: Active immunity produced by so-called balanced or neutral mixtures of diphtheria toxin and antitoxin. *J Exp Med*, 1909; 11: 241–56.
20. Finn R, Clarke CA, Donohoe WTA, McConnell RB, Sheppard PM, Lehane D, Kulke W: Experimental studies on the prevention of Rh haemolytic disease. *Br Med J*, 1961; 1: 1486–90.
21. Pollack W, Gorman JG, Hager HJ, Freda VJ, Tripodi D: Antibody-mediated immune suppression to the Rh factor: animal models suggesting mechanism of action. *Transfusion*, 1968; 8: 134–45.
22. Freda VJ, Gorman JG, Pollack W: Rh Factor: prevention of isoimmunization and clinical trial on mothers. *Science*, 1966; 151: 828–30.
23. Chedid L, Parant M, Parant F, Boyer F: A proposed mechanism for natural immunity to enterobacterial pathogens. *J Immunol*, 1968; 100: 292–301.
24. Braude AI, Douglas H: Passive immunization against the local Shwartzman reaction. *J Immunol*, 1972; 108: 505–12.
25. Braude AI, Douglas H, Davis CE: Treatment and prevention of intravascular coagulation with antiserum to endotoxin. *J Infect Dis*, 1973; 128: S157–64.
26. Ziegler EJ, Douglas H, Sherman JE, Davis CE, Braude AI: Treatment of *E. coli* and *Klebsiella* bacteremia in agranulocytic animals with antiserum to a UDP-Gal epimerase-deficient mutant. *J Immunol*, 1973; 111: 433–38.
27. Ziegler EJ, McCutchan JA, Fierer J, Glauser MP, Sadoff JC, Douglas H, Braude AI: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med*, 1982; 307: 1225–30.
28. Wyke RJ, Tsiquaye KN, Thornton A, White Y, Portmann B, Das PK, Zuckerman AJ, Williams R: Transmission of non-A non-B hepatitis to chimpanzees by factor-IX concentrates after fatal complications in patients with chronic liver disease. *Lancet*, 1979; 1: 520–24.
29. Sugg U, Schnaadt M, Schneider W, Lissner R: Clotting factors and non-A, non-B hepatitis. *N Engl J Med*, 1980; 303: 943.
30. Gellis SS, Neefe JR, Stokes J Jr, Strong LE, Janeway CA, Scatchard G: Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXVI. Inactivation of the virus of homologous serum hepatitis in solutions of normal human serum albumin by means of heat. *J Clin Invest*, 1948; 27: 239–44.
31. Scatchard G, Gibson ST, Woodruff LM, Batchelder AC, Brown A: Chemical, clinical, and immunological studies on the products of human plasma fractionation. IV. A study of the thermal stability of human serum albumin. *J Clin Invest*, 1944; 23: 445–53.
32. Heimburger N, Schwinn H, Gratz P, Lüben G, Kumpe G, Herchenhan B: Faktor VIII-Konzentrat, hochgereinigt und in Lösung erhitzt. *Arzneim-Forsch*, 1981; 31: 619–22.
33. Fernandes PM, Lundblad JL: Pasteurized therapeutically active protein compositions. *Eur Pat Appl*, 1980; 35: 204.
34. Dolana G, Tse D, Thomas W, Kingdon HS: Hepatitis risk reduction in hemophilia; a heated factor VIII preparation. *Blood*, 1982; 60: (suppl. 1), 768 (abstract).
35. Tabor E, Aronson DL, Gerety RJ: Removal of hepatitis-B-virus infectivity from factor-IX complex by hepatitis-B immunoglobulin. *Lancet*, 1980; 2: 68–70.
36. Brummelhuis HGJ, Over J, Duivis-Vorst CC, Wilson-de Sturler LA, Ates G, Hoek PJ, Reerink-Brongers EE: Contribution to the optimal use of human blood. IX. Elimination of hepatitis B transmission by (potentially) infectious plasma derivatives. *Vox Sang*, 1983; 45: 205–16.
37. Prince AM, Stephan W, Brotman B: β -propiolactone/ultraviolet irradiation: a review of its effectiveness for inactivation of viruses in blood derivatives. *Rev Infect Dis*, 1983; 5: 92–107.
38. Prince AM, Stephan W, Brotman B, van den Ende MC: Evaluation of the effect of betapropiolactone/ultraviolet irradiation (BPL/UV) treatment of source plasma on hepatitis transmission by factor IX complex in chimpanzees. *Thromb Haemost*, 1980; 44: 138–42.
39. Newman J, Johnson AJ, Karpatkin MH, Puszkin S: Methods for the production of clinically effective intermediate- and high-purity factor-VIII concentrates. *Br J Haematol*, 1971; 21: 1–20.
40. Weinstein M, Deykin D: Comparison of factor VIII-related von Willebrand factor proteins prepared from human cryoprecipitate and factor VIII concentrate. *Blood*, 1979; 53: 1095–1105.
41. Janson PA, Jubelirer SJ, Weinstein MJ, Deykin D: Treatment of the bleeding tendency in uremia with cryoprecipitate. *N Engl J Med*, 1980; 303: 1318–22.
42. Kasper CK: Thromboembolic complications. *Thromb Diath Haemorrh*, 1975; 33: 640–44.

43. Kingdon HS, Lundblad RL, Veltkamp JJ, Aronson DL: Potentially thrombogenic materials in factor IX concentrates. *Thromb Diath Haemorrh*, 1975; 33: 617-31.
44. Giles AR, Nesheim ME, Hoogendoorn H, Tracy PB, Mann KG: The coagulant-active phospholipid content is a major determinant of in vivo thrombogenicity of prothrombin complex (factor IX) concentrates in rabbits. *Blood*, 1982; 59: 401-7.
45. Magner A, Aronson DL: Toxicity of factor IX concentrates in mice. *Develop Biol Stand*, 1979; 44: 185-88.
46. Dike GWR, Griffiths D, Bidwell E, Snape TJ, Rizza CR: A factor VII concentrate for therapeutic use. *Br J Haematol*, 1980; 45: 107-18.
47. Kosow DP, Behre HE, Orthner CL, Menache D: Preparation and properties of a factor IX concentrate depleted of prothrombin and factor X. *Thromb Haemost*, 1983; 50: 116 (abstract).
48. Hultin MB: Activated clotting factors in factor IX concentrates. *Blood*, 1979; 54: 1028-38.
49. Seligsohn U, Kasper CK, Osterud B, Rapaport SI: Activated factor VII: presence in factor IX concentrates and persistence in the circulation after infusion. *Blood*, 1979; 53: 828-37.
50. Sjamssoedin LJM, Heijnen L, Mauser-Bunschoten EP, van Geijlswijk JL, van Houwelingen H, van Asten P, Sixma JJ: The effect of activated prothrombin-complex concentrate (FEIBA) on joint and muscle bleeding in patients with hemophilia A and antibodies to factor VIII. A double-blind clinical trial. *N Engl J Med*, 1981; 305: 717-21.
51. Lusher JM, Shapiro SS, Palascak JE, Rao AV, Levine PH, Blatt PM and the hemophilia study group: Efficacy of prothrombin-complex concentrates in hemophiliacs with antibodies to factor VIII. A multicenter therapeutic trial. *N Engl J Med*, 1980; 303: 421-25.
52. Stenflo J: A new vitamin K-dependent protein. Purification from bovine plasma and preliminary characterization. *J Biol Chem*, 1976; 251: 355-63.
53. Kisiel W: Human plasma protein C. Isolation, characterization, and mechanism of activation by α -thrombin. *J Clin Invest*, 1979; 64: 761-69.
54. Opie EL, Barker BI: Leucoprotease and anti-leucoprotease of mammals and of birds. *J Exp Med*, 1907; 9: 207-21.
55. Aoki N, Saito H, Kamiya T, Koie K, Sakata Y, Kobakura M: Congenital deficiency of α_2 -plasmin inhibitor associated with severe hemorrhagic tendency. *J Clin Invest*, 1979; 63: 877-84.
56. Sharp HL, Bridges RA, Kravit W, Freier EF: Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognized inherited disorder. *J Lab Clin Med*, 1969; 73: 934-39.
57. Laurell CB, Eriksson S: The electrophoretic α_1 -globulin pattern of serum in α_1 -antitrypsin deficiency. *Scand J Clin Lab Invest*, 1963; 15: 132-40.
58. Vogelaar EF, Brummelhuis HGJ, Krijnen HW: Contributions to the optimal use of human blood. III. Large-scale preparation of human C1 esterase inhibitor concentrate for clinical use. *Vox Sang*, 1974; 26: 118-27.
59. van der Starre P, Sinclair D, Damen J, Brummelhuis H: Inhibition of the hypotensive effect of plasma protein solutions by C₁esterase inhibitor. *J Thorac Cardiovasc Surg*, 1980; 79: 738-40.
60. Egeberg O: Inherited antithrombin deficiency causing thrombophilia. *Thromb Diath Haemorrh*, 1965; 13: 516-30.
61. Thaler E, Lechner K: Antithrombin III and thromboembolism. *Clin Haematol*, 1981; 10: 369-90.
62. Mannucci PM, Boyer C, Wolf M, Tripodi A, Larrieu MJ: Treatment of congenital antithrombin III deficiency with concentrates. *Br J Haematol*, 1982; 50: 531-35.
63. Brandt P: Observations during the treatment of antithrombin-III deficient women with heparin and antithrombin concentrate during pregnancy, parturition, and abortion. *Thromb Res*, 1981; 22: 15-24.
64. Jespersen J, Rasmussen NR, Toftgaard C: Observations during the treatment with antithrombin-III concentrate of a case of tampon-related toxic shock syndrome and disseminated intravascular coagulation. Discrepancies between functional and immunologic determinations of antithrombin. *Thromb Res*, 1982; 26: 457-62.
65. Laursen B, Mortensen JZ, Frost L, Hansen KB: Disseminated intravascular coagulation in hepatic failure treated with antithrombin III. *Thromb Res*, 1981; 22: 701-4.
66. Schipper HG, ten Cate JW: Antithrombin III transfusion in patients with hepatic cirrhosis. *Br J Haematol*, 1982; 52: 25-33.
67. Blauthut B, Necek S, Vinazzer H, Bergmann H: Substitution therapy with an antithrombin III concentrate in shock and DIC. *Thromb Res*, 1982; 27: 271-78.
68. Haak HL, Stolk JC, Gratama JW, van Hulsteyn H, Briet E: Use of antithrombin III concentrate in stable diffuse intravascular coagulation. A case report. *Acta Haematol*, 1982; 68: 28-33.
69. Kakkar VV: The clinical use of anti-thrombin III. *Thromb Haemost*, 1979; 42: 265 (abstract).
70. Alving BM, Hojima Y, Pisano JJ, Mason BL, Buckingham RE Jr, Mozen MM, Finlayson JS: Hypotension associated with prekallikrein activator (Hageman-factor fragments) in plasma protein fraction. *N Engl J Med*, 1978; 299: 66-70.
71. Heinonen J, Peltola K, Himberg JJ, Suomela H: Correlation of hypotensive effect of plasma protein fraction with prekallikrein activator activity: a clinical study in patients having openheart surgery. *Ann Thorac Surg*, 1982; 33: 244-49.
72. van Roosevelt RF, Bakker JC, Sinclair DM, Damen J, van Mourik JA: Bradykinin-mediated hypotension after infusion of plasma-protein fraction. *J Lab Clin Med*, 1982; 100: 288-95.
73. Finlayson JS: Albumin products. *Sem Thromb Hemost*, 1980; 6: 85-120.
74. Finlayson JS: Immune globulins. *Sem Thromb Hemost*, 1979; 6: 44-74.
75. Bowman JM, Chown B, Lewis M, Pollock JM: Rh isoimm-

- munization during pregnancy: antenatal prophylaxis. *Can Med Assoc J*, 1978; 118: 623–27.
76. Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sun TS, Hsieh FJ, Szmuness W: Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Initial report of a randomised double-blind placebo-controlled trial. *Lancet*, 1981; 2: 388–93.
77. Finlayson JS: Immune globulins with special reference to their role in bacterial and viral infections. In: *Medical Microbiology*, vol 1., Easmon CSF, Jeljaszewicz J (eds). Academic Press, London, 1982: 129–82.
78. Schultze HE, Schwick G: Über neue Möglichkeiten intravenöser Gammaglobulin-Applikation. *Dtsch Med Wschr*, 1962; 87: 1643–50.
79. Alving BM, Finlayson JS (eds): *Immunoglobulins: Characteristics and Uses of Intravenous Preparations*. DHHS Publication No. (FDA)-80-9005, U.S. Government Printing Office, Washington, 1980.
80. Römer J, Morgenthaler JJ, Scherz R, Skvaril F: Characterization of various immunoglobulin preparations for intravenous application. I. Protein composition and antibody content. *Vox Sang*, 1982; 42: 62–73.
81. Nolte MT, Pirofsky B, Gerritz GA, Golding B: Intravenous immunoglobulin therapy for antibody deficiency. *Clin Exp Immunol*, 1979; 36: 237–43.
82. Ammann AJ, Ashman RF, Buckley RH, Hardie WR, Krantmann HJ, Nelson J, Ochs H, Stiehm ER, Tiller T, Wara DW, Wedgwood R: Use of intravenous γ -globulin in antibody immunodeficiency: results of a multicenter controlled trial. *Clin Immunol Immunopathol*, 1982; 22: 60–67.
83. Cunningham-Rundles C, Smithwick EM, Siegal FP, Day NK, Cunningham-Rundles S, Koziner B, Lion A, Barandun S, O'Malley J, Good RA: Treatment of primary humoral immunodeficiency disease with intravenous (pH 4.0 treated) gammaglobulin. In: *Immunohemotherapy. A Guide to Immunoglobulin Prophylaxis and Therapy*, Nydegger UE (ed). Academic Press, London, 1981: 283–90.
84. Duswald KH, Müller K, Seifert J, Ring J: Wirksamkeit von i.v. Gammaglobulin gegen bakterielle Infektionen chirurgischer Patienten. Ergebnisse einer kontrollierten, randomisierten klinischen Studie. *Münch Med Wschr*, 1980; 122: 832–36.
85. Kornhuber B: Administration of intravenous immunoglobulin to children with malignant diseases. In: *Immu-* noglobulins: Characteristics and Uses of Intravenous Preparations, Alving BM, Finlayson JS (eds). DHHS Publication No. (FDA)-80-9005, U.S. Government Printing Office, Washington, 1980: 107–10.
86. Condie RM, Hall BL, Howard RJ, Fryd D, Simmons RL, Najarian JS: Treatment of life-threatening infections in renal transplant recipients with high-dose intravenous human IgG. *Transplant Proc*, 1979; 11: 66–68.
87. Winston DJ, Ho WG, Rasmussen LE, Lin CH, Chu CL, Merigan TC, Gale RP: Use of intravenous immune globulin in patients receiving bone marrow transplants. *J Clin Immunol*, 1982; 2: 42S–47S.
88. Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schöni M, Vest M, Wagner HP: High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet*, 1981; 1: 1228–31.
89. Fehr J, Hofmann V, Kappeler U: Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gamma globulin. *N Engl J Med*, 1982; 306: 1254–58.
90. Pirofsky B: Which is the factual basis, in theory and clinical practice, for the use of intravenous gammaglobulin in the treatment of severe bacterial infections? *Vox Sang*, 1979; 37: 126–28.
91. Köhler G, Milstein C: Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, 1975; 256: 495–97.
92. Olsson L, Kaplan HS: Human-human hybridomas producing monoclonal antibodies of predefined antigenic specificity. *Proc Natl Acad Sci USA*, 1980; 77: 5429–31.
93. Croce CM, Linnenbach A, Hall W, Steplewski Z, Koprowski H: Production of human hybridomas secreting antibodies to measles virus. *Nature*, 1980; 288: 488–89.
94. Smith TW, Butler VP Jr, Haber E, Fozard H, Marcus FI, Bremner WF, Schulman IC, Phillips A: Treatment of life-threatening digitalis intoxication with digoxin-specific Fab antibody fragments. Experience in 26 cases. *N Engl J Med*, 1982; 307: 1357–62.
95. Miller RA, Maloney DG, Warnke R, Levy R: Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med*, 1982; 306: 517–22.
96. Cohen S, Pick E, Oppenheim JJ (eds): *Biology of the Lymphokines*. Academic Press, New York, 1979.

8. Clinical significance of tissue antigens

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Since many different epitopes are detectable on all cells of the body and also in various body fluids, this chapter will be confined to a discussion of some of the best-recognized antigen systems whose clinical significance has been established.

The human leukocyte A system (HLA)

The antigens of this system are present on all nucleated cells and are also found in varying quantities on nonnucleated cells and in body fluids. The antigens are glycoprotein in nature and are coded by genes at four loci on the short arm of the sixth chromosome, the entire complex being called the 'major histocompatibility complex' (MHC). The loci are A, B, C, and D/DR, and the system is highly polymorphic. There is still controversy whether the D and DR loci are indeed separate and some evidence has been put forth for both points of view. The antigens coded by genes at the A, B, C, and DR loci are conventionally typed by a microlymphocytotoxicity dye exclusion method [1], although the use of a fluorescence method is gaining wider acceptance. The former method has proved to be reproducible and fairly simple to perform and thus has gained acceptance as the standard method for organ transplantation purposes [2].

To date, most reagent antisera have been obtained by screening the sera of multiparous blood donors against panels of HLA-typed target lymphocytes. Those sera with strong mono- or duo-specificity are readily separated out by their patterns of reactivity. Large volumes of these desired

antibodies are obtained by plasmapheresis or blood donation. Reproducibility from laboratory to laboratory is ensured by the constant exchange of sera and comparison of results on a worldwide scale. The advent of monoclonal antibody techniques has naturally lent itself to the production of monoclonal HLA antisera. Unfortunately, many of the reagents produced to date have not proved as useful as was expected, largely because these antibodies seem to be specific for epitopes that are different from those detected by conventional polyclonal alloantisera.

A, B, and C locus typing is carried out on preparations of peripheral blood mononuclear cells; these are largely T lymphocytes, although monocytes or B-cell preparations also carry the antigens in a readily detectable form. DR typing requires the separation of a relatively pure B-cell preparation and this adds a certain complexity to the procedure. Several methods can be employed for the separation step, such as the rosetting out of T cells by the use of sheep red blood cells and the adherence of B cells to nylon wool. Antisera to DR antigens are also more difficult to obtain since many of them are found in the sera of women who elaborate antibodies to HLA A, B, and C antigens. These 'contaminating' antibodies can be absorbed out by the use of platelets that carry A, B, and C but not DR antigens. The complexity of this polymorphic system of antigens is further compounded by the occurrence of serologic cross-reactivity between antigens coded at the same locus. The antigen specificities currently approved by the World Health Organization are listed in Table 1, and the

patterns of well-recognized cross-reactivity are outlined in Tables 2 and 3.

Typing for antigens coded by the D locus is performed by a mixed lymphocyte culture reaction. Stimulation by one population of lymphocytes of another population requires recognition of 'foreignness' at the D locus by the responding lymphocytes when incubated with the stimulator cells. The test requires about 1 week for adequate *in vitro* stimulation and response, and this length of time precludes its use for routine D locus matching for cadaver renal allografts. However, this test is of critical importance for matching donor/recipient pairs for bone marrow allografts. Typing for HLA antigens is currently used for the following clinical purposes: allograft donor/recipient matching for kidneys, bone marrow, and cornea; donor/recipient matching for blood component therapy in alloimmunized patients; paternity testing; and disease marker studies.

Table 1. WHO-recognized specificities, 1980.

HLA-A	HLA-B		HLA-C	HLA-D	HLA-DR
A1	B5	BW47	CW1	DW1	DR1
A2	B7	BW48	CW2	DW2	DR2
A3	B8	BW49 (W21)	CW3	DW3	DR3
A9	B12	BW50 (W21)	CW4	DW4	DR4
A10	B13	BW51 (5)	CW5	DW5	DR5
A11	B14	BW52 (5)	CW6	DW6	DRW6
AW19	B15	BW53	CW7	DW7	DR7
AW23 (9)	BW16	BW54 (W22)	CW8	DW8	DRW8
AW24 (9)	B17	BW55 (W22)		DW9	DRW9
A25 (10)	B18	BW56 (W22)		DW10	
A26 (10)	BW21	BW57 (17)		DW11	DRW10
A28	BW22	BW58 (17)		DW12	
A29	B27	BW59			
AW30	BW35	BW60 (40)			
AW31	B37	BW61 (40)			
AW32	BW38 (W16)	BW62 (15)			
AW33	BW39 (W16)	BW63 (15)			
AW34	B40	BW4			
AW36	BW41	BW6			
AW43	BW42				
	BW44 (12)				
	BW45 (12)				
	BW46				

Renal transplantation

Allograft success rates in this field have improved steadily as a result of many factors other than HLA matching. Prior transfusions appear to exert the single most important effect on cadaver allograft results [3]. There is also a significant 'center effect',

Table 2. Histocompatibility antigens of the HLA-A, B loci and their most common cross-reactive specificities.*

HLA-A antigens	Cross-reactive specificities
1	3, 11
2	28
3	1, 11
9	(23, 24), 2
23	9 (24)
24	9 (23)
10	(25, 26), 11, 32
25	10 (26), 32, 33
26	10 (25), 11
11	3, 26, 1
28	2
29	19
19	(30, 31, 32, 33), 29
30	19 (31, 32, 33)
31	19 (30, 32, 33)
32	19 (30, 31, 33), 25
33	19 (30, 31, 32), 25
5	(51, 52, 53), 35, 15 (62, 63), 17 (57, 58), 21 (49, 50), 18
51	(5, 52, 53), 35, 15 (62, 63), 17 (57, 58), 21 (49, 50)
52	(5, 51, 53), 35, 15 (62, 63), 17 (57, 58), 21 (49, 50), 18
53	(5, 51, 52), 35, 15 (62, 63), 17 (57, 58), 21 (49, 50), 18
7	22 (54, 56), 27, 40 (60, 61)
8	14
12	(44, 45), 21 (49, 50), 13
44	12 (45), 21 (49, 50), 13
45	12 (44), 21 (49, 50), 13
13	40 (60, 61), 7
14	8, 18
15	(62, 63), 35, 5 (51, 52, 53), 17 (57, 58), 21 (49, 50), 18

* Splits of antigens are listed in parentheses behind antigen. From Moore SB: HLA and blood component therapy. In: Theoretical Aspects of HLA: A Technical Workshop, Hackel E, Mallory D (eds). American Association of Blood Banks, Arlington, Virginia, 1982, pp. 81-102. By permission.

which reflects the general quality of medical diagnostic and therapeutic skills at various medical centers.

HLA markers have clearly demonstrated their usefulness in cases of intrafamilial renal allografts, and in this situation the success with two-haplotype HLA-matched sibling grafts is of the order of 95% at 1 year in most reputable centers. Before the DR typing era, there was some controversy concerning

Table 3. Histocompatibility antigens of the HLA-B loci and their most common cross-reactive specificities.*

HLA-B antigens	Cross-reactive specificities
62	15 (63), 35, 5 (51, 52, 53), 17 (57, 58), 21 (49, 50), 18
63	15 (62), 35, 5 (51, 52, 53), 17 (57, 58), 21 (49, 50), 18
16	(38, 39), 22 (54, 56)
38	16 (39), 22 (54, 56)
39	16 (39), 22 (54, 56)
17	(57, 58), 15 (62, 63), 5 (51, 52, 53), 35, 21 (49, 50), 18
57	17 (58), 15 (62, 63), 5 (51, 52, 53), 35, 21 (49, 50), 18
58	17 (57), 15 (62, 63), 5 (51, 52, 53), 35, 21, (49, 50), 18
18	5 (51, 52, 53), 35, 15 (62, 63), 14, 17 (57, 58)
21	(49, 50), 15 (62, 63), 5 (51, 52, 53), 35, 17 (57, 58)
49	21 (50), 15 (62, 63), 5 (51, 52, 53), 35, 17 (57, 58)
50	21 (49), 15 (62, 63), 5 (51, 52, 53), 35, 17 (57, 58)
22	(54, 55, 56), 7, 27, 40 (60, 61), 38
54	22 (55, 56), 7, 27, 40 (60, 61), 38
55	22 (54, 56), 7, 27, 40 (60, 61), 38
56	22 (54, 55), 7, 27, 40 (60, 61), 38
42	22 ((54, 55, 56), 7, 27
35	5 (51, 52, 53), 15 (62, 63), 17 (57, 58), 18, 21 (49, 50)
37	
40	(41, 42, 47, 48, 60, 61), 7, 13
60	40 (61, 41, 42, 47, 48), 7, 13
61	40 (60, 41, 42, 47, 48), 7, 13
41	40 (60, 61), 7, 13, 42, 47, 48
47	41, 42, 40 (60, 61), 7, 13, 48
48	47, 41, 42, 40 (60, 61), 7, 13

* Splits of antigens are listed in parentheses behind antigen. From Moore SB: HLA and blood component therapy. In: Theoretical Aspects of HLA: A Technical Workshop, Hackel E, Mallory D (eds). American Association of Blood Banks, Arlington, Virginia, 1982, pp. 81-102. By permission.

the role of HLA matching for cadaver grafts, but most studies concluded that matching by the HLA A, B locus was correlated with graft outcome. Recent evidence from Persijn *et al.* [4] has confirmed this finding and, in addition, has demonstrated that the severity of rejection episodes is related to the degree of mismatching of A, B locus antigens. The poorer the match, the worse was patient survival, presumably because of increased requirements for immunosuppressive therapy with its attendant morbidity from infection [4]. HLA-DR matching has already been clearly established as being considerably more significant for cadaver renal grafts than is HLA A, B locus matching. Recent data indicate that graft survival with two DR antigen matches is virtually the same as that with intrafamilial complete HLA haplotype matches [5].

Corneal transplantation

Although most corneal transplants have been performed quite successfully without the benefit of MHC matching, there is now a growing body of evidence that, if a patient's corneal tissue is vascularized as a result of any of a variety of pathologic processes, corneal allograft survival is directly related to the degree of HLA A and B matching [6, 7]. Corneal tissue, which is normally avascular and consists of specialized anucleate cells, is often considered to be immunologically isolated. It is assumed that vascularized corneal margins negate this immunologically privileged status. Because most donors of corneal grafts are also considered potential donors of other organs, such as kidneys, it is likely that HLA typing is available for the majority of such donors. Thus the use of HLA matching for corneal transplants for certain groups of patients is both medically desirable and practical.

Bone marrow transplantation

In recent years allogeneic bone marrow transplantation has emerged as a viable alternative form of therapy for patients with severe aplastic anemia or certain types of leukemia [8]. In fact, it is now recognized as the treatment of choice for certain

subgroups [9] – namely, adults with acute lymphoblastic leukemia (ALL) in first remission, children with ALL in second or third remission, or any patient with poor prognostic features such as T-cell ALL or massive organomegaly. Indeed, the criteria for acceptance for bone marrow transplantation are widening. The appropriate indications and subsequent supportive care of these patients are discussed more fully elsewhere in this text.

Graft acceptance by the host is related to the HLA match between donor and recipient. Although there have been successful transplants between HLA-nonidentical sibling pairs and between parent and child, the vast majority of transplants have been performed between HLA-serologically identical, mixed lymphocyte culture (MLC)-compatible sibling pairs. HLA typing for A, B, and C locus antigens in the patient and family members allows haplotype analysis to be done, and the ideal situation occurs when the recipient and the donor share the same two MHC haplotypes. MLC mutual nonreactivity helps to confirm the fact that haplotype identity extends to the D locus. MLC nonreactivity is not an absolute *sine qua non* for successful marrow transplantation. In animal studies, MLC stimulation is related to severity of graft-versus-host reactions, but in human marrow transplants between MLC-nonreactive pairs, non-MHC factors must play a significant role in determining susceptibility to graft-versus-host reactions.

Approximately one in four siblings will be a two-haplotype match with any given patient. Clearly, many patients are not lucky enough to have such a donor who is capable of donating a marrow. Attempts have been made to use nonfamily members as donors for patients who do not have a suitable family donor. Although the idea is still highly controversial, some transplant centers are requesting that blood banks make their lists of HLA-typed blood donors available for matching with patients who lack a suitable family member. The controversy centers around the questions of cost and ethics. Many blood banks have large numbers of blood donors. In addition, it has been suggested that large numbers of untyped blood donors be typed to increase the 'pool' of such donors. This could be a very costly exercise with a potential for

helping only very few patients, since most transplant centers have long waiting lists of patients who do have a suitably matched related donor. Perhaps a more serious consideration is an ethical one, in that marrow donation requires general anesthesia and hospitalization for a day. The problem is not insurmountable, and several centers in the United States and Europe are in the process of investigating the possibilities in this regard.

The role of HLA antigens in the success of liver, heart, or pancreas transplants has not yet been established. Evidence to date indicates that, to the extent to which the role of these antigens has been examined, no strong indication of their importance has emerged.

Blood component therapy [10]

This section will deal only with components that are associated with a need for some type of matching in relation to tissue antigens other than those commonly thought of as red cell antigens. Before a discussion of the question of HLA and component therapy, it might be wise to review the representation of HLA antigens on various blood cells. Red cells carry minute quantities of HLA A, B, and C antigens but no DR, whereas platelets have A, B, and C strongly represented but again, have no DR. Granulocytes have HLA A, B and C antigens, but they are expressed in considerably less abundance than they are on platelets. It is also of considerable interest that there is a wide variation in the expression of some B-locus antigens on platelets and that C-locus antigens are only weakly expressed on the same cells. It should also be remembered that platelets express recognized systems of platelet-specific antigens (Table 4) and other antigens that are less well defined. Similarly, granulocytes express antigens that are peculiar to that cell type, as outlined on Table 5.

Platelet therapy

For about 25 years, platelet transfusion has been an important supportive therapy for thrombocytopenia of various types. Platelet concentrates are separated from whole blood donations by simple

Table 4. Platelet-specific antigens in caucasoids.

Antigen	Antigen frequency (%)
PL ^{A1}	97
PL ^{A2}	26
KO ^a	15
KO ^b	99
PLE1	99
PLE2	5
BAK ^a	91

Data from Décaire F [11].

centrifugation. The use of plastic closed collection systems has facilitated this separation and has ensured that platelet concentrates could be processed from virtually every unit of donated whole blood. The use of elaborate cell separators in recent years has allowed the collection of larger numbers of platelets from a single donor (equivalent to six to eight platelet concentrates from different donors).

It has generally been recognized that repeated transfusions of platelet concentrates lead to a state of alloimmunization because of the formation of antibodies in the recipient. The alloimmunized patient exhibits progressively smaller posttransfusion increments and indeed may even experience a decrement rather than the expected increment. These progressively less effective transfusions are sometimes associated with fever and rigors, and there is considerable evidence that these reactions are largely secondary to the lysis of leukocytes that contaminate the platelet concentrates. Leukocyte-depleted platelets are considerably less immunogenic, and removal of most of the contaminating

leukocytes before transfusion may significantly prolong the period of satisfactory response to platelets [15]. Because of the frequency of the problem (60 to 70% of recipients of multiple platelet transfusions become alloimmunized), it became rapidly apparent that HLA antibodies played a crucial role in the refractory state, and this led to the dramatic demonstration by Yankee and colleagues [16] that the use of single-donor HLA-matched platelets could circumvent the refractory state. Many subsequent studies have corroborated the Yankee group's results, but nevertheless many problems remain. One of these is why, despite a similar degree of exposure to platelets, some patients become alloimmunized while others do not. Another problem is that of preventing or delaying the onset of the refractory state. The presence of certain types of malignancy and the use of extremely toxic chemotherapy for malignant disease seem to render patients less likely to experience a refractory state [17]. The prevention of alloimmunization would be desirable, particularly if it could be accomplished at a reasonable cost. To this end, it has been suggested that all platelet recipients with leukemia or aplastic anemia be given HLA-matched platelets instead of pooled platelet concentrates. The theory behind this approach is that by limiting exposure to a narrow range of HLA antigens, the onset of alloimmunization can be prevented or delayed. To test this hypothesis in an animal model not complicated by many clinical variables, Slichter [18] exposed nonimmunosuppressed dogs to a variety of platelet therapy regimens: single random dog, pool of six random dogs, DLA (MHC in dogs)-matched sibling, DLA-matched sibling following pool, DLA-mismatched sibling. Her findings were that (1) a pool of donors allows longer effective support than a single random donor, (2) some random donor/recipient combinations do not immunize (~20%), (3) platelet antigens causing alloimmunization are not always defined by the MHC, and (4) about 30% of dogs that had previous exposure to random transfusions were sensitized to an MHC-matched donor. She concluded that the best use of resources is to give random platelet concentrates until alloimmunization occurs and to follow this with support from an

Table 5. Neutrophil-specific antigens in caucasoids.

Antigen	Gene frequency
NA1	0.377
NA2	0.633
NB1	0.83
NC1 (VAZ)	0.80
9a	0.345
ND1	0.88
NE1	0.12

Data from Lalezari P, Radel E [12]; Claas FHJ *et al.* [13]; Helmerhorst FM *et al.* [14].

HLA-matched compatible donor.

A more practical clinical problem is that of the diagnosis of the refractory state. This can be difficult for the clinician and extremely important for the management of the patient. Many factors contribute to posttransfusion increments, only one of which is the refractory state. Some of the most important of these factors are listed in Table 6. Making a clear distinction between the mimicking conditions and the true refractory state is important for a number of reasons. For one, once a patient has been diagnosed as being refractory and given HLA-matched platelets, the usual practice is to try to provide all future platelet needs as HLA-matched apheresis-acquired products, which is extremely costly. For another, incorrectly ascribing poor posttransfusion increments to alloimmunization may result in failure or delay in diagnosing conditions such as sepsis.

A practical approach to the problem is to use ⁵¹Cr-labeled, HLA-compatible, crossmatch-negative platelet transfusions and measure the posttransfusion recovery and survival. It is also wise to keep in mind that true alloimmunization may well coexist with one or several of the factors noted in Table 6. Occasionally, the continued use of non-HLA-matched platelets in a truly refractory patient is associated not only with minimal platelet increments but also with a profound decrease in

circulating granulocytes which may last for several days. The 1-hour posttransfusion platelet increment has been shown to be a practical indication of alloimmunization if the factors outlined in Table 6 are kept in mind.

A wide variety of laboratory tests have been used for this purpose, and these include lymphocytotoxicity tests and also methods designed to detect antibodies directed to platelets per se. Depending on the tests employed, it is often possible to detect or confirm the state of humorally mediated platelet refractoriness and also to select the most appropriate donors. This latter application is a recognition of the fact that HLA A, B locus matching is not sufficient to guarantee an adequate response to platelets since the offending antibodies, though generally directed to HLA, may also include specificities outside those coded by the MHC. A combination of HLA A, B locus matching with tests such as platelet immunofluorescence gives success rates approaching 95% [19].

With respect to HLA matching for platelet therapy, since DR antigens are not found on platelets and C locus antigens do not seem to be important in transfusion outcome, most available data pertain to HLA A, B locus antigen matching. It is of enormous practical value that HLA cross-reactive antigens on platelets seem to be seen as 'self' antigens by the alloimmunized patient. This remarkable finding [20] has allowed successful platelet therapy for up to 80% of alloimmunized patients by the use of a pool of HLA-typed donors considerably smaller in number than would be required if only HLA-perfect matches were successful. The yield from platelets matched by cross-reactivity is only slightly less than that seen with matching for the 'true' antigens. Duquesnoy *et al.* [21] have shown that recipients who are HLA-A2 negative seem to tolerate mismatched HLA antigens on platelets better than those who are positive for HLA-A2.

Despite the best efforts of all concerned, about 20% of alloimmunized patients fail to achieve a satisfactory response to HLA-matched platelets. In at least some of these cases, it is likely that antibodies to non-HLA antigens on platelets may be present. The removal of granulocytes from

Table 6. Factors contributing to posttransfusion platelet increments or function following platelet transfusions.

Platelet factors	Patient factors
Method of collection	Size of patient
Method and duration of storage	Sepsis
Temperature of storage	Splenomegaly
pH	Bleeding
Number of platelets transfused	Disseminated intravascular coagulation
Type of anticoagulant	Patient medications
Donor antiplatelet medication	Circulating immune complexes
	Alloimmunization

Modified from Moore SB: HLA and blood component therapy. In: Theoretical Aspects of HLA: A Technical Workshop, Hackel E, Mallory D (eds). American Association of Blood Banks, Arlington, Virginia, 1982, pp. 81-102. By permission.

HLA-matched platelet preparations that are providing progressively smaller increments has been reported by some authors to result in dramatic reestablishment of satisfactory increments. Not all investigators have found this to occur, and the discrepant reports may be a result of different degrees of leukocyte contamination of the platelet preparations transfused by different authors.

In summary on platelet transfusions, it would seem that the most reasonable approach to platelet therapy is to proceed with random donor platelet concentrates (preferably leukocyte-poor) until alloimmunization occurs. Once this stage has been reached, donors should be selected for HLA compatibility, including cross-reactive antigens, and this should be supplemented with a platelet antibody test (radioimmunoassay or immunofluorescence).

Granulocyte transfusions

It is considerably more difficult to assess the role of HLA, or indeed of any other tissue antigens, in granulocyte transfusion than it is in platelet transfusion. Platelets have long been known to correct abnormalities in the bleeding time and they quickly demonstrated their usefulness in the therapy of thrombocytopenic patients. The platelet has an active role in hemostasis and fulfills this role by circulating for about 8 days. Platelet transfusions can be assessed both by cessation of bleeding episodes and by a more quantitative measure – namely, the posttransfusion increment in platelets. This count is a measure of at least one parameter – the ability to circulate. Granulocytes, on the other hand, cannot be as readily assessed. Normally, these cells spend most of their functioning lives in the tissues, and thus measurement of posttransfusion increments may not in itself adequately reflect their capacity to function. ABO and HLA A, B antigens are present on the surface of these cells, and blood transfusions have long been known to evoke leukoagglutinating antibodies in recipients. These antibodies will agglutinate granulocytes *in vitro* in the presence of lymphocytes and have been found to be associated with febrile nonhemolytic transfusion reactions. The specificity of these leukoagglutinins

may include HLA and non-HLA epitopes. The presence of these antibodies in a patient receiving granulocyte therapy may be associated with febrile reactions.

There is convincing experimental evidence that antibodies to HLA or to granulocyte-specific antigens can interfere with the phagocytic function of the cells [22]. There is some controversy about the question of adverse febrile reactions and incompatibility by lymphocytotoxicity crossmatching. The most authoritative report to date fails to find this association [23]. The basic problem with assessments of matching of various antigen systems for granulocyte therapy has been the considerable controversy surrounding the question of the efficacy of granulocyte therapy *per se*. Early studies of the role of granulocyte transfusions used rather imprecise end points such as 'defervescence' or 'clearing of infection by clinical observation'. In some studies the precise but crude end point of patient death was used. Clearly, these end points are inadequate, either because they lack enough objectivity or because they reflect the interplay of multiple factors. Recently, more refined techniques such as indium-111-labeled granulocyte migration studies have been used with considerable success to trace the location of granulocytes at sites of infection [24].

The debate concerning the indications for and efficacy of granulocytes continues, but there appears to be general acceptance that gram-negative sepsis that is unresponsive to a 48-hour course of appropriate antibiotics is an indication in the severely neutropenic patient. A dose of 0.8 to 3.5×10^{10} granulocytes per day for at least 4 days is usually recommended, and it should be borne in mind that this represents considerably less than the normal physiologic marrow output (unstressed), which is of the order of 10^{11} granulocytes per day. Granulocyte therapy is not without significant risks for certain patients, and these include graft-versus-host reaction (GVHR) from the contaminating T lymphocytes and cytomegalovirus infection. Granulocytes being given to patients who are at particular risk of GVHR (for example, patients who have recently received a marrow transplant) should, of course, be irradiated to eliminate the risk of trans-

fusion-induced GVHR; this applies to all transfusions of cell-containing components.

With regard to HLA matching for granulocyte therapy, this matter is still undecided. It has repeatedly been demonstrated that well-matched granulocytes give a better posttransfusion increment. This parameter is of questionable value, as discussed above, and indeed there has not been a clear indication that good posttransfusion increments are necessary for a good clinical response. It is much easier to define a role for HLA matching in patients who possess antibodies by either lymphocytotoxicity or leukoagglutination assays. In these patients, matching correlates directly with increments and inversely with splenic sequestration of transfused cells. There are even better data that granulocyte agglutinins or anti-granulocyte antibodies as detected by indirect immunofluorescence tests have an adverse effect on transfusion results [25].

In summary, it is generally accepted that for the nonalloimmunized patient, HLA matching is probably not warranted for granulocyte transfusions. However, if the patient is alloimmunized, a combination of HLA matching and immunofluorescent crossmatching should be used to select the most effective donor.

HLA and transfusion reactions

HLA antibodies have been implicated in transfusion reactions in two clearly different situations. Patients with a history of multiparity or of previous transfusions not infrequently experience febrile nonhemolytic reactions to the transfusion of blood products. These reactions are clinically manifested by rigors and fever and usually occur about 2 to 4 hours after the onset of the transfusion. The reaction is caused by recipient antibodies (often HLA) directed to antigens carried by leukocytes of the donor. These transfused white blood cells, given either as granulocyte transfusions or as contaminants in platelet concentrates or red blood cell infusions, are lysed and they release pyrogens, which produce the fever [26]. These reactions can usually be avoided subsequently by the use of leukocyte-poor blood products and by premedication with non-aspirin antipyretics.

A much less frequent but potentially more serious reaction occurs in situations in which the antibody is not in the patient but rather in the plasma that has been transfused. The reaction that ensues is an acute lung injury ranging from noncardiogenic pulmonary edema to frank respiratory distress syndrome. The reaction tends to occur within an hour of the transfusion of blood containing the antibodies and usually compromises the respiratory status of the patient so that mechanical ventilation is required. We have recently reported five such cases in a period during which 21,000 blood products were administered to approximately 3,130 patients, for an incidence of 0.02% per unit and 0.16% per patient transfused. In four cases the antibodies found in the implicated donor unit had a specificity that corresponded to an HLA antigen possessed by the recipient. Although none of these particular patients died, all required mechanical ventilation and intensive respiratory care. Fatal cases have been reported sporadically. The routine screening of sera from multiparous or transfused donors is advised so that antibodies can be detected and plasma products from such donors can be eliminated from blood products for transfusion [27].

Disease associations and HLA

A detailed discussion of the role of HLA as a disease marker is beyond the scope of this chapter, but suffice it to say that the literature is replete with data to this effect. However, the strength of associations found with most diseases has been so weak that HLA typing has not been found useful as a clinical diagnostic tool except for a few conditions that are strikingly associated with HLA-B27, namely, ankylosing spondylitis, Reiter's syndrome, and *Yersinia* arthropathy. On the other hand, certain diseases are *linked* with the MHC, which means that HLA-identical siblings of a proband have a very high risk of manifesting the disease in question, such as idiopathic hemochromatosis and 11-hydroxylase deficiency.

HLA typing is widely used as a means of exclusion in paternity testing, but this topic will not be discussed here.

Platelet-specific antigens

Some of the platelet-specific antigen systems are listed in Table 4. Three clinical conditions appear to be related to the PL^A system and will be discussed briefly here.

Glanzmann's thrombasthenia

This disorder is characterized by a defect in the aggregation of platelets after stimulation by ADP, collagen, or thrombin. In a beautiful study by Van Leeuwen *et al.* [28] of 11 patients, it was shown that 8 of the 11 lacked both PL^A system antigens and that 3 carried only very small quantities of PL^{A1} alone. The term 'Zw null disease' was coined by these authors.

Neonatal isoimmune thrombocytopenia

This condition arises in the neonate as a result of the transplacental passage of maternal alloantibodies directed to paternal platelet antigens carried by the platelets of the fetus. The baby presents with profound thrombocytopenia and is often purpuric, whereas other hematologic parameters are normal. This phenomenon is a rare cause of neonatal thrombocytopenia (1 in 10,000 births); conditions such as neonatal sepsis or disseminated intravascular coagulation should be considered and ruled out before considering neonatal isoimmune thrombocytopenia, which accounts for only about 15 to 20% of cases of neonatal thrombocytopenia. Mortality is in the range of 10% and is usually due to intracranial hemorrhage. The causative antibody is usually anti-PL^{A1}, which arises in PL^{A1}-negative mothers who are exposed to a PL^{A1}-positive fetus. Transfusion of the mother's platelets is the treatment of choice, especially if facilities are available for washing the platelets to remove plasma containing the offending antibody.

Post transfusion purpura

This is a rare phenomenon, which occurs approximately one week after a blood transfusion [29]. The patient presents with profound thrombocyto-

penia or its clinical sequelae (purpura, mucosal bleeding) despite otherwise normal hematologic data. The disorder is thought to be caused by the development of alloantibodies to a platelet-specific antigen following transfusion exposure. Red cell products other than washed or frozen-thawed red cells also contain platelet or platelet membrane fragments and thus can immunize. The majority of the reported cases have been caused by an anti-PL^{A1} antibody. The antibody usually becomes undetectable in about 6 to 8 weeks, but it is unclear whether all such patients should subsequently be transfused with blood products from a PL^{A1}-negative donor. Such a restriction would cause serious problems for a blood bank because of the rarity of PL^{A1}-negative donors.

In this condition it appears that the patient's own PL^{A1}-negative platelets are destroyed. This has been attributed to an 'innocent-bystander' phenomenon whereby the immune complexes of PL^{A1}-positive donor platelets and antibody become attached to the patient's own platelets, and this leads to their removal from circulation. This theory has not been proven. Fascinating new data may throw some light on this question. It seems that platelets may acquire HLA antigens on their surface by absorption [30]. If this phenomenon also applies to PL^{A1}, it might be speculated that the mechanism of posttransfusion purpura is as follows: PL^{A1}-positive donor platelets elicit an anti-PL^{A1} antibody, which destroys the remnants of the transfused platelets. The PL^{A1} antigen elutes from these 'foreign' platelets and is taken up (adsorbed) by the patient's own platelets; this now makes them PL^{A1} positive and, thus, targets for the antibody. The natural history of the phenomenon is that the thrombocytopenia can last for several weeks; this would be compatible with a continued elution/absorption process.

Neutrophil-specific antigens

Some of the neutrophil-specific antigens are listed in Table 5. Certain of these antibodies have been implicated in cases of autoimmune neutropenia and in allo(iso)immune neonatal neutropenia [12–

14]. In the latter condition, the infant usually presents with skin infections and the condition is usually self-limited, with resolution in 6 weeks. Interestingly, a compensatory increase in monocytes has been noted during this period of recovery. Treatment for these patients has generally consisted of antibiotics, but some authors have reported using corticosteroids but with little evidence that it is helpful. On the other hand, corticosteroids have been used successfully in autoimmune neutropenia.

Conclusion

The single most obvious fact gleaned from a study of the literature on many of the antigens discussed in this chapter is that we are woefully ignorant about the chemistry and genetics of many of the systems and often depend on quite flimsy data to establish our theories of pathogenesis of the clinical situations associated with these antigens. It is to be hoped that such will not be the case for long.

References

- Mittal KK, Mickey MR, Singal DP, *et al.*: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation*, 1968; 6: 913-27.
- Moore SB: HLA. *Mayo Clin Proc*, 1979; 54: 385-93.
- Moore SB: The enigma of blood transfusions and kidney transplantation. *Mayo Clin Proc*, 1982; 57: 431-38.
- Persijn GG, Cohen B, Lansbergen Q, *et al.*: Effect of HLA-A and HLA-B matching on survival of grafts and recipients after renal transplantation. *N Engl J Med*, 1982; 307: 905-8.
- Goeken NE, Thompson JS, Corry RJ: A 2-year trial of prospective HLA-DR matching: effects on renal allograft survival and rate of transplantation. *Transplantation*, 1981; 32: 522-27.
- Batchelor JR, Casey TA, Werb A, *et al.*: HLA matching and corneal grafting. *Lancet*, 1976; i: 551-54.
- Kissmeyer-Nielsen F, Ehlers N, Kristensen T, *et al.*: Cited by Morris PJ, Batchelor JR, Festenstein H: Matching for HLA in transplantation. *Br Med Bull*, 1978; 34: 259-62.
- Hoagland HC, Letendre L, Moore SB, *et al.*: Bone marrow transplantation in clinical hematology (editorial). *Mayo Clin Proc*, 1982; 57: 668-69.
- Storb R, Thomas ED: Human marrow transplantation. *Transplantation*, 1979; 28: 1-3.
- Moore SB: HLA and blood component therapy. In: *Theoretical Aspects of HLA: A Technical Workshop*, Hackel E, Mallory D (eds). Arlington, Virginia, American Association of Blood Banks, 1982: 81-102.
- Décaire F: Platelet antigens. *Plasma Ther Transfus Technol*, 1982; 3: 251-58.
- Lalezari P, Radel E: Neutrophil-specific antigens: immunology and clinical significance. *Semin Hematol*, 1974; 11: 281-90.
- Claas FHJ, Langerak J, Sabbe LJM, *et al.*: NE₁: a new neutrophil specific antigen. *Tissue Antigens*, 1979; 13: 129-34.
- Helmerhorst FM, Claas FHJ, van der Plas-van Dalen C, *et al.*: Neutrophil-specific antigen NE₁ is not the antithetical allele of ND₁. *Tissue Antigens*, 1981; 18: 139-40.
- Eernisse JG, Brand A: Prevention of platelet refractoriness due to HLA antibodies by administration of leukocyte-poor blood components. *Exp Hematol*, 1981; 9: 77-83.
- Yankee RA, Graff KS, Dowling R, *et al.*: Selection of unrelated compatible platelet donors by lymphocyte HLA-A matching. *N Engl J Med*, 1973; 288: 760-64.
- Dutcher JP, Schiffer CA, Aisner J, *et al.*: Long-term follow-up of patients with leukemia receiving platelet transfusions: identification of a large group of patients who do not become alloimmunized. *Blood*, 1981; 58: 1007-11.
- Slichter SJ: Controversies in platelet transfusion therapy. *Annu Rev Med*, 1980; 31: 509-40.
- Brand A, van Leeuwen A, Eernisse JG, *et al.*: Platelet transfusion therapy. Optimal donor selection with a combination of lymphocytotoxicity and platelet fluorescence tests. *Blood*, 1978; 51: 781-88.
- Duquesnoy RJ, Filip DJ, Rodey GE, *et al.*: Successful transfusion of platelets 'mismatched' for HLA antigens to alloimmunized thrombocytopenic patients. *Am J Hematol*, 1977; 2: 219-26.
- Duquesnoy RJ, Filip DJ, Aster RH: Influence of HLA-A2 on the effectiveness of platelet transfusions in alloimmunized thrombocytopenic patients. *Blood*, 1977; 50: 407-12.
- Nusbacher J, MacPherson JL, Gore IJ Jr, *et al.*: Inhibition of granulocyte erythrophagocytosis by HLA antisera. *Blood*, 1979; 53: 350-57.
- McCullough J, Wood N, Weible BJ, *et al.*: The role of histocompatibility testing in granulocyte transfusion. *Prog Clin Biol Res*, 1977; 13: 321-27.
- McCullough J, Weible BJ, Clay ME, *et al.*: Effect of leukocyte antibodies on the fate in vivo of Indium-111-labeled granulocytes. *Blood*, 1981; 58: 164-70.
- Verheugt FWA, von dem Borne AEGK, Prins HK, *et al.*: The detection of granulocyte antibodies in relation to granulocyte transfusion. *Exp Hematol (Suppl)*, 1977; 5: 151-55.
- Dinarello CA, Wolff SM: Molecular basis of fever in humans (review). *Am J Med*, 1982; 72: 799-819.
- Popovsky MA, Abel MD, Moore SB: Transfusion-related acute lung injury associated with passive transfusion of anti-

- leukocyte antibodies. *Am Rev Respir Dis*, 1983; 128: 185–89.
28. Van Leeuwen EF, von dem Borne AEGK, von Riesz LE, *et al.*: Absence of platelet-specific alloantigens in Glanzmann's thrombasthenia. *Blood*, 1981; 57: 49–54.
29. Ziegler Z, Murphy S, Gardner FH: Post-transfusion purpura: a heterogeneous syndrome. *Blood*, 1975; 45: 529–36.
30. Lalezari P, Driscoll AM: Ability of thrombocytes to acquire HLA specificity from plasma. *Blood*, 1982; 59: 167–70.

9. Clinical significance of red cell blood groups*

P.D. ISSITT

Since the discovery [1] of the ABO blood group system in 1900, more than 400 additional red cell blood group antigens have been identified [2]. Obviously, in a chapter of this length very few of the known complexities can be described. In clinical medicine the major importance of the antigens is the role they play in red cell transfusions. Accordingly, in this chapter an attempt is made to describe the antigens predominantly in that setting. Other aspects of the red cell blood groups as they pertain to certain disease states are mentioned and in some instances brief details about the biochemistry, genetics, serology and immunology of the blood group systems are given in an attempt to make the role of these systems in transfusion therapy more comprehensible. While the stress of the chapter is placed on the role of the blood groups in transfusion, the contributions made from study of the blood groups, in other areas of science, should not be forgotten. Much has been learned about human genetics from studies on how the blood group antigens are inherited. This knowledge is now applied to cases in which parentage is disputed. By using red and white cell blood group markers it is now possible to exclude individuals from paternity and where no exclusion exists, to use gene frequency calculations to provide strong evidence of parentage. Studies on the biochemical structure of proteins, glycoproteins and glycolipids that carry red blood cell antigens have contributed significantly to the current understanding of the structure and function of cell membranes. The way in which individuals respond to exposure to foreign antigens on red cells has contributed to understandings of

the immune system in man. There is no doubt that the survival of renal allografts is considerably better in patients who have been transfused before engraftment than in those who have not. However, this beneficial effect seems to be related to the general immune response to the transfusion of blood (perhaps to the leukocytes contained therein) and not to involve a response to any particular red cell blood group antigen [3]. Accordingly, this subject is discussed in more detail elsewhere in this book.

As most readers will be aware, the majority of red cell transfusions can be successfully accomplished by providing patients with donor red cells that are of the same ABO and Rh type (Rh identity only in terms of presence or absence of the D antigen) as the recipient. In spite of this, and as discussed below, other tests such as those that detect the presence of antibodies, and/or compatibility tests between the patient's serum and the donor's red cells, are performed in all except the most dire emergencies. The reasons that matching the patient's and donor's ABO and D types are necessary are discussed in appropriate sections below. The reason that most individuals do not need red cells matched for any of the numerous other antigens is that only a minority of them have an antibody against one or more of those antigens. While it is well established that exposure to foreign red cells (transfusion and the trans-placental transfer of fetal red cells into the maternal circulation) can stimulate the production of antibodies directed against red cell antigens, such immunization is comparatively rare. In all persons who have been

transfused and/or pregnant, the incidence of antibodies other than anti-A, anti-B and anti-D, is between 1 and 2%. About another 1.5% of people present with so-called naturally-occurring blood group antibodies in their plasma. That is to say, they have an antibody other than anti-A, anti-B or anti-A,B, although they have never been exposed to foreign red cells. However, as discussed below, the majority of these naturally-occurring antibodies react with red cells only at temperatures below 37°C, so are of no significance in the transfusion of blood.

The normal procedure before red cells are transfused is to determine the ABO and D-type of the patient and donor and to test the serum of the potential recipient to see if antibodies are present that are directed against antigens carried on the donor's red cells. At present there is considerable debate [4] as to how this can best be accomplished. The next chapter in this book presents a discussion of the various philosophies regarding compatibility testing and the range of tests considered necessary by different authorities.

Red cell blood group antibodies found in patients can be divided into two major groups. Some of the antibodies are regarded as clinically-significant, that is they have the potential to cause an overt transfusion reaction and/or accelerated *in vivo* clearance of donor red cells. In the other group, the antibodies cause positive *in vitro* tests but, for a variety of reasons, are incapable of clearing antigen-positive cells *in vivo*, so are regarded as clinically insignificant. There is as yet no universal agreement as to which group some antibodies belong. On the other hand, there are no disagreements about many others. While it is not always an easy task, the responsible individual in the blood bank must decide, from *in vitro* test results, whether or not the antibody present is clinically-significant. Just as it is wrong to transfuse antigen-positive red cells to a patient with an antibody that will bring about their *in vivo* clearance, it is wrong to withhold blood or delay surgery while antigen-negative blood is found for patients in whom the antibody is benign. The division of antibodies into clinically-significant and insignificant in this chapter is based on published reports of transfusions

and *in vivo* red cell survival studies and on the author's experience in providing blood for immunized patients. One other point on this matter is that the IgG versus IgM, or immune versus naturally-occurring divisions of antibodies are generally less reliable in forecasting the *in vivo* behavior of an antibody than is a consideration of the thermal range at which the antibody is active. This subject is discussed again in a later section of this chapter.

The ABO system

Red blood cells can be divided into four major ABO groups A, B, AB and O by tests with two antibodies, anti-A and anti-B. Group O serum (anti-A,B) is often used as an additional typing reagent because it has the ability to detect low levels of A antigen on red cells belonging to some of the rare subgroups of A; an ability that anti-A (group B serum) lacks. In addition to red cell typing, ABO groups can be determined by testing an individual's serum against known A and B red cells. This is possible because almost everyone develops, in the serum, ABO system antibodies against the antigens missing from the red cells. The expected reactions in tests to determine ABO groups are shown in Table 1 as are the frequencies of the ABO groups in a Caucasian population.

The formation of ABO antibodies, whenever an antigen is missing from an individual's red cells, relates directly to the ubiquitous nature of structures that are chemically the same or very similar to the A and B antigens. As an example, a group O individual does not have to be exposed to group A or group B red cells in order to make anti-A,B. Instead, A-like and B-like structures are present in food, bacteria, viruses, vaccines etc., and since the group O individual lacks A and B from his red cells, these are recognized as foreign antigens when encountered. In the resulting immune response, potent anti-A,B may be made. As would be expected, the amount of ABO antibodies made in different individuals is variable. However, the fact that some antibody is present in virtually every person of group O, A or B, means that blood lacking the appropriate antigen must always be transfused.

This does not mean, for example, that group A people can receive only group A blood. Obviously, group O red cells also lack the A antigen and can be transfused to group A (and group B and AB) patients. Because there are some dangers involved with the passive transfer of ABO system antibodies (group O blood will contain anti-A,B, an antibody that will react with a group A, B or AB) the transfusion of compatible but non-identical units should usually involve the use of packed cells. The passive transfer of ABO antibodies is discussed again below.

If red cells carrying an A or B antigen are transfused to a patient with an antibody against one of those antigens, a transfusion reaction is almost guaranteed. There are two major reasons for this.

First, the A and B antigens are present in a very high number of copies (ca. 1×10^6) per red cell. Second, both IgM and IgG anti-A and anti-B are efficient complement activating antibodies. As a result, ABO major side incompatible transfusions (antibody in patient, antigen on donor red cells) frequently result in the immediate intravascular destruction of almost all of the transfused red cells. Clinical signs characteristic of immediate red cell destruction in the blood stream are seen and at their worst, ABO incompatible transfusions can cause death. Although these facts are well known to all involved in the supply of blood for transfusion, ABO incompatibility remains as the most common cause of transfusion-induced fatalities [5]. Indeed, some authorities remain unconvinced that

Table 1. Results and interpretations of ABO grouping tests.

Unknown red cells tested with			ABO Antigens on red cells	Unknown serum tested against			ABO Antibodies in serum	ABO group (interpretation)	% Frequency in Caucasians
Anti-A	Anti-B	Anti-A,B		A Cells	B Cells	O Cells			
+	○	+	A	○	+	○	Anti-B	A	43
○	+	+	B	+	○	○	Anti-A	B	9
+	+	+	A and B	○	○	○	None	AB	3
○	○	○	None	+	+	○	Anti-A,B	O	45

Extended to show use of anti- A_1 * *

Unknown red cells tested with				Unknown serum tested against				Interpretation
Anti-A	Anti- A_1	Anti-B	Anti-A,B	A_1 Cells	A_2 Cells	B Cells	O Cells	
+	+	○	+	○	○	+	○	A_1
+	○	○	+	○	○	+	○	A_2
+	○	○	+	+	○	+	○	A_2 with anti- A_1 in the serum*
+	+	+	+	○	○	○	○	A_1B
+	○	+	+	○	○	○	○	A_2B
+	○	+	+	+	○	○	○	A_2B with anti- A_1 in the serum*
○	○	+	+	+	+	○	○	B
○	○	○	○	+	+	+	○	O

+= Agglutination or hemolysis.

○= No agglutination or hemolysis.

* Different specificity cold-reactive antibodies (such as anti- P_1 , anti-M, etc.) may give the same pattern if the A_1 cells carry the antigen defined and the A_2 cells do not.

** Extension of ABO typing with anti- A_1 shown only for illustrative purposes. Since anti- A_1 in the serum of an A_2 or A_2B person is almost always a clinically-insignificant antibody, there is no need routinely to type donor or patient samples for A_1 , A_2 subgroup.

antibodies other than those of ABO system have ever directly caused the death of a patient. Molaison [6] has estimated that in patients transfused major side ABO incompatible blood, the fatality rate is about 10%. When records of cases in which an ABO incompatible transfusion resulted in the death of a patient are reviewed it is found that only seldom is serological mistyping of the donor or the patient responsible. Most such transfusion accidents involve drawing the original sample from the wrong patient, mislabelling one drawn from the right patient, or because blood intended for one patient was transfused to another. It is impossible to overstress the importance of attention to identification methods and clerical records in transfusion practice.

The considerations discussed above about ABO incompatible transfusions apply to the antibodies anti-A, anti-B and anti-A,B as made by group B, A and O persons respectively. Group A and AB persons can be further divided as belonging to one of several different subgroups. These are characterized from A_1 to A_2 (see Table 1) through various others to A_x and involve the presence of fewer and fewer copies of the A antigen on the red cells. In other words, subgroup differences are quantitative not qualitative. Of group A and AB individuals, about 80% are A_1 and A_1B and about 20% are A_2 and A_2B . All of the other subgroups are rare. On average, about 5% of A_2 and about 20% of A_2B people have anti- A_1 in their serum. This is an antibody that agglutinates A_1 but not A_2 cells. In fact, it is a form of anti-A that requires multiple copies of its antigen on red cells in order to be able to agglutinate them. Unlike the anti-A made by group B persons, the anti- A_1 made by A_2 and A_2B persons is rarely a clinically-significant antibody. In its most usual form, anti- A_1 can agglutinate A_1 red cells at temperatures up to about 22°C (and rarely up to about 32°C) but not at 37°C. Like other antibodies with such a characteristic (see below), the antibody cannot destroy A_1 cells *in vivo* because it cannot bind to them at body temperature. Accordingly, patients with cold-reactive anti- A_1 in the plasma do not need A_2 units when transfused. A_1 red cells survive normally *in vivo* in these patients.

While the consequences of major-side ABO in-

compatible transfusions are usually severe, minor side incompatibility (antibody in donor plasma, antigen on patient's red cells) seldom causes acute problems. The main reason is that the donor plasma, containing the antibody, is rapidly diluted by the patient's blood as it is transfused. Nevertheless, if enough ABO antibody is passively transferred, some *in vivo* destruction of the patient's red cells can result. For this reason, major-side compatible, non-ABO-identical blood should be infused in the form of packed red cells. When large volumes of plasma are to be infused, they should not contain ABO antibodies directed against antigens on the patient's red cells. In platelet transfusions, some workers elect to use platelet concentrates from ABO major-side compatible donors because of the presence of some red cells in the platelet concentrates. Others prefer to use platelets from minor-side ABO compatible donors much of the time, to prevent the accumulation of passively transferred ABO system antibodies in the patient. Factor VIII concentrate may contain, in addition to the factor VIII, a considerable amount of anti-A and anti-B. It has been reported [7] that group A or B patients treated with repeated doses of the concentrate, can accumulate sufficient levels of anti-A,B in their plasma that the direct antiglobulin test becomes positive and *in vivo* destruction of the patient's red cells begins. If this happens, a suitable solution is to discontinue use of the concentrate and to substitute cryoprecipitate prepared from appropriate ABO group donor plasma.

Inheritance of the ABO groups is, in most instance, straightforward. There are three major alleles at the *ABO* locus: *A*, *B*, and *O*. Since the immunodominant structures of the A and B antigens are carbohydrate, and since genes code for the production of protein, it follows that the A and B antigens are not the direct products of the *A* and *B* genes. Instead, these genes code for the production of glycosyl-transferase enzymes that add carbohydrates, N-acetyl-D-galactosamine in the case of *A*, D-galactose in the case of *B*, to preformed structures attached to straight and branched glycosphingolipid and glycoprotein chains that extend from the red cell membrane [8]. In order to accept the A and B immunodominant sugars, the

chains must carry H antigen. The immunodominant sugar of that antigen is L-fucose that is added by a glycosyl-transferase enzyme, production of which is controlled by the *H* gene. Since the *O* gene is silent, no further addition of carbohydrate to the H-bearing structures occurs in individuals who are genetically *OO*. The red cells of group O people are rich in H substance. The *ABO* and *H* genes segregate independently. In the very rare Bombay or *O_h* phenotype, no H antigen is made because, in these individuals the *H* genes are replaced by a rare allele *h* that is silent. Thus, although *O_h* individuals may inherit an *A* or a *B* gene (or both) and although their sera can be shown to contain an *A* and/or a *B* gene-specified transferase enzyme (dependent on which *ABO* genes have been inherited) their red cells are devoid of H, A and B antigens. Such individuals can be transfused only with blood from other *O_h* persons [9].

In some disease states, such as lymphoma, leukemia and rarely in other forms of cancer, the amount of A, B and H antigen on an individual's red cells may be markedly reduced [10]. It is believed that the antigenic depression represents impaired production of the transferase enzymes that normally add the immunodominant monosaccharides of A, B and H.

The Rh system

This system is one of the most complex polymorphisms known in man. Although there are more than 40 known antigens in the system (see Table 2) and a bewildering array of genes that effect the production of different antigens and different amounts of those antigens [11], all of these complexities pale into insignificance in terms of clinical importance, when compared to the first discovered antigen of the system, D (known as *Rh_o* and *Rh1* in other terminologies, again see Table 2). There are two reasons why the D antigen is so important. First, it is very highly immunogenic. Second, anti-D causes the most severe form of hemolytic disease of the newborn (HDN) including, on some occasions, *in utero* death of the fetus. For these reasons, the terminology introduced when D was

discovered, namely that red cells that carry D are called Rh-positive (Rh+ or D+) and those that lack the antigen are called Rh-negative (Rh- or D-), has persisted. In most Caucasian populations 83 to 87% of people have Rh+ red cells.

As stated earlier, Rh- people are normally transfused with Rh- blood. Unlike the ABO system, where the transfusion of blood carrying an antigen to a person whose red cells lack that antigen, almost always results in the immediate destruction of the transfused cells, the transfusion of Rh+ blood to an Rh- recipient not previously exposed to foreign red cells, almost always results in normal survival of the transfused cells. In other words, the overwhelming majority of persons with D- red cells do not have naturally occurring anti-D in their plasma. This time the contraindication for transfusing red cells carrying a foreign antigen is that D- people transfused with D+ red cells have a very high probability of making anti-D. By studying various reports [2, 6, 11] it can be seen that of Rh- people transfused with one unit of Rh+ red cells, some 50 to 70% formed anti-D. When the immunizing dose is much smaller, say 25 to 40 ml, the incidence of production of anti-D is nearly as high. If Rh- people are injected with as little as 1 ml of Rh+ red cells, some 15% of them form anti-D. These figures are in very marked contrast to all of the other Rh and other blood group system antigens combined [11]. As mentioned earlier, there are over 400 different red cell antigens. However, of all persons transfused (and obviously each transfusion will involve the introduction of many foreign antigens) only in the order of 1 to 2% form an antibody against a red cell antigen [2, 6].

In transfusion therapy, D is the only antigen that is considered significant in terms of matching the donor and recipient in order to prevent antibody production. One of the reasons will already be clear, namely the high probability that D will stimulate antibody formation compared to the low probability for any other antigen. The other reasons pertain to the clinical significance of anti-D, once it is made. Since over 80% of people in most populations have D+ red cells, the chances that an Rh- woman will marry an Rh+ man are high. Close to 80% of children born of such matings will

Table 2. The Rh system antigens, their frequency and year of discovery.

Name of antigen		% Caucasian bloods positive	Year antigen reported *1
CDE term	Rh-Hr term	Numerical term	
D	Rh _o	Rh1	85 1939
C	rh'	Rh2	70 1941
E	rh"	Rh3	30 1943
c	hr'	Rh4	80 1941
e	hr"	Rh5	98 1945
ce or f	hr	Rh6	64 1953
CE		Rh22	<1 1961
Ce	rh _i	Rh7	70 1958
cE		Rh27	30 1961
C ^w	rh ^{w1}	Rh8	1 1946
C ^x	rh ^x	Rh9	<1 1954
E ^w	rh ^{w2}	Rh11	<1 1955
E ^t		Rh24	30 1962
G	rh ^G	Rh12	85 1958
C ^G		Rh21	70 1961
V or ce ^s *2	hr ^v	Rh10	*2 1955
VS or e ^s *2		Rh20	*2 1960
	Hr _o	Rh17	100 1950
	Hr or Hr ^s	Rh18	100 1960
	hr ^s	Rh19	98 1960
	hr ^H	Rh28	*3 1964
*4	Rh ^A	Rh13	85 1957
*4	Rh ^B	Rh14	85 1959
*4	Rh ^C	Rh15	85 1959
*4	Rh ^D	Rh16	85 1959
D ^w		Rh23	<1 1962
LW ^a *5		Rh25	100 1961
c-like		Rh26	80 1964
RH		Rh29 *6	100 1967
D ^{Cor} *7		Rh30	<1 1958
	hr ^B	Rh31	98 1972
		Rh32 *8	<1 1971
		Rh33 *9	<1 1971
	hr ^B	Rh34	100 1972
		Rh35 *10	<1 1971
Be ^a *11		Rh36	<1 1953
Evans *11		Rh37 *12	<1 1968
Duclos *11		Rh38 *13	>99 1978
C-like		Rh39 *14	>99 1979
Tar *11		Rh40	<1 1975
Ce-like		Rh41	70 1980
Ce ^s		Rh42	*3 1980

*1 Year shown is year antigen first described. Several of the antigens were not associated with the Rh system until later.

*2 The names ce^s and e^s are not particularly applicable any longer and should probably be discontinued. Each antigen is

have Rh+ red cells (all children of D homozygotes and 50% of those of D heterozygotes). Since the anti-D formed following exposure to red cells is invariably IgG in nature and has the ability to cross the placenta, a woman with anti-D in her plasma, who conceives an Rh+ child, is at risk of having an infant suffering from HDN. As already mentioned, the most severe form of HDN results in the *in utero* death of the fetus. Obviously, Rh- women of child-bearing age, who are transfused with Rh+ blood, are at risk of never being able to bear a live child. The second reason that production of anti-D must be avoided is that once it is present, the patient concerned can be transfused only with Rh- blood. If D+ cells are transfused to a patient with anti-D in the plasma, the donor cells will become coated with IgG anti-D and will be removed from circulation via an extravascular mechanism that involves macrophage sequestration and destruction of antibody-coated red cells in the spleen and the liver [2, 6]. While it may appear that Rh- males could be transfused with Rh+ blood, since the consequences of the formation of anti-D are less significant than in females of child-bearing age, such transfusions should be undertaken only in a clinical emergency when there is an acute shortage of Rh- donor blood that must be reserved for transfusion to girls and women who might later



rare in Caucasians but is present on the red cells of 20–25% of random Black donors.

*3 Rare in Caucasians, more common in Blacks.

*4 Portions of the Rh_o or D mosaic. Similar to, but not correlated with, other terminologies such as Category I to VI people with D+ red cells, who make anti-D.

*5 The gene that controls production of the LW^a antigen segregates independently of those at the Rh locus.

*6 Also called 'Total Rh'.

*7 Also called Go^a.

*8 The low incidence antigen produced by the \bar{R}^N gene.

*9 The low incidence antigen produced by the R^{oHar} gene.

*10 The low incidence antigen produced by a (C)D(e) gene.

*11 Names applied before antigen was shown to be a part of the Rh system.

*12 The low incidence antigen produced by the $\cdot D$ gene.

*13 May be a product of joint actions of Rh and MN system genes.

*14 Thus far, all examples of anti-Rh39 have been autoimmune in nature.

become pregnant and for persons who have already formed anti-D. It must be remembered that the use of D+ blood for a D-recipient will cause the production of anti-D more often than not. Thus, if a Rh- male has been given Rh+ blood and has made anti-D, then later needs transfusion in an acute emergency, the patient will have been put at considerable (unnecessary) risk if Rh- blood is in short supply.

It must also be remembered that small quantities of D+ red cells stimulate the production of anti-D in many Rh- persons. Thus, if anti-D production must be avoided (as in young female patients who have a good chance for recovery so that they may later bear children) platelets given to an Rh- recipient must come from Rh- donors. While platelets themselves do not carry Rh antigens [6, 11], all platelet concentrates (including those that do not appear to be pink) contain some red cells so that antibody production may be stimulated. There is a theoretical risk that plasma products, such as fresh frozen plasma and cryoprecipitate, from Rh+ donors will stimulate the production of anti-D in Rh- recipients. Although these products are red cell-free, there is little doubt that during their production some of the red cells in the units from which they are made, rupture. Thus red cell stroma, that carry D antigen, may be present in plasma products. However, most authorities feel that the risk of stimulating production of anti-D is so small (because of the minute quantities of D present) that it can be ignored in practical transfusion therapy [6].

Because small quantities of D+ red cells can stimulate the production of anti-D, many Rh- women used to become immunized as a result of pregnancy in which the child was Rh+. The escape of fetal Rh+ red cells into the Rh- maternal circulation during pregnancy usually involves volumes of red cells that are too small to be immunogenic. At the time of birth, a larger volume of fetal cells enters the maternal circulation. It has now been shown that if Rh- women who deliver Rh+ infants are injected, intramuscularly, with a preparation containing potent anti-D, within 72 hours of delivery of the child, immunization to D can nearly always be prevented. In the USA the standard dose of Rh immune globulin contains

about 300/ μ g of anti-D. In Europe the standard dose is smaller, usually in the order of 100 μ g of anti-D. There is convincing evidence [12-14] that either dose is protective for a fetal to maternal hemorrhage of up to 15 ml of Rh+ red cells (ca. 30 ml of whole blood) in some 97 to 98% of women. Since the dose of Rh immune globulin required to effect protection is dependent on the volume of Rh+ red cells that enter the maternal circulation, services that issue this product must use one of several methods available to detect fetal-maternal hemorrhages of greater than 30 ml, in order to identify those women who should receive more than one dose of Rh immune globulin.

Unlike the situations in which Rh+ blood is transfused to Rh- recipients, there are no dangers involved in using Rh- blood for transfusion to Rh+ patients unless, of course, compatibility tests reveal that the D+ patient already has an antibody directed against an antigen on the D- red cells.

There is a phenotype in the Rh system in which the D antigen is present on red cells in less than the usual amount. Such red cells are said to be of the D^u phenotype. Observations in the past have suggested that D^u recipients occasionally form anti-D, so should be transfused only with Rh- blood. Conversely, it has been suggested that D^u donor blood given to a D- recipient can stimulate production of anti-D. Accordingly, people with red cells of the D^u phenotype have been traditionally regarded as Rh- if recipients but Rh+ if donors. It is probable that these considerations represent overkill. Both the formation of anti-D by D^u persons, and stimulation of production of anti-D by D^u red cells are extremely rare events. While some workers continue to use the traditional definitions, others now regard persons of the D^u phenotype as Rh+ whether they are recipients or donors.

As mentioned earlier, Rh antigens other than D are not potent immunogens and are of no consequence in transfusion therapy, except in those patients who have already formed antibodies against one or more of them. When such a situation does exist, antigen-negative blood must be provided since most Rh system antibodies are IgG1 and/or IgG3 in nature and effect the clearance of antigen positive red cells in a manner analogous to

that described for the clearance of D+ red cells by anti-D. Table 2 lists the known Rh antigens, gives their names in three different terminologies, lists the frequency with which each is found in a Caucasian population and shows the year in which each was first reported. Because the genes (gene complexes) that code for the production of the Rh antigens are not equal in frequency, certain combinations of the antigens D, C, c, E and e are more frequent than others. Table 3 lists the frequency of the most common *Rh* genotypes in a Caucasian population.

There is an exceedingly rare phenotype the Rh system called Rh_{null} . Red cells of this phenotype lack all of the antigens shown in Table 2. The phenotype can be caused by either of two genetic situations. In one, the individual of the phenotype has normal *Rh* genes that are blocked in terms of the synthesis of Rh antigens, by other genes known as $X^o r X^o r$, at a locus genetically independent of *Rh*. In the other, the phenotype occurs when silent alleles, $\bar{r} \bar{r}$, at the *Rh* locus are inherited. Regardless of the genetic background, Rh_{null} red cells are morphologically abnormal, they are cup-shaped stomatocytes, and do not enjoy a normal *in vivo* life span. The accelerated rate of red cell destruction is not antibody-induced but occurs because of the abnormality of the red cells. The hemolytic disease is often well compensated and only mild anemia, or

none, is seen. Individuals of the Rh_{mod} phenotype, in which partial but not total suppression of Rh antigen expression is effected by the genes $X^o X^o$, that may well be alternate alleles at the $X^o r$ locus, also have red cells with a reduced *in vivo* life span. Heterozygotes for these rare genes, whose second gene is of the common type, that is $X^o r X^o r$, $Rh \bar{r}$, $X^o r X^o$, sometimes have reduced levels of red cell Rh antigens but do not suffer from hemolytic anemia. These findings may well mean that Rh antigens, that are probably protein in nature, play a functional role in red cell membrane integrity.

The Kell system

The Kell system is only slightly less complex than the Rh system, with more than 20 different antigens already recognized [2]. The system is unusual in that among five pairs of alleles at the same locus, or at a series of very closely linked loci, one of each pair is very common, while the other is of much lower frequency. As a result, antigens such as k, Kp^b , Js^b and K11 are present on the red cells of most individuals, while the antigens K, Kp^a , Js^a , $U1^a$ and Wk^a are comparatively rare. Further, *Kell* system genes (gene complexes) thus far found, code for production of only one of the rare antigens. That is, no gene (complex) making Kp^a and Js^a , or Kp^a and $U1^a$, etc., has yet been seen. Since many examples of Kell system antibodies are IgG and since some are known to have caused *in vivo* red cell destruction, virtually all workers agree that a patient who has formed an antibody to one of the antigens mentioned above, should receive only antigen-negative blood. Finding such blood is easy if the patient is immunized to K, Kp^a , Js^a , $U1^a$ or Wk^a , but difficult if the antibody is directed against k, Kp^b , Js^b or K11. The subject of providing blood for patients immunized against very common antigens is discussed later in this chapter. There is as yet insufficient evidence to know whether antibodies directed against some other very common Kell system antigens, such as K12, K13, K14, etc., are always capable of effecting *in vivo* red cell destruction. There are early indications that some of these antibodies may be benign *in vivo*. Accordingly,

Table 3. Frequency of the most common Rh genotypes in Caucasians.

Genotype	Shorthand symbols	Frequency as %
<i>CDe/cde</i>	$R^i r$	31
<i>CDe/CDe</i>	$R^i R^i$	17.5
<i>cde/cde</i>	rr	14
<i>CDe/cDE</i>	$R^i R^2$	12
<i>cDE/cde</i>	$R^2 r$	10.5
<i>CDe/cDe</i>	$R^i R^o$	3.5
<i>cDe/cde</i>	$R^o r$	3
<i>cDE/cDE</i>	$R^2 R^2$	2

The other 28 possible genotypes (such as $R^2 R^o$ and $R^o R^o$, and those in which any of the rare genes R^o (*CDE*), r' (*Cde*), r'' (*cde*) and r^y (*CDE*) is present) are of low frequency in Whites. Between them they comprise the 6.5% of the population not included in this table.

when faced with the problem of providing blood for a patient with one of these antibodies and when compatible donors are not available, *in vivo* red cell survival studies to determine the antibody's significance may well be helpful.

There is one phenotype in the Kell system that is of clinical interest out of all proportion to its extreme rarity. The overwhelming majority of people have an antigen called Kx on their red cells and on their granulocytic series of leukocytes. Although production of Kx is controlled by a gene that is on the X chromosome and not at the *Kell* system locus (that locus being on an autosome) there is a strong phenotypic association between Kx and the Kell system. Very briefly, it seems that Kx may be a membrane-associated protein to which the Kell system antigens are added. On very rare occasions, Kx is missing from red cells, white cells, or both. The lack of Kx from granulocytes is associated [15] with abnormal phagocytic function of those cells and the syndrome of X-linked chronic granulomatous disease (CGD). When Kx is missing from red cells (McLeod phenotype) the cells are morphologically abnormal acanthocytes and do not enjoy a normal *in vivo* life span. Patients with the red cell McLeod phenotype have a red cell deficiency type of hemolytic anemia. It has recently been reported [16] that the McLeod phenotype may be associated with clinical abnormalities other than CGD and hemolytic anemia. In addition to having elevated serum creatinine phosphokinase levels, individuals of this phenotype may present with muscular defects and neurological abnormalities.

The Kidd system

While still genetically simple, the Kidd system is important in transfusion therapy. Two alleles at a single locus between them control production of the antigens Jk^a, Jk^b and Jk3 while a third allele is silent and effects no production of these antigens. Anti-Jk^a and anti-Jk^b are invariably IgG complement-binding antibodies capable of causing pronounced *in vivo* red cell destruction. Further, individuals immunized to Jk^a and Jk^b tend to make

serologically demonstrable antibodies for only limited periods of time. Thus almost half of the delayed transfusion reactions that have been documented in the literature [2, 6] have been caused by Kidd system antibodies. The situation involves one in which the antibody is either present at a level below that at which it can be detected serologically or is so weak that it escapes detection. As a result, antigen-positive red cells are inadvertently transfused and an anamnestic antibody response occurs. The antibody then reaches a level at which it can destroy red cells at a time, usually three to ten days after transfusion, that the transfused cells are still circulating and a delayed transfusion reaction follows. Obviously, careful records need to be kept of patients in whom Kidd antibodies have previously been detected so that the patients are transfused only with antigen-negative blood in subsequent transfusions. Further, pretransfusion tests must be sensitive enough to detect very weak Kidd system antibodies.

The Duffy system

Like the Kidd system, the Duffy system is genetically and serologically simple. The antibodies anti-Fy^a and anti-Fy^b must be taken seriously when present since they are invariably capable of effecting *in vivo* red cell clearance. It is not yet clear whether antibodies to Fy3, Fy4 and Fy5 behave similarly.

It has been shown [17] that the red cell membrane-borne Fy^a and Fy^b antigens act as receptor sites for the attachment of *Plasmodium vivax* malarial parasites. It is certainly possible that a natural selection process has resulted in the phenotype Fy(a-b-) now being the most common one in the system among African Blacks and their descendants in the New World.

The MN system

More is known about this system at the biochemical level than about any other [18]. Two major red cell membrane-borne sialoglycoprotein (SGP) chains

carry the antigens of the system. One of them (glycophorin A) carries M or N or the product of an alternate allele at the *MN* locus. The other (glycophorin B) carries S or s or the product of an alternate allele at the *Ss* locus and U. It is known that some of the more than 40 other antigens of the system represent amino acid substitutions in the protein backbone of the SGP while others represent glycosylation changes, that is, different to usual oligosaccharide side chains attached to the SGPs. Recent studies [19] have suggested that copies of the red cell-borne receptor site for *Plasmodium falciparum* malarial parasites may be present on the MN SGP.

At a clinical level some antibodies, such as those directed against S, s and U, are important since they can cause *in vivo* clearance of antigen positive red cells. Some examples of others, such as anti-M and anti-N, although often IgG in nature, are of little significance. Again, the major difference between clinically significant and insignificant forms is the ability of the antibody to bind to red cells at 37°C (see also below).

The Lutheran, Lewis and P systems

While extremely interesting at the serological, genetic and biochemical levels, these systems are not often of importance in practical transfusion therapy. Only rarely are antibodies such as anti-Le^a (Lewis system) and anti-P₁ (P system) active at 37°C. Accordingly, only in patients with such 37°C active antibodies is transfusion with antigen-negative blood necessary (see below).

Other blood group systems

In addition to the blood group systems mentioned, there are many others [2], such as (antigens in parentheses following the system name) Colton (Co^a, Co^b), Dombrock (Do^a, Do^b), Cartwright (Yt^a, Yt^b), Gerbich (Ge1, Ge2, Ge3), Scianna (Sc1, Sc2) etc. There are many very common antigens such as At^a, Er^a, Jo^a, Jr^a, etc., that at present are not associated with any of the known blood group

systems and a huge number of currently independent antigens of low incidence, such as Bp^a, By, Ls^a, Mo^a, Pt^a, Zd, etc. There is one antigen, Xg^a, the production of which is known to be controlled by an X chromosome-borne gene. Like most of the antigens in the known blood group systems that have already been described, these factors are of importance only when a patient requiring transfusion has a warm-reactive antibody directed against one of them, or when a pregnant woman has an IgG antibody against one of them and the father of her child may have passed the gene coding for production of the antigen, to the child. The provision of blood for patients immunized against blood group antigens or for exchange transfusion in infants suffering from HDN, is discussed below.

Transfusion in patients with blood group alloantibodies

An alloantibody is one that is directed against an antigen that is not present on the antibody-maker's red cells. Some years ago it was felt that any patient with such an antibody in the serum should be transfused only with red cells that lacked the antigen against which the antibody was directed. It is now abundantly clear that the practice of providing antigen-negative blood to all such patients is wasteful of time, effort, money and some difficult-to-obtain blood grouping sera. There is, for example, overwhelming evidence that red cell alloantibodies that are active *in vitro* only at temperatures below 37°C, are incapable of destroying significant amounts of antigen-positive red cells *in vivo*. Red cell survival studies, transfusions of antigen-positive red cells and large series in which patients have been transfused with blood compatible in tests performed at 37°C [20–30] have combined to show that if an antibody cannot bind to transfused red cells at 37°C, it cannot clear those cells *in vivo*. In other words, antibody screening and compatibility testing methods should not [6, 25, 29, 31–33] include tests that involve incubation other than at 37°C. In a compatibility test, it may be desirable to retain an immediate spin procedure so that errors in patient or donor ABO typing can be detected at once and

corrective steps can be taken with no delay. Once the immediate spin test has been read, it should at once be placed at 37°C for incubation. These considerations apply even when the patient is to be subjected to hypothermia during a surgical procedure. There is emerging evidence [6, 29, 34] that cold-reactive alloantibodies are of no more significance in such patients than in those whose body temperature will not be reduced at or near the time of transfusion.

When a patient with a clinically-significant alloantibody in the serum is encountered, blood suitable for transfusion can be found relatively easily most of the time. For example, among random Caucasian blood donors: 70% have red cells that are E-; 20% cells that are c-; 91%, K-; 35%, Fy(a-); and 27%, Jk(b-). Thus, for a patient with an antibody against any one of those antigens, simple typing studies on units in inventory would rapidly identify suitable blood. At worst, an average of 20 donor units would have to be typed to find four that were c- (i.e., lacking the most common of the antigens used in the examples). However, some individuals are good responders to blood group antigens [2, 6] and make multiple antibodies following transfusion or pregnancy. For a group O patient with anti-c, anti-E and anti-Jk^b in the serum, about 250 random donors would have to be tested to find four with red cells phenotypically c-, E-, Jk(b-). For a group O patient with antibodies against c, E, K, Fy^a and Jk^b (and such mixtures are encountered) about 800 units would have to be tested to find four that were compatible. Obviously, in a medical emergency, the time required to find suitable blood would be far too long. To be prepared for such situations, most blood centers develop a core of donors, whose red cells are known to lack different combinations of antigens and who are prepared to come to donate at short notice. The very considerable costs of phenotyping donors' red cells can be kept to a minimum by selecting persons who are willing to donate blood regularly as well as in emergencies. Now that units of red cells can be stored for long periods of time (some have been used after seven years storage) when mixed with a cryopreservative such as glycerol and frozen rapidly by use of a substance

such as liquid nitrogen, many centers keep fully phenotyped units on hand.

Similar considerations apply when the patient's antibody is directed against a very common antigen. Table 4 lists a number of antigens that are present on the red cells of more than 99% of random Caucasian donors. Obviously, when a patient with an antibody against one of these antigens is encountered, providing compatible blood is a difficult task. Again, most blood centers attempt to prepare in advance for such emergencies. Regular screening programs are conducted to identify ultra rare donors and when they are found their blood is frozen each time that they donate. No one blood center can hope to have a supply of all of the different types of rare blood. There are in existence National and International Files of rare donors and those centers participating in these organizations ship blood to others as needs for particular phenotypes arise.

Antibodies directed against the very common antigens listed in Table 4, are usually clinically-significant and their presence mandates that the patient receive appropriate antigen-negative red cells. There are a number of other antibodies to

Table 4. Some antigens, present on the red cells of more than 99.9% of random Caucasian donors, that are defined by antibodies that are usually clinically-significant.

Blood group system	Antigens
ABO	H *1
Rh	Hr _o Hr Rh29 Rh34 Rh38
Kell	Kp ^b Ku Js ^b Kx
P	P *2
MN	En ^a U *3

But see also group 2 and group 3 of Table 5 for many other antigens of high incidence defined by antibodies that can be of clinical significance.

*1 Anti-H as made by O_h (Bombay) individuals is a clinically-significant antibody. The anti-H antibodies made by A₁, B and A₁B persons are not.

*2 P is the antigen absent from Tj(a-) and P^k red cells. Not to be confused with P₁, an antigen defined by an antibody that is usually clinically-insignificant.

*3 No Caucasian who has formed anti-U has yet been reported. The antibody and the U-negative phenotype are more common in Blacks.

very frequent antigens that are not similarly active *in vivo*. *In vitro* studies that showed that some of these antibodies are of low avidity and often detect antigens present in relatively few copies per red cell, suggested that antigen-positive red cells might survive normally. Transfusion of *in vitro* reactive red cells, and ^{51}Cr -labelled cell survival studies have confirmed the benign *in vivo* nature of these antibodies. Table 5 lists a number of very common antigens, the antibodies to which can be ignored in transfusion therapy. When a patient presents with

Table 5. Red cell antigens (many of high frequency) divided into groups based on the clinical-significance of the antibodies that define them.

Group 1. Antigens defined by clinically-insignificant antibodies.

I, i, IH, iH *1; LW^a *2; Cs^a, Yk^a; Cha, Rg^a; JMH; McC^a, McC^b, McC^c, McC^d; Kn^a; S1^a, Sd^a *3; Win, MPD; Vennera.

Group 2. Antigens defined by antibodies, some examples of which are clinically-significant and some, clinically-insignificant.

Lu^b; Yt^a; Vel; Ge; Gy^a, Hy *4.

*Group 3 *5.* Antigens defined by antibodies about which little is known in terms of their ability to cause *in vivo* red cell destruction.

Fy3, Fy5; K11, K12, K13, K14, K16, K18; Lu3, Lu4, Lu5, Lu6, Lu7, Lu8, Lu11, Lu12, Lu13; Do^a; Co^a, Co3; Di^b; Sc1, Sc3; In^b; At^a; Jo^a; Jr^a; Lan; Cr, Er^a.

*1 Autoantibodies to any of these four antigens may cause CHD. In transfusion therapy they can be ignored in CHD patients provided that the patient is kept warm. In other persons they are of no clinical-significance.

*2 It seems that patients with anti-LW^a can be transfused with Rh-, LW(a+) blood. Such cells carry less LW^a antigen than those that are Rh+, LW(a+).

*3 One example of anti-Sd^a caused a transfusion reaction when the patient received red cells carrying a strong expression of the Sd^a antigen. In dozens of other patients with anti-Sd^a, Sd(a+) red cells have survived normally.

*4 The Gy^a and Hy antigens are related. While some examples of anti-Gy^a may be clinically-significant, it is not known if the same applies to anti-Hy.

*5 Antibodies to these antigens should not be ignored. It is probable that some of them, such as those that define Do^a, Co^a, Co3, Sc1, Sc3, Lan etc., will prove capable of *in vivo* destruction of antigen-positive red cells. On the other hand some, such as those that define K18, Cr, Er^a etc., might well be benign *in vivo*.

one of these antibodies, it is extremely important that its benign nature be appreciated. Attempts to find antigen-negative units are time consuming because so many samples must be tested and are expensive in terms of labor costs and reagents (most of the antibodies react only in the indirect antiglobulin test). Thus, if the antibody is not recognized as being active *in vitro* but clinically benign, necessary surgery and/or transfusions may be delayed and money may be wasted, for no good reason. The evidence showing that these antibodies are clinically-insignificant is abundant [2, 6, 33, 35-50].

In addition to the antibodies described above, there are some that vary in terms of their clinical-significance. For example, some examples of anti-Yt^a, an antibody that defines an antigen present on the red cells of 99.8% of random donors, have caused *in vivo* red cell clearance, while others have allowed transfused Yt(a+) red cells to survive normally [51-53]. The antigens defined by antibodies of this type are also listed in Table 5. When blood is needed for a patient with an antibody directed against one of these antigens, there are two choices. One is to honor the antibody and to transfuse only appropriate antigen-negative blood. The other is to perform ^{51}Cr (or similar) cell survival studies to see if the particular example of the antibody is clinically-significant. In cases where transfusion or surgery should not be delayed any longer than absolutely necessary and suitable amounts of antigen-negative blood are not available, the latter choice is worth making since many of these antibodies can be shown to be clinically-insignificant.

There are still more antigens, again listed in Table 5, defined by antibodies about which essentially no information is yet available. That is to say, *in vivo* red cell survival studies or transfusions with antigen-positive blood have not been performed. There is little doubt that some of these antibodies will prove to be clinically-significant while others will prove to be benign *in vivo*. Obviously, when faced with an antibody of one of these specificities and a real need for transfusion, properly performed and interpreted cell survival studies are necessary in order to determine the *in vivo* behaviour of the antibody. Such survival studies,

when performed, should be documented in the literature so that the information becomes generally available.

When faced with an antibody of one of the types described in this section, careful consideration of the needs of the patient and the potential *in vivo* activity of the antibody are necessary. For example, in a patient with anti-Cs^a it was shown [47] that the survival of a 1ml test dose of ⁵¹Cr labelled Cs(a+) red cells was normal at 10 and 60 minutes and at 24 hours (90.6% survival) following the injection of the cells. At 7 days, 63% of the injected Cs(a+) red cells were still present in the patient's circulation. With completely normal survival it would have been expected that 75 to 80% of the test dose would have been present at 7 days. Thus, it seemed that in this case the antibody was capable of effecting slow and limited clearance of a minority of the Cs(a+) red cells. Several points need to be considered. First, if the antibody could clear only 37% of a 1ml test dose in 7 days, it is likely that the transfusion of a whole (or several) unit(s) of Cs(a+) blood would have resulted in even less antibody-mediated red cell clearance. The limited destruction of the small test dose probably represents limitation in the number of antibody molecules present; the antibody titer did not increase after the Cs(a+) red cells were injected. Second, the clinical effects of limited red cell destruction in a patient with normal liver and kidney function are not serious. Indeed, in patients with antibody-induced hemolytic anemia, immune red cell destruction often proceeds at a very brisk rate but it is the resultant anemia and not the by-products of red cell breakdown, that compromise the patient. Third, if the patient with the anti-Cs^a needed blood relatively urgently or had a real need for surgery with cal-transfusion support, a decision to use Cs(a+) blood, or delay the treatment until Cs(a-) units were available, would have to be made. With the earlier mentioned points in mind, this author would not hesitate to use Cs(a+) blood. With only limited red cell clearance, the Cs(a+) blood would support the patient through the emergency or the surgery and by the time that any clearance of those cells might occur, the patient would be through whatever had caused the need for transfusion, and

would be undergoing a reticulocyte response to replace lost cells. Such a course is far preferable to delaying surgery or withholding blood, until Cs(a-) units are found, since the antibody's ability to clear Cs(a+) cells is so limited. Fourth, only in a patient with total red cell aplasia would the use of Cs(a-) cells for a patient with an antibody of this type, seem necessary. In such a circumstance maximum *in vivo* survival of the transfused cells is obviously beneficial. Finally, it should again be stressed that most examples of anti-Cs^a are totally benign *in vivo* [42, 44, 49] and do not have even the limited potential for *in vivo* cell clearance of the example described.

Transfusion of blood from donors with antibodies

Other than the transfusion of very large volumes of plasma containing anti-A or anti-B to patients with A or B on their red cells, there are very few reports of problems caused by the passive transfer of donor antibodies to patients. There is no evidence that cold-reactive or even moderately strong IgG antibodies in donor plasma, cause any harm at all. Accordingly, many blood centers now test donor units for antibodies only by the indirect anti-globulin test. Even then, the tests are often performed on pooled serum samples from five or more donors since the object of the exercise is to detect only very strong donor antibodies. The rationale for such tests is that in four of five reported cases [54-58] in which passively transferred antibodies (from donors) caused transfusion reactions, the antibody had a titer of over 1000. Further, in four of the five cases [54-57] the antibody did not destroy the patient's red cells but those of another donor whose blood was given to the same patient. The findings that antibodies of moderate strength do not destroy the patient's red cells when those antibodies are passively introduced, simply reflects the fact that there are so many antigen sites on the patient's red cells that too few donor antibody molecules are bound, per red cell, to effect *in vivo* red cell destruction. This also explains why passively transferred antibodies are slightly more likely to clear the red cells of another donor when those

cells are transfused. Clearly, in such an instance there are far fewer red cells carrying the antigen so that a higher number of antibody molecules become bound to each red cell. The potential problems that could arise from the passive transfer of donor antibodies to patients and from interdonor incompatibility, can all be overcome by the simple expedient of issuing donor units, in which an antibody is present in the plasma, as packed red blood cells. Since *in vivo* red cell destruction is directly related to the total amount of antibody infused and the subsequent number of antibody molecules bound to each red cell, removal of much of the plasma in the preparation of packed red cells also removes the potential for harm. Because of lack of serious consequences arising from the passive transfer of donor antibodies, this author regards a suggestion [59] that all units with antibodies be given as washed or frozen-thawed red cells as preposterous. Such a procedure is totally unnecessary and would be wasteful of time, money and, because red cells are often washed in an open system and then carry a 24 hour outdate, of some units of blood as well.

Transfusion in patients with blood group autoantibodies

Many of the blood group specificities seen as alloantibodies are also found as causative autoantibodies in acquired immune hemolytic anemia (AIHA). That is to say, the antibody is made in a patient who also has the antigen against which the antibody is directed, on the red cells. Clearly, antigen-antibody reactions may then form *in vivo* and the potential for immune hemolytic anemia exists. In the 'warm' antibody-induced form of the disorder (WAIHA), antibodies to very common antigens in the Rh, Kell, MN and other systems are seen. In cold hemagglutinin disease (CHD or 'cold' antibody-induced hemolytic anemia) many of the autoantibodies belong in the I or Pr blood group system. In drug-induced hemolytic anemias, the antibodies have either a specificity for the drug or one of its metabolites, or are like those seen in WAIHA.

Although patients with WAIHA may present with severe anemia, blood transfusion is far from an ideal form of treatment. First, the anemia is not a simple one and cannot be corrected by transfusion. Second, there is anecdotal and sometimes disputed evidence that premature transfusion adversely affects the patient's long-term prognosis. Red cells given too soon in the course of the disease may act as additional immunogen and may effect a long-term increase in the total level of autoantibody made. Third, because most of these autoantibodies are of a specificity such that they react with almost all different phenotype red cells, it is seldom possible to transfuse compatible blood. Fourth, the effects of transfusion in the disorder are palliative and short-lived and point two above, must be remembered. In spite of these considerations, there are a number of patients with WAIHA who must be transfused if they are to survive. In such instances, transfusion is usually undertaken at the same time as other forms of treatment, such as steroid therapy, are instituted. This and the other forms of treatment are discussed elsewhere in this book. It should be stressed that the decision to transfuse must be based on the patient's clinical condition (i.e., cardiac decompensation) and not on the hemoglobin level. Once the clinical decision to transfuse has been reached, a difficult serological problem remains. In over 80% of patients with WAIHA, free autoantibody is present in the serum. The patient's red cells are saturated (direct antiglobulin test (DAT), strongly positive) with autoantibody. The free autoantibody invariably reacts with all red cell samples tested so that compatibility testing is extremely difficult. The major consideration at this point is that while blood incompatible with the autoantibody will probably be well tolerated, that is it will not cause an overt transfusion reaction, blood incompatible with any clinically-significant alloantibodies present will likely cause such a reaction. It becomes extremely difficult to detect and identify alloantibodies in a serum containing an autoantibody that reacts with all red cells. An attempted resolution is to use one or more of several methods [60], that vary in efficiency in different cases, that attempt to remove autoantibody from the patient's own DAT+ red

cells. Those cells are then used to adsorb autoantibody from the patient's serum so that any alloantibodies simultaneously present, but previously masked by the reactions of the autoantibody, can be detected and identified. While these methods work on most occasions there is always a danger that an alloantibody may be overlooked or may be lost, for example by dilution, during the procedure. In those cases in which no free autoantibody is present in the serum, compatibility tests can be performed more easily. However, it should be remembered that even when those tests are negative, the blood to be transfused is not usually compatible. While it may appear that all of the autoantibody is bound to the patient's red cells *in vivo*, it must be remembered that some of the antibody molecules will elute from the patient's red cells and bind to the donor's cells when they are transfused.

In some 2 to 3% of patients with WAIHA, the autoantibody may have a simple specificity so that donor blood more compatible than the patient's own cells can be used. For example, a patient with C+ D+ E- c+ e+ (R₁r) red cells who has made auto-anti-e, can be transfused with C- D+ E+ c+ e- (R₂R₂) red cells. The risk that the patient will form anti-E is very small (2,61). In an Rh-negative patient with C- D- E- c+ e+ (rr) red cells who has made auto-anti-e, R₂R₂ blood should not be used since the risk that the patient will form anti-D is high. Instead, rr donor red cells, that is those of the same phenotype as the patient, should be used. When the autoantibody is compatible only with Rh_{null}, K_o, U-, etc., red cells, blood of that phenotype is not used. Red cells incompatible with the patient's autoantibody usually survive *in vivo*, as well as the patient's own red cells [6]. That is, they have a reduced life span but are not destroyed immediately after their infusion. Blood of ultra rare phenotypes must be reserved for use in alloimmunized patients who cannot receive incompatible blood. Because there is always some risk that an alloantibody has escaped detection in pretransfusion tests, patients with WAIHA should be watched very carefully for signs of a reaction, as the transfusions are given.

Unlike WAIHA, there are no contraindications for transfusion in patients with CHD. However,

the disease is usually mild enough that transfusions to treat the condition are not necessary. In those rare cases in which transfusion is necessary because of the hemolytic anemia, infusion through a warming coil may be indicated if the autoantibody has a thermal range such that it has some activity near 37°C. If the CHD patient needs a transfusion for another reason, i.e., surgery, blood compatible with any alloantibodies present can usually be identified by performing prewarmed compatibility tests. In tests done at 37°C the cold-reactive autoantibody does not usually interfere. Blood compatible with the autoantibody is not usually available or necessary since *in vivo* hemolysis in this disorder is associated with a drop in the patient's body temperature that, obviously, can be avoided in a hospital setting.

There are no contraindications to transfusion in patients who have formed antibodies as a result of drug therapy. When the antibody is directed against a drug or one of its metabolites (e.g., anti-penicillin) it will not interfere in compatibility tests. When the antibody is directed against a red cell antigen (as in patients receiving alpha-methyl-dopa) the same methods as used in selecting blood for patients with WAIHA are appropriate.

Summary

This chapter has provided a brief description of some of the more important considerations of the red cell blood groups, primarily as they apply to transfusion therapy. Obviously far more is known about blood transfusion and the blood groups than could be included here. Readers seeking additional data are referred to several textbooks available [2, 6, 11, 18, 33, 61, 62].

References

- Landsteiner K: Zur kenntnis der antifermentativen, lytischen und agglutinierenden wirkungen des blutserums und der lymphe. Zbl Bakt, 1900; 27: 357-62 (English translation, Blood Transfusion Division, U.S. Army Medical Research Laboratory, Fort Knox, Kentucky).
- Issitt PD, Issitt CH: Applied Blood Group Serology, 2nd

- ed. Oxnard, Spectra Biologicals, 1975.
3. Cicciarelli J, Terasaki PI: Immunological aspects of HLA, crossmatching and transfusion in kidney transplantation. In: *Blood Group Antigens and Disease*, Garratty G (ed). Arlington, Va., American Association of Blood Banks, 1983: 149-64.
 4. Garratty G: The role of compatibility tests. Report of a meeting sponsored by the Bureau of Biologics for the Blood Products Advisory Committee. *Transfusion*, 1982; 22: 169-72.
 5. Honig CL, Bove JR: Transfusion-associated fatalities: review of Bureau of Biologics reports 1976-1978. *Transfusion*, 1980; 20: 653-61.
 6. Mollison PL: *Blood Transfusion in Clinical Medicine*, 7th ed. Oxford, Blackwell, 1983.
 7. Rosati LA, Barnes B, Oberman HA, Penner JA: Hemolytic anemia due to anti-A in concentrated antihemophilic factor preparations. *Transfusion*, 1979; 10: 139-41.
 8. Watkins WM, Morgan WTJ: Possible genetic pathways for the biosynthesis of blood group mucopolysaccharides. *Vox Sang*, 1959; 4: 97-119.
 9. Davey RJ, Tourault MA, Holland PV: The clinical significance of anti-H in an individual with the O_h (Bombay) phenotype. *Transfusion*, 1978; 18: 738-42.
 10. Salmon C: A tentative approach to variations in ABH and associated erythrocyte antigens. *Ser Haematol*, 1969; II: 3.
 11. Issitt PD: *Serology and Genetics of the Rhesus Blood Group System*. Cincinnati, Montgomery, 1979.
 12. Pollack W, Ascari WQ, Kochesky RJ, O'Connor RR, Ho TY, Tripodi D: Studies on Rh prophylaxis. I. Relationship between doses of anti-Rh and size of antigenic stimulus. *Transfusion*, 1971; 11: 333-39.
 13. McMaster Conference. Prevention of Rh immunization. *Vox Sang*, 1977; 36: 50-64.
 14. Tovey LAD, Murray J, Stevenson BJ, Taverner JM: Prevention of Rh haemolytic disease. *Br Med J*, 1978; 2: 106-8.
 15. Marsh WL: The Kell blood groups and their relationship to chronic granulomatous disease. In: *Cellular Antigens and Disease*, Steane EA (ed). Washington, D.C., American Association of Blood Banks, 1977: 52-66.
 16. Schwartz SA, Marsh WL, Symmans A, Johnson CL, Mueller KA: 'New' clinical features of McLeod syndrome. *Transfusion*, 1982; 22: 404 (abstract).
 17. McGinniss MH, Miller LH: Malaria, erythrocyte receptors and the Duffy blood group system. In: *Cellular Antigens and Disease*, Steane EA (ed). Washington, D.C., American Association of Blood Banks, 1977: 67-77.
 18. Issitt PD: The MN Blood Group System. Cincinnati, Montgomery, 1981.
 19. Pasvol G, Jungery M, Weatherall DJ, Parsons SF, Anstee DJ, Tanner MJA: Glycophorin as a possible receptor for *Plasmodium falciparum*. *Lancet*, 1982; 2: 947-51.
 20. Cutbush M, Mollison PL: Relation between characteristics of blood-group antibodies in vitro and associated patterns of red cell destruction in vivo. *Br J Haematol*, 1958; 4: 115-37.
 21. Mollison PL: Blood group antibodies and red cell destruction. *Br Med J*, 1959; 2: 1035-41.
 22. Mollison PL: Blood group antibodies and red cell destruction. *Br Med J*, 1959; 2: 1123-30.
 23. Mollison PL: Factors determining the relative clinical importance of different blood-group antibodies. *Br Med Bull*, 1959; 15: 92-98.
 24. Mollison PL: Further studies on the removal of incompatible red cells from the circulation. *Acta Hemat* Fasc 10 Basel, Karger, 1959: 495-500.
 25. Gblett ER: Blood group alloantibodies: an assessment of some laboratory practices. *Transfusion*, 1977; 17: 299-308.
 26. Mollison PL, Johnson CA, Prior DM: Dose-dependent destruction of A₁ cells by anti-A₁. *Vox Sang*, 1978; 35: 149-53.
 27. Morel PA, Garratty G, Perkins HA: Clinically significant and insignificant antibodies in blood transfusion. *Am J Med Technol*, 1978; 44: 122-29.
 28. Cronin CA, Pohl BA, Miller WV: Crossmatch-compatible blood for patients with anti-P₁. *Transfusion*, 1978; 18: 728-30.
 29. Issitt PD: Antibodies reactive at 30°C, room temperature and below. In: *Clinically Significant and Insignificant Antibodies*, Butch SH, Beck ML (eds). Washington D.C., American Association of Blood Banks, 1979: 13-28.
 30. Waheed A, Kennedy MS, Gerhan S, Senhauser DA: Transfusion significance of Lewis system antibodies. Success in transfusion with crossmatch-compatible blood. *Am J Clin Pathol*, 1981; 76: 294-98.
 31. Standards for Blood Banks and Transfusion Services, 10th Ed. American Association of Blood Banks, Washington D.C., 1981: 27.
 32. Technical Manual of the American Association of Blood Banks, 8th Ed, Widmann F (ed). American Association of Blood Banks, Washington D.C., 1981: 177-82, 211-13.
 33. Petz LD, Swisher SN: *Clinical Practice of Blood Transfusion*. Churchill Livingstone, New York, 1981: 171, 182, 183, 191.
 34. Kurtz SR, Ouellet R, McMican A, Valeri CR: Survival of MM cells during hypothermia in two patients with anti-M. *Transfusion*, 1983; 23: 37-39.
 35. Helgeson M, Swanson J, Polesky HF: Knops-Helgeson (Kn^a), a high-frequency erythrocyte antigen. *Transfusion*, 1970; 10: 137-38.
 36. Colledge KI, Kaplan HS, Marsh WL: Massive transfusion of Sd(a+) blood to a recipient with anti-Sd^a without clinical complication. *Transfusion*, 1973; 13: 340 (abstract).
 37. Moore HC, Issitt PD, Pavone BG: Successful transfusion of Ch(a+) blood to two patients with anti-Ch^a. *Transfusion*, 1975; 15: 266-69.
 38. Wells RF, Korn G, Hafleigh B, Grumet FC: Characterization of three new apparently related high frequency antigens. *Transfusion*, 1976; 16: 427-33.
 39. Tilley CA, Crookston MC, Haddad SA, Shumak KH: Red blood cell survival studies in patients with anti-Ch^a, anti-Yk^a, anti-Ge and anti-Vel. *Transfusion*, 1977; 17: 169-72.

40. Silvergleid AJ, Wells RF, Hafleigh EB, Korn G, Keller RT, Grumet FC: Compatibility tests using ^{51}Cr -labeled red blood cells in crossmatch positive patients. *Transfusion*, 1978; 18: 8–14.
41. Sabo B, Moulds JJ, McCreary J: Anti-JMH: another high titer low avidity antibody against a high frequency antigen. *Transfusion*, 1978; 18: 387 (abstract).
42. Shore GM, Steane EA: Survival of incompatible red cells in a patient with anti- Cs^a and three other patients with antibodies to high frequency red cell antigens. *Transfusion*, 1978; 18: 387 (abstract).
43. Nordhagen R, Aas M: Survival studies of ^{51}Cr Ch(a+) red cells in a patient with anti-Ch a and massive transfusion of incompatible blood. *Vox Sang*, 1979; 37: 179–81.
44. Garratty G: Clinical significance of antibodies reacting optimally at 37°C. In: *Clinically Significant and Insignificant Antibodies*, Butch SH, Beck ML (eds). Washington, D.C., American Association of Blood Banks, 1979: 29–49.
45. Ryden SE: Successful transfusion of a patient with anti-Yk a . *Transfusion*, 1981; 21: 130–31 (letter).
46. Viggiano E, Ballas SK: Erythrocyte survival studies of 'Kn a /McC a ' incompatible blood in a patient with anti-'Kn a /McC a '. *Transfusion*, 1981; 21: 603 (abstract).
47. Valko DA, Silberstein EB, Greenwalt TJ, Issitt PD: Normal survival of in vitro reactive red cells in one patient with anti-McC a and one with anti- Cs^a . *Transfusion*, 1981; 21: 603–4 (abstract).
48. Grove W, Peters B, Ellisor SS, Vanderbilt-Tate TJ: Normal ^{51}Cr red cell survival of York (Yk a) positive blood in a patient with anti-Yk a . *Transfusion*, 1981; 21: 607–8 (abstract).
49. Issitt PD: The clinical significance of some anti-red cell antibodies. In: *Advances in Pathology, Antatomic and Clinical, Part I*, Levy E (ed). Oxford, Pergamon, 1982: 395–398.
50. Harpool DR: Anti-SI a : lack of effect on transfused SI(a+) red cells. *Transfusion*, 1983; 23: 402–3 (letter).
51. Bettigole R, Harris JP, Tegoli J, Issitt PD: Rapid in vivo destruction of Yt(a+) red cells in a patient with anti-Yt a . *Vox Sang*, 1968; 14: 143–46.
52. Dubbs JV, Prutting DL, Adebahr ME, Allen FH Jr: Clinical experience with three examples of anti-Yt a . *Vox Sang*, 1968; 15: 216–21.
53. Gobel U, Drescher KH, Pottgen W, Lehr H: A second example of anti-Yt a with rapid in vivo destruction of Yt(a+) red cells. *Vox Sang*, 1974; 27: 171–75.
54. Zettner A, Bove JR: Hemolytic transfusion reaction due to interdonor incompatibility. *Transfusion*, 1963; 3: 48–51.
55. Franciosi R, Awer E, Santana M: Interdonor incompatibility resulting in anuria. *Transfusion*, 1967; 7: 297–98.
56. Abbott D, Hussain S: Intravascular coagulation due to inter-donor incompatibility. *Can Med Assoc J*, 1970; 103: 752–56.
57. Morse EE: Interdonor incompatibility as a cause of reaction during granulocyte transfusion. *Vox Sang*, 1978; 35: 215–18.
58. Goldfinger D, Kleinman S, Connelly M, Chaux A, Sacks HJ: Acute hemolytic transfusion reaction (HTR) with disseminated intravascular coagulation (DIC) and acute renal failure (ARF) due to transfusion of plasma containing Rh antibodies. *Transfusion*, 1979; 19: 639–40 (abstract).
59. Anonymous referee of the first draft of this chapter, 1983.
60. Issitt PD: Red cell transfusions in autoimmunized patients. *Cleveland Clinic Quart*, 1981; 48: 289–303.
61. Petz LD, Garratty G: *Acquired Immune Hemolytic Anemias*. New York, Churchill Livingstone, 1980.
62. Race RR, Sanger R: *Blood Groups in Man*, 6th Ed. Oxford, Blackwell, 1975.

* This chapter was completed in December, 1983.

10. The crossmatch: how much is enough?

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While the first transfusion of human blood took place early in the nineteenth century, almost a century elapsed before pretransfusion compatibility testing was performed. Such testing initially consisted of rather cumbersome assessment of both recipient and donor blood, and, by the second decade of this century, included determination of the ABO type of donor and recipient and a crossmatch. Since that time the crossmatch has undergone considerable modification, reflecting changes in antibody detection procedures and recognition of the clinical significance of various antibodies. In recent years there has been general agreement that, at the very least, the crossmatch should consist of a reaction between donor red cells and recipient serum in a manner which would detect clinically significant antibodies. Furthermore, current Standards of the American Association of Blood Banks state that this testing must include an antiglobulin test [1].

During the past several years there has been increasing challenge of this procedure, especially relating to considerations of cost in comparison to the potential risk to the patient of abbreviating the test. The most common proposed modification is exclusion of the antiglobulin phase. However, others believe that the refinement of procedures for blood typing and screening for unexpected antibodies permits elimination of the crossmatch.

Before discussing the appropriate extent of the procedure, a brief review of the changes in this test is appropriate. An historical study on this subject has recently been published [2].

Development of the crossmatch

The dawn of the 'modern era' in pretransfusion testing occurred in the mid-1940s with the advent of methodology which facilitated detection of IgG antibodies. With the discovery of the Rh factor, many testing techniques were described for detection of antibodies in this system. Use of albumin for the enhancement of agglutination was a major advance over the use of saline [3]. In the same year Coombs and colleagues described use of the anti-globulin technique, although this test had been proposed almost four decades earlier by Moreschi [4]. The use of these two techniques, coupled with incubation at 37°C, greatly enhanced detection of 'complete' and 'incomplete' antibodies.

Two years later the use of proteolytic enzymes was described by Morton and Pickles, and this was incorporated by some in pretransfusion testing, although it found greater use in antibody identification [5]. Pretransfusion crossmatching was further modified by the introduction of low ionic strength saline solution. The augmenting properties of this solution had been recognized for many years, yet not until 1974 was it popularized for use in a Transfusion Service by Low and Messeter [6].

The addition of these techniques spawned an era during which numerous new red cell antigenic specificities were defined, based upon discovery of antibodies in patients' sera. Blood banks made a compulsive effort to define all antibodies in a patient's serum which might conceivably interfere with survival of the transfused red cells. As a result, pretransfusion testing became lengthy, and the

need to resolve positive screening tests for recipient antibodies, or incompatibility observed in crossmatching, had the potential for delaying transfusion.

It is of interest that the first edition of Mollison's text, *Blood Transfusion in Clinical Medicine*, advocated a compatibility test involving incubation of a mixture of donor red cells and patient serum with albumin for two hours, although in an emergency the incubation time could be shortened [7]. The antiglobulin reaction was advocated only when the patient had been previously transfused or was pregnant. However, the second edition of the text, which appeared five years later, provided somewhat stronger endorsement of the indirect antiglobulin test. A screening test for unexpected antibodies in donor serum, using a pool of enzyme-treated red cells, was advocated, supplanting the 'minor crossmatch'.

The evolution of compatibility testing since 1958 also is reflected in the description of crossmatching in the Standards for Blood Banks and Transfusion Services of the American Association of Blood Banks. The minor crossmatch has never been required by the Standards. Furthermore, from the earliest days of crossmatching authors have minimized the importance of testing between the patient's red cells and donor's serum. In 1968 Monroe and Jennings, in a discussion of the importance of the minor crossmatch, observed that the increasing acceptance of pretransfusion antibody screening had nullified the importance of this test [8]. The Standards of the American Association of Blood Banks which, in its early editions had suggested that the minor crossmatch was optional, finally, in 1976 stated that it was unnecessary [9].

While the fourth edition of the Standards (1963) stated that the major crossmatch optimally should detect 'complete and incomplete antibodies' [10], by 1970 the objective of the major crossmatch was modified in the Standards to detection of 'agglutinating and coating' antibodies, and also was amended to require that the crossmatch 'shall include an antiglobulin test' [11]. Furthermore, screening of donor and recipient serums for unexpected antibodies was required, and, in addition, tests employing albumin, serum-albumin or pro-

tease treatment of red cells was considered advisable.

Four years later the Standards indicated that pretransfusion compatibility testing must detect hemolysing antibodies in both donor and recipient serum in antibody screening procedures and in the crossmatch [12]. Furthermore, an initial attempt to balance the extent of testing with clinical significance was reflected in the statement that tests should detect 'significant antibodies'. By 1978 the Standards specified that only antibodies active at 37°C need be detected, and the longstanding requirement for noninactivated, or freshly-collected, serum was dropped [13].

The requirement for tests for serological incompatibility in the current Standards has been further simplified to specify that such tests should demonstrate ABO incompatibility and also clinically significant unexpected antibodies active at 37°C [1]. However, an antiglobulin phase continues to be required.

The sensitivity of early crossmatch procedures utilizing antiglobulin serum undoubtedly was compromised through performance on slides or tiles, without centrifugation. The routine crossmatching test in many institutions in the 1950s consisted solely of room temperature incubation of a saline mixture of red cells of the donor with serum of the patient, followed by addition of albumin to the mixture, incubation and centrifugation.

In 1964 Grove-Rasmussen debated the relative importance of the antiglobulin phase in performance of the crossmatch [14]. Obviously, his opinion was stimulated by the advent of screening tests for unexpected antibodies in the 1950s. His belief that this phase of testing was necessary in the crossmatch only when unexpected antibodies had been detected by the screening test is advocated by many today.

Current status of the crossmatch and future trends

Interest in abbreviation or elimination of the crossmatch appears to have been stimulated by two situations. The pressure upon clinical laboratories for cost containment has led to considerable discus-

sion as to the appropriateness and extent of various aspects of clinical laboratory practice. In the Blood Bank this has focused on the crossmatch. It has been proposed that time spent on a procedure, such as the crossmatch, could more productively be utilized for other laboratory duties, thereby avoiding the need for increasing the laboratory staff.

The second factor which has occasioned concern as to the extent of crossmatching has been the need to provide blood more rapidly for transfusion. This has been related, in part, to the advent of surgical blood order schedules, as exemplified by the 'type and screen' concept [15, 16]. These schedules, arrived at by consensus between the Medical Director of the Blood Bank and the surgeon, stipulate the number of units of blood which will be prepared for a common operative procedure, based upon patterns of blood usage in the individual institution. Implicit in such schedules is the obligation of the Blood Bank to provide compatible blood promptly in the event of unexpected hemorrhage. This has occasioned the need for Blood Banks to consider methods which would shorten the crossmatch to enhance cooperation with surgeons and anesthesiologists in implementation of such schedules. To facilitate the latter situation the Standards of the American Association of Blood Banks has set forth guidelines for blood provision in situations of urgent blood requirement, and also has permitted abbreviation of the crossmatch at the behest of the laboratory director [1].

Those who advocate elimination of the crossmatch propose, as a substitute, an expanded and more sensitive screening test for unexpected antibodies, while those who advocate abbreviation of the procedure focus their attention on the antiglobulin phase of the test, and suggest that it be eliminated or made optional.

It must be recognized that the crossmatch is the final procedure performed before blood is issued for transfusion. Therefore, it serves as a final check on the compatibility of the unit of donor blood with that of the patient, especially in the ABO system. Screening tests for unexpected antibodies cannot serve this purpose since they utilize type O red cells. Issuance of improper blood from the Blood Bank, related to erroneous typing of the patient or

accidental selection of an incorrect unit of blood from the refrigerator, can best be averted by the crossmatch, provided that the initial blood specimen submitted for processing from the patient has been properly identified. Therefore, the most important function of the crossmatch is assurance of ABO compatibility.

The second function of the crossmatch is the recognition of clinically significant antibodies which were not detected with pretransfusion screening tests for unexpected antibodies. The antiglobulin reagent plays an important role in this function. Failure of detection of such antibodies may relate to technical error in performance of pretransfusion screening tests, absence of corresponding antigenic specificity on the reagent red cells used in testing, or presence of an antibody in the patient's serum which manifests dosage effect while the corresponding antigenic expression on the reagent red cells is heterozygous.

The first of these issues, ABO compatibility, directly relates to proposals for elimination of the crossmatch. The second function of the crossmatch, detection of unexpected antibodies which were not recognized in pretransfusion screening tests, relates to elimination of the antiglobulin phase of the crossmatch. Let us separate these two elements of the procedure.

Elimination of the crossmatch

As noted above, the most important function of the crossmatch is prevention of issuance of ABO incompatible blood. Most life-threatening hemolytic transfusion reactions are related to ABO incompatibility, and most are due to human error either in the collection of the patient specimen, processing of blood in the laboratory, or institution of the transfusion at the bedside. Obviously, the crossmatch will prevent only the second of these three possibilities. Were the crossmatch eliminated, this final confirmatory test would be lost. Proposals for elimination of the crossmatch rely on the accuracy of typing of donor and patient blood and on the error-free performance of Blood Bank technologists [17].

In an effort to test the potential risk of this procedural modification, we surveyed a group of experienced technologists in our laboratory to determine the frequency with which the major crossmatch had alerted them to inadvertent administration of ABO-incompatible units of blood. An anonymous questionnaire was completed by 20 technologists, who had a collective work experience of 99 years. Each technologist was asked to indicate the number of times that the major crossmatch had alerted them to an error of this type. Sixteen such examples were noted by the group [18]. These technologists worked solely in the Blood Bank, were all registered medical technologists and several were specialists in blood banking. One might anticipate that the frequency of this potential error might even be greater in a laboratory that employed less experienced technologists, and especially in an institution wherein the technologists also had responsibility for performance of tests in laboratories other than the Blood Bank.

As noted above, the screening test for unexpected antibodies utilizes type O red cells, and therefore would not detect inadvertent administration of ABO incompatible blood. Therefore, elimination of the crossmatch, even if accompanied by an extremely detailed and complex screening test for unexpected antibodies, seems inappropriate and potentially dangerous.

Abbreviation of the crossmatch

Most suggestions for abbreviation of the crossmatch focus on the deletion of the antiglobulin phase [19]. If one relies solely on an 'immediate spin' reaction between donor red cells and patient's serum, eliminating incubation at 37°C and the addition of antiglobulin reagent, there will be considerable savings in technologist effort and reagent cost. However, what will be the risk of such abbreviation to the patient? The answer to this question centers on the frequency with which the crossmatch detects unexpected antibodies which had been overlooked by the screening test.

Several years ago, we studied this issue in an effort to determine the safety of abbreviating the

crossmatch in the event of a massive transfusion [20]. Our report surveyed a two-year interval during which our routine crossmatch procedure consisted of a 15 to 20 minute room temperature incubation, addition of albumin and incubation at 37°C for 30 min, followed by addition of antiglobulin reagent. It was noted that a high level of safety was present when the antiglobulin phase of the crossmatch was deleted. Only eight clinically significant unexpected antibodies were detected by the major crossmatch among 82,000 tests performed on blood samples which previously had manifested nonreactive antibody screening tests. These antibodies were all weakly reactive, and had specificities in the Rh, Kell and Kidd systems. Almost 150 unexpectedly reactive crossmatches noted in the study did not result in identification of antibodies of clinical significance. Nevertheless, provision of blood for transfusion of the latter patients was delayed until antibody identification procedures could be completed.

Therefore, it might be concluded that some degree of accelerated removal of transfused red cells from the patient's circulation may have been prevented for eight patients; however, delay in provision of blood for transfusion for almost 150 other patients, related to clinically irrelevant reactive crossmatches, resulted in increased cost to the patient. Furthermore, this delay in transfusion potentially increased morbidity.

Because of procedural changes in our laboratory, we performed a comparable study three years later [2, 18, 21]. We incorporated low ionic strength saline solution into both our crossmatch and antibody screening procedures, and also eliminated use of albumin and of room temperature incubation. These changes were in line with modifications in the Standards of the American Association of Blood Banks [13]. This procedural change also permitted more rapid provision of blood for transfusion, as the total time for testing could be completed within 15 min. Once again we examined instances wherein the crossmatch detected unexpected antibodies which were not recognized by pretransfusion screening tests.

In this second study of 81,400 crossmatches we found only nine clinically significant antibodies

among 150 unexpectedly positive tests performed on approximately 4,000 patient specimens. In both studies all of the clinically significant antibodies were detected solely in the antiglobulin phase of the technique.

The major crossmatch, in both studies, permitted recognition of neonatal patients with anti-A in their serum, undoubtedly of maternal origin. It is obvious that the antibody screening test, which utilizes type O red cells, cannot detect such antibodies. Because of a recent procedural modification in our laboratory, wherein only type O red blood cells are provided for transfusion of these patients, the crossmatch no longer serves to detect such antibodies. It also may be argued that there is little reason to attempt to detect passively transferred anti-A in these infants provided that type O blood is being transfused. Anti-A in an infant's serum, without clinical manifestations of hemolytic disease of the newborn, is of little importance.

Our results contrast with other reports. Several years ago Heisto indicated that the antiglobulin phase of the crossmatch could be eliminated provided that a sensitive pretransfusion antibody screening test was employed [22]. He incorporated papain in the screening tests, as well as an anti-globulin phase, and utilized a 45-minute incubation. This procedure was of sufficient sensitivity that only one example of anti-Le^a unexpectedly was discovered by the indirect antiglobulin phase of the crossmatch.

More recently, Mintz *et al.* found that only ten unexpectedly positive crossmatches, related to clinically significant alloantibodies, were discovered among approximately 200,000 major crossmatches following a nonreactive conventional pretransfusion screening test for unexpected antibodies [23]. This study yielded a slightly lower incidence of unexpected detection of clinically significant antibodies in the antiglobulin phase of the major crossmatch than did our own study. Nevertheless, it seems evident that, even when optimal pretransfusion screening tests are employed, an occasional clinically significant antibody will be detected solely by the major crossmatch.

Optimal extent of the crossmatch

It must be appreciated that severe hemolytic transfusion reactions usually are related to ABO incompatibility, and these will not be prevented by the expanded screening test. However, this would also increase the number of antibody identification procedures which would need to be performed, thereby delaying transfusion and increasing the cost of medical care. Therefore, elimination of the crossmatch, even if accompanied by an extremely detailed and complex screening test for unexpected antibodies, seems inappropriate and potentially dangerous. The question of eliminating the anti-globulin phase of the crossmatch relates to the acceptable risk of transfusion.

Some have considered this issue to be an appropriate model for risk: benefit discussions; however, it should be evident that there is a broader dimension to the subject. In this situation the individual subjected to the risk does not control the procedure and cannot participate actively in the decision of risk level to be assumed.

Our studies, and those of Mintz, indicate that an occasional previously unrecognized clinically significant antibody will be identified solely by the major crossmatch. This is an uncommon occurrence, as clinically significant antibodies were detected by this procedure in only 17 of over 160,000 major crossmatches in the two studies reported from our institution. Nevertheless, if the goal of pretransfusion testing is the detection of *every* clinically significant unexpected antibody, it is clear that the antiglobulin phase of the crossmatch must be retained. Furthermore, it must be appreciated that even this procedure will not detect *every* unexpected antibody. Augmentation of the screening test for unexpected antibodies with enzyme-treatment of reagent red cells and extension of incubation times would further enhance the sensitivity of the test.

Rather than basing conclusions on the relationship between number of specimens tested and unexpectedly positive crossmatches, it may be more appropriate to assess the appropriateness of abridging the crossmatch in terms of potential serologic incompatibilities (P.S.I.), referring to the

total number of reactive antibody screening tests and crossmatches, regardless of the clinical significance of subsequently identified antibodies. During the 30-month interval of the last phase of the study noted above, 5.2% of all patient specimens submitted to the Blood Bank for pretransfusion studies manifested a positive screening test for unexpected antibodies. Therefore, when combined with the positive crossmatches, in the face of a nonreactive pretransfusion screening test, 6.5% of specimens contained P.S.I. In this context, it is seen that 5% of the P.S.I. would have been missed were it not for inclusion of the antiglobulin phase of the crossmatch. Consideration of this issue in terms of P.S.I. casts a different light on this issue than basing conclusions on the number of clinically significant antibodies discovered solely with the crossmatch. Pursuing the latter line of reasoning, one might question performance of antibody screening tests, relating the number of positive tests to the number of clinically significant antibodies discovered.

As noted above, one of the most pressing reasons for abbreviating the crossmatch is to facilitate response to urgent requests for blood from the operating room, related, in part, to acceptance of such new concepts as assigned blood order schedules. Elimination of the antiglobulin phase of the procedure will reduce the time required for the crossmatch; however, with the current procedure utilized in our laboratory we are able to provide blood within an acceptable time for all elective clinical requirements. Workload figures in our laboratory indicate that elimination of the antiglobulin phase of the crossmatch would save approximately five minutes of procedural time. This amount of time is not significant in provision of blood for elective transfusion.

Urgent provision of blood

As noted above, routine compatibility testing of blood for transfusion implies performance of ABO and Rh typing, as well as testing for the presence of unexpected antibodies on specimens from both donor and recipient. The crossmatch serves as the

final step in the compatibility testing process. Provided that all of the preliminary steps have been completed previously, performance of a crossmatch, using the method described above, can result in provision of blood within 30 minutes, including all requisite paperwork. If preliminary screening of recipient blood for unexpected antibodies is not included in the compatibility testing procedure, a more extended crossmatch may be desired, lengthening the time required for routine provision of blood. It must also be appreciated that the time required for *routine* provision of blood from any Blood Bank usually will be lengthened by other functions of the laboratory, such as more pressing needs for blood by other patients.

In most emergency situations a hypovolemic patient may initially receive volume expanders until the blood bank can complete routine compatibility testing. However, there are situations wherein delay in providing blood may jeopardize the patient's welfare. This risk must be balanced against the potential risk of a hemolytic reaction when incompletely tested blood is issued. It must be recalled that most patients who manifest an urgent requirement for blood either are anesthetized or comatose. Therefore, they cannot complain of early symptoms of a transfusion reactions, such as chills or back pain.

Patients who require emergency transfusion of blood, and whose ABO and Rh types have not previously been determined by the blood bank, may receive type O, Rh-negative red blood cells until proper typing has been performed. The latter should require no more than 5 min. Most patients can be sustained with volume expanders, rather than blood, during this interval. Indiscriminate use of type O, Rh-negative red blood cells compromises availability of this component for other patients, and also does not take into consideration the possibility that the recipient may have an unexpected antibody which reacts with an antigen on the transfused red cells.

Hospitalized patients whose blood previously has been typed, and in whom no unexpected antibodies have been detected through previous testing, may receive type-specific blood with far greater safety than those patients in whom such

testing has not been performed. In this situation it is essential that the physician in the Blood Bank define the level of emergency through consultation with the patient's physician. Each step of the compatibility test which can be completed increases the safety of the transfusion. The time required for such testing must be weighed against the potential risk to the patient resulting from delay of transfusion. Only a physician can make this decision.

While 30 min should be adequate to provide fully crossmatched blood under emergency circumstances, an 'immediate spin' crossmatch may be appropriate for patients with an emergency need for blood whose red cells have previously been typed and screened for unexpected antibodies. Using such an abbreviated crossmatch technique, without incorporation of an antiglobulin reaction, blood can be released for transfusion within approximately 10 minutes after notification of the Blood Bank that an emergency exists.

It is the author's belief that the greatest risks to the patient in emergencies relate to inappropriate delay in provision of blood and errors in identification of the unit of blood with the appropriate patient due to haste in implementing the transfusion.

Conclusions

The primary role of the major crossmatch is the detection of ABO incompatibility, and the prevention of transfusing ABO-incompatible blood. Elimination of the crossmatch will preclude this most important function; therefore, it may be viewed as a potentially dangerous proposal. Screening tests for unexpected antibodies, performed in advance of the crossmatch, will not serve this purpose, regardless of their extent.

Elimination of the antiglobulin phase of the major crossmatch will result in relatively little additional risk for most patients receiving transfusion, especially when preliminary ABO and Rh typing, and screening for unexpected antibodies, in both patient and donor, has been performed.

Current pretransfusion testing is imperfect, and heavily laden with compromise. An ideal test might incorporate longer incubation times, use of en-

zymes in the antibody detection test, as well as the use of the antiglobulin phase of the crossmatch. However, while this might detect increased numbers of clinically significant unexpected antibodies, it would be far too expensive to be practical. Therefore, the unresolved question is: 'Where do we draw the line?', as we attempt to relate potential risk to benefit and to cost for our patient population.

References

1. Standards for Blood Banks and Transfusion Services. Oberman HA (ed). Ed. 10. Washington, D.C., Am Assoc Blood Banks. 1981.
2. Oberman HA: The crossmatch: past, present and future. In: A Seminar on Immune-mediated Cell Destruction, Bell C (ed). Washington, D.C., Am Assoc Blood Banks, 1981: 29-44.
3. Diamond LK, Denton RL: Rh agglutination in various media with particular reference to the value of albumin. *J Lab Clin Med* 1945; 30: 821.
4. Coombs RRA, Mourant AE, Race RR: A new test for the detection of weak and 'incomplete' Rh agglutinins. *Br J Exp Path* 1945; 26: 225.
5. Morton JA, Pickles MM: Use of trypsin in the detection of incomplete RH antibodies. *Nature* 1947; 159: 779.
6. Low B, Messeter L: Antiglobulin test in low ionic strength salt solution for rapid antibody screening and crossmatching. *Vox Sang* 1974; 26: 53.
7. Mollison P: Blood transfusion in clinical medicine. Eds. 1 and 2. London, Blackwell Scientific Publ., 1951 and 1956.
8. Monroe CH, Jennings ER: The significance of the minor crossmatch - after 10 years. In: A Seminar on Compatibility Testing. Washington, D.C., Am Assoc Blood Banks, 1968.
9. Standards for Blood Banks and Blood Transfusion Services. Oberman, HA (ed.) 8. Washington, D.C., Am Assoc Blood Banks, 1976.
10. Standards for a Blood Transfusion Service. Ed. 4. Washington, D.C., Am Assoc Blood Banks, 1963.
11. Standards for Blood Banks and Blood Transfusion Services. Ed. 5. Washington, D.C., Am Assoc Blood Banks, 1970.
12. Standards for Blood Banks and Blood Transfusion Services, Oberman HA (ed). Ed. 7. Washington, D.C., Am Assoc Blood Banks, 1974.
13. Standards for Blood Banks and Blood Transfusion Services, Oberman HA (ed.) Ed. 9. Washington, D.C., Am Assoc Blood Banks, 1978.
14. Grove-Rasmussen M: Routine compatibility testing. *Transfusion* 1964; 4: 200.
15. Friedman BA, Oberman HA, Chadwick AR, Kingdon KI: The maximum surgical blood order schedule and surgical

- blood use in the United States. *Transfusion* 1976; 16: 380.
- 16. Henry JB, Mintz R: Optimal blood ordering for elective surgery. *JAMA* 1977; 237: 451.
 - 17. Winn LC, Svoboda R, Hafleigh EB, Grumet FC: An extended antibody screen to replace the crossmatch. Annual Meeting. Am Assoc Blood Banks. Washington, D.C., 1980.
 - 18. Oberman HA, Barnes BA, Steiner EA: Role of the crossmatch in testing for serologic incompatibility. *Transfusion* 1982; 22: 12.
 - 19. Garratty G: The role of compatibility tests. *Transfusion* 1982; 22: 169.
 - 20. Oberman HA, Barnes BA, Friedman BA: The risk of abbreviating the major crossmatch in urgent or massive transfusion. *Transfusion* 1978; 18: 137.
 - 21. Oberman HA: Abbreviation or elimination of the crossmatch. *Clin Lab Med* 1982; 2: 181.
 - 22. Heisto H: Pretransfusion blood group serology. Limited value of the antiglobulin phase of the crossmatch when a careful screening test for unexpected antibodies is performed. *Transfusion* 1979; 19: 761.
 - 23. Mintz PD, Haines A, Sullivan M: Incompatible crossmatch following nonreactive antibody detection test: frequency and cause. *Transfusion* 1982; 22: 107.

11. Isoimmunisation in pregnancy: diagnosis, treatment and prevention

J. BENNEBROEK GRAVENHORST and D.G. WOODFIELD

1. Haemolytic disease of the newborn

Forty years of investigation of Rhesus haemolytic disease of the newborn (Rh-HDN) has resulted in major advances in our understanding of the aetiology, diagnosis and management of the disorder. In 1966 it was exciting to realise that most cases of immunisation to the Rhesus D antigen could be prevented by the administration of Anti D immunoglobulin (Anti D-Ig) to Rh D negative mothers after delivery of a Rh positive infant. It was also recognised that it was important to immunise following any stillbirth or abortion. This discovery led to much activity to ensure that adequate supplies of Anti D-Ig could be produced and to find ways in which treatment could be made available to every woman in need. The result has been an over 90% reduction in the frequency of new Rhesus immunisations and a related decrease in the number of children developing Rh-HDN [1].

In the USA between 1970 and 1975, the incidence of Rh-HDN decreased by 53%, from 45 to 21 cases per 10,000 total births, and infant mortality due to Rh-HDN fell by 59% from 2.2 to 0.9 per 10,000 live births. Similarly in England and Wales perinatal death from haemolytic disease of the newborn (all causes) fell by 62.5% from 8 to 3 per 10,000 live births. Similar decreases were noted in countries such as Australia, New Zealand, Finland and Canada. The problem then became one of investigating the reasons for some patients still developing Rh-HDN. In many cases this was due to Anti D not being given when clinically indicated or by the antibody appearing during pregnancy. The latter

cause promoted trials in the use of antenatal administration of Anti D-Ig, and evidence eventually accumulated that this procedure could prevent almost all cases of Rh-HDN albeit at considerable additional cost. For this reason antenatal Anti D prophylaxis has not been widely introduced even in countries whose capability for producing additional supplies of Anti D-Ig is undoubted.

At the present time increased emphasis is now being placed on ensuring that all mothers at risk receive Anti D-Ig at delivery or at the time of termination of pregnancy. This is not as easy as it sounds, as abortions for social, marital or ethical reasons may be concealed and the need for immunoprophylaxis not considered. In some countries transfusion of Rh positive blood to an Rh negative woman still remains a cause of immunisation.

Sufficient evidence now exists to show that a relatively low dose of Anti D-Ig (100 mcg) will protect against immunisation in most women. In a few patients where more than 5 ml of Rh positive foetal red cells can be demonstrated in the mother's circulation a larger dose of Anti D-Ig may be required.

Not all the improvements in the prognosis of this disease can be attributed to immunoprophylaxis. Other advances have occurred simultaneously, such as improved serological diagnosis, systematic antenatal screening of all pregnant women, manoeuvres such as intrauterine transfusion and a greatly improved handling of premature neonates. Despite these advances problem patients still remain. Special management is still required of highly immunised Rhesus negative mothers who

have developed high titer or multiple antibodies, and there is still an appreciable morbidity from antibodies outside the Rhesus system. Haemolytic disease of the newborn has not gone away but the picture has been clearly modified leaving behind a range of intellectually and clinically exacting problem patients.

Various reviews of the Rhesus blood group system are available, with perhaps the most recent and informative being that of Issitt [2]. In his book he outlines 40 defined Rhesus antigens, and at least 12 of these have been implicated in Rhesus disease. A larger variety of antibodies have been the cause of mild, moderate or severe haemolytic disease and these are listed in Table 1.

Rh haemolytic disease

The pathogenesis of Rh haemolytic disease is well known. Immunisation of the primigravida mother most commonly occurs during labour when Rh D positive red cells cross the placenta and enter the maternal circulation. The risk of immunisation is related to the size of foeto-maternal haemorrhage, with 75% of women having a transplacental haemorrhage of less than 0.1 ml while 0.3% have a haemorrhage of greater than 15 ml. The overall risk of Rh D immunisation in ABO compatible first

Table 1.

Blood group system	Antibodies implicated in haemolytic disease of the newborn
ABO	-A, -B, -A ^B
Rhesus	-D, -C, -E, -c, -e, -C ^w , -C ^x , -E ^w , -Go ^a , -f(ce), -Be ^a , -Ce ^s
MNSs	-M, -N, -S, -s, -U, -Mt ^a , Mi ^a , -Vw, -Mur, -Hil, -Hut
P	-Tj ^a
I	-i
Xg	-Xg ^a
Kell	-K, -k, Kp ^a , -Kp ^b , -Js ^a , Js ^b , Ku
Duffy	-Fy ^a , -Fy ^b , -Fy ^y
Kidd	-Jk ^a , -Jk ^b , -Jk ³
Lutheran	-Lu ^a , -Lu ^b
Diego	-Di ^a , -Di ^b
Others	-Hy, -Do ^a , Yt ^a , -Yt ^b , -Co ^a , -Wr ^a , -Ge, -Jr ^a , -Rd, -Livesey, -Fr ^a

pregnancies is about 15%, and about 3% when ABO incompatible. Immunisation can also take place during a late pregnancy, and foetal cells have been found in the maternal circulation in 14% of patients, but in two-thirds of these subjects the haemorrhage had been less than 0.1 ml a volume, often insufficient to cause immunisation except in highly susceptible individuals [3].

Once immunisation has occurred the subsequent clinical course of any further pregnancy can be very variable. Factors such as the phenotype of the father and the titer and IgG subclass [4] of the antibody can affect the outcome of the pregnancy and determine the severity of the disease.

Since widespread Anti D immunoprophylaxis was introduced, the number of new Rh (D) immunisations has been reduced, and most centres now manage a higher proportion of 'non D' immunised patients. Additionally, immunoprophylaxis appears to have selected out a small group of patients who are high responders to antigen immunisation and who have either developed Anti D in their first pregnancy or multiple antibodies. Such patients require very specialised management and there is still morbidity in this group.

Other rhesus antibodies

The D antigen is the most highly immunogenic of the Rh antigens, as even small volumes of Rh D positive cells are reasonably efficient in stimulating Anti D production in a Rhesus D negative individual. The CcEe antigens are far less immunogenic, as in routine transfusion practice many Rh (D) positive persons have been transfused with these antigens, with immunisation rates of only 1 to 2% in susceptible persons even after the infusion of numerous units of red cells. Thus it is not surprising to find that 'non D' Rh antibodies assume less importance in Rh-HDN, although when they present they can at times cause serious clinical problems. Despite their low immunogenicity an appreciable proportion of Rh D immunised women develop other antibodies within the Rhesus system, which is surprising in view of the relatively small volume of red cells entering the maternal circulation during pregnancy and delivery.

Non Rh antibodies in HDN

Although antibodies to antigens in the ABO and Rh system cause 98% of all HDN, antibodies to other blood group antigens can cause disease. If an IgG antibody is detected, examination of the paternal red cells to determine zygosity of the antigen is advisable. Most clinicians now advise that not only Rhesus (D) negative but all pregnant patients should be screened for Rh and other atypical antibodies. Such antibodies, especially if IgG, have the potential for causing HDN and must be investigated with care and the appropriate precautions taken with the patient.

ABO haemolytic disease

It is well recognised that cases of ABO haemolytic disease may go unrecognised unless the disease is considered in the differential diagnosis of neonatal jaundice. Few cases require exchange transfusions. However, in some tropical countries ABO haemolytic disease has been found to be a significant clinical problem and it is recognised that there is a higher frequency of ABO HDN in patients of black or Polynesian extraction as compared with Europeans [5, 6]. In this disease there is a predominance of group O mothers, but the frequency and severity of the disease in infants of group A and B probably does not differ. Antenatal diagnosis and predictions of severity of ABO HDN are unreliable, and diagnosis depends on a high index of clinical suspicion in patients with the appropriate ABO combination along with careful interpretation of cord blood results.

II. Diagnosis of haemolytic disease

Haemolytic disease is manifested in one of the following forms: foetal or neonatal anaemia; icterus gravis neonatorum; hydrops foetalis. In the mildest form, pallor, slight hepatosplenomegaly and anaemia are the only signs. In the most severe cases the foetus may be hydropic and die *in utero* as early as 18–20 weeks of gestation. The excessive quantities of bilirubin produced in the foetus cross

the placenta and are conjugated to direct bilirubin in the mother's liver. After birth, severe icterus neonatorum may develop. Low neonatal levels of glucoronyl transferase prevent adequate conjugation and excretion of bilirubin by the liver and hence results in high plasma bilirubin levels in the neonate. This may lead to deposition of pigment complexes in the basal ganglia of the central nervous system (kernicterus). Lethargy, hypertonia, opisthotonus and spasticity may develop, often resulting in respiratory failure and death. Surviving infants are spastic and mentally retarded.

Prematurity, acidosis, sepsis and dehydration predispose to kernicterus. Keeping serum bilirubin concentration below critical levels by exchange transfusion or phototherapy can prevent this debilitating disease.

Antibody titers

If antibody is detected, the strength of immunisation should be determined by serial dilutions. A dilution of 1:8 or more suggests active Rh immunisation. During the first immunised pregnancy there usually is a good relation between the height of the antibody titer and the severity of the disease, and rise of antibody titer should be taken as a bad prognostic omen. If monthly antibody determinations do not show a rise beyond 1:16, amniocentesis should be omitted [7]. If the patient was immunised previously, the antibody titers do not give reliable information regarding the degree of haemolysis present in the foetus, and unless the antibody is extremely low an amniocentesis is indicated [8].

Amniocentesis

The first amniocentesis is usually performed at 28–30 weeks unless obstetric history or antibody titer suggests that the procedure should be done earlier. Amniocentesis done prior to 20 weeks gestation has little value since therapy before this time will have no effect on the clinical outcome. Subsequent amniocentesis may be necessary depending on the amount of indirect bilirubin in the amniotic fluid (AF). To measure the amount of indirect bilirubin, an 'arbitrary' line is drawn from

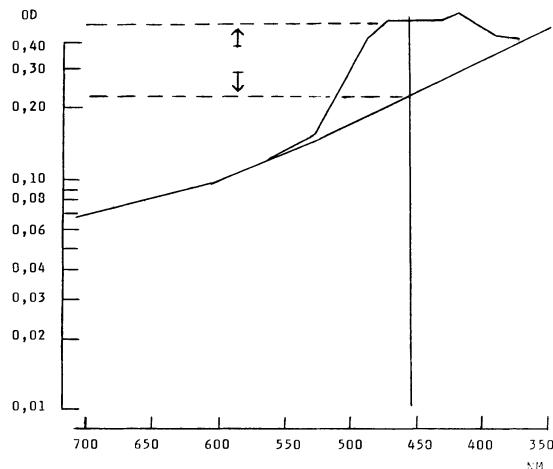


Fig. 1. Spectral absorption curve of liquor: the method of measuring $\Delta\text{OD}450$ is illustrated.

365 nm to 550 nm. The difference between that line and the peak at 450 nm is called $\Delta\text{OD}450$ (Fig. 1).

William Liley of Auckland, New Zealand, was the first to establish that there is a relationship between the duration of gestation and the bilirubin concentration in the amniotic fluid [9]. Liley empirically developed three zones: a lower, a middle and an upper zone. The middle and upper zone were also divided into two. The $\Delta\text{OD}450$ is placed on the graph in one of the prognostic zones according to the duration of gestation (Fig. 2).

The prognosis of the foetus depends on the magnitude of the deviation. If the $\Delta\text{OD}450$ stays in zone 1, the foetus is not at risk and the pregnancy should be allowed to term. A horizontal or rising trend in the lower part of zone 2 can usually be managed by induction of birth at 38 weeks of gestation. Bilirubin values in the upper half of zone 2 signify that the foetus is at risk and pregnancy should be terminated at 34 weeks' gestation. Daily non stress test (CTG) and ultrasound scanning every two days are very useful in determining the 'right moment' of intervention. Horizontal or rising bilirubin trends in zone 3 predict a severely diseased foetus. Between 22 and 32 weeks intrauterine transfusions should be done. After 32 weeks' gestation preterm delivery is advisable.

Several investigators have presented alternative methods of AF analysis, including protein-bilirubin ratios and chemical bilirubin determina-

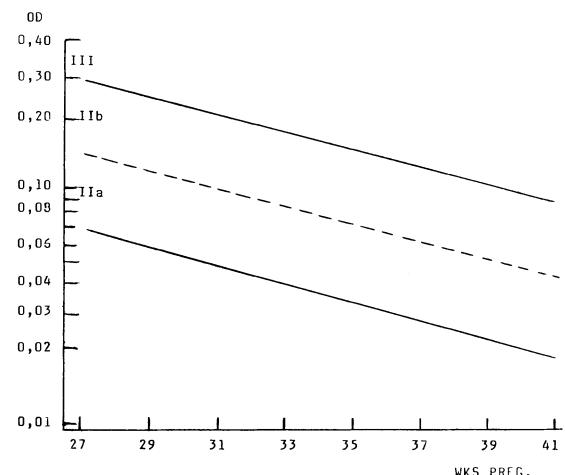


Fig. 2: The significance of the $\Delta\text{OD}450$ peaks recorded at different maturities.

tions [10, 11]. Many modifications of Liley's graph have been proposed, but in the end the Liley method appears to be the most convenient and reliable means for measuring foetal outcome in haemolytic disease.

III. Therapeutic measures in haemolytic disease

Preterm delivery

Although errors still occur, the data obtained from amniotic fluid determinations, ultrasound scanning and antepartum CTG give a sufficient degree of accuracy to be used in determining the optimal time of intervention in severe haemolytic disease. Foetal monitoring, scalp pH determinations and improved neonatal intensive care facilities including exchange transfusions and phototherapy have had a beneficial influence on the survival of preterm infants. In well-equipped neonatal units a baby born alive after 32 weeks of gestation, even when it is suffering from severe HDN, has at least 80% chance of survival.

Intrauterine transfusion

Since the introduction of intrauterine intra-peritoneal transfusion (IUT) by Liley in 1963, refinements in technique and improved neonatal care

have increased the survival rate of infants treated by this procedure [12]. This procedure is based on the fact that red blood cells are absorbed from the foetal peritoneal cavity and enter the circulation. Foetal anaemia may thus be corrected with Rh negative packed cells and delivery postponed to a more mature stage of the pregnancy.

Technique

The introduction of real time sonography has made the IUT procedure much more easy and less dangerous. Radiation exposure can be reduced to a minimum [13]. With real time ultrasound the uterus is scanned to assess foetal position, placental localisation and the presence of foetal oedema or ascites. Posterior position of the spine facilitates the procedure. If the spine is anterior, the foetus sometimes can be moved by external manipulation. If not, it is usually wise to postpone the transfusion.

Visualisation of the enlarged liver, the bladder and the insertion site of the umbilical cord, provides landmarks to guide the needle. A sterile abdominal skin preparation is performed and the patient is covered with a sterile slit drape. In our departments we use a real time transducer containing a slit through which the needle can be accurately guided [14]. The slit forms a sound-free shadow which is projected on the screen and enables accurate positioning of the needle. Under local anaesthesia a 16 gauge blunt needle with a sharp obturator is brought into the foetal abdominal cavity guided by real time ultrasonography. When the foetal abdomen is entered, a dimpling of the foetal skin may be seen. Correct positioning of the needle is checked by fluoroscopy or a radiograph following infusion of a small amount of water-soluble contrast material. A characteristic spread of the contrast between the foetal bowel and along the diaphragm should be observed. Type O negative packed cells with a haematocrit of about 80% are infused through the needle at a rate of 5–10 ml/min. Leucocyte poor, irradiated blood is recommended to prevent possible graft versus host reactions. The blood should be cross matched with the mother's blood. The amount of blood trans-

fused is determined by the formula: (gestation in weeks – 20) × 10 ml [15].

If ascites is present it is wise to remove a quantity of fluid equal to the amount of blood to be transfused in order to prevent excessive increase in intra abdominal pressure which may cause cardiac failure in the foetus. During transfusion, foetal heart rate is continuously monitored and in the case of persistent bradycardia the procedure is stopped. Acceleration of the foetal heart rate is a favourable sign, whereas decelerations and persistent bradycardia are bad omens and usually lead to foetal death.

Complications

Maternal morbidity occurs in 1–2% of the cases but is usually not severe. The major complications from IUTs are infection, trauma to the placenta and preterm labour [16, 17].

The risk of IUT to the foetus is much greater. Foetal death occurring within 48 hours of the procedure is considered to be a direct result of the IUT. In recent series initial mortality rates of 3.6–9.3% are reported [14, 17, 18, 19]. The main risk is puncture of a foetal vessel resulting in exsanguination. Some foetuses die within 2 days of the transfusion, usually from cardiac failure, without traumatic lesions being found.

Survival

Comparison of the reported data of several studies is difficult because differences in indication, technique, experience of the operator and standard of neonatal intensive care are involved. Also, the foetal age and condition at the moment of the first IUT are of major importance to the outcome. In initial published series overall survival rates were 24–56% [12, 14, 21, 22], whereas in recently published studies they were 67–75% [16, 18, 19, 20]. The survival rates in hydropic infants range from 25 to 60% [14, 16, 17]. Larkin presented a small series where foetal transfusions done between 23 and 26 weeks of gestation resulted in a survival rate of 73% [17]. From follow-up studies it appears that the prognosis for quality of life is good and may be com-

pared with that of preterm children of comparable gestation age.

Direct techniques

Intrauterine exchange transfusions using foetoscopy in pregnancies from 16 to 23 weeks of gestation have been described and a direct foetal transfusion via the hepatic part of the umbilical vein under ultra sound guidance has been reported [23, 24, 25]. These techniques are still in an experimental stage, but may have some promise for the future.

Plasmapheresis

Plasmapheresis offers the possibility of removing a volume of plasma, thus reducing the concentration of noxious substances in the maternal circulation. According to several authors, frequent plasmapheresis of large volumes of plasma can significantly lower antibody levels in women carrying babies severely affected by Rh-haemolytic disease. Plasmapheresis is often carried out in combination with IUT [26, 27, 28].

The largest series have been published by Fraser and coworkers of the Bristol/Liverpool group [27]. They report an overall success rate of 61.5% [59–96%]. In one of our clinics 27 severely immunized Rh (D) negative women were treated by small volume (600 ml) plasmapheresis once a week [28]. This scheme of treatment was based on Rubenstein's observation that antibody levels, when measured daily with sensitive auto-analyser techniques, follow a cyclical pattern [29]. During a period of five days the level will gradually decline followed by a rise to the original level. When a smaller amount of antibody is removed, the same pattern is seen but the original level is not reached [29]. Of the 27 treated mothers, four gave birth to a Rh (D) negative infant. Of the remaining 23 Rh (D) positive children 19 survived (56%). Plasmapheresis alone without additional IUT was successful in 12 of 16 pregnancies, resulting in a 75% survival of these children. Since the results of both types of treatment are similar, it is dubious whether there is anything to be gained by carrying out frequent large volume plasmapheresis.

Immunosuppressive drugs

Promethazine hydrochloride. Gusdon and colleagues recently treated 72 severely Rh sensitised patients with doses of promethazine hydrochloride ranging from 25–500 mg daily, beginning as early as 14 weeks gestation. An improvement in perinatal outcome and reduction in the need for exchange transfusions was found [30].

Similar results were obtained by Charles and Blumenthal [31]. Other authors found no beneficial effect of promethazine hydrochloride (in doses of 100–150 mg daily) in treated patients compared with matched controls [32, 33].

Series using higher doses of promethazine have not included prospective controls, so the actual clinical effect of this drug remains controversial.

Steroids. Corticosteroids were used in the 1950's to treat erythroblastosis foetalis. Some beneficial effect was reported in isolated cases but no randomised clinical trials were performed. Maternal administration of dexamethasone in weekly doses of 24 mg over a period of 2–7 weeks to enhance foetal lung maturity showed a gradual decline of the $\Delta OD450$ [34]. It has been suggested that steroid therapy may alter antigen antibody affinity and splenic macrophage sequestration reducing erythrocyte destruction. Changes in amniotic fluid optical density after corticosteroid administration may well be due to alterations in foetal bilirubin metabolism [32]. This treatment could therefore falsely lower the amniotic fluid bilirubin concentration and mislead the clinician.

Oral desensitisation. It has been shown in animal studies that parenteral administration of antigen may result in a failure of subsequent parenteral antigen administration to elicit an immune response by stimulating specific T cells and thus inhibiting secondary immune response [35]. Bierme has administrated capsules containing Rh positive cell membranes in trying to desensitize severely Rh sensitized pregnant patients. The results so far are not convincing and further studies are needed to confirm these findings.

IV. Prevention of Rh (D) immunisation

A parenteral dose of 10–20 μg of anti (D) immunoglobulin offers complete protection where transplacental haemorrhage is 1 ml or less [36]. The routine administration of different doses of anti (D) immunoglobulin, based on the volume of TPH determined by the Kleihauer test, would be highly impracticable. Therefore, in most countries a standard dose of 100–300 μg anti (D) immunoglobuline is administered to every Rh (D) negative mother after the delivery of a Rh (D) positive baby.

Post abortion

The incidence of TPH in spontaneous abortion has been estimated at about 3–6% [37]. In induced abortion the incidence is much higher [37, 38, 39, 40]. There seems to be sufficient evidence to warrant the administration of anti (D) immunoglobulin to Rh (D) negative women following induced and spontaneous abortion. In the first trimester abortion a dose of 50–75 μg seems to be sufficient; in the second trimester abortion a dose of 200–300 μg is recommended [41].

Ectopic pregnancy

Katz and Marcus have pointed out that in ruptured ectopic pregnancies foetal blood is resorbed from the abdominal cavity and enters the maternal circulation [42]. Therefore in Rh (D) negative women, anti (D) immunoprophylaxis is mandatory following an ectopic pregnancy.

During pregnancy

Trials with antepartum administration of anti (D) immunoglobulin have been started in many countries. Data presented at the McMaster University conference in 1977 showed an incidence of Rh immunisation of 0.37% in mothers given antenatal anti (D) prophylaxis compared to 1.53% of the control group. A dose of 300 μg given at 28 weeks of gestation to all Rh (D) negative pregnant women at risk appears to be sufficient [43]. Although the results reported have been good, there is con-

siderable disagreement concerning the advisability of an antenatal prophylaxis program. Such a program would involve a tremendous increase in the cost of Rh prevention. Except for the very few women who lose their first-born child, immunisation occurring during the first pregnancy is generally benign and thus immunoprophylaxis during the first pregnancy will have only a limited value in terms of lowering perinatal mortality due to haemolytic disease of the newborn.

Abdominal trauma and bloodloss in the third trimester of pregnancy

Abdominal trauma, placenta praevia, abruption or bleeding from the margin of the placenta may cause transplacental haemorrhage. If foetal erythrocytes are detected in the maternal circulation by the Kleihauer test, anti (D) immunoglobulin should be administrated [44].

Foetal or intrapartum death, the birth of an anaemic child

When a foetal or intra partum death occurs, it is useful to search for the presence of foetal cells in the maternal circulation. This also applies to anaemia in the newborn baby [44]. In cases where a TPH is found in Rh (D) negative mothers at risk, anti (D) immunoglobulin administration is indicated.

Amniocentesis

Amniocentesis, according to several authors, is associated with an increased risk of TPH and Rh immunisation. In second trimester amniocentesis a dose of 50–100 μg anti (D) immunoglobulin seems sufficient; later in pregnancy a dose of 200–300 μg is recommended [41, 45, 46].

Immunoprophylaxis after inadvertent transfusion of Rh (D) positive blood

There is evidence that administration of an adequate dose of anti (D) immunoglobulin after a Rh incompatible blood transfusion will prevent Rh im-

munisation [47, 48, 49]. For calculation of the anti (D) immunoglobulin dose required, the volume of Rh (D) positive cells in the circulation of the recipient should be determined. A dose of 20 µg anti (D) immunoglobulin per ml red cells found should be administered, preferably intramuscularly. The intravenous route of anti (D) treatment is sometimes recommended especially if a large amount of anti (D) immunoglobulin is needed. Unfortunately more side effects are encountered when anti (D) immunoglobulin is administered by this route [49].

Optimal time of administering anti (D) immunoglobulin

Anti (D) immunoglobulin should be administered within 72 hours of the antigenic stimulus. But even when this arbitrary time limit has been exceeded, immunoprophylaxis should not be withheld. According to some leading investigators, anti (D) immunoglobulin can be given as late as 14 days after foeto-maternal haemorrhage with some evidence that it produces antibody depression [50]. The administration of anti (D) immunoglobulin is generally regarded as ineffective once antibody production has developed. Tovey has suggested the possibility of suppressing early Rh sensitisation and reducing the severity of Rh haemolytic disease in subsequent children by the administration of anti (D) immunoglobulin [50, 51, 52]. The evidence presented so far is not conclusive.

Failure to prevent immunisation

Failure to prevent immunisation is mainly due to first pregnancy immunisation or massive transplacental haemorrhage. Until the postnatal anti (D) immunoprophylaxis program has been fully implemented it seems unjustifiable to start an antenatal program which is likely to increase the cost of immunoprophylaxis 3–4 times. When the loopholes in the postnatal program have been closed, the administration of one dose of 200–300 µg anti (D) immunoglobulin in the 28th week of the first pregnancy to all women at risk as suggested by Tovey and Taverner, might be considered [53]. Massive placental haemorrhage has been reported

after Caesarean section, manual removal of the placenta and traumatic births, and may also occur sometimes after uncomplicated deliveries [36]. Determination of the volume of transplacental haemorrhage and adaption of the anti (D) immunoglobulin dose is suggested to prevent immunisation.

References

1. Davey MG, Zipursky A: McMaster conference on prevention of Rh immunisation. *Vox Sang*, 1979; 36: 50–64.
2. Issitt PD: Serology and genetics of the Rhesus blood group system. Montgomery Scientific Publications, Cincinnati, 1979.
3. Bowman JM: Rh erythroblastosis fetalis. *Haematology*, 1975; 12: 189–207.
4. Schanfield MS: Immunoglobulin (IgG) subclasses and their biological properties. In: *Blood Bank Immunology*, Dawson RB (ed), Washington D.C., American Association of Blood Banks, 1977; 97–112.
5. Bucher KA, Patterson AM, Elston RC, Jones CA, Kirkman HN: Racial difference in medicine of ABO haemolytic disease. *Am J Pub Health*, 1976; 66: 854–8.
6. Woodfield DG: ABO haemolytic disease. *NZ Med J*, 1979; 90: 310–12.
7. Queenan JT: Erythroblastosis fetalis. In: *Management of High Risk Pregnancy*, Queenan JT (ed). Oradell, N.Y., Medical Economics, 1980.
8. Queenan JT: Current management of Rh sensitized patient. *Clin Obstet Gynaec*, 1982; 25: 293–301.
9. Liley TAW: Liquor amnii analysis in management of pregnancy complicated by rhesus sensitization. *Am J Obstet Gynaec*, 1961; 82: 1359–1370.
10. Queenan JT, Goetchel E: Amniotic fluid analysis for erythroblastosis fetalis. *Obstet Gynaec*, 1968; 32: 120–33.
11. Cherry SH, Kochwa S, Rosenfield RE: Bilirubin – protein ratio in amniotic fluid as an index of the severity of erythroblastosis fetalis. *Am J Obstet Gynaec*, 1965; 26: 826–28.
12. Liley AW: Intra uterine transfusion of the fetus in haemolytic disease. *Br Med J*, 1963; 11: 1107–1109.
13. Hobbins JC, Davis CD, Webster J: A new technique utilizing ultra sound to aid in intra uterine transfusion. *J Clin Ultrasound*, 1976; 4: 135–39.
14. Bennebroek Gravenhorst J, Woudenberg J van: Intra uterine foetal transfusion in severe Rh iso immunisation. In: *Paediatrics and Blood Transfusion*, Smit Sibinga CTh, Das PC, Forfar JO (eds). Martinus Nijhoff Publishers, The Hague, 1982: 171–76.
15. Bowman JM: Rh erythroblastosis. *Semin Haematol*, 1975; 12: 189–207.
16. Bowman JM: The management of Rh isoimmunisation. *Obstet Gynaec*, 1978; 52: 1–16.

17. Larkin RM, Knochel JQ, Lee TG: Intra uterine transfusions: new techniques and results. *Clin Obstet Gynaec*, 1982; 25: 303-12.
18. Berkowitz RL, Hobbins JC: Intrauterine transfusion utilizing ultrasound. *Obstet Gynaec*, 1981; 57: 33-36.
19. Clewell WH, Dunne MG, Johnson ML, *et al.*: Fetal transfusion with real time ultrasound guidance. *Obstet Gynaec*, 1981; 57: 516-20.
20. Hamilton EG: Intra uterine transfusion: safeguard or peril? *Obstet Gynaec*, 1977; 50: 255-60.
21. Whitfield CR, Thompson W, Armstrong MJ, Mac Reid M: Intra uterine foetal transfusion for severe rhesus haemolytic disease. *J Obstet Gynaec Br Cwth*, 1972; 79: 931-40.
22. Robertson EG, Brown A, Ellis ML, Walker W: Intra uterine transfusion in the management of severe rhesus isoimmunisation. *Br J Obstet Gynaec*, 1976; 83: 694-97.
23. Rodeck CH, Holman CA, Karnicki J, *et al.*: Direct intra vascular fetal blood transfusion by fetoscopy in severe rhesus isoimmunisation. *Lancet*, 1982; 1: 625-27.
24. Mackenzie IZ, MacLean DA, Fry A, Evans SL: Midtrimester intra uterine exchange transfusion in the fetus. *Am J Obstet Gynaec*, 1982; 143: 555-59.
25. Bang J, Bock JE, Trolle D: Ultra sound guided fetal intra venous transfusion for severe rhesus haemolytic disease. *Br Med J*, 1982; 284: 373-74.
26. Clarke CA, Bradley J, Elson CJ, Donohoe WTA: Intensive plasmapheresis as a therapeutic measure in rhesus immunised women. *Lancet*, 1970; 1: 793-98.
27. Fraser ID, Bennet MO, Bothamley JE, Airth GR: Intensive antenatal plasmapheresis in severe rhesus isoimmunisation. *Lancet*, 1976; 1: 6-8.
28. Eernisse JG, Bennebroek Gravenhorst J: Prevention of severe haemolytic disease of the newborn by weekly small volume plasmapheresis during pregnancy. In: *Paediatrics and Blood Transfusion*, Smit Sibinga CTh, Das PC, Forfar JO (eds). Martinus Nijhoff Publishers, The Hague, 1982, 164-70.
29. Rubinstein P: Cyclical variations in anti Rh titer detected by automatic quantitative haemagglutination. *Vox Sang*, 1972; 23: 508-22.
30. Gusdon JP: The treatment of erythroblastosis with promethazine hydrochloride. *J Reprod Med*, 1981; 26: 454-58.
31. Charles AG, Blumenthal LS: Promethazine hydrochloride therapy in severely Rh sensitized pregnancies. *Obstet Gynaec*, 1982; 60: 627-30.
32. Caudle MR, Scott JR: The potential role of immunosuppression plasmapheresis and desensitisation as treatment modalities for Rh immunisation. *Clin Obstet Gynaec*, 1982; 25: 313-19.
33. Stenchever MA: Promethazine hydrochloride: use in patients with Rh isoimmunisation. *Am J Obstet Gynaec*, 1978; 130: 665-68.
34. Navot D, Rozen E, Sadovsky E: Effect of dexamethasone on amniotic fluid absorbance in Rh sensitized pregnancy. *Br J Obstet Gynaec*, 1982; 89: 456-58.
35. Bierme SJ, Blanc M, Abbal M, Fournie A: Oral Rh treatment for severely immunised mothers. *Lancet*, 1979; 1: 604-5.
36. Zipurski A: Rh haemolytic disease of the newborn, the disease eradicated by immunology. *Clin Obstet Gynaec*, 1977; 20: 759-72.
37. Mathews JH, Mathews AEB: Transplacental haemorrhage in spontaneous and induced abortion. *Lancet*, 1969; 1: 694-95.
38. Voigt JC, Britt RP: Feto maternal haemorrhage in therapeutic abortion. *Br Med J*, 1969; 4: 395-96.
39. Lakoff KM, Bolognese RJ, Corson SL: Transplacental haemorrhage during voluntary interruption of pregnancy. *J Reprod Mod*, 1971; 6: 19.
40. Hensleigh PA, Dixon LW, Hall E, Kitay DZ, Jackson JE: Reduced dose of Rh (D) immuno globulin following induced first trimester abortion. *Am J Obstet Gynaec*, 1977; 129: 413-16.
41. Bennebroek Gravenhorst J: Prevention of Rhesus (D) isoimmunisation after abortion. In: *Second Trimester Pregnancy Termination*, Keirse MJNC (ed). Leiden University Press, The Hague, 1982: 168-73.
42. Katz J, Marcus RG: The risk of Rh isoimmunisation in ruptured tubal pregnancy. *Br Med J*, 1972; 3: 667-69.
43. McMaster Conference on prevention of Rh immunisation. September 1977. *Vox Sang*, 1979; 36: 50-64.
44. Renaer M, Putte I vd, Vermijlen C: Massive feto maternal haemorrhage as a cause of perinatal mortality and morbidity. *Eur J Obstet Gynaec*, 1976; 6: 125-40.
45. Blajchman MA, Mandsley RG, Uchida I, Zipursky A: Diagnostic amniocentesis and fetal maternal bleeding. *Lancet*, 1974; 1: 993-94.
46. Hill LM, Platt LD, Kellogg B: Rh sensitisation after genetic amniocentesis. *Obstet Gynaec*, 1980; 56: 459-61.
47. Keith L, Berger GS, Pollack W: The transfusion of Rh positive blood into Rh negative women. *Am J Obstet Gynaec*, 1976; 125: 502-5.
48. Bowman H: Effectiveness of prophylactic Rh immuno suppression after transfusion with D-positive blood. *Am J Obstet Gynaec*, 1976; 124: 80-84.
49. Emker J, Maas DHN, Schneider J: Immunoprophylactic anti Rh (D) treatment after mismatched transfusions. *Eur J Obstet Reprod Biol*, 1979; 9: 117-24.
50. Samson D, Mollison PL: Effect on primary Rh immunisation of delayed administration of anti Rh. *Immunology*, 1975; 28: 349-57.
51. Tovey LAD, Scott JS: Suppression of early rhesus sensitization by passive anti D immunoglobulin. *Vox Sang*, 1980; 39: 149-52.
52. Tovey LAD, Robinson AE: Reduced severity of Rh haemolytic disease after anti D immuno globulin. *Br Med J*, 1975; 4: 320-22.
53. Tovey LAD, Taverner JM: A case for the antenatal administration of anti D immunoglobulin to primigravidae. *Lancet*, 1981; 1: 878-881.

12. Immune plasma for immunoglobulin

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Introduction

Although the organisation of transfusion services varies from country to country, and indeed some countries remain partially or wholly dependent on commercial organisations for immunoglobulin products, there are certain common paths which all producers must follow.

Immunoglobulin, normal and specific. Any transfusion service approaching self-sufficiency for factor VIII and albumin will most probably be fractionating 8 kg of plasma or more per thousand population per annum [1]. The supply of normal immunoglobulin will be greater than any reasonable demand. Because of the different infective agents prevalent in different countries it is generally preferable to use indigenous plasma to prepare normal immunoglobulin (Table 1).

Immunoglobulin concentrates have a number of advantages over plasma. They contain an antibody concentration 20 to 25 higher than that of plasma, permitting intramuscular injection. They have a well-documented but unexplained reduction in hepatitis risk [2]. The batch mode of production allows single dose vials of a well-standardised product to be produced and the concentrates are stable for long periods at 4°C.

In certain clinical circumstances an immunoglobulin product is required to contain a very high level of a specific antibody [3]. The specific immunoglobulins produced from high titre plasma will, of course, contain the other antibody activities in concentrations similar to those in normal immunoglobulin.

Production target. Some rational projection must be available to indicate the likely demand for a particular specific immunoglobulin preparation. Similarly, there must be some indication of the potency required in the final product.

Processing losses may range from 60% to 90% of the initial activity. With experience it is possible to arrive at a working estimate of the plasma requirements of a community for each of the specific immunoglobulins. The clinical demand for an immunoglobulin product does not necessarily reflect either the incidence of infection in the community or the prevalence of high titre immunity.

Screening, collection and local conditions. The first essential is to establish the prevalence of high titre antibody in the donor community. Once the availability of the antibody can be estimated, decisions can be made on *methods of plasma collection*. The choice between harvesting plasma from whole blood donations and using plasmapheresis depends on a number of variables which will be discussed later.

If an immunogen is available and is suitable for hyperimmunising donor volunteers many of the problems can be avoided. Small volumes of highly potent antibody can be collected quite quickly with low testing costs and low collection costs. Boosting of donors does have problems and in particular the ethics must be carefully considered [3].

Table 1. Estimated specific immunoglobulin and plasma requirements in the population.

Specificity	Dose	Doses/10 ⁶	Plasma required/10 ⁶	Acceptable titre	Screening method
Anti-D	500 iu	1500	150 kg	50 iu/ml	Autoanalyser
Anti-tetanus	250 iu	1000	250 kg	10 iu/ml	CIEP
Anti-HBs	500 iu	50	25 kg	10 iu/ml	RIA

Note: Anti rubella 10,000 iu/vial made from plasma containing 350 iu/ml. Demand is unknown.

Anti vaccinia 2,000 iu/vial is made from plasma containing 25 iu/ml. Demand is unknown.

Anti zoster and anti CMV are not fully standardised and demand is not clearly known. However, fractionators should compare the potency of new products with ones already in use.

Reference assays

If possible the screening assay should be calibrated against a reference method. The best reference methods are animal protection methods such as are used for anti-rabies and anti-tetanus or *in vitro* neutralisation tests which are more precise and more humane. These assays (*in vivo* or *in vitro*) have the outstanding advantage that they measure only protective antibody activity. In the course of an infection, antibodies are produced to a variety of antigenic determinants, many of which are irrelevant to the biological properties of the pathogen. A neutralisation test is the best test (short of human experimentation) of the ability of the donor's plasma or IgG to interfere with the disease process in a non-immune individual. This issue has been emphasised because it has important practical bearings on the screening assays used.

A recent multi-centre study within the Scottish Transfusion Service compared counter immuno electrophoresis (CIEP), passive haemagglutination (HA) and radial immunodiffusion (RID) for anti-tetanus against each other and against the mouse protection assay. After allowance for a degree of imprecision in a protection assay using a small number of animals at each dose level, it was found that each of the assay methods occasionally produced results totally different from the consensus. Thus a screening assay should not only be able to give a comparable dose response curve on dilutions of a standard to that of the reference assay, it should also produce a minimum number of unacceptably bad results.

For many antibody activities, neutralisation

methods are either unavailable (e.g. anti-hepatitis B) or excessively difficult (e.g. anti-zoster). This makes validation more difficult. In the case of anti-HBs this difficulty has been overcome by the radioimmunoassay of Gerrey and Barker [4]. Their assay uses a commercial anti-HBs test kit to test three replicate dilution series of standard and unknown. It achieves a high level of precision, and products assayed by their method are satisfactory in use.

However, it is possible that some of the antibody measured has no protective activity. We have found modest concentrations of anti-HBs in the serum of HBsAg carriers on a number of occasions.

The reader will not be surprised by the fact that in many cases plasma screening assays can only be validated by the clinical performance of the immunoglobulin product. Zaia and his colleagues [5] set out to determine whether there was any difference between a zoster immunoglobulin prepared only from the plasma of varicella convalescents and a zoster immunoglobulin prepared from the plasma of blood donors with persistent high titre antibody. The latter was found marginally better, which is fortunate as we have found varicella convalescents a very poor source of high titre plasma as have Heijas *et al.* [6].

Screening assays

As indicated before, these must be validated by reference methods (if available) and by the usefulness of the final product. In addition they must be *inexpensive* and *robust* enough for large-scale use,

they must be at least *semi-quantitative* and they must be reasonably *precise* at the high antibody concentration being studied. If the assay is imprecise, some substandard plasmas will be submitted for fractionation while others which are just acceptable will be rejected. This will not seriously affect the overall quality of the plasma but donors may be recruited for plasmapheresis and then suspended from the programme because of the vagaries of the assay.

Because of the labour involved in working with high dilutions of serum it is often useful to select a test which is intrinsically insensitive but which uses neat serum. To minimise the amount of plasma held awaiting tests, the screening tests should ideally be completed the day after collection.

A further consideration is the mixture of methods in a laboratory. We find it economic to screen for anti-HBs with a home-made inhibition radioimmunoassay (RIA) based on spare reagents from HBsAg screening. However, for tetanus antibody we screen by CIEP. This avoids overloading our RIA capacity. Other laboratories will prefer to use a single technology. Our colleagues in the Protein Fractionation Centre are able to provide a wide range of screens for viral antibodies which are done almost entirely by immunofluorescence [7]. If automated multi-channel sample dilutors are available [8] it is possible to do a large number of different tests on each specimen by enzyme immunoassay.

The donor population

Once a test has been established it is necessary to screen large numbers of donors to determine how commonly high titre antibody is present. We have found in the west of Scotland that for a number of antibodies (e.g. tetanus, rubella and CMV) 3–5% of donors are suitable. With other antibodies such as anti-HBs, suitable donors are extremely uncommon, about 0.1%. If possible, subgroups within the community should be studied in the hope that they may have a higher prevalence of potent antibody. This policy has been described elsewhere [9].

Because the performance of any test varies be-

tween laboratories and because the performance of fractionation centres is not standard it is not possible to define the level of antibody which is acceptable. Some generalisations can be made. The plasma should contain, if possible, 10% or more of the antibody concentration required in the final product. A specific immunoglobulin product should contain at least five times the antibody titre found in normal immunoglobulin [10], but this figure is a minimum and useful products are generally much more potent.

Methods of collection

As we mentioned before the choice of method depends on the nature of the resources available. When there is extreme pressure to collect large amounts of antibody from a limited panel of donors, the arguments in favour of boosting may outweigh the ethical objections. The specific advantages and disadvantages will be discussed for each method but the reader must remember that the correct policy for a service will depend at least as much on the facilities and expertise available as on theoretical considerations.

Whole blood. As an antibody collection programme must start with screening, it follows that the plasma from the donations used for screening should be kept unpooled until the assay results are to hand. Screening may be continued and used as the source of continuing supplies of antibody. When an antibody is known to be one of those where titres fall rapidly it is more effective to screen donations than to attempt to recruit donors for plasmapheresis.

Where an antibody is uncommon, like anti-HBs, large-scale screening is also required. In this case it is necessary to supplement supplies by plasmapheresis. However, it will not be possible to plasmapheresis all high titre donors, and it is an advantage to have a computer record which identifies their subsequent blood donations [11].

Plasmapheresis. This method is more costly than whole blood collection. However, it is often

cheaper to collect 500 ml of plasma by double plasmapheresis than to collect *two* donations of whole blood, especially if the red cells are not needed. The procedure has now been automated. The cost of the machines, and that of the disposables has to be set against the shorter time spent by each donor on the couch.

Although we have experimented with mobile plasmapheresis teams using facilities at outlying hospitals [12], we find that plasmapheresis is most appropriate for those donors who live or work within easy travelling distance of our donor centre.

A variable proportion of plasmapheresis donors will show falling titres. These donors must be removed from the programme to ensure that only donors with persistent high titres are plasmapheresed. The rate of fall depends on the antibody and anti-HBs tends to persist for many years.

When the plasmapheresis capacity is fully utilised there is a range of options. Extra antibody can be collected from whole blood donations at the cost of more screening. Exact quantitation of antibody can allow adequate supplies of some antibodies to be collected from a smaller number of donors by selecting those with the highest titres. Automated plasmapheresis may be cost-effective in allowing existing staff to collect more plasma in the same time. If none of these options is acceptable the programme must be expanded or donors must be boosted.

Boosting. This is an area where opinions differ particularly widely. The issues are explained in a World Health Organisation publication [2].

In general, the risks of immunisation are justified by the expected advantage to the patient, but hyperimmunisation (for instance to tetanus) involves producing antibody titres of the order of a thousand times higher than those required for immunity. The risks of vaccine are recognised [13] to be higher in hyperimmune individuals, and the majority of reactions to tetanus toxoid which we have studied have been in inadvertently hyperimmunised patients. Before boosting can be permitted there must be a well-documented need for the immunoglobulin product, and the standards of safety required of the vaccine may be stricter than

those imposed by the national licencing authorities for general clinical use.

It is best to consider an extreme example – anti-D immunoglobulin. There can be no doubt that post natal anti-D immunoglobulin, given according to accepted criteria, is the principal means of preventing Rhesus haemolytic disease of the newborn. The more effective the anti-D programme becomes, the fewer accidentally immunised donors will be available. The Rhesus negative donor volunteer has no hope to benefit from immunisation. The blood donor who volunteers to have booster injections to produce anti-D takes on a number of risks. Future transfusions may be made more difficult especially if non Rhesus antibodies are accidentally stimulated. Fisher [14] has reported failures of sterilisation leading to accidental pregnancy in female donors whose anti-D had been boosted. However carefully the boosting cells are chosen there is always a remote risk of transmitting hepatitis and other infections.

Other risks can be minimised by restricting the plasma collected to 13 litres per year and by monitoring plasma protein levels. The number of boosting injections given to each donor must be strictly limited. The practice of hyperimmunising donors is justified on the grounds that there is no alternative source of anti-D.

Conclusion

No difficulty should be encountered in providing supplies of normal immunoglobulin. The demand for specific immunoglobulin products should be estimated as should the prevalence of high titre antibody in the donor population. Local circumstances will dictate the most appropriate method of plasma collection. Intentional hyperimmunisation is the only way of obtaining some anti-bodies in sufficient quantities.

References

1. Hassig A, Lundsgaard-Hansen P: The procurement of blood and plasma for the production of components and

- derivatives within the frame of an integrated national blood program. *Vox Sang*, 1978; 34: 257–60.
2. Watt J: Epidemic hepatitis B caused by commercial human immunoglobulin. *Lancet*, 1979; 1: 1399–1400.
 3. World Health Organisation. The collection, fractionation, quality control, and uses of blood and blood products. Geneva WHO, 1981.
 4. Barker LF, Gerety RJ, Lorenz DE, Rastogi SC, Seligman EB: Biological standardization in viral hepatitis. In: *Viral Hepatitis*, Vyas GN, Cohen SN, Schmid R (eds). Philadelphia Franklin Institute Press, 1978: 581–87.
 5. Zaia JA, Levin MJ, Preblud SR: The status of passive immunisation for herpes virus infections. In: *Immuno-globulins: Characterisations and Uses of Intravenous Preparations*, Alving BA, Finlayson JS (eds). United States Dept. of Health and Human Services, Washington D.C., 1980: 111–21.
 6. Heijas M, Salkier R, Barbara JA: Screening blood donors for high titre antibody to herpes varicella zoster. *Vox Sang*, 1980; 39: 335–38.
 7. Cuthbertson B: PhD thesis, University of Glasgow, 1982.
 8. Barbara JA: In: *Proceedings of the Second Hepatitis Workshop*, Stirling, Scotland, 1 – 3 September, 1982. Nuclear Enterprises 1983.
 9. Crawford RJ: Immunoglobulin procurement. *Biotest Bulletin*, 1982; 1: 119–24.
 10. World Health Organisation Memorandum. Appropriate use of human immunoglobulin in clinical practice. *Bulletin of the World Health Organisation*, 1982; 60: 43–47.
 11. Crawford RJ: In: *Proceedings of the Second Hepatitis Workshop*, Stirling, Scotland, 1 – 3 September, 1982. Nuclear Enterprises 1983.
 12. Crawford RJ, Morgan M, Mitchell R: A regional programme to collect plasma for preparation of human immunoglobulin anti-rabies. *J Clin Path*, 1980; 33: 180–82.
 13. Edsall G, Elliott MW, Peebles TC, Levine L, Eldred MC: Excessive use of tetanus toxoid boosters. *JAMA*, 1967; 202: 111–13.
 14. Fisher MM: Pregnancy in anti-D donors. *Lancet* 1982; 2: 618.

Part Two

Clinical practice

1. Blood and blood components: uses and abuses

M.E. CONRAD

‘... for the blood is the life ...’ [1]

Blood has been associated with mysticism and pseudoscience for centuries. The letting of blood of either animals or humans was used by many ancient societies as a sacrificial offering. Transfusion was described by Ovid as a means of restoring youthful vigor. Blood baths were employed by the Egyptians to treat elephantiasis and the drinking of blood of dying gladiators was tried to cure epilepsy [2]. The Bible contains prohibitions against the consumption of blood which continue to be the basis of certain kosher laws for the preparation of meat [3] and which are also cited by certain religious groups as a moral reason to refuse blood transfusion.

While there are a number of anecdotal reports of blood transfusion during the fifteenth and sixteenth century, the first public demonstration of transfusion is credited to Richard Lower [4, 5]. In 1665 he showed the Royal Society in London that he could resuscitate exsanguinated dogs by transfusion from canine donors. Jean Denis, a staff physician of Louis XIV, read Lower’s experiments and performed the first documented human transfusions [6]. He used bovine blood and provided the first description of a hemolytic transfusion reaction. Denis’ practices led to a ban of transfusion by France, England and the Pope [7]. Transfusion remained dormant until the early nineteenth century, when it was revivified by James Blundell, an English obstetrician for the treatment of postpartum hemorrhage [8]. He appreciated the importance of using species-specific blood and is usually credited with first transfusing humans with human blood. Subsequently considerable debate regard-

ing the risk-benefit ratio occurred as transfusion was employed more widely [9]. Keeping blood in a liquid form while it was being transferred from one human to another was recognized as a major problem. This decreased the volume of blood which could be transfused and limited storage time and exposure to foreign surface. These limitations diminished mortality.

The modern era of blood transfusion began with the discovery of the ABO blood groups by Landsteiner at the turn of the century and the development of sodium citrate as an anticoagulant at the beginning of World War I [10, 11]. Citrated stored type O blood was used by both British and U.S. forces for resuscitation of wounded soldiers in the World Wars [12, 13]. During the interval between the two World Wars, developments in blood banking were slow. In the Spanish Civil War, the practicability of supplying wounded men near the battlefield with stored blood and plasma secured from a civilian population was demonstrated [14]. The British Ambulance Unit in Spain obtained information which was valuable in establishing the British Blood program for World War II [14]. The high priority placed upon the research and development of blood programs and transfusion first by Britain and later by the United States led to idealized storage solutions and conditions, recognition of complications such as hepatitis and non-ABO blood group reactions and was responsible for making the transfusion of blood and its components an important therapeutic modality on a worldwide basis [13].

The impetus established in World War II for

development of better and safer programs of blood transfusion has not abated. The development of the antiglobulin (Coombs) test [15], the discovery of a multiplicity of blood group systems, identification of reactions caused by white blood cells, platelets and plasma; the discovery of hepatitis B antigen (HB_sAg) have each added methods of improving the safety of transfusion due to basic scientific discoveries. Technologic developments such as automation of records and laboratory equipment improved quality and diminished error. The substitution of plastic for glass containers, the development of newer blood preservative solutions which provide a better product and longer shelf life, the exclusion of non-volunteer donors, the implementation of component therapy and the advent of quality control programs and minimum performance standards with associated inspection programs have each added to the safety of blood transfusion and the quality of the product.

Most vices are virtues carried to an extreme'

The capability to transfuse blood and its components is one of the major achievements of modern medicine. It has markedly diminished the mortality of trauma and prolonged and improved the quality of life of numerous people with a multiplicity of disorders. Surgical procedures and medical therapies have been developed which could not exist without the availability of blood and blood products for transfusion. Indeed, these benefits have been so great that physicians often fail to consider the limitations and hazards of transfusion therapy and tend to indulge in overuse.

Blood transfusion is not a definitive therapy. It is short-lived transplantation of one or more blood elements to improve a deficiency state. It is most valuable in providing temporary support of a patient until an underlying problem can be resolved. When transfusion therapy is used chronically, it is generally because definitive treatment is unavailable. The hazards of transfusion are appreciable and obligate the clinician to make careful clinical judgments regarding anticipated benefits versus possible complications. The risk of transfusing a single

unit of whole blood is comparable to the mortality encountered with an exploratory celiotomy. As advances have occurred in medicine and surgery, the safety of each procedure has improved in proportion to one another. Even in the best of circumstances, certain hazards remain. Therefore, transfusions should be avoided whenever this is reasonable and when they cannot, the fewer the better.

In addition to avoiding and discouraging the use of unnecessary transfusions, the clinician can diminish the complications of transfusions in other ways. The use of appropriate blood components rather than whole blood is not only in the best interest of the patient but also the entire community. It diminishes the probability of an undue reaction to transfusion of an unneeded blood component and makes components which are not required to treat one patient's deficiency available for others. Minimizing the number of units cross-matched in blood banks for potential but improbable use diminishes the chance of administrative and laboratory error. Careful labeling of pilot tubes and proper identification of recipients decrease the probability of a transfusion reaction. Proper preparation and care of the skin of the recipient at venipuncture and infusion sites will diminish bacterial complications. Surveillance of the patient during and following transfusion can markedly lessen the morbidity and mortality of adverse reactions. Early identification of a reaction with cessation of transfusion can be lifesaving. Blood containers which may have been involved in a reaction must be saved and appropriate laboratory studies initiated to identify the etiology of the reactions so that specific therapy can be initiated promptly. Careful documentation of clinical observations, laboratory data and facts but not surmises should be incorporated into the clinical record. This is not only beneficial for the patient but also for proper use in any litigation. The victim of a transfusion reaction is owed a factual description of the complication from his physician. In my experience, obfuscation encourages and justifies litigation rather than averting it.

Many physicians assume that blood banking is not their responsibility. This apathy on the part of

practicing physicians discourages donor recruitment and maintenance of quality personnel in the areas of blood recruitment and processing. In the United States it unduly prolonged the development of a totally volunteer blood program and permitted the transfusion of millions of units of blood containing hepatitis virus which could have been avoided during the last quarter century. Solely from a selfish viewpoint, involved knowledgeable physicians are recognized by blood bank staffs and this encourages efficient delivery of better and appropriate services for their patients.

Uses

In general, the transfusion of blood and blood products should be reserved for situations in which the benefits clearly outweigh the risks. Usually component therapy is preferable to the use of whole blood because it diminishes complications and is economic by saving components which are not needed by one patient for use in another with a different specific need.

Hemorrhage is probably the most clearcut indication for blood transfusion. While the acute loss of a liter of blood from an adult can usually be managed by restoration of an adequate blood volume with saline or plasma expanders, greater losses require the administration of blood. Massive hemorrhage is the most important legitimate use for whole blood transfusion today. Replacement of both red blood cells and blood volume are needed to resuscitate the patient in hypovolemic shock. While packed red cell preparations may be re-expanded with albumin or plasma, this significantly increases cost and in the latter instance doubles the risk of developing hepatitis. Chronic blood loss can often be managed by iron therapy, and when this is inadequate because of the rate of bleeding by the use of packed red cell preparations.

Anemia is probably the most common reason for transfusing blood but the indications for transfusion are often not as clearcut as they may seem. There is no established hematocrit or hemoglobin concentration at which transfusion should be automatically employed. Younger patients with chronic

anemia tolerate hemoglobin concentrations of 7 g/dl without much deficit in performance. On the other hand, people with coronary insufficiency, chronic pulmonary disease, fever or an acute anemia may become symptomatic such that they require transfusion at a higher hemoglobin concentration. Generally, transfusion should be employed for anemia when it is sufficiently profound to produce symptoms and/or physical findings and is not correctible by other more specific method of therapy (vit. B 12, folate, iron for specific deficiency states). Packed red cell preparations and not whole blood should be used to treat chronic anemias unassociated with massive blood loss. The use of whole blood increases the risk of circulatory overload and pulmonary edema in addition to wasting a valuable resource.

Exchange transfusion is an established therapeutic procedure for hemolytic disease of the newborn. This is another situation in which whole blood is indicated rather than the use of reconstituted components. Exchange transfusion has been extended to other situations to include a few forms of poisoning, infants with respiratory distress syndrome, Reyes' syndrome and the treatment of disseminated intravascular coagulation in infants. Use remains experimental in most of the latter conditions and controlled clinical trials are needed.

Bone marrow suppression is a rationale for the use of packed red cell transfusions in patients with severe complications of sickle cell disease during pregnancy or prior to a major surgical procedure. Transfusion of normal red blood cells suppresses the production of the patient's abnormal cells and prevents the complications associated with sickle cell crisis. Increasing usage of chronic transfusion therapy to maintain a hemoglobin of 10-11 g/dl is being employed in thalassemic children and to a lesser extent in sickle cell disease to permit more normal growth and development with fewer complications. Unfortunately, this mode of therapy produces iron overloading which must be counterbalanced with chronic chelation therapy.

Platelet transfusions are most useful in the treatment of bleeding patients with non-immune thrombocytopenia. These patients can usually be identified by the virtual absence of megakaryocytes in

the bone marrow. In immune thrombocytopenias, transfused platelets usually rapidly disappear from the circulation and are of questionable benefit, their use is usually reserved for the treatment of serious and life-threatening hemorrhagic events in this circumstance. An exception to this dictum are newborn infants with immune neonatal thrombocytopenia because maternal platelets will survive normally in the infant. Beneficial results have been ascribed to the use of prophylactic platelet transfusions in patients with acute leukemia and platelet counts less than $20 \times 10^9/l$ either before or during induction therapy. However, most patients develop alloantibodies with repeated platelet transfusions and then have a shortened survival of transfused platelets. This probably markedly decreases the efficacy of the donor platelets and has led some physicians to question the use of prophylactic platelet transfusions in the non-bleeding patient. The development of alloantibodies can be decreased by obtaining platelets from single donors by thrombapheresis rather than using platelets harvested from single units of blood (random donors) because it decreases the number of antigenic exposures.

Granulocyte transfusions have been advocated for the treatment of the infected severely neutropenic patient. The use of a genetically similar donor (HLA compatible) for granulocyte transfusions markedly increases the duration of survival of the transfused cells. For obtainement of sufficient numbers of granulocytes from normal donors, leukapheresis must be employed. While the transfusion of granulocytes may aid in combination with appropriate antibiotic therapy in the treatment of bacterial infections in the severely granulocytopenic patient, it is associated with an enhanced transmission of cytomegalovirus infection and possibly other viruses [16].

In the past, plasma was used mainly as a volume expander for the treatment of acute traumatic shock. However, whole blood is usually the therapy of choice and other plasma expanders have largely replaced plasma in an emergency until whole blood can be obtained. Fresh frozen or freeze dried plasma is a useful source of labile coagulation factors (factors V and VIII) and the

treatment of deficiencies of the coagulation system has become one of the major uses of this blood component. Plasma also has been used in the therapy of hypogammaglobulinemia, as a source of C1 esterase inhibitor in the treatment of angio-neurotic edema and for the treatment of thrombotic thrombocytopenic purpura. Whenever possible, units of plasma from single donors should be employed rather than pooled plasma because this markedly reduces the number of donor exposures for the recipient and as a result diminishes the risk of contracting viral hepatitis and other infections transmitted by blood.

Plasma fractions are in general preferable to the use of whole plasma because they usually contain the desired component in higher concentration and thus reduce problems associated with volume overload. Most plasma fractions are prepared by a cold ethanol method developed by Cohn during World War II. Except for albumin (or plasma protein fraction, PPF) and immune human serum globulin, all blood and plasma fractions are capable of transmitting viral hepatitis and possibly other disorders such as acute immune deficiency syndrome (AIDS) [17]. Albumin has been used for the treatment of patients in shock due to hemorrhage, trauma or burns. It has been employed for the treatment of hypoalbuminemia due to cirrhosis or chronic renal disease but is only of transient value. Immuno-globulins represent another plasma fraction which have been useful in patients with specific immune deficiency disorders and also as a method for passive protection against certain viral disorders such as hepatitis. Cryoprecipitate and plasma concentrates are employed in the prophylaxis and treatment of patients with specific coagulation disorders such as the hemophilias and von Willebrand's disease. Recent reports of the transmission of acute immune deficiency syndrome (AIDS) in association with the use of factor VIII concentrates have led to more conservative use in the last year and the substitution of cryoprecipitate from single donors rather than the use of pools prepared from a large number of donors. Plasma enzymes are being isolated, which would be valuable in providing specific therapy for patients with deficiency states.

References

1. The Bible: Deuteronomy, 12: 22.
2. Brown HM: The beginnings of intravenous medication. *Ann Med Hist*, 1917; 1: 177.
3. The Bible: Leviticus, 17: 11-12.
4. Maluf MFR: History of blood transfusion. *J. Hist Med*, 1954; 9: 59.
5. Lower R: *Philos Trans R Soc Lond*, 1666; 1: 353.
6. Denis J: *Philos Trans R Soc Lond*, 1667; 3: 489.
7. Hoff HE, Guillemin R: The tercentenary of transfusion in man. *Cardiovasc Res Center Bull*, 1967; 6: 47.
8. Blundell J: Some account of a case of obstinate vomiting in which an attempt was made to prolong life by the injection of blood into the veins. *Med Chir Trans*, 1819; 10: 296.
9. Routh C: Remarks statistical and general on transfusion of blood. *Med Times*, 1849; 20: 114.
10. Landsteiner K: Über agglutinationserscheinungen normalen menschlichen blutes. *Wien, Klin, Wochenschr*, 1901; 14: 1132.
11. Weil R: Sodium citrate in the transfusion of blood. *JAMA*, 1915; 64: 425.
12. Robertson O: Transfusion with preserved red blood cells. *Br Med J*, 1918; 1: 691.
13. Kendrick DB: Blood Program in World War II. Office of the Surgeon General. Dept of the Army, Wash, D.C., 1964.
14. Saxton RS: The Madrid Blood Transfusion Institute. *Lancet*, 1937; 2: 606.
15. Coombs RRA, Mourant AE, Race RR: Rh agglutinins. *Br J Exp Pathol*, 1945; 26: 225.
16. Rook AH, Quinan GV: Cytomegalovirus infections following blood transfusion. In: *Infections Complications of Blood Transfusions*, Tabor E (ed.) Acad. Press, New York, 1982: 45-59.
17. Curran JW, Lawrence DN, Jaffe H: Acquired immuno deficiency syndrome (AIDS) associated with transfusions. *N Engl J Med*, 1984; 310: 69-75.

2. Massive transfusion and its effects in clinical practice

L.C. STEHLING and H.L. ZAUDER

Management of the exsanguinating patient involves more than the administration of blood. In the majority of cases blood loss is due to disruption of vascular integrity. The goal of the surgeon is identification and treatment of bleeding sites. The anesthesiologist is concerned with maintenance of circulating blood volume and delivery of oxygen to the tissues. Blood bank personnel are occupied with provision of adequate supplies of compatible blood. Consultation with a hematologist or clinical pathologist is often indicated if disorders of coagulation are to be diagnosed and treated appropriately. Unfortunately, this team approach is infrequently employed. More often, transfusion therapy is administered on the basis of emotion and habit.

Massive transfusion is arbitrarily defined as the administration of one blood volume equivalent within 24 hours. The rapidity with which replacement is required as well as the total amount infused govern many of the decisions to be made and the complications which occur. Several theoretical complications such as hyperkalemia and acidosis do not, in fact, occur. However, administration of the wrong unit of blood and transmission of disease are life-threatening problems which influence morbidity and mortality.

Infusion of crystalloid and colloid solutions

Restoration and maintenance of circulating volume by the administration of crystalloid or colloid solutions is critical to the survival of patients who

require massive transfusion. Controversy over the relative advantages and disadvantages of the two types of solutions is extensive, heated and likely to continue. Proponents of crystalloid therapy point out that saline and Ringer's lactate solution are inexpensive, readily available, and easy to administer. Unlike colloids containing complex carbohydrates, crystalloids are not antigenic and do not affect coagulation. Colloids are administered in an attempt to restore plasma oncotic pressure. The amount required is one-fourth to one-half that of crystalloid and the effect is longer-lasting. The controversy revolves around whether either solution is better for maintaining hemodynamic stability or is associated with a lower incidence of pulmonary or renal dysfunction. Despite numerous clinical reports claiming the superiority of one or the other, they are probably equally effective and associated with a similar incidence of organ failure. Maintenance of tissue perfusion depends upon the speed, adequacy and completeness of resuscitation, not the type of acellular fluid administered.

Whole blood versus packed cells

The second great debate involves the choice of red cell product. Whole blood, modified whole blood or red blood cells can be administered to ensure oxygen carrying capacity. If appropriate component therapy is to be available for patients receiving chemotherapy and those with such hereditary disorders of coagulation as hemophilia, a large proportion of the donor blood must be fractionated.

Approximately 86% of whole blood collected in the United States in 1983 was processed into red cells (Blood Services Operations Report 1982-83, American Red Cross). Objections to packed red cells include the viscosity, absence of coagulation factors, and increased exposure to infectious disease in patients who require multiple transfusions. While it is possible to administer red cell concentrates through micropore filters at rapid rates if an external pressure bag is used [1], packed cells should always be diluted with saline prior to infusion to minimize hemolysis [2]. Admittedly, a patient who receives 10 units of red cells, 4 units of fresh frozen plasma, and 10 units of platelets sustains 24 potential exposures to hepatitis. However, administration of the latter two products is unnecessary in the previously healthy patient who requires 10 units of packed cells. In addition, it must be recognized that 'whole blood,' unless fresh, is an incomplete product deficient in platelets and labile coagulation factors.

The storage lesion

The biochemical and cellular changes that occur as a result of the collection, storage, continued metabolism, and transfusion of bank blood are termed the 'storage lesion.' Storage of blood in currently licensed anticoagulants results in an altered affinity of hemoglobin, decreased pH, impaired deformability of the red cells, loss of platelets, hemolysis, elevated potassium, sodium, phosphate and ammonia, accumulation of denatured proteins and vasoactive substances, and leaching of plasticizers from the blood bags (Table 1) [3, 4].

The addition of adenine results in acceptable levels of ATP, assuring red cell viability. Theoretically, the depletion of 2,3-DPG in transfused red cells, associated with diminished capacity to release oxygen to the tissues is disadvantageous; however, the clinical significance of this shift in the oxygen-hemoglobin dissociation curve is questionable. Furthermore, transfused red cells regain the ability to exchange oxygen rapidly and to regenerate stores of 2,3-DPG within hours to days.

Electrolyte changes

The active transport of potassium and sodium virtually ceases in stored blood and intracellular and extracellular concentrations approach equilibrium. Although the potassium level may reach 20 to 27 mEq/L in blood stored for 35 days, the adverse effects are more imagined than real. It must be remembered that potassium levels are reported in terms of mEq/L of plasma. In addition, the patient who requires transfusion is losing potassium with the shed blood. The loss of a unit of blood in a patient with a hematocrit of 40% and a serum potassium of 5 mEq/L results in the loss of 1.5 mEq of potassium. The patient gains approximately 7 mEq of potassium per unit transfused. Under the worst of circumstances, a patient transfused with 10 units of blood will gain 70 mEq of potassium. This presupposes that all of the blood infused is 35 days old, an unlikely possibility. The potassium level in

Table 1. Characteristics of whole blood stored in CPDA-1.

Parameter	Storage time (days)	
	0	35
Plasma dextrose (mg/dl)	432 ± 15*	282 ± 27
	440 ± 57**	229 ± 51
Plasma sodium (mEq/L)	169 ± 31	153 ± 2
	169 ± 9	155 ± 9
Plasma potassium (mEq/l)	3.3 ± 0.3	17.2 ± 3.6
	4.2 ± 1.8	27.3 ± 4.6
Whole blood pH	7.16 ± 0.03	6.73 ± 0.04
	7.6 ± 0.13	6.98 ± 0.16
Whole blood lactate (mg/dl)	19 ± 5	202 ± 30
	—	—
Plasma hemoglobin (mg/dl)	0.5 ± 0.5	45.6 ± 32.7
	8.2 ± 8.5	46.1 ± 28
Red blood cell ATP (μmol/gHb)	—	—
	4.18 ± 0.68	2.40 ± 0.76
Red blood cell 2,3-DPG (μmol/gHb)	—	—
	13.2 ± 3.0	1.6 ± 0.7
RBC (×10 ⁶)	4.0 ± 0.5	3.9 ± 0.4
	—	—
RBC (% survival)	—	—
	—	79 ± 10

* Upper numbers in each series from Ref. 3

** Lower numbers in each series from Ref. 4

the recipient is determined by (1) the absolute amount of potassium infused, (2) the degree of alkalosis resulting from hyperventilation and the metabolism of bicarbonate precursors in bank blood and balanced salt solutions, and (3) renal excretion of potassium. Potassium readily reenters transfused red cells, and hypokalemia rather than hyperkalemia, frequently occurs in patients who receive large quantities of homologous blood.

Acid-base disturbance

The pH of blood collected into acid CPD or CPDA-1 solution, which has a pH of 5.5, immediately decreases to approximately 7.0. Continued red cell metabolism results in a further lowering of the pH to 6.6–6.8 toward the end of the storage period. This is accounted for in part by the accumulation of fixed acids as well as continued generation of carbon dioxide. Although the elevated PaCO_2 poses no problem in the adequately ventilated patient, the accumulation of fixed acids was formerly assumed to be deleterious. Consequently, the administration of sodium bicarbonate, 44.6 mEq per 4–5 units of blood was recommended. The introduction of arterial blood gas monitoring, as well as recognition that significant tissue buffering occurs, and that citrate, acetate and lactate in bank blood and balanced salt solutions are bicarbonate precursors, has led to abandonment of this practice.

Extensive experience with combat casualties as well as civilian victims of trauma indicates that the impact of transfusion on the acid-base status of the recipient is not simply the result of buffering and titration in a closed system. The rate of transfusion, volume of balanced salt solution administered, adequacy and speed of resuscitation, and presence or absence of sepsis all influence the metabolic removal of fixed acids [5, 6]. The patient's blood pressure at the end of transfusion, rather than the initial base deficit, is a more reliable guide to outcome. The patient whose blood pressure is readily restored with transfusion can usually handle the infused acid load and reverse preexisting metabolic acidosis. It must also be remembered that the ad-

ministration of sodium bicarbonate may impair delivery of oxygen to the tissues, especially by red cells depleted of 2,3-DPG. With adequate blood and fluid replacement and rapid restoration of hemodynamic stability, alkalosis rather than acidosis is the rule rather than the exception. Sodium bicarbonate may also interfere with the clotting process [7]. Finally, it is unwise to administer a significant sodium load to patients already prone to retention of salt and water.

Citrate intoxication and hypocalcemia

With the exception of heparin, all anticoagulants used in storage of bank blood depend on the chelation of calcium by an excess of citrate. Although citrate levels are elevated in recipients of large volumes of transfused blood, citrate intoxication does not occur. The average normothermic adult with adequate hepatic function is capable of metabolizing citrate at a rate equal to the transfusion of 20 units of blood per hour. Since citrate is also metabolized by skeletal muscle, compromised hepatic function does not of necessity predispose to citrate intoxication.

The ready availability of ionized calcium measurements has led to the realization that ionized calcium often falls to 40–50% of normal when blood is infused at a rate of 50–150 ml per minute. However, the hypocalcemia is more of a laboratory curiosity than a clinically significant event. Values return toward normal shortly after the termination of transfusion. Cardiac performance is rarely impaired, as long as the circulating volume is maintained. Calcium should not be administered empirically on the basis of volume of transfusion or changes in the Q-T interval evident on the electrocardiogram [8]. Transfusion in excess of one or two blood volume equivalents may result in a prolonged decrease in ionized calcium which is associated with a marked change in the contour of the arterial pressure curve on the oscilloscope. Intravenous administration of calcium salts in this circumstance frequently restores the pressure curve to normal. Patients undergoing debridement, excision, and grafting of extensive thermal injuries

appear to benefit from the judicious administration of calcium. However, the need for continuous monitoring of the electrocardiogram and serial ionized calcium determinations cannot be over-emphasized. It must be stressed that a decrease in ionized calcium will first be manifest as an effect on the myocardium, not coagulation. Calcium chloride is administered for its inotropic effects, not to facilitate coagulation.

Hypothermia

With the exception of platelets, blood is stored at a temperature of 4°C to maintain cellular integrity and prevent the growth of microorganisms. Administration of large quantities of cold blood is arrhythmogenic. Depression of metabolic functions such as biotransformation, and increased oxygen consumption also result. A number of blood warmers have been devised and marketed to prevent the hypothermia that accompanies infusion of large quantities of unwarmed bank blood. Most achieve an efficiency of 80% or less at flow rates of 100–150 ml per minute. Nevertheless, their use is mandatory when large volumes of cold blood are infused rapidly. Placing the infusion tubing in a bottle or basin of warm water is ineffective and is mentioned only to be condemned. Use of microwave ovens to warm blood never gained popularity because of the high incidence of hemolysis.

The moderate hypothermia that follows the infusion of partially warmed blood in air conditioned operating rooms may, in part, be offset by surface warming with water perfused blankets and heating lamps, increasing the ambient temperature, and by heating and humidifying inspired gases. It is possible that mild hypothermia offers some protection during the critical perioperative period by reducing metabolic demands, if shivering, which increase oxygen consumption, is prevented.

Pulmonary dysfunction

There has been considerable speculation that massive transfusion and post-transfusion pulmonary

dysfunction are causally related. While stored blood contains microaggregates of platelets, leukocytes, fibrin and other debris, it is apparent that the microaggregates *per se* are not the culprit. Mechanical occlusion of the pulmonary vessels probably plays a relatively minor role in initiating the pathologic changes. Use of microaggregate filters when administering blood does not prevent development of the respiratory distress syndrome. The interference of these filters with flow rate may be of greater consequence to patients requiring massive transfusion than is the particulate matter. Microaggregate filters appear to be a treatment in search of a disease. They may be useful in preventing (1) arterial emboli during cardiopulmonary bypass and (2) febrile transfusion reactions in patients with chronic transfusion requirements who experience febrile episodes due to granulocyte reactions [9].

Transfusion of microaggregate debris has been shown to decrease *in vivo* levels of fibronectin, an opsonic glycoprotein which augments reticuloendothelial phagocytic clearance of nonbacterial particulate matter [10]. If infusion of fibronectin proves to be beneficial, removal of microaggregates prior to transfusion may be warranted.

The etiology of pulmonary dysfunction in patients who are massively transfused is complex. The injured patient requiring large volumes of blood is often the one who sustains pulmonary contusion, fat embolism or thromboembolism. He is frequently at greater risk of aspiration, systemic and pulmonary sepsis, and volume overload. In addition, the use of pulmonary artery catheters has revealed that many patients, previously thought to be the victims of transfusion-induced pulmonary dysfunction, are actually suffering from left ventricular failure.

Coagulopathy

Abnormal bleeding has traditionally been ascribed to the infusion of large volumes of bank blood. While stored blood is an imperfect clotting package lacking platelets and ionized calcium and containing reduced concentrations of the labile clotting

factors, coagulopathy secondary to dilution of the clotting factors may not be as common as once believed. Although the platelet count decreases linearly during massive transfusion, it is only after 15 to 20 units of blood have been infused that platelet therapy is required in most patients [13]. If possible, the platelet count should be monitored after the transfusion of each five units of blood. However, this is not always feasible. Even in the best of laboratories there is a delay between the time a platelet count is requested and the results are available and between the time platelets are requested and received. In general, it is recommended that 10 units of platelets be administered when 15–20 units of blood are transfused and further transfusion is anticipated. Abnormal platelet function may also be the cause of bleeding. While a bleeding time is the best screening test of platelet function, it may be impossible to perform accurately in the patient who has sustained multiple trauma.

Although bank blood is deficient in the labile clotting factors V and VIII, [14] it is unlikely that a clinically significant deficiency of these factors will occur in previously healthy patients until at least 80% of the original blood volume has been replaced because these factors exist far in excess of the amount normally required for coagulation. The mathematics of transfusion, extrapolated from exchange transfusion of newborns, indicates that 1.5–2 blood volumes must be replaced before a deficiency of labile clotting factors is manifest [6]. The administration of fresh frozen plasma should not be routine. Formulae prescribing 'X' number of units of fresh frozen plasma per 'X' units of red cells are irrational, impractical, and may subject the patient to unnecessary risks. Although patients receiving 15 or more units of blood almost invariably have prolongation of both the prothrombin time and partial thromboplastin time, transfusion of fresh frozen plasma returns the numbers to the normal range, but does not usually alter an existing bleeding tendency [15].

Measures to decrease transfusion-associated morbidity

The administration of homologous blood is not without hazard. The fact that patients do not require 10 g of hemoglobin for everyday existence, or even to safely undergo anesthesia and surgery [15] is apparently difficult for many clinicians to accept. Nevertheless, it is a fact and patients should not arbitrarily be transfused to some magic end point. While autologous blood cannot meet all of the needs of patients requiring massive transfusion, its usefulness should not be forgotten. Even potentially 'high risk' surgical candidates can safely pre-donate blood for elective surgery [16]. Intraoperative scavenging and reinfusion of shed blood is appropriate in some patients who require massive transfusion. Blood substitutes may one day become the preferred therapy in the patient who sustains significant blood loss. Finally, the necessity of carefully verifying the identity of the patient prior to administration of blood is essential. The patient who requires massive transfusion is far more likely to suffer serious morbidity from administration of the wrong unit of blood than from the use of type-specific blood.

References

1. Cullen DJ, Kunsman J, Caldera D, *et al.*: Comparative evaluation of New Finescreen filters: Effects on blood flow rate and microaggregate removal. *Anesthesiology*, 1980; 53: 3.
2. Calkins JM, Vaughan RW, Cork RC, *et al.*: Critical importance of diluting packed red blood cells for transfusion. *Anesthesiology*, 1980; 53: S169.
3. Latham JT, Bove JR, Weirich FL: Chemical and hematologic changes in stored CPDA-1 blood. *Transfusion*, 1982; 22: 158.
4. Moore GL, Peck CC, Sohmer PR, *et al.*: Some properties of blood stored in anticoagulant CPDA-1 solution. A brief summary. *Transfusion*, 1981; 21: 135.
5. Collins JA, Simmons RL, James PM *et al.*: Acid-base status of seriously wounded combat casualties. II. Resuscitation with stored blood. *Ann Surg*, 1971; 173: 6.
6. Collins JA: Problems associated with the massive transfusion of stored blood. *Surgery*, 1974; 75: 274.
7. Wong DW, Mishkin FS, Tanaka TT: The effect of bicarbonate on blood coagulation. *JAMA*, 1980; 244: 61.

8. Kahn RC, Jascott D, Carlon GC *et al.*: Massive blood replacement: Correlation of ionized calcium, citrate and hydrogen ion concentration, *Anesth Analg*, 1979; 58: 274.
9. Snyder El, Bookbinder M: Role of microaggregate blood filtration in clinical medicine. *Transfusion*, 1983; 23: 460.
10. Saba TM, Blumenstock FA, Shah DM *et al.*: Reversal of fibronectin and opsonic deficiency in patients. *Ann Surg*, 1984; 199: 87.
11. Collins JA: Does a relationship exist between massive blood transfusion and the adult respiratory distress syndrome? If so, what are the best preventive measures? *Vox Sang*, 1977; 32: 311.
12. Counts RB, Haisch C, Simon TL *et al.*: Hemostasis in massively transfused trauma patients. *Ann Surg*, 1979; 190: 91.
13. Nilsson L, Hedner U, Nilsson IM, *et al.*: Shelf life of bank blood and stored plasma with special reference to coagulation factors. *Transfusion*, 1983; 23: 377.
14. Bove JR: What is the factual basis, in theory and clinical practice for the use of fresh frozen plasma. *Vox Sang*, 1978; 34: 428.
15. Allen JB, Allen FB: The minimum acceptable level of hemoglobin. *Int Anes Clin*, 1982; 20(4): 1.
16. Mann M, Sacks HJ, Goldfinger D: Safety of autologous blood donation prior to elective surgery for a variety of potentially 'high-risk' patients. *Transfusion*, 1983; 23: 229.

3. Diagnosis of bleeding disorders

M. BROZOVIĆ

Assessment of haemostasis is undertaken for diagnostic purposes under two sets of circumstances:

1. to diagnose and treat acute haemostatic failure
2. to investigate an individual with a suspected bleeding disorder.

The diagnostic approach in these two situations is different and they will be considered under separate headings.

1. Acute haemostatic failure

Acute haemostatic failure is a common medical, surgical and obstetric emergency. There is invariably a need for rapid diagnosis to enable optimal treatment. The tests used must be simple, quick and capable of distinguishing between various causes of haemostatic failure described below. Emergency staff, laboratory and clinical, must be familiar with their significance and interpretation. The tests usually employed are: one stage prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen determination (f) and platelet count. Using the four tests, the common causes of haemostatic failure can usually be distinguished without difficulty, as shown in Table 1 [1].

1.1. Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) is a term used to describe a profound derangement of haemostasis that occurs in oligaemic or septicaemic shock, obstetric catastrophes and after massive injury, as well as in many other conditions [2].

There is usually a generalized bleeding tendency, often associated with ischaemic damage to kidneys, brain and other organs. All tests of haemostasis are grossly abnormal reflecting the combination of bleeding and thrombosis throughout the body [3]. DIC is not a disease per se: it always arises as a result of another severe disorder which must be corrected first if haemostasis is to be restored. For example, blood volume must be repleted, acidosis corrected, appropriate antibiotic given, or uterus evacuated, before specific haemostatic measures (administration of fresh frozen plasma and/or platelets) are likely to take effect [2].

Serial tests [2] of haemostasis, usually platelet count and fibrinogen concentration, are required during the acute stage of DIC to monitor the success of therapy, as shown in Fig. 1 for a patient with septic abortion and DIC. Additional tests that may be useful in diagnosis or monitoring of DIC are shown in Table 2.

1.2. Massive transfusion with stored blood

Stored blood contains no viable platelets, and is deficient in factors V, VIII:C and XI [4]. When one or two blood volumes are replaced by stored blood over a short period of time, definitive haemostatic abnormalities become apparent on laboratory testing (Table 1) and may lead to a clinically significant bleeding tendency or aggravate the existing haemostatic defect. In general, fresh frozen plasma promptly corrects the defect [5]. Lim [6] has reported that there is a defect of platelet function in massive transfusion; thus platelet transfusion may

Table 1. Emergency tests in the laboratory diagnosis of acute haemostatic failure.

Condition	Tests			
	PT	PTT	f	Platelet count
DIC	↑↑	↑↑	↓↓	↓↓
Massive transfusion	↑	↑	N	↓
Liver disease	↑↑	↑	↑ or N	↓ or N
Vit. K deficiency, overdose of oral anticoagulants	↑↑	↑	N	N
Bleeding in renal failure	↑	N	N	↓ or N
Bleeding due to heparin	↑ or N	↑↑	N	N
Haemophilia	N	↑↑	N	N
Thrombocytopenia	N	N	N	↓↓

N: normal
↑: prolonged
↑↑: very prolonged

↓: low
↓↓: very low

be indicated in patients who fail to respond to fresh frozen plasma.

1.3. Liver disease

Liver disease is associated with multiple haemostatic defects [7]. The pattern of abnormalities ob-

served on emergency testing may resemble DIC; indeed DIC may have supervened due to massive GI tract bleeding or septicaemia in a patient with an underlying liver condition. The haemostatic abnormalities are difficult to correct with blood products [8]. Other tests of possible help in diagnosis or prognosis are shown in Table 2.

1.4. Vitamin K deficiency and overdose of oral anti-coagulants

Acute vitamin K deficiency is not uncommon in patients on parenteral feeding not given vitamin K supplements, especially if wide spectrum antibiotics are administered at the same time. As the turnover of vitamin K is very rapid [9], vitamin K deficiency with bleeding manifestation may occur within days of surgery. Characteristically, one stage prothrombin time is very long whereas partial thromboplastin time is less prolonged. Platelet count and fibrinogen concentration are normal. The haemostatic defect is rapidly corrected on infusion of fresh frozen plasma and administration of vitamin K₁.

Identical laboratory findings are observed in patients who are bleeding due to an overdose of oral anticoagulants [10]. Fresh frozen plasma is given to arrest the bleeding. Vitamin K₁ is administered

Table 2. Additional tests in the diagnosis of acute haemostatic failure.

Condition	Test	Comment
DIC	FDP concentration. Paracoagulation tests (ethanol, protamine gelation).	Useful contributory test. Normal or negative results do not exclude DIC.
Massive transfusion	Bleeding time	If prolonged, confirms the presence of platelet defect.
Liver disease	Liver function tests	Useful contributory tests; also measure of the extent of liver damage.
Bleeding in renal failure	Factor assays	Required to prove factor deficiencies, occasionally found in nephrotic syndrome.
Bleeding due to heparin	Ancrod or Reptilase time	A method of recognizing the presence of heparin in plasma.
Hemophilia	Assay of VIII:C and IX	Required to prove which of the two factors is deficient when the only defect is a prolonged PTT.
Thrombocytopenia	Bone marrow cytology	To establish whether megakaryocytes are present. To exclude malignancy.

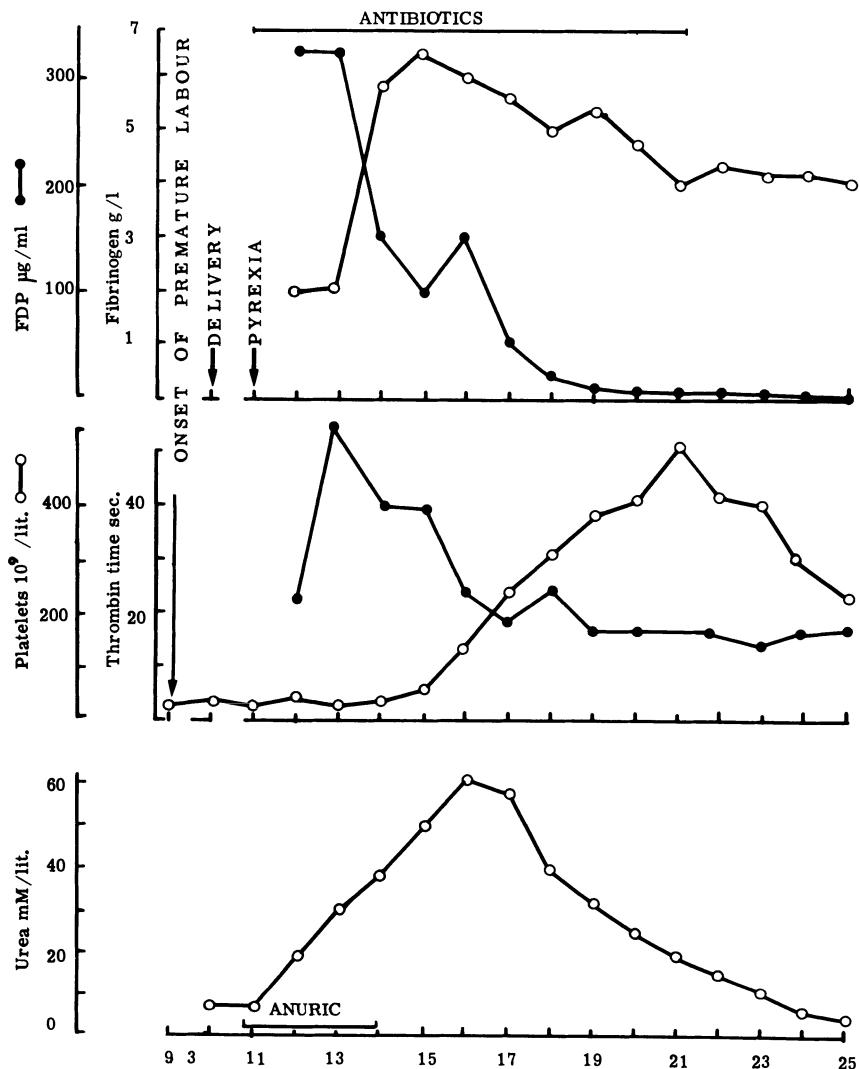


Fig. 1. Acute DIC in a patient with septic abortion in the 26th week of pregnancy. The arrows indicate the onset of labour, the delivery and the onset of pyrexia. *E. coli* was grown from blood cultures, fetal and vaginal swabs.

(a) Fibrinogen ○—○, FDP ●—●; (b) platelets ○—○, thrombin time ●—●; (c) urea ○—○. The patient recovered completely.

only if the patient no longer requires treatment with oral anticoagulants or if the bleeding is life threatening, i.e. cerebral or retroperitoneal [11].

1.5. Bleeding in renal failure

Patients with blood urea exceeding 25 or 30 mmol/l may bleed profusely from a single site, usually GI tract, or ooze from mucosal membranes and catheter sites. Emergency tests disclose few abnormalities apart from slightly prolonged one stage prothrombin time and sometimes marginally re-

duced platelet count. The principal abnormality is a reversible defect of platelet function caused by retained metabolites in plasma [12]. Transfused donor platelets quickly acquire the same defect and lose their haemostatic function. The only way to correct this defect and arrest bleeding is to ensure regular and effective dialysis.

In rare instances patients in renal failure may have a selective defect of one coagulation factor, usually factor IX [13], or develop DIC. The laboratory tests will reflect these abnormalities: in factor IX deficiency, partial thromboplastin time will be

disproportionately prolonged; in DIC all tests will be abnormal.

1.6. Bleeding due to heparin

Heparinized patients may experience severe bleeding, especially after vascular surgery. It then becomes important to establish whether excess heparin is the sole cause of bleeding. If the most marked abnormality is a very long partial thromboplastin time, the Reptilase time is normal and platelet count within the normal range, heparin is the likely culprit. Other causes of bleeding, such as DIC, thrombocytopenia and acute renal failure, must always be considered if the pattern of abnormalities on emergency testing is atypical.

1.7. Undiagnosed mild haemophilia

Mild haemophilia A and B can remain undiagnosed through life only to become manifest as intractable bleeding after trauma or major surgery [14]. The emergency tests usually disclose an isolated, often only slight prolongation of partial thromboplastin time (see 1.9.2). Specific assays must be carried out to establish the definitive diagnosis and allow specific treatment (Chapter 4).

1.8. Thrombocytopenia

Autoimmune, drug-induced or amegakaryocytic thrombocytopenia may give rise to acute haemostatic failure. Very low platelet count with normal coagulation tests is invariably found. Bone marrow aspirate must be carried out to exclude leukaemia or malignant infiltration, as well as to establish whether megakaryocytes are present. Drug history should be carefully reviewed and all drugs known to cause thrombocytopenia (such as quinidine, gold salts, sulphonamides, penicillin, rifampicin, frusemide, etc.) discontinued [15]. Platelet transfusion and high dose steroids are given in life-threatening situations [16].

1.9. Common pitfalls in laboratory diagnosis of acute haemostatic failure

The diagnosis of acute haemostatic failure can usually be made without undue difficulty and the underlying cause elucidated using the tests shown in Table 1. Occasionally, additional tests (see Table 2) may be required. Nevertheless, technical difficulties and errors of interpretation may cause potentially serious problems.

1.9.1. Technical difficulties. It is often difficult to obtain adequate venous sample from a severely ill, peripherally collapsed patient. Partially clotted samples, samples obtained through indwelling catheters or containing traces of heparin used for flushing such catheters, find their way to the laboratory and may give rise to results suggestive of DIC or excess heparin in systemic circulation. Staff collecting blood samples must be constantly reminded that only blood obtained by good venepuncture can be used for tests of haemostasis.

It is also important to ensure that emergency techniques, reagents and controls are subjected to the same quality control as the routine tests.

1.9.2 Interpretation errors. All emergency tests are global and of low specificity, and errors can occur if this is not kept in mind. Some common problems are outlined below.

In early stages of DIC activated clotting factors may be present, free, in circulation and give rise to normal or even shorter than normal clotting times. Minutes or hours later all tests are grossly prolonged with the fully blown picture of DIC. The early, apparently 'normal' tests may lull the clinician into a false sense of security, because he or she does not appreciate the dynamic aspects of DIC and the global nature of the tests used.

Most reagents used for partial thromboplastin time are only sensitive to factor VIII:C deficiency below 20 iu/dl [17]. It is thus possible for a mild haemophiliac with factor VIII:C level of ~25 iu/dl to experience post-operative bleeding, yet have normal or nearly normal partial thromboplastin time. Thus, normal partial thromboplastin time does not exclude a mild haemophilia.

Many additional examples can be listed. Such errors of interpretation can only be minimized with close cooperation between laboratory and clinical staff.

2. Investigation of a suspected bleeding disorder

When investigating a suspected bleeding tendency the laboratory has usually adequate time and full facilities, as well as access to a relatively healthy individual who can be (if necessary) recalled for further studies. A detailed clinical, family and drug history is usually taken first, then a number of 'screening' tests carried out, finally followed by more complex 'second-line' investigations.

2.1. History

It is diagnostically more efficient and a more economical use of resources to take a discriminating personal and family history of the incidence of spontaneous bleeding and the duration of post-traumatic bleeding, and to carry out detailed laboratory investigation only on those patients describing abnormal symptoms, than to perform two or three global tests on a large number of patients [14]. Detailed drug history should always be included; patients should be asked specifically about aspirin intake.

2.2. Screening tests

Screening tests vary from laboratory to laboratory but generally include blood and platelet count, bleeding time, one stage prothrombin time, partial thromboplastin time, thrombin time, quantitation of fibrinogen and a clot solubility test. In individuals who give history of recent onset of bleeding tendency, other tests, such as urea, electrolytes, liver function tests and search for autoantibodies against various tissues, should also be carried out, to exclude systemic disease.

2.3. Second line tests

When the results of screening tests are available, it is usually clear what type of second-line test is required, as shown in Table 3. There remains a small number of patients with completely normal screening tests who have a convincing personal and family history of bleeding. A selection of second-line tests (usually assays of factor VIII:C, VIII: RAg and RiCoF, and platelet function tests) are carried out to exclude the congenital defects that may present with normal screening tests, such as mild haemophilia A, von Willebrand's syndrome and some platelet defects [18].

2.3.1. Technical problems associated with second-line tests. Second-line tests require careful collection of samples, meticulous techniques, stable and standardized reagents, reliable normal and abnormal controls and accurate end-point assessment. Coagulation factor assays (biological and immunological) require in addition reliable standards, preferably freeze dried and calibrated by more than one laboratory. Platelet function tests are also subject to many variations; this is particularly true for the platelet aggregation tests which are affected by small alterations in pH, length of storage of blood prior to testing, aggregometer temperature, type, size and even shape of the stir-bar used [19].

2.3.2. Interpretation of second-line tests. For many second-line tests the dividing line between normal and abnormal is poorly defined. Plasma concentration of many coagulation factors varies with age, sex, race, oral contraceptive usage and the presence of stress or acute phase reaction [20]. All these variables must be taken into account when assessing the concentration of any coagulation factor and especially in the study of possible heterozygotes.

Many unexplained oddities of platelet behaviour are due to drugs such as antihistamines, tranquilizers or β blockers, often not mentioned by the patient unless specifically asked about. The normality is even harder to define with platelet tests, many of which have only just emerged from research laboratories to be used for diagnostic purposes.

Table 3. Second line tests in the investigation of a suspected bleeding disorder.

Screening abnormality	Second line test	Likely diagnosis if second line test low or abnormal
Isolated long PTT	Assay of 1. F VIII:C (normal RAg) 2. F IX 3. F XI 4. other contact factors	Haemophilia A F IX deficiency F XI deficiency F XII, Fletcher, Fitzgerald deficiency.
Isolated long PT	1. F VII	F VII deficiency
Long PT and PTT	1. F X; stypven time 2. F V; long bleeding time 3. prothrombin (biological and immunological).	F X deficiency F V deficiency Prothrombin deficiency or dysprothrombinaemia
Long PT, PTT and TT, low or absent fibrinogen	1. clot weight, polymerization studies, FPA, and FPB release Immunological studies	Afibrinogenaemia Dysfibrinogenaemia
Increased clot solubility	1. Biochemical and immunological assay of F XIII	F XIII deficiency
Long bleeding time	1. F VIII:C, VIII R:Ag RiCoF CIE of VIII R:Ag	von Willebrand syndrome
Long bleeding time	1. Platelet morphology 2. Platelet aggregation 3. Membrane glycoproteins 4. ADP, ATP content 5. Prostaglandin pathway 6. Release reaction 7. Assay of F VIII:C, R:Ag, RiCoF 8. Electron microscopy	Platelet function defects including Glanzmann's, Bernard-Soulier, storage-pool defects

Finally, if the pattern of abnormalities obtained on second-line testing does not fit one of the conditions listed in Table 3 even after excluding the possibility of technical or clerical error, one of the rare combined defects may be present [21].

3. Conclusions

Simple and quick tests for the diagnosis of acute haemostatic failure must be available in all hospitals with acute admissions. There must be a clear cut policy on the interpretation of these tests and the ensuing therapeutic measures.

When investigating a patient with bleeding tendency, two types of tests are required: the screening tests and the more elaborate 'second-line' tests. In the majority of patients, screening tests with a small battery of second-line tests (factor VIII:C,

RAg, RiCoF and factor IX assays, and platelet aggregation tests) can establish the diagnosis. The remaining patients require further, often complex investigations, available in specialized centres only.

References

1. Brozović M, Machin S: Coagulation tests. Hospital Update, 1977; 3: 203-12.
2. Brozović M: Disseminated intravascular coagulation. In: Bleeding Disorders, Ingram GIC, Brozović M, Slater NGP. Blackwell Scientific Publications, Oxford, 1982: 88-102.
3. Minna JD, Robboy JS, Colman RW: Disseminated Intravascular Coagulation in Man. Charles C Thomas, Springfield, Illinois, 1973.
4. Collins JA: Massive blood transfusion. Clin Haematol, 1976; 5: 201-22.
5. Brozović M: Haemostatic defect from massive transfusion

- with stored blood. In: Bleeding Disorders, Ingram GIC, Brozović M, Slater NGP. Blackwell Scientific Publications, Oxford, 1982: 105-7.
6. Lim RC, Olcot C, Robinson A, Blaisdell FW: Platelet response and coagulation changes following massive blood replacement. *J Trauma*, 1973; 13: 577-82.
 7. Brozović M: Bleeding complications of systemic illness. In: Bleeding Disorders, Ingram GIC, Brozović M, Slater NGP. Blackwell Scientific Publications, Oxford, 1982.
 8. Silk DBA, Williams R: Acute liver failure. *Br J Hosp Med*, 1975; 22: 437-47.
 9. Bjorsson TD, Blaschke TF: Vitamin K₁ disposition and therapy of warfarin overdose. *Lancet*, 1978; ii: 846.
 10. Kelton IG, Hirsh J: Bleeding associated with antithrombotic therapy. *Semin Hematol*, 1980; 17: 259-91.
 11. Brozović M: Bleeding due to antithrombotic therapy. In: Bleeding Disorders. Ingram GIC, Brozović M, Slater NGP. Blackwell Scientific Publications, Oxford, 1982: 109-16.
 12. Hardisty RM: Disorders of platelet function. *Br Med Bull*, 1977; 33: 207-12.
 13. Handley DR, Lawrence JR: Factor IX deficiency in nephrotic syndrome. *Lancet*, 1967; i: 1079-81.
 14. Ingram GIC: Haemophilia. In: Bleeding Disorders, In-
 - gram GIC, Brozović M, Slater NGP. Blackwell Scientific Publications, Oxford, 1982: 23-31.
 15. Slater NGP: Drug induced immune thrombocytopenia. In: Bleeding Disorders, Ingram GIC, Brozović M, Slater NGP. Blackwell Scientific Publications, Oxford, 1982: 191-98.
 16. Karpatkin S: Autoimmune thrombocytopenic purpura. *Blood*, 1980; 56: 329.
 17. O'Brien PF, North WRS, Ingram GIC: The diagnosis of mild haemophilia by the partial thromboplastin time test. *Thrombosis and Haemostasis*, 1981; 45: 162.
 18. Brozović M: Blood coagulation: Laboratory practice. In: *Blood Transfusion and Problems of Bleeding*, Smit Sibinga C Th, Das PC, van Loghem JJ, (eds.) Martinus Nijhof, The Hague, 1982; 15-22.
 19. Bowie EJW, Owen CA: The significance of abnormal postoperative hemostatic tests. In: *Progress in Hemostasis and Thrombosis*, vol 5, Spaet TH (ed.). Grune & Stratton, New York, 1981: 179-210.
 20. Brozović M: Physiological mechanisms in coagulation and fibrinolysis. *Br Med Bull*, 1977; 33: 231-38.
 21. Bloom AL: Inherited disorders of blood coagulation. In: *Haemostasis and Thrombosis*, Bloom AL, Thomas DP, (eds.) Churchill Livingstone, Edinburgh, 1981: 321-70.

4. Treatment of the haemophilias

C.A. LUDLAM

Prompt treatment, or prophylaxis, with blood products allows the majority of patients with haemophilia A and B the prospect of leading relatively normal lives. Along with von Willebrand's disease (vWD), these disorders are numerically and clinically the most important congenital bleeding conditions. The prevalence of haemophilia A is approximately five per hundred thousand of population whereas vWD is probably about half this and haemophilia B (Christmas disease) one fifth; other congenital coagulation deficiencies are rare. Although clinically severe congenital platelet disorders are uncommon, it is becoming increasingly acknowledged that minor abnormalities of platelet function may be relatively common and on occasion clinically important.

Haemophilia A is due to a deficiency of factor VIII coagulant activity (FVIIIC), whereas vWD is primarily due to a defect in an associated part of the molecule, termed factor VIII related antigen (VIIIRAG), which when polymerised to high molecular weight multimers has ristocetin cofactor activity (FVIII RCF). This latter activity is essential for platelet adhesion to subendothelium and when reduced, primary platelet plug formation in damaged vessels does not occur normally. In Christmas disease there is a reduced plasma factor IX level [1].

Clinical presentation

Haemophilia A and Christmas disease are sex-linked disorders, clinically indistinguishable and

characterised by recurrent joint and muscle bleeds [1]. In general the clinical severity of bleeding is related to the plasma level of the deficient factor. 'Severe' disease, resulting in 'spontaneous' bleeds is associated with factor VIIIC or IX levels of 0–2% normal; patients with 'moderate' severity (2–5%) usually only experience bleeds after minor trauma, and patients with mild disease (>5%) only have bleeds after trauma or surgery. Babies, even with severe haemophilia, do not usually present with haemorrhagic episodes until approximately six months of age. Moderately or mildly affected individuals may not present until later in childhood or as adults either with post traumatic bleeds or haemorrhage following surgery. Female carriers of haemophilia may have reduced factor levels and these should be checked prior to surgery. Haemarthroses of knees, elbows and ankles are the most common joints to be affected and muscle haematoma of the calf, thighs, arms and psoas are characteristic. Repeated haemarthroses in severe haemophilia are not only extremely painful if untreated, but lead to hypertrophy of the synovium, destruction of the joint, and secondary osteoarthritis [2]. This along with recurrent muscle haematoma can result in severe physical disability.

Clinically vWD is quite distinct from haemophilia in that, in most instances, it is dominantly inherited and is associated with epistaxis, gastrointestinal bleeds and menorrhagia. Like mild haemophilia, however, bleeding episodes are uncommon and are often precipitated by trauma or surgery. In severe vWD, which is a rare disorder, factor VIII activities are very low or undetectable, and such

patients bleed like severe haemophiliacs, e.g. haemarthroses.

Therapy for haemophilia A

Ideally, intravenous therapy with factor VIII should be available promptly as soon as a bleed begins. In most instances, particularly with haemarthroses, the patient is aware of the discomfort of early haemorrhage long before the classical signs of swelling and warmth become apparent. Furthermore, early treatment not only requires a lower dose of therapy but the bleed settles more quickly and the patient is less inconvenienced. In the past 10 years, with the development of lyophilised factor VIII concentrates, treatment for individual bleeding episodes has been increasingly given at home or work by the patient instead of in hospital as formerly. This allows the child to remain at school, to receive a conventional education, and the adult to stay at work so being able to support himself and his family. Increasingly, patients are receiving factor VIII prophylactically to prevent bleeding episodes particularly when these are frequent or recurrent in a single or 'target' joint. For this, factor VIII, because of its short 12 hour half-life, needs to be given daily or every second or third day; the frequency being the minimum necessary to keep the patient bleed free. During an acute bleed the importance of resting the affected part is often underestimated by the patient. In severe painful haemarthroses, resting in a splint will not only lessen haemorrhage but also reduce pain.

Treatment regimes

On demand

The dose of factor VIII necessary to control a bleed depends upon its site, severity, whether it is spontaneous or post traumatic, and the time before therapy is given. Ideally, treatment should be available immediately after the patient feels the bleed begin. For a minor spontaneous bleed a dose of 5–10 iu/kg is often sufficient, but if the haem-

arthrosis follows trauma, or there is a delay of several hours, or the bleed develops rapidly, then two to four times this dose may be required [3]. Some patients consistently seem to need higher doses than others for apparently similar haemorrhagic episodes. The aim of treatment is to arrest bleeding with the minimum dose of factor VIII but the necessity for a second injection 12–24 h later is related to the size of the initial dose. A dose of 5–10 iu/kg will be satisfactory for 80% of bleeds; the remaining 20% require further treatment. For one injection to be sufficient to treat 95% of bleeds, the initial dose has to be increased to 15–20 iu/kg; if all haemarthroses were treated with this size of dose, three-quarters of the episodes would be overtreated and this would be wasteful of factor VIII [4, 5, 6].

Muscle haematoma are often slower to settle than haemarthroses and may require larger and repeated doses to maintain a factor VIII level of 0.2–0.3 iu/ml. Gastrointestinal bleeding should be treated for several days with factor VIII. Haematuria usually settles spontaneously after several days; for treatment to be effective it is often necessary to give large doses, e.g. 20–30 iu/kg twice daily for several days. Haemophiliacs have minor abnormalities of renal function that appear not to be progressive and haematuria is not associated with progressive renal damage [7]. Intracranial haemorrhage, so often a fatal event in young adults, has decreased in frequency presumably because patients are receiving frequent treatment for minor bleeding episodes. Treatment should aim for a plasma factor VIII level of 1.00 iu/ml and the usual investigations for intracranial haemorrhage instituted, e.g. CT scanning, before surgery is contemplated.

Prophylactic treatment

Besides giving therapy for haemophilia 'on demand' there has been an increasing trend to transfuse patients prophylactically. If a particular physical activity frequently results in a haemarthrosis, factor VIII can be administered prophylactically; a dose of 5–15 iu/kg daily, or on alternate days, is usually sufficient to prevent most haemorrhages. If

a patient has recurrent bleeds at a single site, regular prophylaxis, sometimes for several months, may allow a joint to become quiescent but best results are obtained if it is accompanied by intensive physiotherapy to build up the muscles around the affected joint [8, 9].

Surgery

Prior to operation it is important to confirm the diagnosis of the specific bleeding disorder and test the blood for the presence of an antifactor VIII inhibitor [10]. At no time should intra-muscular injections be given. Immediately before major surgery the dose of factor VIII calculated to achieve a level of 1.00 iu/ml should be given. This should be checked by factor VIII assay before the surgery commences. Following operation factor VIII should be transfused initially thrice, but subsequently twice daily to maintain a pretransfusion level of at least 0.50 iu/ml. Over a 7–21 day period, depending on the severity of the surgery, factor VIII doses are gradually reduced. At operation, careful attention to securing haemostasis and subsequent immobilisation lessens the chance of bleeding. For dental surgery a single dose sufficient to raise the factor VIII level to 0.50 iu/ml prior to surgery along with 0.5 g i.v. tranexamic acid followed by 1 g tid and penicillin, both orally, for 7 to 10 days [11] is usually adequate.

Therapeutic materials

The provision of factor VIII from donor plasma stretches to capacity the resources of the blood transfusion services. Although the factor VIII level can be raised by 0.2–0.3 iu/ml for a short period by transfusion of fresh plasma, the level cannot be maintained at this concentration because of the large volumes that would have to be administered. Furthermore, it is wasteful of the many other plasma proteins e.g. albumin and immunoglobulins.

Cryoprecipitate

This is a product with a high yield of factor VIII from the starting plasma. The development of this product by Pool *et al.* in 1965 [12] revolutionised the treatment of haemophilia and her original technique has since been modified by others to increase both the yield from 350 iu to 500 iu per litre of plasma and the purity of the product [13, 14]. Cryoprecipitate can be stored for up to 6 months frozen at -20°C and each donation contains approximately 70–100 iu factor VIII. This product can also be freeze dried to give a lyophilised product which can be stored at 4°C . Although cryoprecipitate can be used to treat most bleeds in haemophilia it has a number of limitations. Its variable factor VIII content and the high incidence of mild allergic reactions especially in severe haemophiliacs, who have received many thousands of units, make it a less than an ideal product. At present it is the treatment of choice for patients with mild haemophilia and von Willebrand's disease (being preferentially enriched in factor VIIIRAG compared to FVIIIC) because these patients only require infrequent transfusions and the risk of developing hepatitis is less than with factor VIII concentrates prepared from plasma pools of several thousand donors. The advantage, so far as hepatitis B is concerned, does not extend to severe haemophiliacs because over the years they become exposed to many thousands of individual donations some of which contain HBsAg at doses which confer infectivity but at concentrations below the detection limit of the radioimmunoassay [15, 16].

DDAVP

For minor external bleeding episodes in mild haemophiliacs and patients with vWD, e.g. epistaxis, and minor surgical procedures such as dental extraction, the vasopressin analogue DDAVP (deamino-8-D-arginine vasopressin) may be useful [17]. Given slowly intravenously at a dose of $0.3\text{ }\mu\text{g/kg}$ it will raise factor VIII 2–4 times the basal level for several hours which is often sufficient to normalise haemostasis. It also increases plasma fibrinolytic activity but this can be inhibited

by tranexamic acid (10 mg/kg i.v. slowly and then as this dose orally three times per day until wound healing is complete). Further injections of DDAVP can be given at 6–12 hourly intervals, but the rise in factor VIII after each dose becomes progressively less. It is usually possible to get useful rises in factor VIII for 2–3 injections, but this tachyphylaxis limits its long-term use. Apart from facial flushing, DDAVP is remarkably free from significant side effects. Its antidiuretic activity lasts 24 h, and unless the patient is discouraged from drinking this can result in water intoxication. The major advantage of DDAVP is that it prevents patients with mild bleeding disorders, who may previously have received no transfusions, from being exposed to blood products and the risk of hepatitis.

Factor VIII concentrates

From bulk fresh frozen plasma, factor VIII can be fractionated to give a product varying in purity from cryoprecipitate (0.2 iu/mg protein), through 'intermediate purity' factor VIII concentrate (0.3–1.0 iu/mg protein) to a 'high purity' product (2–3 iu/mg protein). The yield of factor VIII is approximately 250–350 iu per litre plasma for intermediate purity but significantly less for high purity products. These concentrates have many advantages over cryoprecipitate in that the exact content of factor VIII per vial is known and allergic reactions are rare. Intermediate purity concentrate is suitable for the majority of bleeding episodes; the high purity product should be reserved for patients who have developed acquired haemostatic abnormalities secondary to transfusion of low purity products or when patients with antifactor VIII inhibitors are to be treated with large doses of factor VIII. The main drawback of these large pool concentrates is the high incidence of hepatitis following therapy with a single batch [18]. For this reason they should be reserved for treating severe and moderate haemophiliacs who, even if treated with cryoprecipitate, would be exposed to many thousands of donors. Patients are at risk of developing both B and non A non B hepatitis. The immunisation of all new haemophiliacs against hepatitis B

virus [19] and the development of hepatitis 'free' concentrates may reduce the future incidence of these infections and the resultant chronic and potentially serious liver pathologies [20, 21].

Acquired immunodeficiency syndrome, originally described in promiscuous male homosexuals and drug addicts in the USA, has recently been reported in several haemophiliacs [22, 23]. Circumstantial evidence has accumulated to suggest that it may be transmitted by blood products. A prodromal stage of malaise associated with lymphadenopathy and splenomegaly is followed by generalised wasting and a predisposition to opportunistic infections, e.g. pulmonary aspergillosis and pneumocystis carinii. A profound disturbance of the immune system is demonstrable with lymphopenia and depressed T cell mitogen responses along with an inversion of the helper/suppressor T cell ratio in the peripheral blood. At post-mortem atrophy of lymph nodes and spleen are observed. These cases may, however, merely reflect the tip of an immune deficiency iceberg, for recent reports suggest that some asymptomatic, otherwise apparently healthy haemophiliacs may have altered T cell function as demonstrated by monoclonal antibodies and mitogen stimulations [24, 25].

Other side effects of factor VIII preparations are uncommon but include haemolytic anaemia due to transfused isohaemagglutinins, acquired haemostatic abnormalities if patients receive large amounts of relatively impure products and the development of antifactor VIII antibodies.

Factor VIII inhibitors

The prevalence of antifactor VIII antibodies in haemophiliacs has remained remarkably constant in the UK during the past 20 years despite the introduction of progressively more purified factor VIII containing therapeutic products. They are usually of IgG₄ K subtype and detectable in approximately 6% of patients of whom the majority have severe haemophilia [26]. Antifactor VIII antibodies occasionally develop in non-haemophiliacs often in association with the puerperium, lymphoreticular, autoimmune, or skin disorders; the patients presenting with extensive bruising or bleed-

ing. Such acquired haemophilia may resolve spontaneously, particularly if developing after pregnancy but in some patients it is remarkably resistant to treatment [27].

Therapy for haemophiliacs with inhibitors is controversial and there is no universally applicable treatment. At low levels of inhibitor e.g. <10 Bethesda units, treatment with factor VIII in larger doses than for a patient without an inhibitor may transiently raise the circulating factor VIII level and arrest haemorrhage. For a serious life-threatening bleed plasmapheresis daily for several days to wash out the antifactor VIII immunoglobulin from the extravascular fluid, along with high dose factor VIII therapy, may allow for a more sustained rise in its plasma level [28, 29]. In some patients, 'low responders', the inhibitor level rises only slightly after therapy, but in a 'high responder' who has not received treatment for some time an anamnestic rise in the inhibitor may occur about the 5th day of therapy with a rapid and sharp rise in the inhibitor concentration making factor VIII treatment much less effective. Following such a rise, or in a patient presenting with a bleed and a high level of inhibitor, treatment with larger than usual doses of factor VIII e.g. 2–5,000 iu may produce clinical improvement.

Treatment with porcine factor VIII is having a renaissance following the development of a new poly-electrolyte purified product that does not produce thrombocytopenia or stimulate the development of antifactor VIII antibodies to the same extent as the less purified material containing the platelet aggregating factor. With this new porcine factor VIII in patients with 10–50 Bethesda units, prolonged rises of factor VIII coagulant activity can be observed in the patients, accompanied by an arrest of haemorrhage [30].

Evidence is accumulating that treatment with either factor IX concentrates as used for patients with Christmas disease, or 'activated' factor IX products e.g. FEIBA (factor eight inhibitor bypassing activity) or Autoplex, may stop bleeding particularly if factor VIII is administered immediately afterwards [31, 32, 33, 34]. The mechanism by which they work is unknown. These concentrates contain small amounts of activated clotting frac-

tions, e.g. Xa, and these could potentiate the haemostatic pathway augmenting the generation of thrombin at the site of haemorrhage. Small amounts of phospholipid are present in these products and it is possible that the factor VIIICAG associated with this may be protected from inactivation by antifactor VIII inhibitors and able to promote haemostasis at a bleeding site [35]. As the active ingredient is not yet identified it is impossible to monitor therapy effectively. For the same reason there may be considerable batch to batch variation in the amount of the active constituents in the product arising during production and hence quality control is less than ideal.

These factor IX containing products are not as effective as factor VIII in non-inhibitor patients at stopping bleeding. It is difficult to measure their clinical efficacy except by controlled clinical trials, although many physicians treating such patients have impressive anecdotes apparently exemplifying their efficacy. A well-designed Dutch trial has reported that FEIBA is marginally more effective than a non-activated factor IX product [31]. Studies by Lusher *et al.* have demonstrated that non-activated factor IX products are more effective than an albumin placebo [36].

The type of treatment these patients with inhibitors should receive is determined by the site and severity of the bleed. Rest and splinting alone for a minor haemarthrosis may be adequate whereas a major psoas or calf haematoma will require urgent therapy. If the antibody level is low, e.g. less than 10 Bethesda units, then large doses of factor VIII may be effective. If the patient's inhibitor does not cross react with porcine factor VIII then its use can be considered. Patients with levels greater than 10 Bethesda units can be treated in the first instance with nonactivated factor IX, but if this fails to secure haemostasis then FEIBA should be used. To enhance the efficacy of these factor IX products their infusions can be followed by factor VIII. This should be done slowly as the risk of thrombosis or disseminated intravascular coagulation is probably increased over the use of factor IX products alone. However, these products are potentially thrombogenic even when used alone and can give rise to DIC and myocardial infarction [37, 38],

39]. It is probably prudent not to give tranexamic acid with any of these factor IX containing materials as they may enhance the risk of thrombosis.

Von Willebrand's disease

Although most patients with vWD rarely experience haemorrhagic episodes; the commonest being epistaxis, gastrointestinal and menorrhagia, many individuals experience post-operative bleeding. Only patients with very low factor VIII activities, many of whom are homozygous for vWD, suffer from haemarthroses as in haemophilia. These patients may develop an antibody against predominantly the higher molecular weight fractions of factor VIII. Occasionally acquired vWD is found in association with autoimmune and lymphoproliferative disorders due to the presence of an antibody against FVIII RCF activity. Haemostasis is usually easier to control with cryoprecipitate infusions than in haemophilia because of the secondary rise in factor VIIIC following transfusion. It is customary to control replacement therapy by monitoring factor VIIIC levels despite the fact that the factor VIII RCF is often the most reduced factor VIII activity. Although cryoprecipitate raises the FVIII RCF-levels substantially, this usually only results in a transient reduction in the bleeding time. Although intermediate purity factor VIII concentrates can be used to treat vWD, these are not ideal because they do not contain as much of the high molecular weight polymers as cryoprecipitate and their use is followed by a higher incidence of hepatitis [40]. DDAVP is often the preferred therapy (vide supra) as it avoids the necessity for transfusions of any blood product. Factor VIII concentrates, however, may be the treatment of choice in vWD associated with anti FVIII RCF antibody because they contain more of the low molecular weight polymers which do not react so well with the antibody [41].

Christmas disease (haemophilia B)

Clinically this disorder is indistinguishable from

haemophilia A and is due to a deficiency of factor IX coagulant activity. Indications for treatment with factor IX concentrates are similar to those in haemophilia A. The therapeutic level of factor IX required to obtain a similar degree of clinical haemostasis is approximately two-thirds that for haemophilia A. Of the infused dose of factor IX only approximately 40–50% is recovered in the circulation, the remainder probably being bound to endothelial cells. Thus, the doses of factor IX, per kilogram body weight, for particular bleeds and to cover surgery, are very similar to doses of factor VIII used in the treatment of haemophilia A (vide supra). As the half life of factor IX *in vivo* is 18–24 h, effective prophylaxis can often be achieved by a once weekly injection. This form of therapy is becoming more generally accepted in the UK, particularly as the supplies of concentrate are adequate. There are two principal types of factor IX containing concentrates, those that also contain factors II and X (3 factor concentrates) and those in which there is in addition factor VII (4 factor concentrates).

Organisation of haemophilia services

In the UK definitive diagnosis and treatment of congenital bleeding disorders is organised by approximately 100 Haemophilia Centres and Reference Centres spread throughout the country [42]. These Centres offer a broad range of services with the assistance of particularly interested orthopaedic surgeons, rheumatologists, paediatricians, physiotherapists, nurses, dental surgeons, and social workers. This UK Haemophilia Service also collects statistics on factor VIII/IX consumption, which not only document current clinical practice but are vital for resource planning by Blood Transfusion Services. In the UK, on average, each treated haemophilia A or B patient receives approximately 30,000 iu and 23,000 iu of factor respectively per annum, but this is increasing each year. There are, however, marked differences in therapeutic practices between various countries, which to some extent are determined by the available resources [43, 44].

Future developments

With the increasing availability of factor VIII and IX concentrates, the quality of life and survival of patients with haemophilia has improved dramatically in the past twenty years. Home and prophylactic therapy have enabled haemophiliacs to lead almost normal lives and hopefully delay the onset of chronic arthropathy. Those individuals with inhibitors still pose unresolved therapeutic dilemmas. Although all factor VIII is currently derived from blood plasma, in the foreseeable future its synthesis *in vitro* may be achieved. This will not only increase the supply of factor VIII but the synthetic material is likely to be devoid of hepatitis viruses and is unlikely to transmit AIDS. If hepatitis 'free' concentrates can be developed, these latter complications of therapy may be avoidable. The antenatal diagnosis of haemophilia may reduce the incidence of severe and moderate haemophilia, but many women are reluctant to undergo amniocentesis and foetal blood sampling by foetoscopy. Furthermore, approximately half of the new patients with haemophilia are born to mothers without a family history of haemophilia; any small reduction in the number of children born with haemophilia because of antenatal diagnosis will be more than compensated for by the increased life expectancy of the patients, so that the prevalence of patients with these congenital bleeding disorders is likely to increase. Not only do these patients require therapy for every-day bleeds as they grow older but additional factor VIII will be needed to cover orthopaedic and general surgical operations. This increasing demand for factor VIII poses a major challenge to the economic development of health care resources.

References

1. Bloom AL: Inherited Disorders of Blood Coagulation. In: *Haemostasis and Thrombosis*, Bloom AL, Thomas DP, (eds). Churchill Livingston, 1981; 321-70.
2. Arnold WD, Hilgartner MW: Haemophilic arthropathy. *J Bone Joint Surg*, 1977; 59: 287-305.
3. Rizza CR: Management of patients with inherited blood coagulation defects. In: *Haemostasis and Thrombosis*, Bloom AL, Thomas DP (eds). Churchill Livingston, 1981; 371-94.
4. Aronstam A, Choudhury DP, Wassef M, Turk PM, McLellan DS: Double blind controlled trial of three dosage regimens in treatment of haemarthroses in haemophilia A. *Lancet*, 1980; i: 169-71.
5. Allain JP: Dose requirement for replacement therapy in haemophilia A. *Thrombosis and Haemostasis*, 1979; 42: 825-31.
6. Aronstam A, Wassef M, Hamad Z: Low doses of factor VIII for selected ankle bleeds in severe haemophilia A. *Br Med J*, 1982; 284: 790.
7. Small M, Rose PE, MacMillan N, Belch J, Rolfe EB, Forbes CD, Stuart J: Haemophilia and the kidney assessment after 11 year follow-up. *Br Med J*, 1982; 285: 1609-11.
8. Jones P, Fearn M, Forbes C, Stuart J: Haemophilia A home therapy in the United Kingdom 1975-7. *Br Med J*, 1978; i: 1447-50.
9. Aronstam A, McLellan DS, Turk P: Transfusion requirements of adolescents with severe haemophilia A. *J Clin Path*, 1979; 32: 927-30.
10. Duthie RB, Matthews JM, Rizza CR, Steel WM: The Management of Musculoskeletal Problems in the Haemophiliacs. Blackwell Scientific Publications 1972.
11. Walsh PN, Rizza CR, Mathews JM, Eipe J, Kernoff PBA, Coles MD, Bloom AL, Kaufman BM, Beck P, Hanan CM, Biggs R: Epsilon-amino caproic acid therapy for dental extraction in haemophilia and Christmas disease: a double blind trial. *Br J Haematol*, 1971; 20: 463-75.
12. Pool JG, Shannon AE: Production of high potency anti-haemophilic factor concentrate prepared from cryoglobulin precipitate. *Nature*, 1971; 203: 312.
13. Mason EC: Thaw-siphon technique for production of cryoprecipitate concentrate of factor VIII. *Lancet*, 1981; ii: 15-17.
14. Smit Sibinga CTh, Welbergen H, Das PC, Griffin B: High yield method of production of freeze-dried purified factor VIII by blood banks. *Lancet*, 1981; ii: 61-62.
15. Rickard KA, Batey RG, Dovity P, Johnston S, Campbell J, Hodgson J: Hepatitis and Haemophilia Therapy in Australie. *Lancet*, 1982; ii: 146-40.
16. Stirling ML, Murray J, MacKay P, Black SH, Peutherer JF, Ludlam CA: Incidence of infection with hepatitis B virus in 56 patients with haemophilia A, 1971-1979. *J Clin Path*, 1983; 36: 577-80
17. Mannucci PM, Ruggeri ZM, Parietti FI, Capitanio A: De-amino-8-D-arginine vasopressin; a new pharmacological approach to the management of haemophilia and von Willebrand's disease. *Lancet*, 1977; i: 869.
18. Bamber M, Murray A, Arborgh BA, Scheuer PJ, Kernoff PB, Thomas HC, Sherlock S: Short incubation non A non B hepatitis transmitted by factor VIII concentrates in patients with congenital coagulation disorders. *Gut*, 1981; 22: 854-9.
19. Szmuness W, Stevens CE, Harley EJ, Zang EA, Oleszko WR, William DC, Sadovsky R, Morrison JM, Kellner A: Hepatitis B vaccine. Demonstration of efficacy in a control-

- led clinical trial in a high-risk population in the United States. *N Eng J Med*, 1980; 303: 833–941.
20. Preston FE, Triger DR, Underwood JCE, Bardhan G, Mitchell VE, Stewart RM, Blackburn EK: Percutaneous liver biopsy and chronic liver disease in haemophiliacs. *Lancet*, 1978; ii: 592–94.
 21. Spero JA, Lewis JH, Van Thiel DH, Hasiba U, Rabin BS: Asymptomatic structural liver disease in haemophilia. *N Engl J Med*, 1978; 298: 1373–78.
 22. Macek C: Acquired immunodeficiency syndrome cause still elusive. *JAMA*, 1982; 248: 1423–31.
 23. Ragni MV, Lewis JH, Spero JA, Bontempo FA: Acquired immunodeficiency-like syndrome in two haemophiliacs. *Lancet*, 1983; i: 213–14.
 24. Menitove JE, Aster RH, Casper JT, Lauer SJ, Gottschall JL, Williams JE, Gill JC, Wheeler DV, Piaskowski V, Kirchner P, Montgomery RR: T-Lymphocyte subpopulations in patients with classic haemophilia treated with cryoprecipitate and lyophilised concentrates. *N Eng J Med*, 1982; 308: 79–83.
 25. Lederman MM, Ratnoff OD, Scillian JJ, Jones PK, Schacter B: Impaired cell-mediated immunity in patients with classic haemophilia. *N Engl J Med*, 1982; 308: 79–83.
 26. Allain JP, Gaillandre A, Lee H: Immunochemical characterization of antibodies to factor VIII in hemophilic and nonhemophilic patients. *J Lab Clin Med*, 1981; 97: 791–800.
 27. Green D, Lechner K: A survey of 215 non-hemophilic patients with inhibitors to factor VIII. *Thrombosis and Haemostasis*, 1981; 45: 200–208.
 28. Wensley RT, Stevens RF, Burn AM, Delamore IW: Plasma exchange and human factor VIII concentrate in managing haemophilia A with factor VIII inhibitors. *Br Med J*, 1980; 281: 138–9.
 29. Slocombe GW, Newland AC, Colvin MP, Colvin BT: The Role of Intensive plasma exchange in the prevention and management of haemorrhage in patients with inhibitors to factor VIII. *Br J Haematol*, 1981; 47: 577–85.
 30. Kernoff PBA, Thomas ND, Lilley PA, Tuddenham EGD: Clinical experience with polyelectrolyte-fractionated porcine factor VIII concentrate. *Br J Haematol*, 1981; 49: 131.
 31. Sjamsoedin LJM, Heijnen L, Nauser-Bunschoten EP, Van Geijswijk JL, Van Houwelingen H, Van Asten P, Sixma JJ: The effect of activated prothrombin-complex concentrate (FEIBA) on joint and muscle bleeding in patients with haemophilia A and antibodies to factor VIII. *N Eng J Med*, 1981; 305: 717–21.
 32. Abildgaard CF, Penner JA, Watson-Williams EJ: Anti-inhibitor coagulant complex (autoplex) for treatment of factor VIII inhibitors in haemophilia. *Blood*, 1980; 56: 978–84.
 33. Hanna WT, Madigan RR, Miles MA, Lange RD: Activated factor IX complex in treatment of surgical cases of haemophilia A with inhibitors. *Thrombosis Haemostasis*, 1981; 46: 638–41.
 34. Bloom AL: Factor VIII inhibitors revisited. *Br J Haematol*, 1981; 49: 319–24.
 35. Barrowcliffe TW, Kemball-Cook G, Gray E: Factor VIII Inhibitor bypassing activity: A suggested mechanism of action. *Thrombosis Res*, 1981; 21: 181–86.
 36. Lusher JM, Shapiro SS, Palascak JE, Vijaya Rao A, Levine PH, Blatt PM, Haemophilia Study Group: Efficacy of prothrombin complex concentrates in haemophiliacs with antibodies to factor VIII: a multicenter therapeutic trial. *N Engl J Med*, 1980; 303: 421–25.
 37. Rodeghiero F, Castronovo S, Dini E: Disseminated intravascular coagulation after infusion of FEIBA (Factor VIII Inhibitor Bypassing Activity) in a patient with acquired haemophilia. *Thrombosis Haemostasis*, 1982; 48: 339–40.
 38. Schimpf KL, Zeltsch CH, Zeltsch P: Myocardial infarction complicating activated prothrombin complex concentrate substitution in patient with haemophilia A. *Lancet*, 1982; ii: 1043.
 39. Abildgaard CF: Hazards of Prothrombin-complex concentrates in treatment of haemophilia. *N Eng J Med*, 1981; 304: 670.
 40. Nilsson IM, Hedner U: Characteristics of various factor VIII concentrates used in treatment of haemophilia A. *Br J Haematol*, 1978; 37: 543.
 41. Bloom AL, Peake IR, Furlong RA, Davies BL: High potency factor VIII concentrate, more effective than cryoprecipitate in a patient with von Willebrand's disease and inhibitor. *Thrombosis Res*, 1979; 16: 847.
 42. Jones P: Organisation of a haemophilia service. *Haemostasis and Thrombosis*, Bloom AL, Thomas DP (eds). Churchill Livingstone 1981; 389–93.
 43. Temperley I: National Resources and the Use of factor VIII. In: *Bloodtransfusion and problems of bleeding*, Smit Sibinga CTh, Das PC, Vanloghem JS (eds). Martinus Nijhoff, 1982; 243–47.
 44. Rizza CR, Spooner RJD: Treatment of haemophilia and related disorders in Britain and Northern Ireland during 1976–80: report on behalf of the directors of haemophilia centres in the United Kingdom. *Br Med J*, 1983; 236: 929–33.

5. Advances in the treatment of thrombocytopenia

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1. Introduction

Thrombocytopenia is defined as a subnormal number of circulating platelets. It is manifested clinically by an abnormal bleeding tendency, most characteristically of the purpura type. The diagnosis of thrombocytopenia depends on the observation of abnormal bleeding and the coexistent finding of low platelet counts. The platelet count should always be checked by counting directly from capillary blood and by observation of the number of platelets in a blood smear. Pseudothrombocytopenia due to agglutination or other abnormalities may be excluded in this way [1]. This is especially important when thrombocytopenia is suggested from a routine platelet count and no bleeding is observed.

Various degrees of bleeding tendency may be recognized:

- Minor degree: excessive bleeding following trauma (e.g. surgery)
- Moderate: spontaneous bleeding without trauma (e.g. purpura in the dependent parts)
- Serious: active bleeding (e.g. from mucous membranes).

A number of examinations is required in order to establish the cause of thrombocytopenia, such as full blood cell count, examination of the bone marrow, determination of the size of the spleen, coagulation studies and studies of platelet survival. However, an impression of platelet survival can be inferred from the other data collected. The cause of thrombocytopenia may thus be classified into one of two groups, depending upon normal or short-

ened platelet survival. They are listed in Table 1.

The treatment of thrombocytopenia is dependent upon the underlying disease. Some disorders are self-limiting or remit following the elimination of certain exogenous factors. Others are persistent and require medical or surgical treatment. Treatment potentials have improved in recent years, especially during intensive chemotherapy for malignant disorders and bone marrow transplantation for aplastic anaemia and leukemia. Thrombocytopenia often occurs in these disorders or it may be caused by the treatment given. Platelet transfusions will then be needed temporarily. The problems raised by repeated transfusions will be discussed at the end of this chapter.

2. Immune thrombocytopenia

The terminology on the subject of immune thrombocytopenia has been quite confusing. The term is used here as a designation of all forms of thrombocytopenia due to destruction of platelets by immune mechanisms. Immune thrombocytopenia may thus be divided into drug-dependent immune thrombocytopenia (DITP), idiopathic thrombocytopenia (ITP) and secondary immune thrombocytopenia, in the course of other disorders such as systemic lupus erythematosus (SLE) or malignant lymphoma [2].

A substantial quantity of evidence in favour of the immune character of platelet destruction has been presented [2]. Serum anti-platelet antibodies have been demonstrated in 30–65% and platelet-

Table 1. Causes of thrombocytopenia.

Platelet survival	Cause	Examples
Normal	Production impaired	Leukemia Aplastic anaemia
Shortened	Production ineffective	Megaloblastic anaemia
	Loss	Massive bleeding
	Sequestration	Splenomegaly
	Consumption	DIC TTP
	Accelerated destruction	
	non-immune	Prosthetic heart valves Extracorporeal circulation
	immune	ITP

associated IgG (and sometimes IgA and/or IgM) in 78–100% of patients by various techniques [2, 3]. Complement fixation (C3) to patient platelets has been reported in about half the cases [4, 5]. The platelet antigens that are targets for immune attack are still unknown, although certain membrane glycoproteins may be involved [6].

The course of ITP may be chronic, intermittent or acute. The duration of acute ITP ranges from one week up to three months [7]. In children the acute form predominates and is self-limiting in 90% of cases [7]. Initially acute and chronic ITP, DITP and secondary immune thrombocytopenia may be clinically indistinguishable. The diagnosis of chronic ITP may often emerge by exclusion of the other disorders and by the clinical course.

2.1. *Conservative treatment*

Medical or surgical treatment is indicated only if the patient has bleeding manifestations or the platelet count ranges at a level that is considered to be potentially dangerous. As there are no prospective studies available on this subject, the level of safety has to be estimated from retrospective data. There is great individual variation: bleeding has been reported to appear with platelet counts from very low values up to $50-70 \times 10^9/l$ in children and adults [7, 8]. In later stages in patients with refractory thrombocytopenia, a platelet count over $30 \times 10^9/l$ may be considered safe [2].

In all patients a vigorous search should be made for exogenous factors that might have provoked the thrombocytopenia. Acute ITP is preceded by a viral infection in about 70% of cases [7]. Chronic ITP preceding infections occur much less frequently, but viral or focal bacterial infections may provoke a first attack or an exacerbation of thrombocytopenia [9]. Thus, effective treatment of any existing infections is required.

DITP is usually reversible after withdrawal of the offending drug, usually within four weeks and frequently in less than one week. An extensive list of potentially harmful drugs has been given by Wintrobe [10]. If thrombocytopenia persists following the discontinuation of a suspected drug, all drugs should be withheld if possible. When a remission is then still not attained another diagnosis is likely, most often chronic ITP.

2.2. *Corticosteroids*

In patients with significant bleeding tendency, especially from mucosal or retinal origin, or in patients with ITP and a platelet count well below $30 \times 10^9/l$, active treatment is indicated and should be started immediately. Current treatment modalities are given in Table 2. Sustained remissions are obtained in 80–90% of patients with bleeding tendency.

The initial treatment of choice is still corticosteroids. Prednisone is usually prescribed in relatively high doses, 1 mg per kg of body weight daily, in single or divided doses. In resistant cases higher doses, 2 mg per kg orally or even higher intravenous intermittent doses have been recommended [11], but in our experience additional remissions are rarely obtained. In 70–90% of patients

Table 2. Treatment of ITP.

Corticosteroids
Splenectomy
Immunosuppressive drugs
Infusion of vinca-alkaloid loaded platelets
Infusion of high-dose gammaglobulin
Plasmapheresis
Platelet transfusions

treated with corticosteroids the bleeding tendency subsides. A normal platelet count is obtained in about a third of patients, but a sustained remission exceeding one year is rare, probably less than 25% [2].

Once a remission has been obtained the dose of prednisone should be tapered off and stopped within half a year. If remission is not attained or relapse occurs, other treatment is indicated. Prednisone should not be continued for long periods because of its serious side effects, especially in doses over 10 mg daily.

The short-term effects of corticosteroids probably result partly from reduced vascular fragility and from inhibition of platelet destruction by the mononuclear phagocytic system (MPS). Reduction of antibody production only occurs after a protracted administration of many weeks [12].

ITP in children is likely to run an acute and limited course. Treatment with corticosteroids has not been shown to shorten the natural course of the disease [11]. The mortality rate is very low [13]. Treatment with corticosteroids is recommended only to reduce bleeding, thus possibly preventing consequent fatal haemorrhage.

2.3. *Splenectomy*

If a sustained remission is not obtained by corticosteroid therapy, splenectomy is indicated. In patients with serious bleedings, the operation should be performed early, within four weeks. In milder cases the effect of corticosteroids may be observed for three months. If remission or definite improvement are not attained, splenectomy is the treatment of choice [2, 7]. In all cases the risks and the benefits of the operation should be carefully considered. In a recent study of 78 patients with ITP, postoperative morbidity was 14 per cent and one patient died [14]. Remission occurred in 77% and improvement in a further 8% of the patients. The overall operation mortality in the literature is slightly below 1% [11]. Remission rates of 60–80% have been reported [11]. If operation is contraindicated on clinical or technical grounds, one of the other treatment modalities mentioned in Table 2 may be given. In selected cases splenectomy may

be performed early on professional or social grounds (e.g. sailors).

The spleen is considered to be the most important site of antibody production and of platelet destruction in patients with ITP [12], especially in the early phase of the disease. In long-standing cases other parts of the MPS may become increasingly involved [12]. Thus, splenectomy should not be postponed for too long, preferably not exceeding half a year. In children with chronic ITP (about 10 percent of children with ITP), splenectomy is usually postponed for up to a half to one year if possible. There is a considerable risk of septicaemia by Gram-positive bacteria in children, especially below the age of eight years. Recently accumulated evidence suggests that this risk in adults has been underestimated in the past. Therefore, at present the administration of pneumococcal vaccine is recommended for all patients who have been or will be splenectomized [15]. If infections are suspected, prompt antibiotic treatment should be started.

In 10–20% of patients improvement but not a complete remission is attained after splenectomy. In another 10–20% no apparent effect is produced by the operation. If these patients still have bleeding manifestations one of the other treatment modalities (Table 2) is indicated. When a relapse occurs after splenectomy the possibility of accessory spleens should be kept in mind. They are easily detectable by scintigraphy and may be removed surgically. Sometimes splenectomy has to be performed in patients with serious bleeding. The operation may then be prepared by one of the other treatment modalities given in Table 2.

2.4. *Immunosuppressive drugs*

Treatment with immunosuppressive drugs (ISD) is much less established than treatment with corticosteroids or splenectomy. The data are obtained from reports on relatively small groups of patients with refractory ITP, and controlled studies have been lacking. The most important ISD in current use are vinca alkaloids, cyclophosphamide and azathioprine. Long-term corticosteroid therapy has an immunosuppressive effect as well. All these

ISD have specific side effects which will not be discussed in detail here. Once more the potential benefit of the drug should be weighed against the risks and the patient should be observed carefully during the treatment.

Vinca alkaloids are the first choice if a remission is to be attained in a short time. Vincristine 0.025 mg per kg (maximum 2 mg) or Vinblastine 0.125 mg per kg (up to 10 mg) are given intravenously once weekly. Remission is obtained usually within two weeks in about 40% of patients with refractory ITP [16, 17]. In one study [16] thirteen refractory patients were treated with vincristine. Six of them attained complete remission which persisted for six years without maintenance therapy in four of these patients. In four other patients a favourable response could only be maintained by continued use of vincristine. However, this kind of maintenance therapy should be avoided because severe neuropathy is apt to occur sooner or later. Much less is known about vinblastine treatment, but the results seem to be comparable to those of vincristine [2, 18]. The choice between the two drugs may be largely determined by their side effects, leukopenia being more often induced by vinblastine and neuropathy by vincristine. The effect of vinca alkaloids probably is a reduction of platelet-antibody production [19] and of platelet destruction by the MPS [18, 19].

Infusion of vinca alkaloid-loaded platelets. Recently Ahn *et al.* devised a new method for the administration of vinca alkaloids: intravenous infusion of vinblastine-loaded platelets [18]. The delivery of vinblastine to macrophages is supposed to be enhanced by its being loaded on to platelets that are prone to rapid destruction by antibodies and phagocytosis in the MPS. Patient or donor platelets were used depending on the level of thrombocytopenia. Six out of 11 patients with refractory ITP achieved a complete remission, three of whom relapsed within four months [18]. These results have been confirmed by others [19, 20]. The patients mentioned in the three reports together amount to 27, six of whom obtained a complete remission lasting for up to seven months [18, 20–22]. The side effects have been considerable: neuropathy and transfusion reactions, including severe anaphylac-

tic reactions [21]. Only some of the patients had been splenectomized. Comparable results have been obtained using vincristine-loaded platelets in non-splenectomized patients, with fewer side effects reported [22].

The benefit of loading these drugs on to platelets is not yet clear. The patient groups described were heterogeneous with respect to previous therapy. Many patients were on prednisone during treatment and may thus have had impaired MPS function. Most patients had probably not been treated with vinca-alkaloids alone previously. Moreover, vinblastine has been shown to elute rapidly from rabbit platelets [21]. The results reported [18, 20–22] may in fact be equal to those obtained with vinca-alkaloid treatment alone. In our own modest experience (unpublished) five splenectomized but otherwise untreated patients did not respond to vincristine intravenously and subsequently did not do so to vinblastine-loaded platelets.

Prednisone may be continued or reintroduced when a patient proves to be refractory to splenectomy. In many cases relatively safe platelet counts and control of bleeding can be achieved. Remission may occur in 20% of patients, even when they had been refractory to corticosteroids before splenectomy. Unfortunately most remissions cannot be maintained on the acceptable prednisone dose level of 5–10 mg daily or preferably on alternate days.

Azathioprine has been used since 1967 in the treatment of refractory ITP [23]. It is given in a dose of 1–3 mg per kg daily. Response occurs slowly, after several months. Improvement has been reported in about half the cases, but remissions have been rarely sustained [2]. Combination therapy with corticosteroids may be given, but the value of such therapy is as yet unclear because extensive studies are not available. Possibly a reduction of the dose of prednisone may be achieved.

Cyclophosphamide may be given in a dose of 2–3 mg per kg daily. Response is obtained more quickly than by azathioprine: usually within 6–8 weeks. Complete remission is achieved in 30–40% of cases and in part of them remission may be persistent without maintenance therapy [24–26]. Combination therapy with vincristine and pred-

nisone has been given successfully to a few patients with serious bleeding [2, 25], but this combination has not been studied extensively. The long-term side effects of cyclophosphamide, especially hemorrhagic cystitis and the risk of development of myelodysplasia or acute leukemia should be kept in mind while considering its application to patients with a chronic disease.

Frentizole has been used successfully in the treatment of one patient with refractory ITP [27] but of course more data are required before it can be recommended for the treatment of ITP.

If treatment with immunosuppressive drugs is considered, the first choice will usually be prednisone, the second vincristine, and the other drugs should be reserved for resistant cases. A summary of data on the treatment of chronic ITP is given in Table 3.

2.5. Infusion of high dose gammaglobulins

The observation of disappearance of severe thrombocytopenia in two children with congenital agammaglobulinaemia after intravenous infusion of high dose gammaglobulin (as prepared for intravenous administration) prompted Imbach *et al.* to apply this therapy to children with ITP [28]. It was infused in a daily dose of 0.4 g per kg in one to two hours on five consecutive days. Maintenance therapy was given by the same daily dose once

every 1–6 weeks if necessary. Six children with acute and seven with chronic or intermittent ITP (three splenectomized) were treated. In all patients a complete initial response was obtained. Four of the children with acute ITP remained in complete remission without maintenance therapy. In the children with chronic ITP, remission persisted in one without treatment. In the other six the platelet count decreased within a month and maintenance therapy had to be given, with improvement occurring in all, but remission was sustained in only one patient.

In adults, results have been encouraging though less impressive than in children. In one study [29] two splenectomized patients had a complete response, while two non-splenectomized patients responded only partially. In a larger study by Newland *et al.* [30] 25 patients were treated. In all ten refractory patients with recent disease (within 16 weeks of presentation) a complete response was obtained. In 15 patients with long standing disease (10 splenectomized) complete response was achieved in six and partial response in nine patients. In all patients the response was transient, with reversion usually within four weeks. In our own experience (unpublished) a transient partial or complete response was obtained in about two-thirds of the patients and response has often not been permanent.

The effect of high dose gammaglobulin could be

Table 3. Results of therapy in chronic idiopathic thrombocytopenic purpura.*

Therapy	No. of cases	Average dose	Response time (days)	Response**			
				Excellent	Good	Fair (percent)	Poor
Splenectomy	756	–	1–14	80		← 20 →	
Steroids	253	60–100 mg/day	14	19	← 34 →		47
Cyclophosphamide	61	50–200 mg/day	14–56	42	14	12	32
Vinblastine	20	10 mg/wk	10	5	← 56 →		39
Vincristine	21	2 mg/wk	10	← 28 →		48	24
Azathioprine	92	100–250 mg/day	60–120	8	18	26	48

* Adapted from McMillan R: N Engl J Med, 1981; 304: 1143. with permission of the author.

** Response definitions are as follows: excellent, normal platelet count after therapy; good, normal platelet count during therapy; fair, improved platelet count during therapy; poor, no response. In some cases, details of the reports did not permit accurate placement, and in these cases percentages may apply to more than one response category (arrows).

due to overloading of the MPS or simply to competitive inhibition of macrophage IgG-Fc receptors by the IgG, leading to reduced phagocytosis of antibody-coated platelets. This hypothesis has been reinforced by Fehr *et al.* [31], who showed transient responses of thrombocytopenia and decreased clearance of radiolabelled IgG-anti D coated autologous red cells which had been injected simultaneously with high dose gammaglobulin. Untoward effects of high dose gammaglobulin infusions have been rarely observed, probably dependent upon the method of preparation of the product [32].

Children with ITP have been treated in the past with infusions of 10–30 ml of plasma per kg body weight. Permanent or transient responses have been reported in 19 out of a total of 33 patients [33]. Comparable results have been reported more recently [34]. Although responses in children with ITP should not be attributed too loosely to any treatment given, the data suggest that the active principle in plasma could be IgG, and that comparable results could be obtained using lower doses of IgG-than applied up to now. In view of the transient effect of high dose gammaglobulins this therapy is only indicated in case of life-threatening bleeding or as a preoperative measure.

2.6. Plasmapheresis

Platelet antibodies may be removed from a patient's circulation by plasmapheresis. It may be supposed that as a consequence antibody will be bound to circulating platelets to a lesser extent. Indeed short-lived partial responses have been reported in a few children [35] and adults with ITP [36] following plasma exchange. In other cases the procedure has not been successful [36, 37]. In these studies 5–10 l of plasma were exchanged in 1–10 days. However, controlled studies have not been performed. Most patients received various modes of therapy before plasmapheresis. If an elevation of the platelet count occurred it lasted for only a few days. As only about half the patients with chronic ITP have antibodies in the serum and most of the antibody is often platelet-associated, the failures of treatment by plasmapheresis are readily

explained. The risks of hypocoagulability and hypotension during the procedure are considerable, especially in bleeding patients. Thus, the role for this kind of treatment in life-threatening bleeding episodes remains to be established.

2.7. Androgens

Recently *danazol*, a synthetic steroid with weak androgenic and anabolic activity has been introduced as a drug of possible value in the treatment of refractory ITP [38]. In 15 out of 22 patients on corticosteroid therapy partial or complete remission was obtained. Five of the responding patients had not been splenectomized. Corticosteroid dosage could be reduced in many responding patients. In six of them corticosteroids could even be stopped. The duration of remissions ranged from 2 to 13 months. Most patients tolerated danazol well. Confirmation of these results and more experience with the drug are required before it might be applied as a routine treatment.

2.8. Platelet transfusions

Autologous platelets are rapidly broken down in patients with serious ITP. Unfortunately all kinds of donor platelets applied up to now have shared this fate. This is to be expected because platelet antibodies do not show specificity for certain platelet antigens. However, they may be directed against determinants present on platelet membrane glycoproteins (IIb, IIIa), absent on platelets from patients with Glanzmann's thrombasthenia [6]. Thus, selection of donor platelets with a better survival in the patient is not yet possible. Platelet transfusions are indicated only for the treatment of life-threatening bleeding and for support during operations. A well-delineated indication is the supportive treatment during splenectomy. If the patient is at risk of bleeding and no improvement is obtained after treatment with corticosteroids, platelet concentrates are given following the clamping of the splenic blood vessels at laparotomy.

2.9. ITP and pregnancy

As ITP has some predilection for young women, ITP and pregnancy may often coincide. Some women may acquire ITP during pregnancy, others with ITP may become pregnant. A separate group is formed by women with ITP in remission. Platelet antibodies may still be present in the blood of splenectomized patients in remission [4, 11]. There is no convincing evidence that the clinical course of ITP is directly influenced by pregnancy [39]. During early pregnancy the risk of abortion is slightly increased, in the later stages the risk of bleeding for the fetus is largely dependent on the transplacental passage of antibodies. This will occur only if the antibodies are IgG and are present in the mother's plasma.

Treatment during pregnancy is indicated only when there is a bleeding tendency in the mother, as little is known about the effect of treatment on the fetus. The modes of treatment are largely similar to those for ITP generally, but immunosuppressive drugs are contraindicated. Splenectomy should be avoided if possible, because of the considerable risks for mother and child [2]. Corticosteroids may be tried first in high doses. If remission or improvement are achieved, the dose should be reduced as soon as possible. In many cases an acceptable condition can be maintained on low (preferably alternate days) doses of prednisone without harm to mother and fetus. Infusion of high dose IgG may serve as an emergency treatment, but data on this subject are not available.

Most difficulties arise at the time of delivery. Some of the infants will have acquired antibodies. In a recent study by Cines *et al.* and by van Leeuwen *et al.* [40, 41], no relation was observed between maternal and neonatal platelet count in mothers with active ITP or ITP in remission. The level of antibody in the mothers' serum appeared to correlate with the presence and extent of neonatal thrombocytopenia. Platelet-bound IgG was detected in the blood of all mothers and its level did not identify the neonates at risk of thrombocytopenia. Another approach for the determination of fetal thrombocytopenia is to measure directly the platelet count from fetal scalp blood early in labour

[42]. These counts proved to be fairly reliable and the procedure could be carried out without much risk for the fetus. Recently Karpatkin *et al.* [43] showed in a controlled study that the administration of corticosteroids in the last 10–14 days of pregnancy was effective in raising the platelet count to acceptable levels at the time of delivery. The dose of prednisone varied from 10 to 60 mg according to the maternal condition.

Delivery should be well controlled, in a clinical setting. The following procedure could be used: platelet bound IgG and serum antibodies ought to be assessed in mothers with active ITP or a past history of ITP. If antibodies are found in the serum, prednisone may be given if the drug is not contraindicated (as in hypertension or diabetes mellitus), from two weeks before delivery. The dose should probably be 40 mg or more, as only 13% of corticosteroids is transferred to the fetus in the active form [39]. After the onset of labour platelet counts are obtained from fetal scalp blood. If the platelet count is safe and the mother is not at risk of bleeding, vaginal delivery may be induced. If the fetal platelet count is below $50 \times 10^9/l$ or if the mother is at risk of bleeding, including a platelet count below $100 \times 10^9/l$, Caesarean section is to be preferred [2, 44]. The operation may be prepared, as mentioned earlier, with high-dose IgG infusions. If indicated splenectomy may be performed during the same laparotomy, following delivery.

2.10 Neonatal thrombocytopenia

This may be due to *autoimmunity in the mother*, as discussed above. The disorder is of course self-limiting, but it may be serious. It usually lasts for up to two weeks [2]. Treatment should be given according to the bleeding tendency. Prednisone may be given in a dose of 1 mg per kg, after an initially higher dose of 20 mg daily. If bleeding persists the infant may be treated with (easily achievable) high-dose IgG or occasionally with platelet transfusions or exchange transfusions.

Neonatal *alloimmune thrombocytopenia* may result from antibodies produced by the mother during pregnancy in response to a fetal platelet antigen not shared by the mother. Usually anti-Zw^a (Pl^{A1})

is the offending antibody, but others have been rarely described [45]. The clinical course resembles neonatal ITP. Treatment is indicated and should be started immediately in cases with active bleeding. This is often observed because bleeding manifestations point to the diagnosis of thrombocytopenia in most cases. The therapeutic tools are the same as for ITP, but even less data are available. Good results have been obtained by transfusion with maternal platelets [46]. At present, this is the treatment of choice. Maternal platelets are readily obtained and are highly effective in restoring the level of circulating platelets as they are lacking the offending antigen.

2.11. Other forms of immune thrombocytopenia

Drug-induced immune thrombocytopenia (DITP)
By definition the disorder should be reversible on withdrawal of the offending drug. In most cases remission occurs within four weeks, rarely longer, often within one week [47]. If thrombocytopenia persists either the drug has not been effectively withdrawn (e.g. long-acting drugs, such as gold salts) or the diagnosis should be reconsidered, most often in fact being ITP. A large number of *in vitro* tests for drug-dependent antibodies has been devised [47, 48], usually based on the current technique for the detection of platelet antibodies in serum. Unfortunately the diagnosis of DITP cannot yet be made or excluded reliably by their detection.

Active treatment is rarely required for patients with DITP. If the bleeding tendency is serious corticosteroids may be given. They may reduce bleeding and shorten the recovery period [47]. Emergency treatment is the same as mentioned for ITP. In long-standing cases due to long-acting drugs specific measures may be taken to accelerate the elimination of such drugs (e.g. chelating agents for gold salts).

Symptomatic immune thrombocytopenia

In some of the cases thrombocytopenia will disappear after appropriate treatment for the underlying disease. However, specific therapy as for ITP is often required because the thrombocytopenia may run an independent course.

Post-transfusion purpura (PTP)

Thrombocytopenia may occasionally occur about one week after a blood transfusion. The disorder is usually observed in women and is supposed to be due to platelet specific alloantibodies, most often anti-Zw^a(PI^{A1}). Probably complexes of Zw^a antigen on donor platelets and anti-Zw^a antibodies are formed in the blood of the Zw^a negative recipient. These immune complexes then sensitize the patients' platelets thus shortening their survival [49]. Bleeding may be serious and may persist for several weeks. Therapy has been documented in only a few cases as the disease is very rare. Corticosteroids may reduce the bleeding tendency, but do not influence the natural course. Plasmapheresis has been successful in reducing bleeding, presumably due to the removal of immune complexes [11].

3. Thrombocytopenia due to consumption of platelets

Platelet consumption is observed in thrombotic thrombocytopenic purpura (TTP), haemolytic uraemic syndrome (HUS) and in diffuse intravascular coagulation (DIC). The former two disorders share microangiopathy, formation of platelet aggregates, variable degrees of thrombocytopenia and disturbance of the function of various organ systems. DIC is a complicated pathological condition defined by consumption coagulopathy and secondary multiple organ dysfunction. It may result from various initiating events concerned with activation of coagulation, platelets or endothelial surfaces. The clinical course of DIC may be acute (e.g. solutio placentae) or chronic (e.g. malignant disorders). TTP and HUS are probably basically similar, but they tend to involve different organs and affect different age groups. As thrombocytopenia is not a constant finding in HUS or DIC the therapy of these disorders will not be discussed here.

Thrombotic thrombocytopenic purpura is further characterized by fever, microangiopathic haemolytic anaemia and neurological disturbances [50, 51]. Its etiology is unknown, but it is likely that vascular

damage is the central event and that platelet aggregates are formed secondarily [51]. The vascular damage may be caused by infections or toxic agents or possibly by immune mechanisms [52]. Immune complexes have been demonstrated in the vascular lesions [53] and in a few cases elevation of platelet-bound IgG has been found [52]. The treatment of TTP has been largely empirical and based on case reports [53]. As the disease is very rare, controlled studies are difficult to perform. If the disease is left untreated 80% of patients will die within three months [54]. Thus, survival is usually attributed to the therapy given, but it does not prove the value of such therapy. Survival has been reported in patients treated with corticosteroids, splenectomy, heparin and antiplatelet drugs (aspirin, dipyridamole, sulfipyrazone) or some combination of these [51]. Exchange transfusion or plasma exchange appeared to be successful in some cases [50]. By plasma exchange toxic factors, antibodies or immune complexes could be removed. In one case study remission of TTP did not occur after plasma exchange if washed red cells and albumen were substituted, while fresh plasma rapidly did so. It was suggested that plasma contains an inhibitor of platelet aggregation that is lacking in patients with TTP [55]. Further evidence suggested that this plasma factor could be a stimulator of the potent platelet aggregation inhibitor prostacyclin [50, 56]. Whether the resulting prostacyclin deficiency is a primary or a secondary effect remains to be established. Substitution therapy with prostacyclin has not been found successful up to now [56]. The limited available evidence points to the treatment of TTP with plasmapheresis and antiplatelet drugs, for example dipyridamole and aspirin in low dosage (avoiding the undesirable inhibition of prostacyclin).

4. Thrombocytopenia due to insufficient production of platelets

4.1. *Ineffective thrombopoiesis* may be a part of some deficiency disorder and may be treated by appropriate substitution therapy.

4.2. *Impaired thrombopoiesis* in aplastic anaemia or

by bone marrow infiltration may be treated by the appropriate therapy for the underlying disease. The general supportive treatment of leukemia will be discussed in Chapter 8 and of bone marrow transplantation in Chapter 11. Platelet transfusions are frequently indicated in the course of these disorders and some aspects on the indications and selection will be discussed in the next part of this chapter.

5. Platelet transfusion

Platelet transfusion is indicated in case of severe haemorrhage due to thrombocytopenia or disorders of platelet function. In many cases it is a life-saving procedure. Its success depends on the amount of platelets transfused, the functional integrity of the transfused platelets, the cause of the thrombocytopenia or platelet disorder, and the presence or absence of alloantibodies against platelets.

5.1. Indications for platelet transfusion

General aspects.

Platelet transfusions are given either to stop or to prevent bleeding. From clinical experience it is known that significant bleeding rarely occurs if the platelet count is above $50 \times 10^9/l$ [58]. In fact, life-threatening haemorrhage rarely occurs at a platelet count above $10 \times 10^9/l$, provided that platelet function is normal [59]. In thrombocytopenia due to impaired platelet production, the template bleeding time was even found to be abnormal only in patients with a platelet count below $10 \times 10^9/l$ [5]. Moreover, in patients with thrombocytopenia due to destruction of platelets, many young active platelets are circulating, and the bleeding time may remain normal at platelet counts as low as $5 \times 10^9/l$. In contrast, the bleeding tendency of a thrombocytopenic patient is severely aggravated in the presence of concurrent disease, such as infection, capillary damage or exposure to drugs that affect platelet function. Thus, a careful clinical and haematological evaluation of the patient is necessary when a platelet transfusion is considered. In-

dications for platelet transfusion are listed in Table 4.

Special aspects

Aplastic anaemia. This is a chronic disorder and the risk of bleeding may persist for months or years. A restricted transfusion policy is required in view of possible alloimmunization. Transfusion should be given only in case of severe bleeding, preparation for surgery, or of severe infections. However, a prolonged platelet transfusion programme may be possible using platelets from HLA matched donors [61]. In some patients a state of immune unresponsiveness may occur, thus permitting protracted treatment with random platelet transfusions [62].

Leukemia. Life-threatening bleeding due to thrombocytopenia is common and should be treated vigorously with platelet transfusions. Prophylactic treatment with platelet transfusions in thrombocytopenic patients has been found effective in reducing the incidence and severity of haemorrhage [63]. It is usually indicated when the platelet count is below $10 \times 10^9/l$. Effective pro-

phylaxis may often be obtained by transfusion on alternate days.

Immune thrombocytopenia. The indications for platelet transfusions have been discussed in part 2 of this chapter.

Platelet loss or consumption. In patients with serious bleeding who have been transfused with platelet-poor fluids or blood products the platelet count may fall below $20 \times 10^9/l$. Within a short time the patient's platelet production is not able to compensate losses, and platelet transfusion is indicated. Sometimes so many platelets are consumed in the course of DIC or during and after extracorporeal circulation that platelet transfusion is indicated.

Hereditary disorders of platelet function. In general a conservative approach is necessary in order to avoid and/or delay alloimmunization and refractoriness.

5.2. Collection and preservation of platelets for transfusion

Platelet concentrates are preferred to platelet-rich plasma (PRP) in order to avoid volume overload. They may be prepared from ACD or CPD-blood and are usually delivered as pools of 4–8 donors. Commonly the concentrates are harvested from freshly drawn blood from random donors by differential centrifugation. The units of platelets produced in this way should contain at least 5.5×10^{10} platelets in a volume of 20–50 ml of plasma, in 75% of the units pooled. They should not contain aggregates and the pH should be above six after 72 h storage [58]. Multiple units of platelets may be obtained from one selected donor by hand cytapheresis or by machine cytapheresis using cell separators. This may be carried out many months without harm to the donor [64].

Platelet concentrates should be used as fresh as possible. When storage is necessary, this is preferably done at room temperature and as PRP, in order to maintain maximal function and viability [65]. Cryopreserved autologous- or donor platelets may

Table 4. Indications of platelet transfusions.

A. Therapeutic
1. Thrombocytopenia due to impaired platelet production
– Primary bone marrow failure (aplastic anaemia)
– Secondary bone marrow failure (e.g. leukemia)
2. Thrombocytopenia due to increased platelet destruction
– Immune (e.g. ITP, neonatal): exceptionally!
– Non-immune (e.g. prosthetic heart valves)
3. Thrombocytopenia due to increased consumption or loss
– Sepsis
– DIC
– Extracorporeal circulation
– Massive bleeding and inadequate transfusions
4. Disorders of platelet function
– Hereditary (e.g. Glanzmann's disease)
– Acquired (e.g. drugs, paraproteinaemia)
B. Prophylactic
1. Thrombocytopenia of transient nature (platelet count below $20 \times 10^9/l$)
– Bone marrow failure, primary or secondary
2. Preparation for surgery

be applied [66], but cryopreservation is still an expensive procedure, and platelet loss is high.

5.3. Administration and dosage

Transfusion frequency depends on the increment of the patient's platelet count and on platelet survival. Following transfusion the increase of the platelet count should be about $12 \times 10^9/l/m^2$ of body surface per unit platelets transfused [67]. Since the actual yield often varies and in fact may be regularly low in febrile patients, it is important to check platelet count 1/2–1 h after transfusion and subsequently every day in order to get an impression of transfusion effectivity. In case of serious bleeding platelets should be transfused immediately, in amounts sufficient to stop the bleeding, usually requiring maintenance of the platelet count above $50 \times 10^9/l$. Transfusion should be continued until the bleeding has definitely stopped. During and after major surgery the maintenance of the same platelet count is required prophylactically as long as risks of bleeding persist.

5.4. Immunological aspects of platelet transfusion and donor selection

Platelets carry the antigens of the blood group ABO, the HLA system and some platelet specific systems (Zw or Pl^A, Ko, Bak), but not of the Rhesus system. Since ABO incompatibility diminishes platelet recovery the administration of ABO incompatible platelets is usually not recommended. Rhesus compatibility is only necessary in young Rh negative patients with a curable disease in order to avoid alloimmunization due to contaminating Rh positive red cells. If administration of platelets from Rh positive donors is inevitable, IgG anti-D should be given prophylactically.

Resistance to infused platelets develops in many patients on prolonged platelet transfusion support from random donors and is attributed mainly to HLA alloimmunization. Although certain patients may continue to show good responses to unmatched platelets for months, many will become refractory by eight weeks, some even within two weeks [58]. However, several authors have shown

that alloimmunization to HLA antigens may be short-lived or does not occur at all in at least half the patients with acute leukemia who were tested after the induction therapy [69, 70]. Moreover, the number of platelet transfusions given was not related to the extent of alloimmunization [71]. Thus, this therapy should not be withheld for prophylaxis of alloimmunization in leukaemic patients. In refractory patients not only HLA antibodies but also platelet specific- and even granulocyte-specific antibodies may have formed. Platelet-specific antibodies may then be responsible for a low recovery and increased destruction of donor platelets [72–74].

These results support the following policy in the (potentially) refractory patient: HLA typing of the patient and possibly of family members is performed. As soon as the one hour corrected platelet increment decreases below $10 \times 10^9/l/m^2$ per 10^{11} platelets transfused [74], serum is obtained, tested for HLA and platelet-specific antibodies and used in a cross-match with the cells of selected donors (family or unrelated). The cross-match should include a cytotoxicity test on lymphocytes and an immunofluorescence test on platelets [72–74, 76]. In one study [77] it was found that the development of refractoriness and HLA-alloimmunization occurred more slowly by transfusion of platelets from single than from multiple random donors. In another study [78] these results have been challenged and the inpracticability of this approach has been emphasized.

5.5. Complications of platelet transfusion therapy

These are largely the same as for blood transfusion in general, such as infections (e.g. hepatitis B, non A non B, CMV, AIDS) and transfusion reactions due to alloimmunization. The risks of infection due to platelets are considerable, as multiple donors are usually involved. Febrile reactions are frequently encountered and may often be due to alloimmunization to contaminating granulocytes [73]. More careful differential centrifugation may help to prevent this reaction.

Posttransfusion purpura due to Zw^a(Pl^{A1}) immunization may rarely develop. Graft versus host re-

actions may occur in the immunodeficient recipient, by contaminating lymphocytes. To prevent this, irradiated platelets should be supplied in this case.

References

- Veenhoven WA, van der Schans GS, Huiges W, Metting-Scherphuis HE, Halie MR, Nieweg HO: Pseudothrombocytopenia due to agglutinins. *Am J Clin Pathol*, 1979; 72: 1005-8.
- McMillan R: Chronic idiopathic thrombocytopenia. *N Engl J Med*, 1981; 304:1135-47.
- Von dem Borne AEG, Helmerhorst FM, van Leeuwen EF, Pegels HG, von Riesz E, Engelfriet CP: Autoimmune thrombocytopenia: detection of platelet antibodies with the suspension immunofluorescence test. *Br J Haematol*, 1980; 45: 319-27.
- Cines DB, Schreiber AD: Immune thrombocytopenia: use of a Coombs antiglobulin test to detect IgG and C3 on platelets. *N Engl J Med*, 1979; 300: 106-11.
- McMillan R, Martin M: Fixation of C3 to platelets in vitro by antiplatelet antibody from patients with immune thrombocytopenic purpura. *Br J Haematol*, 1981; 47: 251-56.
- van Leeuwen EF, van der Ven JThM, Engelfriet CP, von dem Borne AEGK: Specificity of autoantibodies in autoimmune thrombocytopenia. *Blood*, 1982; 59: 23-28.
- Baldini MG: Idiopathic thrombocytopenic purpura. *N Engl J Med*, 1966; 274: 1245, 1301, 1360.
- Lammi AT, Albert L: Idiopathic thrombocytopenic purpura: an epidemiologic study. *J Paediatr*, 1973; 83: 31-36.
- Veenhoven WA, Halie MR, Stijnen PJ, Nieweg HO: Bacterial infections and thrombocytopenia in chronic idiopathic thrombocytopenic purpura. *Acta Haematol*, 1979; 62: 159-66.
- Winthrobe MM: Haematology. 8th ed. Lea and Febiger, Philadelphia, 1981; 1100.
- Koller CA: Immune thrombocytopenic purpura. *Med Clin N Am*, 1980; 64: 761-73.
- McMillan R: The pathogenesis of immune thrombocytopenic purpura. *Crit Rev Clin Lab Sci*, 1977; 8: 303-31.
- Luscher JM, Zuelzer WW: Idiopathic thrombocytopenic purpura in childhood. *J Paediatr*, 1966; 68: 971-77.
- Mintz SJ, Petersen SR, Cheson B, Cordell LJ, Richards RC: Splenectomy for immune thrombocytopenic purpura. *Arch Surg*, 1981; 116: 645-50.
- PHS Advisory committee on immunization procedures. Pneumococcal polysaccharide vaccine. *Morbid Mortal Weekly Rep*, 1978; 27: 25-31.
- Ahn YS, Harrington WJ, Seelman RC, *et al.*: Vincristine therapy of idiopathic and secondary thrombocytopenias. *N Engl J Med*, 1974; 291: 376-80.
- Ries CA: Vincristine treatment of refractory autoimmune thrombocytopenia. *N Engl J Med*, 1976; 295: 1136.
- Ahn YS, Byrnes JJ, Harrington WJ, *et al.*: The treatment of idiopathic thrombocytopenic purpura with vinblastine-loaded platelets. *N Engl J Med*, 1979; 298: 1101-7.
- Rosse WF: Selective chemotherapy of macrophages in the treatment of idiopathic thrombocytopenic purpura. *N Engl J Med*, 1979; 298: 1139-40.
- Slichter SJ, Schwartz K: Mechanism of action of vinblastine-loaded platelets in the treatment of chronic idiopathic thrombocytopenic purpura. *Clin Res*, 1979; 27: 307 A.
- Kelton JG, McDonald JWD, Barr RM, *et al.*: Reversible binding of vinblastine to platelets: implications for therapy. *Blood*, 1981; 57: 431-437.
- Agnelli G, DeCunto M, Gresele P, Nenci CG: Vincristine-loaded platelets for autoimmune thrombocytopenic purpura. *Blood*, 1982; 60: 1235-36.
- Sussman LN: Azathioprine in refractory idiopathic thrombocytopenic purpura. *JAMA*, 1967; 202: 259-63.
- Laros RK, Penner JA: Refractory thrombocytopenic purpura treated successfully with cyclophosphamide. *JAMA*, 1971; 215: 445-49.
- Lacey JV, Penner JA: Management of idiopathic thrombocytopenic purpura in the adult. *Semin Thromb Hemost*, 1977; 3: 160-74.
- Caplan SN, Berkman EM: Immunosuppressive therapy of idiopathic thrombocytopenic purpura. *Med Clin N Am*, 1976; 60: 971-86.
- O'Duffy JD, Colgan JP, Phyllyk RL, Ferguson RH: Frentizole therapy of thrombocytopenia in systemic lupus erythematosus and refractory idiopathic thrombocytopenic purpura. *Mayo Clin Proc*, 1980; 55: 601-5.
- Imbach P, Barandum S, d'Apuzzo V, *et al.*: High dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet*, 1981; i: 1228-30.
- Schmidt RE, Budde U, Schäfer G, Strochmann I: High dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura. *Lancet*, 1981; ii: 475-76.
- Newland AC, Treleaven JG, Minchinton RM, Waters AH: High-dose intravenous IgG in adults with autoimmune thrombocytopenia. *Lancet*, 1983; i: 84-87.
- Fehr J, Hoffmann V, Kappeler U: Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gammaglobulin. *N Engl J Med*, 1982; 306: 1254-58.
- Bierling P, Faracet JP, Dvedain N, Rochant H: Gamma-globulin for idiopathic thrombocytopenic purpura. *N Engl J Med*, 1982; 307: 1150.
- Lehoczy D, Kelemen E: Gammaglobulin for idiopathic thrombocytopenic purpura. *N Engl J Med*, 1982; 307: 1150-51.
- Cohen JI, Gammaglobulin for idiopathic thrombocytopenic purpura. *N Engl J Med*, 1982; 307: 1151.
- Novak R, Wilimas J: Plasmapheresis in catastrophic complications of idiopathic thrombocytopenic purpura. *J Paediatr*, 1978; 92: 463-66.
- Branda RF, Tate DY, McCullough JJ, Jacob HS: Plasma

- exchange in the treatment of fulminant idiopathic thrombocytopenic purpura. *Lancet*, 1978; i: 688-90.
37. Weir AB, Poon MC, McGowan EI: Plasma exchange for idiopathic thrombocytopenic purpura. *Lancet*, 1978; ii: 689.
38. Ahn YS, Harrington WJ, Simon SR, Mylvaganam R, Pall LM, Go AG: Danazol for the treatment of idiopathic thrombocytopenic purpura. *N Engl J Med*, 1983; 308: 1396-99.
39. Handin RI: Neonatal thrombocytopenia- the doctors dilemma. *N Engl J Med*, 1981; 305: 951-53.
40. Cines OB, Dusak B, Tomaski A, Mennuti M, Schreiber AD: Immune thrombocytopenia and pregnancy. *N Engl J Med*, 1982; 306: 826-31.
41. van Leeuwen EF, Helmerhorst FM, Engelfriet CP, von dem Borne AEGKr: Maternal autoimmune thrombocytopenia and the newborn. *Br Med J*, 1981; 283: 104.
42. Scott JR, Cruikshank DP, Kochenour NK, Pitkin RM, Warenski JC: Fetal platelet counts in the obstetric management of immunologic thrombocytopenic purpura. *Am J Obstet Gynaec*, 1980; 136: 495-99.
43. Karpatskin M, Porges RF, Karpatskin S: Platelet counts in infants of women with autoimmune thrombocytopenia: effect of steroid administration to the mother. *N Engl J Med*, 1981; 305: 936-39.
44. Carloss HW, McMillan R, Crosby WH: Management of pregnancy in women with autoimmune thrombocytopenic purpura. *JAMA*, 1980; 244: 2756-58.
45. von dem Borne AEGKr, van Leeuwen EF, von Riesz LE, van Boxtel CJ, Engelfriet CP: Neonatal alloimmune thrombocytopenia: detection and characterization of the responsible antibodies by the platelet immunofluorescence test. *Blood*, 1981; 57: 649-56.
46. Vain NE, Bedros AA: Treatment of isoimmune thrombocytopenia of the newborn with transfusion of maternal platelets. *Paediatrics*, 1979; 63: 107-9.
47. Karpatskin S: Drug-induced thrombocytopenia. *Am J Med Sci*, 1971; 262: 68-78.
48. van Leeuwen EF, Engelfriet CP, von dem Borne AEGKr: Studies on quinine-and quinidine-dependent antibodies against platelets and their reaction with platelets in the Bernard-Soulier syndrome. *Br J Haematol*, 1982; 51: 551-60.
49. Pegels JG, Bruijnes ECI, Engelfriet CP, von dem Borne AEGKr: Post-transfusion purpura: a serological and immunological study. *Br J Haematol*, 1981; 49: 521-30.
50. Editorial. Plasma exchange in thrombotic thrombocytopenic purpura. *Lancet*, 1979; i: 1065-66.
51. Aster RH: TTP: New clues to the etiology of an enigmatic disease. *N Engl J Med*, 1977; 297: 1400-1.
52. Morrison J, McMillan R: Elevated platelet associated IgG in thrombotic thrombocytopenic purpura. *JAMA*, 1977; 238: 1944-45.
53. Weisenburger DD, Frij GI, Hoak JC: Thrombotic thrombocytopenic purpura: conflicting results of in-vitro studies. *Lancet*, 1980; i: 99-100.
54. Amorosi EL, Ultman JE: Thrombotic thrombocytopenic purpura. *Medicine*, 1966; 45: 139-59.
55. Byrnes JJ, Khurana M: Treatment of thrombotic thrombocytopenic purpura with plasma. *N Engl J Med*, 1977; 297: 1386-89.
56. Hensby CN, Lewis PJ, Hilgard P, Mufti GJ, Hows J, Webster J: Prostacyclin deficiency in thrombotic thrombocytopenic purpura. *Lancet*, 1979; ii: 748.
57. Budd GT, Bukowski RM, Lucas FV, Cato AE, Cocchetto DM: Prostacyclin therapy of thrombotic thrombocytopenic purpura. *Lancet*, 1980; ii: 915.
58. Hack JC, Kaephe JA: Platelet transfusions. *Clin Haematol*, 1976; 5: 69-79.
59. Gaydos LA, Freireich EJ, Mantel N: The quantitative relation between platelet count and haemorrhage in patients with acute leukemia. *N Engl J Med*, 1962; 226: 905-9.
60. Harber LA, Slichter SJ: The bleeding time as a screening test for evaluation of platelet function. *N Engl J Med*, 1975; 287: 155-59.
61. Yankee RA, Graft KS, Dowling R, Henderson ES: Selection of unrelated compatible platelet donors by lymphocyte HLA matching. *N Engl J Med*, 1973; 288: 760-64.
62. Pegels JG, Bruyns ECE, Korhals Altes HR, Engelfriet CP, von dem Borne AEGKr: Immune unresponsiveness to platelets, a case study. *Vox Sang*, 1982; 42: 211-16.
63. Highby DJ: The prophylactic treatment of thrombocytopenic leukemic patients with platelets: a double blind study. *Transfusion*, 1974; 14: 440-44.
64. Bongiovanni MB, Katz RS, Wurzel HA: Long term plateletpheresis of a donor. *Transfusion*, 1980; 20: 465.
65. Schiffer CA: Some aspects of recent advances in the use of blood cell components. *Br J Haematol*, 1978; 79: 289.
66. Lazavious HM, Kanicki-Green EA, Warm SE, Aikawa M, Herzig RH: Therapeutic effectiveness of frozen platelet concentrates for transfusion. *Blood*, 1981; 57: 243-49.
67. Freireich AJ: Platelet transfusion procedures. *Cancer Chemother Rep*, 1968; 1: 1.
68. Aster RH: Effect of anticoagulant and ABO incompatibility on recovery of transfused human platelets. *Blood*, 1965; 26: 732.
69. Ditcher JP, Schiffer CA, Aisner J, Wiernik PH: Long term follow-up of patients with leukemia receiving platelet transfusions: identification of a large group of patients who do not become allo-immunized. *Blood*, 1981; 58: 1007.
70. Kutt J, Zaroulis CG, Dinsmore RE, Reich L, Clarkson BD, Good RA: A prospective study of platelet transfusion therapy administered to patients with acute leukemia. *Transfusion*, 1982; 22: 44-47.
71. Ditcher JP, Schiffer CA, Aisner J, Wiernik PH: Allo-immunization following platelet transfusion: the absence of a dose-response relationship. *Blood*, 1981; 57: 395.
72. Brand A, van Leeuwen A, Eernisse JG, van Rood JJ: Platelet transfusion therapy. Optimal donor selection with a combination of lymphocytotoxicity and platelet fluorescence tests. *Blood*, 1978; 51: 781-88.
73. Waters AH, Minchinton RM, Bell R, Ford JM, Lister TA: A crossmatching procedure for the selection of platelet

- donors for allo-immunized patients. *Br J Haematol*, 1981; 48: 59-68.
74. Pegels JG, Bruynes ECE, Engelfriet CP, von dem Borne AEGKr: Serological studies in patients on platelet- and granulocyte substitution therapy. *Br J Haematol*, 1982; 52: 59-68.
75. Daly RA, Schiffer CA, Aisner J, Wiernik PH: Platelet transfusion therapy. One-hour posttransfusion increments are valuable in predicting the need for HLA-matched preparations. *JAMA*, 1980; 243: 435-38.
76. Myers TJ, Kim BK, Steiner M, Baldini MG: Selection of donor platelets for allo-immunized patients using a platelet-associated IgG assay. *Blood*, 1981; 58: 444-50.
77. Sint Nicolaas K, Sizoo W, Haye WG, Abels J, Vriesendorp HM, Stenfert Kroese WF, Hop WCJ, Löwenberg B: Delayed allo-immunization by random single donor platelet transfusions. *Lancet*, 1981; i: 750-54.
78. Schiffer CA, Slichter SJ: Platelet transfusion from single donors. *N Engl J Med*, 1982; 307: 245-48.

6. Red cell therapy

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Introduction

Red cell therapy is indicated in conditions of anemic tissue hypoxia where the patient's oxygen carrying capacity is reduced to the extent that it produces symptoms which are so severe that immediate correction is necessary. Oxygen delivery to metabolizing tissues depends mainly on blood flow, hemoglobin concentration (i.e. oxygen carrying capacity) and oxygen unloading. In oxygen transport several systems are involved, each with its own reserve capacity. Important in oxygen unloading is the affinity of hemoglobin for oxygen as expressed by the P_{50} ; for example, an anemic patient with 55% reduction of the circulating hemoglobin suffers only a 27% reduction in oxygen availability by a considerable shift of the oxygen-hemoglobin dissociation curve to the right [1]. One of the most important compensatory mechanisms of the body for a lowered hemoglobin concentration is the increase of the cardiac output as the result of a lowered afterload due to the diminished viscosity of the blood.

Also of importance are further concomitant diseases and integrity of vital organs. In general it can be said that transfusion of red blood cell products is indicated if there are signs, or symptoms, resulting from tissue hypoxia or when these are anticipated. Acute blood loss is not automatically an indication for blood transfusion. It should be realised that many manifestations of acute blood loss are caused by hypovolemia and not by a decrease in the oxygen carrying capacity of the blood; the first goal of administration of fluids should be the restoration of tissue perfusion. This is initially much more important than the restoration of the oxygen delivery capacity; adequate perfusion in fact restores the delivery of oxygen to the periphery in the most effective and immediate manner [2]. Chronic anemia, without signs or symptoms of tissue hypoxia, which can be treated with iron, vitamin B_{12} or folate as appropriate is no indication for blood transfusion. Blood transfusion is not indicated as 'a booster', to speed up convalescence after operation, to accelerate wound healing or to treat hypoalbuminemia.

Patient consideration

In deciding if or when an anemic patient should be transfused there is no rule of thumb about the optimal hemoglobin concentration or hematocrit. First, one must define the type of anemia and try to establish a diagnosis, then consider how well a patient can compensate for his low hemoglobin, for instance as expressed in his exercise tolerance.

Blood preservatives

Citrate-Phosphate-Dextrose (CPD) or CPD-Adenine (CPDA-1) is mostly used as preservative to store blood (Table 1). Citrate is used as anti-coagulant, dextrose provides a source of energy for the synthesis of ATP and adenine is added to hold the ATP content of the red cells at an acceptable level during the period of storage. Red cell 2.3

Table 1. Composition of anticoagulant preservatives: Citrate-Phosphate-Dextrose Solution (CPD) and Citrate-Phosphate-Dextrose-Adenine Solution (CPDA-1).

	CPD	CPDA-1
Na ₃ citrate	26.3 g	26.3 g
Citric acid	3.27 g	3.27 g
Dextrose	25.2 g	31.9 g
NaH ₂ PO ₄ · H ₂ O	2.22 g	2.22 g
Adenine	—	0.275 g
Water to make	1000 ml	1000 ml
Volume per 100 ml blood	14 ml	14 ml
Red cell storage limit	21 days	35 days

DPG is better maintained in CPD than in acid citrate dextrose solution (used previously). Normally red cells are stored at about 4°C. The shelf life for whole blood in CPD is 21 days and in CPDA-1 35 days [3, 4]. In the formulation called CPDA-2, where both dextrose and adenine concentration have been further increased, whole blood and red cells can be stored for 42 days, but such preparations are not yet routinely available. With CPDA-2 blood the mean post-transfusion red cell survival is increased to more than 75%. When large transfusions must be given quickly the high viscosity of red cell concentrates might be a problem. This can be solved if the red cells are suspended in a special suspension preservative medium containing sodiumchloride, adenine, glucose and mannitol (SAGM); another advantage of this special medium is that more plasma can be removed [5].

Component therapy or whole blood

Since the introduction in the 1960's of plastic bags for the collection of blood it has been possible to separate a unit of whole blood into its components. From a unit of whole blood it is possible to obtain packed red blood cells, platelet rich plasma or fresh frozen plasma and platelet concentrates. The red cells can be processed as washed or frozen cells, as leucocyte poor red cells or can be provided genotyped. Compositions of some of the red cell preparations are shown in Table 2. The platelet rich plasma can be separated to produce platelet concentrates for transfusion and platelet poor plasma, which can be used either as a plasma volume expander or subjected to further fractionation. Fresh frozen plasma may be further processed to cryoprecipitate (rich in factor VIII and fibrinogen) or fractionated to produce one or more of the following products – antihemophilic factor (VIII) concentrate, other clotting factors (II, VII, IX, X complex), immunoglobulin preparations, salt poor albumin and plasma protein solution. The argument for component therapy is that many patients need only a fraction of whole blood. A patient with chronic anemia who requires blood has a shortage of oxygen carrying capacity; there is no deficit in blood volume, coagulation factors, thrombocytes or proteins. Thus transfusion of red blood cells will be an adequate and optimal therapeutic modality. In so doing many risks accompanying transfusion of whole blood are preventable in these patients.

It should be remembered that within 24 h of donation 90% of the activity of the thrombocytes and 50% of the activity of factor VIII are lost. It should also be noted that most of the first 24 h after

Table 2. Commonly available red blood cell components.

	Whole blood	Modified whole blood	Red blood cells	Washed cells	Leucocyte poor	Frozen resuspended
Volume of CPD (ml)	63					
PCV (%)	40	40	70	as required	60	as required
Volume of cells (ml)	200	200	200	200	160	180
Volume of plasma (ml)	250	230	60	<0.5	10	0

Modified from ref. 36.

collection are taken up with grouping, testing for HBV and transportation. Instead of conventional whole blood, modified whole blood can be used. This consists of 200 ml of red cells to which supernatant plasma has been returned after the removal of platelets or cryoprecipitate (factor VIII). Another argument is one of logistics: there is a limited amount of blood available and a very great need for plasma products, especially for factor VIII concentrates, which require a considerable amount of plasma to fulfill the national requirements.

The main indication for whole blood is exsanguinating hemorrhage, that is when serious hypovolemia is associated with a significant deficiency of oxygen carrying capacity. Other indications are disorders requiring exchange transfusion for neonatal hyperbilirubinemia or, rarely, in life-threatening sickling crisis [6] or fulminant plasmodium infections [7]. However, our experience in a major University Hospital having its usual quota of cardiac surgery, traumatology and liver transplants suggests that some 70% of all blood transfusions could be managed with some form of red cell concentrates.

Red blood cells (packed cells)

Packed red cells are prepared by sedimenting or centrifuging whole blood in such a way that most of the plasma (with or without thrombocytes or buffy coat) is removed. In practice it means that about two-thirds of the plasma is removed, leaving a product with a hematocrit of about 70%. In general, enough plasma, leucocytes and platelets are left so that sensitization to plasma proteins, HLA antigen, or graft versus host disease (GVHD) in severe immune depressed patients may occur. Therefore red blood cells may not be suitable for patients who have developed antibodies to plasma proteins or to HLA antigens or in those in whom it is important to avoid sensitization to those antigens, such as patients who may need multiple transfusions or who will undergo bone marrow transplantation. The storage time is the same as for whole blood as described above.

Indication for red blood cell transfusion

Generally, it can be said that all those circumstances in which there is a deficit in oxygen carrying capacity not attended with hypovolemic hypotension are indications for red blood cells. This would include chronic anemia, slow or moderate continuing hemorrhage, pre- or post-operative transfusion needs, peri-operative blood loss and acute and chronic leukemia. The advantages of red blood cell transfusion compared to whole blood are (a) only that fraction of blood is replenished which is deficient; (b) the risk of hypervolemia is minimized and (c) for the same volume transfused more erythrocytes can be delivered.

If compatible ABO blood is not available, rbc's from group O donors can be used universally for all patients including group A or B recipients. The use of whole blood as universal donor blood carries considerable risk in this situation because of the presence of large amounts of allo antibodies in the donor plasma which could be directed against the erythrocytes of the recipients. The product may not be suitable in some immunodeficiency patients or in patients where prevention of sensitization to HLA, white cell and platelet antigens is desired.

Leucocyte poor red blood cells

In patients receiving multiple blood cell transfusions, non-hemolytic febrile transfusion reactions (NHFTR) are commonly seen. The majority of these reactions are caused by leucocyte antibodies [8] and can be prevented by minimizing the amount of leucocytes transfused [9]. Most of the NHFTR can be prevented in sensitized patients when the blood transfused contains less than 0.5×10^9 leucocytes [10].

This means that more than 80% of the leucocytes must be removed from the donor blood. There are several methods of removing leucocytes, each of which is attended by some degree of loss of red blood cells. These are:

1. Following centrifugation, the buffy coat is removed from the blood or the erythrocytes are separated by aspiration. Using this easy and in-

- expensive technique 65–88% of the leucocytes can be removed although 12–42% of the red cells may be lost [11].
2. Using a blood cell processor (e.g. IBM) the blood can be centrifuged and the packed cells then washed. More than 90% of the leucocytes are removed, but 11–25% of the red cells are also lost. The method is expensive and time consuming.
 3. Sedimentation with high molecular weight polymers such as dextran, gelatin or hydroxyethyl starch provides a leucocyte depletion of 82–95% with a red cell loss of 3–9%. The method can be time consuming.
 4. Filtration of blood through specific leucocyte depleting filters [12] made of cotton-wool, nylon or cellulose acetate fibers. More than 90% of the leucocytes are removed together with about 10% of the red cells. It is a simple but moderately expensive method, which can be completed within 20 min.
 5. Freezing and thawing of the red cells removes more than 90% of the leucocytes and less than 10% of the red cells. The method is expensive and time consuming.

All these methods are limited, however, by the fact that a closed and sterile system has to be broken, which increases the risk of contamination; therefore the prepared blood must be used as soon as possible but certainly within 24 hours. Recently it has been shown that the use of microaggregate filters can deplete blood of leucocytes and moreover reduce non-hemolytic febrile transfusion reactions drastically [14, 15].

With such filters 40–90% leucocyte depletion is obtained with 6–10% loss of red cells. The major future role for microaggregate filters could well be in the prevention of febrile transfusion reactions [16] because: (a) it does not have the limitation of the open system processing; (b) it is a bedside method; (c) it appears to be cheaper than the leucocyte depleting filters.

However, the use of microaggregate filters does not seem to be an appropriate method for preventing HLA or white cell sensitization. Microaggregate filters are advised for the prevention of post-transfusion adult respiratory distress syndrome

(ARDS). However, although no doubt exists about the efficiency with which these filters prevent the infusion of microaggregates, the relation between microaggregates from banked blood and ARDS still remains controversial.

Indications for leucocyte poor red cells

(a) When there is a history of non-hemolytic febrile transfusion reaction. In patients with high titres of leucoagglutinins it may be necessary to give washed or frozen and thawed red blood cells. However, it should be remembered that even with the use of frozen and thawed red cells leucoagglutinins are formed in 5–10% of the recipients [17].

(b) To minimize or prevent HLA sensitization leucocyte poor red cells are used prophylactically in patients who are likely to receive multiple transfusions over a prolonged period of time, and in patients expected to need platelet and granulocyte transfusion, or bone marrow transplantation.

Washed red blood cells

Manual or mechanical (with the use of blood cell processor) washing of red blood cells with isotonic salt solution can remove most of the plasma components and leucocytes and thrombocytes. The advantage of this product over unwashed red blood cells could be a reduction in the incidence of febrile non-hemolytic transfusion reactions and a reduced chance of immunization against HLA antigens because of the near absence of leucocytes and thrombocytes.

The product is virtually free of microaggregates. The risk of urticarial and anaphylactoid reaction is minimized because of the virtual elimination of plasma proteins, and the depletion of lymphocytes may prevent the risk of GVHD. The disadvantage of the product is that it is time consuming and expensive to prepare. It remains to be seen if there will be a significant reduction in transfusion reactions occurring in patients receiving washed rbc [18], thus justifying the costs and efforts required if they are to be widely used.

Indications for washed red blood cells

(a) In paroxysmal nocturnal hemoglobinuria (PNH) it is often recommended to use washed red cells to avoid the administration of complement. Sirchia *et al.* [19] have shown that in patients with PNH lysis of the red cells is the result of the interaction between the leucocyte antigens and their antibodies which activate complement and so damage the rbc but Sherman *et al.* [20] could not demonstrate any difference in the occurrence of transfusion reactions when patients with PNH received whole blood, red cell suspensions or washed red cells. However, it seems justified to use leucocyte poor red blood cells to transfuse a patient with PNH [21].

(b) When there is a history of allergic reactions to plasma constituents varying from hives to generalized urticaria.

(c) Patients with IgA deficiency. These patients may have specific IgA antibodies leading to serious anaphylactoid reactions if unwashed rbc are transfused.

(d) Patients highly sensitized against HLA antigens may need washed rbc to prevent NHFTR.

Frozen red cells

The procedure to freeze and thaw red blood cells is as follows: Plasma is separated from a fresh unit of whole blood by centrifugation and is replaced by a cryoprotectant preservative solution, usually 40% glycerol; the product is transferred to a plastic container able to withstand temperatures below -80°C . The unit is then frozen and stored in the freezer. When required it is thawed in a 37°C waterbath and the contents transferred for washing to remove glycerol; this requires several washings and then the product is transferred to the transfusion bag.

During the procedures of freezing and thawing 10–15% of the red cells are lost. The viability of the thawed red cells is 85–90%. Taken together it means that 1 unit of frozen and thawed rbc finally contains 75% of the red cells present in fresh whole blood [22]. Frozen and thawed rbc contains less

than 5% of leucocytes and thrombocytes, and virtually no plasma proteins [22]. The content of 2.3 DPG and ATP of the thawed rbc is nearly normal if fresh blood has been kept frozen. The cost of a unit of frozen and thawed rbc is 2–3 times more than the cost of a unit of rbc.

Indications for frozen rbc

This product was used very successfully by the United States armed forces during the Vietnam War. Although its potential clinical applications are many in civilian practice there are only a few situations where it is used as a first choice. These include

(a) patients with allo antibodies to high incidence blood group antigens (public antigens);

(b) patients who for several specific reasons require stored autologous blood especially for elective surgery; and

(c) in some centres, well documented Rh genotyped blood is kept frozen for 6 months prior to injection in volunteers for the production of anti D immunoglobulin.

In patients who suffer frequent anaphylactoid reactions to donor IgA plasma, multiple washed red cells (6 times) are more appropriate. In patients sensitized to leucocyte and platelet antigens the use of leucocyte poor blood is generally satisfactory.

Irradiation of blood products

Graft versus host disease (GVHD) is a well known complication after bone marrow transplantation. GVHD has been defined as the clinical and pathological syndrome which results from the reaction initiated by a 'graft' of immunologically competent lymphocytes introduced into a 'host' that confronts the graft with a histocompatibility difference, yet it is unable to mount a similar immunological attack against the intrusive donor lymphoid cells [23]. Manifestations of the syndrome are an erythematous skin rash, liver function disturbances and diarrhoea. Diagnosis is confirmed by biopsy of the involved organs.

GVHD also has been reported as a complication

of transfusion therapy. Fliedner *et al* [24] analysed 38 reported cases in the literature since 1965; they include 13 infants with severe combined immunodeficiency (SCID) or cellular immunodeficiency, 7 young adults with Hodgkin's disease, 3 patients with advanced non Hodgkin lymphomas, 12 patients with acute leukemia, one with aplastic and one with chronic lymphatic leukemia. In these cases various blood products were used. The time between the first implicated transfusion and the onset of GVHD ranged from 4 to 30 days. The overall mortality was 68% but in patients with SCID and advanced Hodgkin's disease the figure was 88% and in those with leukemia 23%. The difference is probably due to the severe impairment of cell mediated immunity in the first group.

Since there is no satisfactory therapy for GVHD, attempts have to be made to prevent the syndrome. Based on the 'in vitro' observations of lymphocyte sensitivity to radiation, irradiated blood is transfused when GVHD can be expected. Doses of 1500–10,000 rads to the donor blood are used. Button [25] used the uptake of ^3H thymidine by mitogen stimulated lymphocytes as a measure of their mitotic capacity. When 1500 rad was used there was still an uptake of 15% but with 5000 rad it was reduced to 1.5%. The functional qualities of cellular blood components other than lymphocytes do not seem to be compromised by 5000 rad.

Indications for irradiated blood products

The use of irradiated blood products in the period after bone marrow transplantation is without any doubt the most important indication; opinions vary as regards its value in immune compromised and myelosuppressed patients. It is not clear how severe immune deficiency must be for GVHD to occur. Based on the published reports some authors [26] advise use of irradiated blood products for all severely immuno-compromised and myelosuppressed patients until investigations allow for more specific recommendations to be made. It should be remembered that unless the blood bank possesses its own radiation sources, a close liaison with the radiotherapy unit is necessary to cover emergency treatment during weekends and holi-

days, otherwise the quality of the product is unnecessarily compromised.

Autologous blood transfusion

In this chapter the term autologous blood transfusion will be used for those situations where blood taken from an individual, after a period of storage, is transfused into the same individual. The subject of autologous transfusion to replace blood lost during trauma or surgery is discussed elsewhere.

If the patient is medically fit to donate blood and there is no limitation in the production of erythrocytes, blood can be collected every 3–10 days depending on the regenerative capacity of the bone marrow. Every phlebotomy must be preceded by careful evaluation of the patient in order to decide if another 500 ml blood should be collected. During the whole procedure the patient should receive iron therapy. The time between collection and operation will be determined by whether the blood is stored in the frozen or liquid state. In the latter case, CPDA-1 anticoagulated blood could be stored for 35 days.

Another simple but effective way of collecting a number of donations from the same person in a short time is the 'leap-frog' technique [27]. In the first visit one unit is collected and during the second visit, having collected one fresh unit the patient receives back the blood collected from the first visit and a second fresh unit is then collected. Subject to the patient's state of health and bone marrow reserve capacity, 5–6 units of blood can be collected over the 3 weeks prior to planned surgery. We have done this in patients having multiple allo antibodies requiring coronary bypass surgery and in one requiring a major hip operation.

Indications for autologous blood transfusion

(1) If the patient has a rare blood group, for example if he lacks public antigens or if he is already alloimmunized.

(2) If the patient's religion only permits him to receive transfusion of homologous blood or blood products.

It should be clear that with this form of blood transfusion the risk of transmission of infectious agents (HBV, CMV and HTLV) and adverse effects due to immunological mechanisms are significantly reduced and therefore it should be considered more often in elective surgery.

Transfusion and transplantation

An excellent review of this subject has been published recently by Storb and Weiden [28]

Renal transplantation

Patients with chronic renal failure usually suffer from a normochromic, normocytic anemia which is often so severe that it limits their activities and means that before transplantation these patients require transfusion of red blood cells. In the past, physicians were reserved in transfusing such patients before transplantation, mostly because of the risk of sensitizing them against HLA antigens. It was known that the more transfusions were given the more lymphocytotoxic alloantibodies were produced. Further, a positive cross-match between the recipients sera and donor lymphocytes correlated well with hyperacute graft rejection. For these reasons the transfusion policy for patients waiting for transplantation was, until a few years ago, that no transfusions were given unless absolutely necessary. If a blood transfusion was required, frozen and thawed red blood cells were usually given because they contained the fewest leucocytes.

Opelz *et al* [29] were the first to report a beneficial effect of pretransplant transfusions on kidney graft survival. Although this study was retrospectively done the results were confirmed by others [30]. There seemed to be a strong dose effect of transfusion: the graft survival rate at one year without prior transfusion was 38%, with 1–5 transfusions the graft survival was $50 \pm 4\%$, 6–10 transfusions $59 \pm 9\%$, 11–20 transfusions $60 \pm 10\%$ and with more than 20 transfusions $72 \pm 12\%$ [31]. The transfusion transplant interval was irrelevant, and frozen and thawed red blood cells were the least effective.

On the basis of the above findings a policy of deliberate transfusions seems justified. For a patient waiting for renal transplantation who has never been transfused, it seems in the patient's best interest to transfuse 2–3 units of leucocyte poor red blood cells.

While there are several working hypotheses to explain the phenomenon the exact mechanism by which prolongation of graft survival is effected remains to be strictly defined [32]. That this phenomenon might have implications for patients who receive blood products for other reasons was proposed by Woodruff and van Rood [33]. They considered the possibility that infusion of blood products might weaken the response to antigens other than those of the major histocompatibility complex.

Bone marrow transplantation

Presently, the most important indications for bone marrow transplantation (BMT) are aplastic anemia, acute leukemia and chronic myeloid leukemia. Due to the disease or its treatment with chemotherapeutic agents, bone marrow failure occurs before transplantation. After bone marrow grafting it takes a few weeks before the graft is functioning and in this period support with red blood cells is often necessary.

There seems to be a relationship between pretransplant transfusion of blood products and graft rejection in patients with aplastic anemia [34]. Graft rejection occurs in 25–59% in the case of aplastic anemia, but in leukemia patients the rate is low: 1–2%. The explanation for this seems to be that blood transfusions in leukemic patients are given when they are immunosuppressed by their disease and by the chemotherapeutic treatment. Storb *et al.* [35] transplanted 30 patients who had never been transfused before, or transfused only 1–3 days before the cyclophosphamide treatment. Ninety percent showed sustained engraftment compared to 65% who had been transfused in the past. Eighty six percent of the rejecting patients died. It is therefore recommended that aplastic anemia patients who have compatible and related

bone marrow donors should not receive blood transfusion unless absolutely necessary. In the latter instance it is better to prescribe leucocyte poor or frozen and thawed red cells in order to prevent sensitization and to transfuse just before the cyclophosphamide conditioning regimen. For leukemia patients there are no such recommendations. In the post transplant period for both aplastic anemia and leukemic patients it is important, as mentioned earlier, to irradiate all blood products to prevent graft versus host disease.

References

- Allan JB, Allen FB: The minimum acceptable level of hemoglobin. In: *Techniques of Blood Transfusion*, Stehling LC (ed). Boston, Little, Brown and Company, 1982: 1-22.
- Collins JA: The pathophysiology of hemorrhagic shock. In: *Massive Transfusion in Surgery and Trauma*, Collins JA, Murawska K, Shafer AW (ed). New York, Alan Liss Inc., 1982: 5-29.
- Mollison PL: The storage of red cells in the liquid state. In: *Blood Transfusion in Clinical Medicine*, Mollison PL, Oxford, Blackwell, 1983: 130-49.
- Nusbacher J: Transfusion of red blood cell products. In: *Clinical Practice of Blood Transfusion*, Petz LD, Swisher SN (eds). New York, Churchill Livingstone, 1981: 289-308.
- Högman CF, Rosen I, Andreen M, Akerblom O: Hemotherapy with red cell concentrates and a new red cell storage medium. *Lancet*, 1983; 1: 269-72.
- Pette J vd, Pearson T, Slater N: Exchange transfusion in life threatening sickling crisis. *J Roy Soc Med*, 1982; 75: 777-80.
- Kramer S, Campbell C, Moncrieff R: Fulminant plasmodium falciparum infection treated with exchange blood transfusion. *JAMA*, 1983; 249: 244-45.
- Payne R: The association of febrile transfusion reactions with leucoagglutinins. *Vox Sang*, 1957; 2: 233-41.
- Brittingham TE, Chapling H: Febrile transfusion reactions caused by sensitivity to donor leucocytes and platelets. *JAMA*, 1957; 165: 819-25.
- Perkins HA, Payne R, Ferguson J, Wood M: Non-hemolytic febrile transfusion reactions: quantitative effects of blood components with emphasis on isoantigenic incompatibility of leucocytes. *Vox Sang*, 1966; 11: 578-600.
- Hughes ASB, Brozovic B: Leucocyte depleted blood: an appraisal of available techniques. *Br J Haematol*, 1982; 50: 381-86.
- Mijovic V, Brozovic B, Hughes MB, Davies TD: Leucocyte depleted blood: a comparison of filtration techniques. *Transfusion*, 1983; 23: 30-32.
- Diepenhorst P, Sprockholt R, Prins HK: Removal of leucocytes from whole blood and erythrocyte suspension by filtration through cotton wool. *Vox Sang*, 1972; 23: 308-35.
- Wenz B, Gurtlinger KF, O'Toole AM, Dugan EP: Preparation of granulocyte poor red blood cells by microaggregation filtration: a simplified method to minimise febrile transfusion reactions. *Vox Sang*, 1980; 39: 282-87.
- Wenz B: Microaggregate blood filtration and the febrile transfusion reaction. *Transfusion*, 1983; 23: 95-98.
- Snijder EL, Bookbinder M: Role of microaggregate blood filtration in clinical medicine. *Transfusion*, 1983; 23: 460-70.
- Polesky HF: Leucocyte poor blood: a study in the evaluation of component therapy. In: *A Seminar on Blood Components*. American Association of Blood Banks, 1977: 53-74.
- Goldfinger D, Lowe C: Prevention of adverse reactions to blood transfusion by the administration of saline washed red blood cells. *Transfusion*, 1982; 21: 277-80; 22: 82-83 and 400.
- Sirchia G, Ferrone S, Mercuriali F: Leucocyte antigen-antibody reaction and lysis of paroxysmal nocturnal hemoglobinuria erythrocytes. *Blood*, 1970; 36: 334-36.
- Sherman SP, Taswell HF: The need for transfusion of saline washed red blood cells to patients with paroxysmal nocturnal hemoglobinuria. *Transfusion*, 1977; 17: 683.
- Mollison PL: Haemolytic reactions in patients with paroxysmal nocturnal hemoglobinuria. In: *Blood Transfusion in Clinical Medicine*, Mollison PL (ed). Oxford, Blackwell, 1983; 566-67.
- Chaplin H: Clinical uses of frozen-stored red blood cells. In: *Clinical Practice of Blood Transfusion*, Petz LD, Swisher SN (eds). New York, Churchill Livingstone, 1981; 333-44.
- Billingham RE: Harvey lecture, 1966, 62, 21, cited by Weiden P.L. *et al.*, Graft versus host disease in allogeneic marrow transplantation. In: *Biology of Bone Marrow Transplantation*, Gale RP, Fox FE (eds). New York, Academic Press, 1980; 37-48.
- Fliedner V, Higby D, Kim U: Graft versus host reaction following blood product transfusion. *Am J Med*, 1982; 72: 951-61.
- Button L, de Wolf W, Newburger P, Jacobson N, Kevy S: The effects of irradiation on blood components. *Transfusion*, 1981; 21: 419-26.
- Brubaker DB: Human posttransfusion graft versus host disease. *Vox Sang*, 1983; 45: 401-20.
- Silver H: Banked and fresh autologous blood in cardio-pulmonary bypass surgery. *Transfusion*, 1975; 15: 600.
- Storb, R, Weiden PL: Transfusion problems associated with transplantation. *Semin Hematol*, 1981; 18: 163-96.
- Opelz G, Sengar DPS, Mickey MR: Effect of blood transfusion on subsequent kidney transplantation. *Transplant Proc*, 1973; 5: 253-9.
- Sypkens YWJ, Koep LJ, Persijn GG, van der Werf BA: Pros and cons of donor specific blood transfusions in living related kidney transplantation. *Neth J Med*, 1984; 27: 1-5.
- Opelz G, Mickey MR, Terasaki P: Blood transfusions and

- kidney transplants. Remaining controversies. *Transplantation Today*, 1981; 6: 136-41.
32. Editorial: Blood transfusion and allograft survival. *Lancet*, 1984; 1: 830-31.
 33. Woodruff MFA, van Rood JJ: Possible implications of the effect of blood transfusion on allograft survival. *Lancet*, 1983; 1: 1201-3.
 34. Storb R, Prentice RL, Thomas ED, *et al.*: Factors associated with graft rejection after HLA identical marrow transplantation for aplastic anemia. *Br J Haematol*, 1983; 55: 573-85.
 35. Storb R, Thomas ED, Buckner CD, *et al.*: Marrow transplantation in thirty 'untransfused' patients with severe aplastic anemia. *Ann Int Med*, 1980; 92: 30-6.
 36. Da Costa J: Red cell therapy. *Br J Hosp Med*, 1973; 9: 681-86.

7. Blood transfusion in sickle cell disease

G.R. SERJEANT

1. Pathophysiology

Sickle cell disease is characterised by two basically independent but closely interrelated pathophysiological processes, haemolysis and vaso-occlusion. Polymerisation of deoxygenated sickle haemoglobin molecules leads to cell deformity and secondary membrane change. Such cells have a high internal viscosity and relatively inelastic membranes rendering them less likely to negotiate the small vessels in capillary beds. These processes bring about either destruction of the red cell itself or impairment of flow in a capillary bed with avascular necrosis of the tissue supplied.

1.1. Haemolysis

Although most genotypes constituting sickle cell disease have evidence of shortened red cell survival, the distribution of steady state haemoglobin levels varies markedly between genotypes. Sickle cell-haemoglobin C (SC) disease and sickle cell $- \beta^+$ thalassaemia ($S\beta^+$ thalassaemia) have similar haemoglobin levels mostly between 12 and 15 g/dl whereas homozygous sickle cell (SS) disease and sickle cell $- \beta^0$ thalassaemia ($S\beta^0$ thalassaemia) have similar levels characteristically between 6 and 9 g/dl (Figs. 1 & 2).

These haemoglobin levels do not equate with oxygen carrying capacity because sickle haemoglobin (HbS) within the cell behaves as a low affinity haemoglobin, loading oxygen well in the high oxygen tensions present in the lungs but releasing oxygen more readily than Hb A at the low oxygen

tensions present in the periphery. This shifted oxygen dissociation curve may vary markedly between patients with SS disease, and the degree of shift is negatively correlated with the total haemoglobin level, patients whose blood demonstrates the lowest oxygen affinity tending to have the lowest haemoglobin levels. Indeed this relationship occurs in a variety of abnormal haemoglobins [1], suggesting that the oxygen affinity is one factor controlling the haemoglobin level maintained by the marrow, and compensating, in part, for the severe anaemia [2].

Generally, the chronic haemolytic process and steady state haemoglobin levels in sickle cell disease are well tolerated and infrequently require medical attention. The classical symptoms of anaemia are rare but may appear in adults over 40 years of age when a falling haemoglobin level is not uncommon. The commonest cause of this is the onset of chronic renal failure although declining haemoglobin levels may occur in the presence of apparently normal renal function raising the possibility of a primary marrow failure. Factors associated with haemolysis which may lead to medical complications include a high incidence of gall stones from increased bilirubin excretion, megloblastic change from a relative deficiency of folic acid, and occasionally bone pathology from the medullary expansion and cortical thinning caused by erythropoietic expansion.

Transfusion of patients while at their steady state haemoglobin levels achieves little in terms of oxygen delivery, the effect of the increased haematocrit being offset by the higher affinity of transfused blood (see Section 4.1).

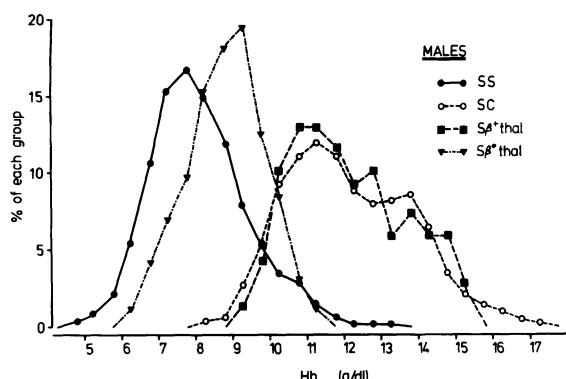


Fig. 1. Distribution of steady state haemoglobin levels in the four principal genotypes of sickle cell disease – Male patients.

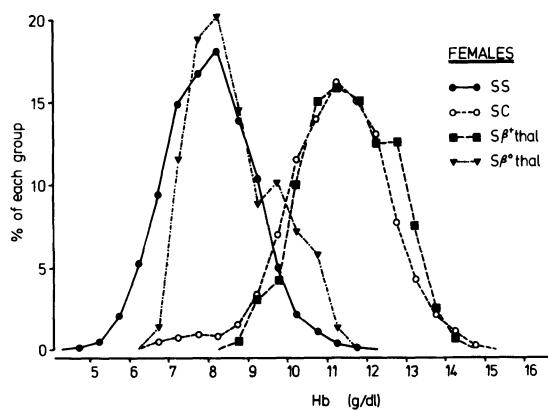


Fig. 2. Distribution of steady state haemoglobin levels in the four principal genotypes of sickle cell disease – Female patients.

1.2. Vaso-occlusion

Most of the morbidity of sickle cell disease may be attributed to vaso-occlusion. In areas of high oxygen extraction such as the bone marrow, even temporary reduction of blood flow may allow demand to exceed supply, and the resulting marrow necrosis causes dactylitis in young children, and the juxta articular pain typical of the painful crisis in adolescents and adults.

There is progressive renal impairment from early childhood ending in chronic renal failure (see below). This is thought to be due to sickling infarcts within the renal tissue. Inappropriate loss of fluid due to impairment of tubular reabsorption of water increases the risk of dehydration and prolonging

sickling crises, and also leads in many patients to persistent enuresis.

Areas of sluggish circulation may become static as in the obstruction to splenic venous outflow in acute splenic sequestration and in the corpora cavernosa during priapism. Vaso-occlusion in the skin contributes to chronic leg ulceration, in the brain to strokes and other CNS lesions, in the eyes to proliferative retinopathy, in the lungs to infarction and pneumonia, and in the placenta to increased foetal morbidity and mortality.

Transfusion reduces the population of cells capable of sickling partly by diluting the circulating HbS containing cells, but also by suppressing marrow activity and hence the synthesis of new HbS containing cells.

2. Indications for transfusion – falling haemoglobin levels

There are a variety of conditions in which the haemoglobin may fall below steady state levels. Falls to symptomatic or life threatening levels are an absolute indication for simple replacement transfusion. These conditions include:

2.1. Acute splenic sequestration (ASS)

This is one of the most dramatic early manifestations of sickle cell disease, and causes appreciable mortality [3]. For reasons not yet understood, the spleen undergoes acute enlargement, trapping a considerable part of the circulating red cell mass. The haemoglobin falls precipitately and may reach levels of 1–2 g/dl only 4–6 h after the onset of the complication. Compensatory marrow activity is unable to maintain haemoglobin levels and death from peripheral circulatory failure associated with the low haemoglobin levels is not uncommon. Acute splenic sequestration (ASS) was the commonest single factor contributing to be 10% mortality observed in the first year of life in the Jamaican cohort study [4].

If the diagnosis can be made early enough, treatment is simple and consists of blood transfusion, following which the rise in haemoglobin is fre-

quently greater than anticipated because of auto-transfusion of cells trapped within the spleen. Episodes of ASS are frequently recurrent and further mortality may be prevented by splenectomy. Episodes of ASS become less frequent with advancing age and are uncommon after the age of 5 years.

2.2. Aplastic crisis

In the aplastic crisis there is a temporary, self-limited cessation of erythropoiesis which, as a result of the shortened red cell survival, is attended by a progressive fall in haemoglobin level. It is common for the haemoglobin to fall by 1 g/day, and since aplasia may last 5–10 days, patients may become severely anaemic and occasionally die from this complication.

Attacks frequently affect members of the same family and occur in epidemics. The most recent epidemic in Jamaica (1979–1980) appears to have been associated with infection by a parvovirus-like agent [5]. Ultimately it may be possible to protect children with a vaccine but in the meantime transfusion is the mainstay of therapy and only needs to raise the haemoglobin to a viable level until marrow activity spontaneously resumes. Aplastic crises are rarely, if ever, recurrent.

2.3. Hypoplastic episodes

A variety of infections impair the compensatory activity of the bone marrow and result in a falling haemoglobin level. Reticulocyte counts fall to 1–5% from mean levels of 10–15%. Treatment should be directed at the underlying infection although transfusion may be given if the haemoglobin falls very low or to augment treatment of the infection.

2.4. Renal failure

Progressive renal failure is a common problem among older patients with SS disease and is associated with a falling haemoglobin level probably secondary to reduced erythropoietin production [6]. Many patients adjust well to low haemoglobin lev-

els but when the haemoglobin falls below 5 g/dl, shortage of breath, ankle swelling, and cardiac failure may occur. Raising the haemoglobin level by transfusion relieves these symptoms and a chronic transfusion programme may be capable of maintaining such patients in reasonable health for years.

2.5. Megaloblastic change

Requirements for folic acid are increased as a result of haemolysis in SS disease. If the dietary supply is insufficient, megaloblastic change may occur resulting in macrocytosis and a slowly falling haemoglobin level. The manifestation is almost confined to childhood in Jamaica and patients develop increasing pallor, shortage of breath and occasionally congestive cardiac failure. Although transfusion may be necessary to restore oxygen carrying capacity more promptly, less severely affected patients respond well and dramatically to folate supplementation only.

3. Indications for transfusion – vaso-occlusive complications

There are a variety of conditions in which indications for transfusion would be considered relative rather than absolute. Since the primary purpose is to reduce the concentration of HbS containing cells, partial exchange transfusion is usually the method of choice. The duration of such exchange transfusion programmes depends on the indications, but may be acute (perioperative management), short term (pregnancy), or long term (prophylaxis of strokes and other chronic or recurring pathology). The conditions for which these programmes have been advocated are discussed in order of their general acceptance.

3.1. Preoperative preparation

In the past, increased morbidity and mortality has been associated with surgery and anaesthesia in patients with sickle cell disease. Sudden unexplained intra-operative or post-operative deaths have occurred and an increased prevalence of

pneumonia, pulmonary embolism, and other vaso-occlusive complications. This perioperative mortality and morbidity has shown marked improvement in recent years, much of which is attributable to increasing awareness and better management of patients with sickle cell disease, but the coincident increasing use of preoperative transfusion may have contributed. Techniques of transfusion have included acute transfusion, repeated transfusion, and partial exchange transfusion either immediately before [7], or 10–15 days before surgery [8, 9]. Comparison of transfusion techniques failed to demonstrate differences in operative performance [10]. Some form of preoperative transfusion has become almost routine in the United Kingdom and the United States and perioperative morbidity following this procedure has been minimal. However, transfusion is not routinely practised in Jamaica where perioperative morbidity is also minimal [11], once again raising the question of whether the improving statistics reflect generally improving standards of medical care or a specific effect of transfusion. It is possible that the minimal morbidity experienced in Jamaica could be improved by routine preoperative transfusion, and theoretical considerations suggest that the lower the number of circulating HbS containing cells, the greater the margin of safety in the event of complications or anaesthetic accidents. However, the cost, inconvenience and potential complications of such pre-operative transfusion make it regrettable that its effectiveness has not been indisputably confirmed in controlled trials.

3.2. *Stroke*

Occlusion of vessels in the central nervous system may cause devastating symptoms, the commonest major syndrome being hemiplegia. Such CNS lesions occur most frequently in children before the age of 10 years, cluster in time [12], and have a high morbidity and mortality. Chronic transfusion programmes with transfusion at approximately 3-weekly intervals reduces the risk of recurrence [13–15] although termination of such programmes after 1–2 years has been attended by a high recurrence of CNS lesions [16].

3.3. *Pregnancy*

Pregnancy in sickle cell disease, especially SS disease, has been associated with increased morbidity and mortality in both mother and child. For the mother, painful crises become more frequent especially during the third trimester and post partum period, toxæmia has been claimed to be more common, pneumonia and pulmonary embolism occur more frequently and maternal mortality has occurred in 1–2% in recent series (reviewed by Charache *et al.* [17]). For the foetus, there is an increased chance of abortion, still birth, neonatal death, and low weight.

Experience has varied markedly between different centres in the United States but has generally shown a marked improvement since the early 1970's. This improvement may be attributable to an increased standard of general medical care or to the increasing use of exchange transfusion in the management of pregnancy. Partial exchange transfusion from the 28th week of pregnancy appears to have been associated with improved maternal and fetal outcome [18, 19], but no adequate control observations are available. The use of historical controls from an earlier period characterised by different methods of management is clearly unsatisfactory.

The obviously necessary controlled trials on the effectiveness of prophylactic transfusion in the management of pregnancy [20] still have not been performed with the result that the role of transfusion in pregnancy in sickle cell disease has not yet been defined [17, 21–24].

3.4. *Prophylaxis and treatment of acute recurring complications*

In addition to long-term or chronic complications, several acute, severe and recurring complications assumed to result from vaso-occlusion, have also been considered appropriate for chronic transfusion programmes.

Acute splenic sequestration (ASS) may manifest acute and repeated life-threatening episodes in young children. There may be reluctance to perform splenectomy in very young children and a

chronic transfusion programme is occasionally advocated as an alternative therapy in such cases. Since the natural history of ASS indicated attacks to be infrequent after the age of 5 years, it has been assumed that chronic transfusion to this age will protect children from this complication. The problem with this approach is that during such a transfusion programme, it is probable that the continued vaso-occlusion causing progressive splenic fibrosis is inhibited and the assumption of a normal natural history of splenic involution is unjustified. Indeed it is feasible that on termination of a transfusion programme at the age of 5 years, the spleen commences to behave as it did at the beginning of the programme. Despite these theoretical considerations and claims that chronic transfusion reduces the risk of episodes of ASS, no published data on the efficacy of transfusion, or on the effect of transfusions on the natural history of splenic involution are available.

Stuttering episodes of priapism are recurrent, transient, and painful events which frequently herald a major episode of priapism [25]. Stuttering episodes cluster in time and may occur nightly for 3–4 h. In such patients, it is probable that a chronic transfusion programme would reduce the sickling and improve drainage of the static reservoirs of the corpora cavernosa. No published experience of prophylactic transfusion in this condition is available although there are claims that transfusion may enhance resolution of major episodes [26, 27]. The use of transfusion in prevention or treatment of the painful crisis is highly controversial. Improvement of established painful crises has been claimed in uncontrolled studies [28, 29] and its prophylactic use has been suggested in patients frequently affected by severe painful crises. However, controlled observations are essential to determine the risk/benefit ratio of such therapy.

Transfusion has also been reported to be beneficial in other life-threatening complications where vaso-occlusion is presumed to occur [30]. In acutely and severely sick patients with sickle cell disease, complex pathologies may rapidly develop. In this situation, exchange transfusion may inhibit secondary vaso-occlusive complications and is only likely to be beneficial. Controlled data are not

available but it is perhaps unrealistic to require this in such situations.

3.5. *Delayed puberty*

Delay in physical and sexual development is common in SS disease and chronic transfusion programmes have been advocated to improve growth during this critical period. Although acceleration of growth is well documented in children with homozygous β thalassaemia, no published studies, controlled or otherwise, are available in children with SS disease.

3.6. *Chronic leg ulceration*

This is an important complication affecting approximately 75% of Jamaican adults with SS disease and probably the single most major determinant of morbidity in Jamaica. Ulcers tend to run a chronic healing/relapsing course and the healing rate has been shown to be improved by oral zinc sulphate [31]. Although not tested in formal studies, there is a consensus of opinion that complete bed rest is also associated with more rapid healing. Blood transfusion has also been proposed as beneficial [32] but has not been subject to study, controlled or otherwise.

4. Methods of transfusion

Transfusion with normal (AA) blood will increase the proportion of HbA and decrease the proportion of HbS present in the circulation. This is achieved partly by dilution of the circulation HbS containing cells, but also by suppressing the formation of new HbS containing cells by the marrow. The method of transfusion is dictated by the speed of blood change required, the duration of the transfusion programme, the sophistication of equipment, and the blood resources available.

4.1. *Simple transfusion*

This is the simplest and the method of choice for the acutely lowered haemoglobin levels in acute splenic sequestration and the aplastic crisis, or in

chronically lowered haemoglobin levels as in chronic renal failure. Either whole blood or packed red cells may be used, and white cell depleted blood reduces the frequency of transfusion reactions to white cell or platelet antigens. Transfusion with normal (AA) blood increases the haematocrit but shifts the oxygen dissociation curve back towards normal because of the increased oxygen affinity of AA cells, increased further by depletion of 2,3-DPG on storage [33]. The final effect on oxygen delivery of increasing both haematocrit and oxygen affinity may be difficult to predict, but there is some evidence that the increasing blood viscosity actually causes a fall in venous oxygen tension when the haematocrit exceeds 35% [34].

4.2. Exchange transfusion

This procedure allows the removal of SS blood and its replacement by normal donor (AA) blood. It is useful if rapid blood exchange is required as in the treatment of certain complications or preparation for emergency surgery. It also has advantages in decreasing the amount of iron accumulation in patients on chronic transfusion programmes.

A simple early method consisted of removal of 500 ml blood followed by infusion of 2 units of donor cells and achieved a 33% exchange in 90 minutes [35]. More recent techniques generally involve the use of two intravenous lines with simultaneous withdrawal of SS blood and its replacement by donor AA cells [18, 36, 37]. Mathematical formulae are available for predicting the required conditions for exchange transfusion [37]. However, although manual procedures are effective in conducting exchange, they are usually tedious, time consuming and may cause potentially dangerous fluctuations in the patient's blood volume.

4.3. Cell separators

Automatic machines, performing centrifugal separation of red blood cells, allow a rapid and efficient method of performing partial exchange transfusion [27, 38-43]. Two types of machine are in common use, the discontinuous and continuous flow models.

The discontinuous flow cell separators act as batch processors, pumping blood from the patient's arm, centrifuging it to allow separation of red cells from plasma, and then discarding the red cells and returning the plasma to the patient along with donor cells. Five to six such cycles are required for a standard procedure which allows a 50% exchange in approximately 140 minutes [43].

Continuous flow separators use two intravenous lines, blood being continuously pumped from the patient's arm, processed, and returned to the other arm. Control of inflow and outflow means that the patient's blood volume need not vary during the procedure. A 6 unit exchange can be completed in 70 minutes [43] and a 90% exchange in 3.5 hours [38]. Such a rapid exchange invariably results in a rapid increase in oxygen affinity, which may cause problems [38] although no ill effects have been observed in other series [40, 42, 43].

Discontinuous flow separators are small, mobile, simple to operate, relatively cheap and require only one intravenous line. Continuous flow separators are more complex and expensive, more efficient, but require two intravenous lines with large needles or catheters capable of carrying the large flows.

4.4. Type of blood used in transfusion programmes

Restoration of oxygen carrying capacity in acute or chronic anaemia superimposed on SS disease may be achieved by whole or packed cells, preferably but not essentially from normal (AA) donors. In chronic transfusion programmes where the object is to lower the proportion of HbS containing cells, it is essential that donor blood be screened by methods for the detection of HbS, preferably haemoglobin electrophoresis. Blood recently obtained from donors is desirable since it would be expected to survive for longer in the recipient's circulation. Occasionally frozen red blood cells have been used [42] since the lesser depletion of 2,3-DPG results in more normal oxygen affinity.

A further refinement of especial value in chronic transfusion programmes has been the use of the cell separator to concentrate populations of young cells (neocytes) which last much longer in the recipient's

circulation, and reduce the frequency of required blood exchanges. Originally developed for chronic transfusion programmes in thalassaemia [44] this procedure would also have application to patients with sickle cell disease. However, the resources of donors required from which to separate neutrophils will limit the use of this procedure.

5. Problems with transfusion programmes

5.1. Adequate superficial veins

Repeated transfusion or partial exchange transfusion in which two veins are required on each occasion may be limited by the availability of adequate superficial veins, especially in young children.

5.2. Social dislocation of hospital attendance

Most patients on chronic transfusion programmes need transfusions repeated at 3–4 week intervals with consequent repeated hospital admissions. It is arguable whether the improved health claimed for chronic transfusion programmes reduces hospital admissions for symptomatic episodes to the same extent. Use of the cell separator allowing exchange transfusion in 3–4 hours may allow such therapy on an outpatient basis.

5.3. Alloimmunisation

Use of repeated blood transfusion inevitably results in an increased risk of acquiring antibodies to antigens from red cells, white cells and platelets. Use of washed red cells may reduce the frequency of problems resulting from white cell and platelet antigens. Alloimmunisation to red cell antigens appears to be common in multiple transfused patients with SS disease [45, 47, 51].

Long-term follow-up in the United Kingdom of regular transfusion in sickle cell disease is lacking, but a recent publication [51] suggested that the problem of frequent allo-immunisation resulted from the donor population frequently carrying different minor red cell antibodies from the recipients with sickle cell disease in pregnancy. The immune

antibodies which cross the placenta have implications in pregnancy, both for the mother (who may require blood transfusion at delivery) and the fetus.

Although it is preferable to avoid the development of antibodies by careful matching, this may not always be possible, and a chronic transfusion programme may have to be terminated in alloimmunised patients. Should such patients develop acute anaemia it may be impossible to locate compatible blood and the use of autologous frozen stored blood has been proposed as a life-saving short-term measure [47, 48].

5.4. Iron overload

Impairment of cardiac and endocrine function consequent on gross iron deposition in the tissues is well recognised in thalassaemic patients on chronic transfusion. Such iron deposition is much less likely in SS disease because of the shorter duration of transfusion programmes and the use of exchange transfusion which removes the iron in the patient's cells. However, although iron stores are frequently low in SS disease, increased stores are not uncommon in multiple transfused adults [49] and the possibility of impaired function remains a theoretical hazard of chronic transfusion.

5.5. Hepatitis

The risks of serum hepatitis following transfusion are well known but prevalence figures are unavailable. One study reported two cases of HBs Ag positive hepatitis among 36 pregnant patients on an exchange transfusion programme in pregnancy [18] and another reported one case of HBs Ag positive hepatitis and six cases who were seropositive among 27 patients transfused prophylactically for strokes [15]. Screening of donor blood for HBs Ag dramatically decreases the risk of this form of hepatitis.

5.6. Logistics of chronic transfusion programme

In devising transfusion programmes it has been necessary to make many arbitrary decisions. The

objective of such programmes is to lower the amount of circulating HbS containing cells, and theoretical considerations would favour total replacement. In practice, this is hardly possible and maintaining patients with less than 50% of their own SS cells has been suggested [36] and received some experimental justification [50]. In determining an arbitrary level of HbS in such programmes it must be recognised that the situation in a transfused patient with SS disease is quite different from that in the sickle cell trait. Although both may have a 40% HbS level, in the sickle cell trait this is distributed throughout the red cell population whereas in a transfused patient with SS disease, it is concentrated within 40% of the red cells which therefore contain close to 100% HbS. Such cells are clearly capable of sickling and causing vaso-occlusion and greater reductions in HbS are likely to confer greater protection in such patients. The other major unknown factor in chronic transfusion programmes is when to stop. This problem may not arise in self-limited events such as pregnancy, but was well illustrated by Wilimas *et al.* [16], who noted a high recurrence of strokes within a short period of stopping transfusion programmes which had lasted 1–2 years.

6. Conclusion

Theoretically, blood transfusion can achieve the total replacement of HbS containing cells by normal AA cells and so cure the condition. In practical terms, however, maintenance of such replacement is almost impossible. Problems which attend such transfusion programmes vary in different parts of the world as do the physical facilities and resources available. In Jamaica and probably most of equatorial Africa, sophisticated equipment such as cell separators would be difficult to obtain and maintain. Furthermore, the transfusion services are relatively undeveloped and lack the financial resources to obtain blood from elsewhere. The technical expertise required for blood matching and monitoring of the levels of HbS or HbS containing cells may also be lacking. It is inevitable then, that the indications for transfusion should

vary not only with the natural history of the disease, but also with the technical resources of the particular area.

Transfusion to maintain oxygen delivery in acute exacerbations of anaemia such as the aplastic crisis or acute splenic sequestration would be universally acknowledged as necessary. Similarly there would be little dispute about maintaining haemoglobin levels in patients limited by low haemoglobins secondary to renal failure. The necessity for routine preoperative transfusion and the claims for most chronic transfusion programmes, however, are more controversial. It may well be that such programmes are beneficial and the data on prevention of further CNS lesions is the most compelling, although still uncontrolled. The use of blood transfusions has important placebo effects for both patient and doctor, and against this background it is essential that incontrovertible evidence be obtained in controlled trials. Such data are not available, and until they are, the debate will continue. In the meantime the communities with greater resources are likely to continue such programmes, while those with lesser resources will eventually have to conduct the necessary controlled trials.

References

1. Bellingham AJ, Huehns ER: Compensation in haemolytic anaemias caused by abnormal haemoglobins. *Nature*, 1968; 218: 924–26.
2. Rossi-Bernardi L, Luzzana M, Samaja M, Rossi F, Perrella M: The functional properties of sickle cell blood. *FEBS Letters*, 1975; 59: 15–19.
3. Topley JM, Rogers DW, Stevens MCG, Serjeant GR: Acute splenic sequestration and hypersplenism in the first five years in homozygous sickle cell disease. *Arch Dis Child*, 1981; 56: 765–69.
4. Rogers DW, Clarke JM, Cupidore L, Ramlal AM, Sparke BR, Serjeant GR: Early deaths in Jamaican children with sickle cell disease. *Br Med J*, 1978; 1: 1515–16.
5. Serjeant GR, Topley JM, Mason K, Serjeant BE, Pattison JR, Jones SE, Mohamed R: Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. *Lancet*, 1981; 2: 595–97.
6. Morgan AG, Gruber C, Serjeant GR: Erythropoietin and renal function in sickle-cell disease. *Br Med J*, 1982; 285: 1686–88.
7. Janik J, Seeler RA: Perioperative management of children

- with sickle hemoglobinopathy. *J Pediatr Surg*, 1980; 15: 117-20.
8. Burrington JD, Smith MD: Elective and emergency surgery in children with sickle cell disease. *Surg Clin N Am*, 1976; 56: 55-71.
 9. Bentley PG, Howard ER: Surgery in children with homozygous sickle cell anaemia. *Ann Roy Coll Surg Eng*, 1979; 61: 55-58.
 10. Fullerton MW, Philippart AI, Sarnak S, Lusher JM: Pre-operative exchange transfusion in sickle cell anemia. *J Pediatr Surg*, 1981; 16: 299-300.
 11. Homi J, Reynolds J, Skinner A, Hanna W, Serjeant GR: General anaesthesia in sickle-cell disease. *Br Med J*, 1979; 1: 1599-1601.
 12. Powars DR, Wilson B, Imbus C, Pegelow C, Allan J: The natural history of stroke in sickle cell disease. *Am J Med*, 1978; 65: 461-71.
 13. Lusher JM, Haghishat H, Khalifa AS: A prophylactic transfusion program for children with sickle cell anemia complicated by CNS infarction. *Am J Hematol*, 1976; 1: 265-73.
 14. Russell MO, Goldberg H, Reis L, Freidman S, Slater R, Reivich M, Schwartz E: Transfusion therapy for cerebrovascular abnormalities in sickle cell disease. *J Pediatr*, 1976; 88: 382-87.
 15. Sarnak S, Soorya D, Kim J, Ravindranath Y, Lusher J: Periodic transfusions for sickle cell anemia and CNS infarction. *Am J Dis Child*, 1979; 133: 1254-57.
 16. Wilimas J, Goff JR, Anderson HR, Langston JW, Thompson E: Efficacy of transfusion therapy for one to two years in patients with sickle cell disease and cerebrovascular accidents. *J Pediatr*, 1980; 96: 205-8.
 17. Charache S, Scott J, Niebyl J, Bonds D: Management of sickle cell disease in pregnant patients. *Obstet Gynecol*, 1980; 55: 407-10.
 18. Morrison JC, Wiser WL: The use of prophylactic partial exchange transfusion in pregnancies associated with sickle cell hemoglobinopathies. *Obstet Gynecol*, 1976; 48: 516-20.
 19. Morrison JC, Wiser WL: The effect of maternal partial exchange transfusion on the infants of patients with sickle cell anemia. *J Pediatr*, 1976; 89: 286-89.
 20. National Institutes of Health Consensus Development Conference. Transfusion therapy in pregnant patients with sickle-cell disease. *Ann Intern Med*, 1979; 91: 122-23.
 21. Cunningham FG, Pritchard JA, Mason R, Chase G: Prophylactic transfusions of normal red blood cells during pregnancies complicated by sickle cell hemoglobinopathies. *Am J Obstet Gynecol*, 1979; 135: 994-1003.
 22. Morrison JC, Schneider JM, Whybrew WD, Bucovaz ET, Menzel DM: Prophylactic transfusions in pregnant patients with sickle hemoglobinopathies: benefit versus risk. *Obstet Gynecol*, 1980; 56: 274-80.
 23. Morrison JC, Probst MG, Blake PG: Sickle hemoglobin and the gravid patient: a management controversy. *Clin Perinatol*, 1980; 7: 273-84.
 24. Miller JM, Horger EO, Key TC, Walker EM: Management of sickle hemoglobinopathies in pregnant patients. *Am J Obstet Gynecol*, 1981; 141: 237-41.
 25. Emond AM, Holman R, Hayes RJ, Serjeant GR: Priapism and impotence in homozygous sickle cell disease. *Arch Intern Med*, 1980; 140: 1434-37.
 26. Seeler RA: Intensive transfusion therapy for priapism in boys with sickle cell anemia. *J Urol*, 1973; 110: 360-61.
 27. Rifkind S, Waisman J, Thompson R, Goldfinger D: RBC exchange pheresis for priapism in sickle cell disease. *JAMA*, 1979; 242: 2317-18.
 28. Brody JI, Goldsmith MH, Kwan Park S, Soltys HD: Symptomatic crisis of sickle cell anemia treated by limited exchange transfusion. *Ann Intern Med*, 1970; 72: 327-30.
 29. Green M, Hall RJC, Huntsman RG, Lawson A, Pearson TC, Wheeler PCG: Sickle cell crisis treated by exchange transfusion. *JAMA*, 1975; 231: 948-50.
 30. van de Pette JEW, Pearson TC, Slater NGP: Exchange transfusion in life-threatening sickling crisis. *J Roy Soc Med*, 1982; 75: 777-80.
 31. Serjeant GR, Galloway RE, Gueri MC: Oral zinc sulphate in sickle cell ulcers. *Lancet*, 1970; 2: 891-93.
 32. Chernoff AI, Shapleigh JB, Moore CV: Therapy of chronic ulceration of the legs associated with sickle cell anemia. *JAMA*, 1954; 155: 1487-91.
 33. Valeri CR: Oxygen transport function of preserved red cells. *Clin Haematol*, 1974; 3: 649-54.
 34. Jan K, Usami S, Smith JA: Effects of transfusion on rheological properties of blood in sickle cell anemia. *Transfusion*, 1982; 22: 17-20.
 35. Anderson R, Cassell M, Mullinax GL, Chaplin H: Effect of normal cells on viscosity of sickle-cell blood: In vitro studies and report of six years' experience with a prophylactic program of 'partial exchange transfusion'. *Arch Intern Med*, 1963; 111: 286-94.
 36. Charache S: Treatment of sickle cell anemia. *Ann Rev Med*, 1981; 32: 195-206.
 37. Nagey DA, Garcia J, Welt SI: Isovolumetric partial exchange transfusion in the management of sickle cell disease in pregnancy. *Am J Obstet Gynecol*, 1981; 141: 403-7.
 38. White JM, White YS, Buskard N, Gillies ADS: Increasing whole blood oxygen affinity during rapid exchange transfusion: a potential hazard. *Transfusion*, 1976; 16: 232-36.
 39. Kernoff LM, Botha MC, Jacobs P: Exchange transfusion in sickle cell disease using a continuous-flow blood cell separator. *Transfusion*, 1977; 17: 269-71.
 40. Key TC, Horger EO, Walker EM, Mitchum EN: Automated erythrocytophoresis for sickle cell anemia during pregnancy. *Am J Obstet Gynecol*, 1980; 138: 731-37.
 41. Keeling MM, Lavery JP, Clemons AU, Schefer RL, Blandford PD, Harris EA: Red cell exchange in the pregnancy complicated by a major hemoglobinopathy. *Am J Obstet Gynecol*, 1980; 138: 185-88.
 42. Klein HG, Garner RJ, Miller DM, Rosen SL, Statham NJ, Winslow RM: Automated partial exchange transfusion in sickle cell anemia. *Transfusion*, 1980; 20: 578-84.
 43. Klein HG: Cell separators for red cell exchange, In *Ad-*

- vances in the Pathophysiology, Diagnosis, and Treatment of Sickle Cell Disease.* Alan R Liss, New York, 1982; 109-16.
- 44. Corash L, Klein H, Deisseroth A, Shafer B, Rosen S, Beman J, Griffith P, Nienhuis A: Selective isolation of young erythrocytes for transfusion support of thalassemia major patients. *Blood*, 1981; 57: 599-606.
 - 45. Orlina AR, Unger PJ, Koshy M: Post-transfusion alloimmunization in patients with sickle cell disease. *Am J Hematol*, 1978; 5: 101-6.
 - 46. Coles AM, Klein HG, Holland PV: Alloimmunization in two multitransfused patient populations. *Transfusion*, 1981; 21: 462-65.
 - 47. Castro O: Autotransfusion: a management option for alloimmunized sickle cell patients? In: *Advances in the Pathophysiology, Diagnosis and Treatment of Sickle Cell Disease.* Alan R Liss, New York, 1982: 117-127.
 - 48. Castro O: Viability and function of stored sickle erythrocytes. *Transfusion*, 1980; 20: 695-703.
 - 49. Peterson CM, Graziano JH, de Ciutiis A, Grady RW, Cerami A, Worwood M, Jacobs A: Iron metabolism, sickle cell disease, and response to cyanate. *Blood*, 1975; 46: 583-90.
 - 50. Lessin LS, Kurantsin-Mills J, Klug PP, Weems HB: Determination of rheologically optimal mixtures of AA and SS erythrocytes for transfusion. *Prog Clin Biol Res*, 1978; 20: 124-34.
 - 51. Tuck SM, Studd JWJ, White JM: Pregnancy in sickle cell disease in the UK. *Br J Obstet Gynaecol*, 1983; 90: 112-17.

8. Supportive care in leukemia treatment

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Supportive care in leukemia treatment

The introduction of new cytostatic drugs and their clinical use in combination of radiotherapy and of bone marrow transplantation procedures have changed the clinical picture of leukemia. The prognosis for children with acute lymphoblastic leukemia (ALL) has already improved considerably since 1960. Nowadays, a first complete remission is achieved in 85 to 95% of these children with two to four drug induction schemes [1, 2, 3].

The first complete remissions and the survival times became longer. Sixty to 65% of children with ALL, with initial leucocyte counts lower than $50 \times 10^9/l$, survive five years and longer [4]. The prognosis for children with higher leucocyte counts [5], with leukemia of the central nervous system [6], pre B type leukemia [7] and of adults with ALL [8] is less favourable. Despite intensive cytostatic schemes, adults with lymphoblastic leukemias have a 5 year survival rate of less than 15%. The so-called CNS prophylaxis, either with irradiation of the brain combined with intrathecal administration of cytostatics or with spinal irradiation and in other schemes with systemic high dosages of cytostatics, has decreased the number of CNS leukemias from 70% to $\pm 10\%$ [9, 10, 11].

Acute non-lymphoblastic leukemias (AML) are more frequent in adult patients. Intensive cytostatic schemes result in complete remission in 55 to 85% of the patients [12]. However, the survival time with or without maintenance therapy is short; only 15% of the patients survive more than two years. Mayer *et al.* [13] published better results.

Median survival time of 26.1 months of 105 patients was reported with intensive induction and intensification regimes

Bone marrow transplantation in children and adults under 40 years of age with AML who have HLA identical donors has improved the prognosis for patients transplanted during the first remission. Sixty-five percent survive more than two years with no signs of disease [14, 15]. Chronic myeloid leukemia (CML) without the Philadelphia chromosome, the so-called infantile type, has a very poor prognosis; no patient survives, unless transplantation is possible. Children and adults with a Philadelphia chromosome positive CML survive for several years; a concomitant blastic crisis is fatal, unless bone marrow transplantation is possible [16]. A blastic crisis may be treated with bone marrow transplantation of autologous chronic phase cells.

Chronic lymphatic leukemia (CLL) is not treated with aggressive regimens of cytostatic drugs.

The supportive care in leukemia treatment is directed to the handling of the direct complications of the disease and the treatment of the effects and side-effects provoked by cytostatic drugs and irradiation.

Mitigation of the symptoms of the disease

In ALL the clinical signs are related to the hematological disturbances and to the infiltration of organs. Mediastinal infiltration may lead to tracheal and bronchial obstruction or to a vena cava

superior syndrome. Simultaneously with disappearance of the blasts, this infiltrate may shrivel within a few days.

Infiltration of the kidney sometimes impairs the kidney function, often complicated by hyperuricaemia. The infiltration and the urate crystal deposits should be treated before cytostatics are applied, the former with a low dosage of irradiation, the latter with enforced diuresis and alkalinisation of the urine and if necessary the use of allopurinol. Primary infiltration of the CNS results in headache, vomiting, and rarely, to seizures and hyperphagia. When the intracerebral pressure is very high the immediate implantation of an Ommaya reservoir is advised [17]. This facilitates the administration of cytostatics and guarantees their therapeutic levels in the cerebrospinal cavity, but is seldom practised. Enlargement of the lymph nodes, the liver and the spleen does not cause major complaints. With high numbers of blasts sludging of blood occurs in the vessels. Leukapheresis or an exchange transfusion is indicated in the case of an impending danger of a cerebrovascular accident [18].

AML shows the signs of aplasia and its complications. In AML and particularly in acute pro-myelocytic leukemia, primary diffuse intravascular coagulation (DIC) brings about a bleeding tendency. Drapkin *et al.* [19] advise heparin therapy for the diffuse intravascular coagulation. DIC can also occur as a complication of gram-negative bacterial infections.

In chronic myeloid leukemia, the enlargement of the spleen leads to mechanical problems especially in young children. Rupture of the enlarged spleen is dangerous. Forty-eight percent of patients who do not undergo splenectomy following rupture of the spleen, die, but with splenectomy most patients survive. If the CML is not sensitive to cytostatics, irradiation of the spleen can solve the mechanical problems. The priapism of some CML patients, on account of high peripheral blood counts, is an embarrassing sign. Leucapheresis may relieve the problem.

Cytostatics and side effects

The induction treatment initiates a rapid destruction of blast cells and as a result an enormous amount of phosphate is released. The serum calcium is bound to these phosphates and the clinical signs of tetany and seizures may arise due to hypocalcemia.

Slow administration of calcium gluconate restores the serum calcium level [21]. Destruction of the cells causes hyperuricaemia; allopurinol (Zyloric®) given 24 hours before the start of cytostatic therapy, forced diuresis and alkalinisation of the urine restrict the kidney function disturbance. In the case of kidney function impairment, the cell destruction leads to hyperkalaemia. Both serum uric acid and potassium levels should be monitored.

In ALL, vincristin, prednisone, rubidomycin or doxorubicin and L-asparaginase are in use. The most prominent side effects of vincristin are paralytic ileus, inappropriate ADH syndrome and, rarely, seizures. The paralytic ileus is unpredictable and may happen despite a regular use of laxatives. Total withdrawal of oral feeding, postponement of vincristin dosages and intravenous fluid administration would be treatment for the ileus.

The inappropriate ADH syndrome is manifest by a hyponatremia and, if not recognized, with water intoxication and convulsions. Patients with a history of seizures should receive anti-epileptic therapy during the induction therapy. The neuropathic effect of vincristin gives a stumbling gait with absent knee tendon reflexes. Ptosis and dropping hands are other signs of vincristin neuropathy; however, usually hypo- and paraesthesia of fingers or toes are the first symptoms. All disappear after a prolonged withdrawal of the drug.

The corticosteroids lead to more or less severe Cushing signs which disappear without complications after a longer cessation of the drug. The *anthracyclines* are potent remission induction drugs; they have severe side effects including vomiting and cardiac toxicity. In the presence of diffuse intravascular coagulation and thrombocytopenia the danger from vomiting is cerebral haemorrhage; Motilium® controls this vomiting partially.

The cardiac toxicity of the anthracyclines threatens the patient when the total dosage exceeds 450 mg/m² [22], the status of the myocardium should be monitored with echocardiography, electrocardiography and possibly with myocardial biopsy in case of higher dosages [23]. Generally, these high dosages are not necessary during leukemia treatment.

Special care should be paid to intravenous administration of vincristin and the anthracyclines; extravascular injection of vincristin leads to induration in the surrounding tissue and to nearly irreparable necrosis after the anthracyclines. The development of alopecia during the use of vincristin and the anthracyclines may lead to psychological problems, but fortunately the hair growth returns after the cessation of the drugs within about 3 months.

L-asparaginase has to be applied daily over a period of several weeks. When used for the first time it is generally tolerated well. A second or third course can be complicated by anaphylactic reactions. As the fibrinogen and antithrombin III levels have a tendency to drop to very low levels, one has to monitor these parameters. Generally, the hypofibrinogenaemia does not lead to withdrawal of the drug. Dural sinus thrombosis due to L-asparaginase induced antithrombin III deficiency has been reported [24].

Haemorrhagic pancreatitis is diagnosed with high amylase levels in blood and urine, and pancreatic oedema with echography.

After the induction therapy of ALL, prophylaxis against CNS leukemia is performed both in children and in adult patients. Presently CNS prophylaxis is carried out with irradiation of the skull with 24 Gy combined with intrathecal methotrexate [25], 18 Gy combined with intrathecal methotrexate [11] or high dosages of systemic cytostatics, often combined with intrathecal therapy [10]. The use of an Ommaya reservoir is sometimes advocated for the intrathecal route. Although the reported therapies seem to have comparable beneficial results [26]. Freeman *et al.* [27] have reported that systemic medium dosage methotrexate is inferior to irradiation in the prevention of CNS leukemia.

The direct effects of irradiation are vomiting; 6–8 weeks later a somnolence syndrome may appear with anorexia and sleepiness. The lumbar puncture sometimes causes pain in the legs and back. Paresis is only seen when chemical arachnoiditis arises due to a technically unsufficient lumbar puncture. The late effects of 24 Gy are a slight grade of intellectual impairment; in rare cases this can appear 2–5 years after irradiation. Prolonged use of methotrexate seems to provoke this effect as well, particularly in the presence of a later CNS leukemia.

The maintenance therapy may be complicated by granulocytopenia and lymphocytopenia and some degree of impairment of the immune response later in the treatment [28]. 6-Mercaptopurin (6-MP) administration is complicated by liver function disturbances, that make it necessary to discontinue the drug. Mouth ulcers due to methotrexate are treated with local application of Ledervorin®. A long-standing treatment with methotrexate might lead to osteoporosis, pain in the bones and a tendency to fractures. One has to be aware that allopurinol inhibits the catabolism of 6-MP, necessitating dose reduction of 6-MP, when both drugs are given simultaneously.

In AML cytosine arabinoside and cyclophosphamide are most widely used. Cytosine arabinoside is applied in various dosages.

Vomiting is one side effect; a far more serious one is an intestinal syndrome in older and infected patients with symptoms of watery stools, distension of the abdomen, oedema and ascites due to the marked atrophy of the gastrointestinal mucosa [8].

A second less threatening but inconvenient complication is a photophobia and keratitis punctata. It occurs when high dosages are administered. The photophobia disappears spontaneously and can be controlled with corticosteroid containing ophthalmic drops.

Cyclophosphamide is widely used in the field of solid tumours as well as in leukemia treatment and bone marrow transplantation. Denudation of the bladder epithelium due to the direct influence of acrolein, a breakdown product of cyclophosphamide, complicates the use of this drug. The resulting haemorrhagic cystitis is prevented by the use of 2-mercapto-ethane sulphonate sodium

(mesna) during and after the cyclophosphamide. An irreversible side effect of cyclophosphamide can be male sterility; less is known about female sterility and sterility of children who received cyclophosphamide before puberty. Cyclophosphamide in high dosages is cardiotoxic.

6-Thioguanine (Lanvis[®]) is in use in combination with ara C in some therapeutic schemes; it has the same side effects as 6-mercaptopurin, although generally less severe.

Doxorubicin instead of daunorubicin is also used in AML. The maintenance treatment of AML is controversial; it seems that intensive consolidation schemes with induction drugs prolongs the complete remission [13], but the bone marrow aplasia is prolonged although reversible after each course.

Special attention has to be paid to the dosage of cytostatics in blastic crisis of CML. As CML is a stem cell disease [29] a very long lasting and severe aplasia may follow a relatively mild cytostatic scheme. The same concerns the use of busulphan in CML; when the number of $20 \times 10^9/l$ leucocytes is reached the cytostatic therapy with busulphan has to be interrupted because of its long lasting cytostatic effect.

Application of blood and blood products

Red blood cell and platelet transfusions restore the haemoglobin level and stop the bleeding in thrombocytopenia. It is advisable to perform chromosomal and cell kinetic studies before any transfusion.

As patients with leukemia have a normal circulating volume, blood components can raise the haemoglobin without overloading the circulation. For children, 10 ml/kg body weight of packed cells are usually acceptable. During the induction treatment Hb levels of at least 6 mmol/l (95 g/l) should be maintained. On the basis of experimental study and findings in children with ALL, Smith *et al.* [52] advise hypertransfusion in order to encourage the stem cell to granulocytogenesis and megakaryocytopoiesis. Another positive effect of a well-executed transfusion management is a possible decrease in cardiac toxicity of the anthracyclines. The haemoglobin recovers spontaneously when a re-

mission approaches. In AML and blastic crisis of CML more red blood cell transfusions may be necessary. In comparison with aplastic anaemia and chronic kidney disease, the frequency of alloimmunisation to minor red cell groups is very low and does not become a problem; compatibility for A, B and Rhesus factor is sufficient.

In the light of platelet transfusion requirement and potential later bone marrow transplantation, alloimmunisation against HLA antigens should be prevented. Leucocyte poor blood, made by filtering through cotton wool filters or using differential centrifugation methods, postpones alloimmunisation. Irradiation of the blood is of no help in the prevention of alloimmunisation.

The effect of platelet transfusion is dependent on the number transfused, the size of the spleen, the presence of diffuse intravascular coagulation and the state of alloimmunisation. With severe bleeding tendencies, 2–4 donor units of platelets are given to children, and 6–8 units for adults. Platelet transfusions may be necessary during the whole remission induction period, however early alloimmunisation should be avoided in order to provide support during relapse and bone marrow transplantation.

Alloimmunisation to HLA antigens is far more important than to thrombocyte specific antigens (Ko_a, Bac and Zwa); thus leucocyte poor platelets are preferable. Dutcher *et al.* [30] have shown that 12% of their patients had been immunised upon admission, 30% became alloimmunised during remission induction and 58% showed no alloimmunisation. The normal survival time of platelets *in vivo* is about 8 days [31]; however, in patients with severe bleeding tendency the platelet consumption is increased and platelet transfusion may be necessary each 2 to 3 day interval.

The application of platelet transfusions during DIC is questionable, as donor platelets could accelerate the clotting process. However, in the presence of a severe bleeding tendency, platelet transfusion must be given.

The majority of patients with AML and ALL are treated with random, non HLA typed platelets. Only in cases of an early alloimmunisation is HLA-identical donor or platelets of a family member used.

Red cell and platelet transfusions contain immunological competent cells, which could lead to a graft versus host disease in severely immunocompromised patients [33, 34]. Irradiation with 15 Gy kills the immunocompetent cells, but irradiation is only used if red cell and platelet transfusions are contaminated with many nucleated white cells. Altered platelet functions and circulation of transfused platelets during amphotericin therapy have been suggested [35].

Granulocyte suspensions are applied when persistent bacterial or fungal infections fail to respond to optimal antibiotic and antimycotic treatment for 48 hours. The infections can be life threatening when the granulocyte number is under $0.5 \times 10^9/l$. Winston *et al.* [36] performed a study on 46 AML patients. With granulocyte support there was more pneumonia and higher cytomegalic virus infections. Even when severe granulocytopenia was present the beneficial effect of granulocyte suspensions was not significant. Pflieger *et al.* [37] treated 40 leukemic patients with granulocytes. Twenty-six patients showed clinical improvement. Only patients with regeneration of granulopoiesis had a definite benefit. If given, the granulocyte transfusions are continued during 4 to 5 days. But the maximal danger of alloimmunisation and other side effects, especially CMV infections, restrict the use of granulocyte transfusions. And in a recent study [38] the advantages of granulocyte transfusions are doubted. An effective prophylaxis of infections and adequate decontamination procedures significantly reduce the need for granulocyte suspensions.

Prevention of menstrual bleeding

During thrombocytopenia and intravascular coagulation, the menstruation may be postponed. Orgametril® 5 mg daily and 10–15 mg during menstruation restricts the loss of menstrual blood.

Prevention, mitigation and treatment of infection

Healthy children and adults live in a symbiotic

relationship with the bacteria of the gut, the skin, nose, mouth and throat. At times pathogenic bacteria overwhelm the normal bacterial flora and an infection occurs.

In patients with leukemia the normal symbiotic relation between the body and bacteria is disrupted [39]. When leukemia occurs the number of normal granulocytes falls; cytostatic therapy has a similar outcome. The cytostatic drugs affect the humoral and cellular immunity as well, thus breaking down another barrier. The patient's own bacteria are replaced by bacteria present in the hospital, many of these resistant to a variety of antibiotics. With the use of broad spectrum antibiotics the patient's own bacteria are affected, so the whole balanced symbiosis is disturbed. The debilitated patient is threatened with potentially fatal infections. In particular, AML patients suffer from multiple bacterial infections with gram negative organisms. In 200 patients with acute myeloblastic leukemia a significantly worse survival rate was seen [40] when a severe granulocytopenia with fever and a bacterial infection were present. Gram positive bacteria may be the origin of septicaemia as well. Siegel *et al.* [41] found 88 episodes of septicaemia in 221 children with leukemia; thirty-one of these episodes (35%) were gram positive septicaemias. Of 72 localized infections, 28 were due to gram positive organisms. Prevention of infections due to bacteria and fungi must be the primary aim. The natural barrier is broken after iatrogenic lesions caused by blood sampling, intramuscular and intravenous injections, infusions and catheters, bone marrow punctures, biopsies and lumbar punctures. Other 'portes d'entrée' are gastric tubes *in situ* for a prolonged time, rectal injury with a clysm or a thermometer. Drugs like methotrexate lead to mouth ulcers, and malfitting dentures may give pressure ulcers. The careful observation of sterile precautions in iatrogenic measures prevents the breakdown of the natural barriers.

Contact with infected persons should be avoided. In order to decrease the number of pathogenic bacteria, food and other orally taken products are made bacteria-poor by sterilisations, pasteurisation and the use of fresh, well boiled food and drinks. The procedures are labour intensive,

and sterilized food is only used during periods of granulopenia under $0.5 \times 10^9/l$. During remission one should be careful with raw meat, fish and vegetables, because of salmonella contamination. Although salmonellosis was not fatal in a published series [42], salmonellosis can have a severe clinical course.

Microbiological inventarisation of the nose, throat, skin and anal, preputial and vaginal regions, and cultures of the faeces could contribute to the prevention of septicaemia. A positive culture of pathogenic bacteria before septicaemia makes the treatment more appropriate. Newman *et al.* [43], however, do not advise microbiological inventarisations in a non-research setting. The change of the flora in the throat and faces during hospitalisation has been demonstrated [44]: there was change in 68% of the flora in the throat, and of 57% of the faecal flora during induction therapy in 33 patients receiving at least one antibiotic course.

Long-term granulocytopenia is expected during cytostatic therapy and this leads to more infections. In a series of 221 children with ALL, the number of infection was closely related to the intensity of chemotherapy. Two drug induction schemes lead to 6.5% serious infections, four drug induction to 20% [45].

During intensive cytostatic treatment, decontamination of the gut of potential pathogenic micro-organisms with nonresorbable antibiotics is advised. This so-called selective decontamination is possible in the open ward [46] and decreases the fatal outcome of acquired infection. The selective decontamination consists of a combination of polymyxin B and cotrimoxazole or cotrimoxazole alone [47, 48, 49]. When these drugs are not well tolerated, pipemidic acid or quinoline derivatives can be used. Neomycin and colistin are the third choice [48]. During the decontamination procedure it is advisable to undertake faecal culture twice a week and to ascertain bacterial sensitivity pattern [50]. With these measures it is possible to eliminate susceptible enterobacteria and pseudomonas from the gastrointestinal tract. Faecal cultures are free from gram negative rods one week after the start of nalidixic acid or cotrimoxazole; with polymyxin this is achieved in a few days [50].

Total decontamination is under discussion; in our opinion this kind of decontamination should not be applied in the open ward because it destroys the total flora. Kurrle [51] is of the same opinion. When bone marrow transplantation is to be undertaken, total decontamination plays a more important role [15].

Early death in acute myeloid leukemia is due, in most cases, to an infection with *Klebsiella pneumoniae*, *Pseudomonas* species and *Escherichia coli* [52, 53]. The bacterial infections should be treated with bactericidal antibiotics. Schimpff *et al.* [59] advise ticarcillin and gentamycin until the culture results are known. Other potent combinations are aminoglycosides together with one of the cephalosporins; one must keep in mind that aminoglycosides are nephrotoxic. The therapy with antibiotics should be continued until the number of granulocytes is over $1.5 \times 10^9/l$. As soon as the resistance pattern of the cultured micro-organism is known, the suitable antibiotic treatment should be initiated.

When epidemics of chickenpox occur in school, parents should be warned to keep their child with leukemia at home until at least three weeks after the last manifestation of such a disease in the school. In the case of a direct infection contact, zoster hyperimmune globulin given within 72 hours prevents the outbreak of the disease; the protection lasts 3–4 weeks. Measles in children is becoming more rare because of the routine vaccination. Still measles is dangerous in children with leukemia [54]. The clinical course is not always typical, pulmonary symptoms may be predominant without the typical exanthema. A special danger is subacute sclerosing panencephalitis: the patient dies with a clinical picture of decerebration. There is no successful treatment available. German measles and mumps generally have an uncomplicated course. Cytomegalovirus leads to fever, hepatic and cardiac involvement and interstitial pneumonia. Bussel *et al.* [15] describe 25 patients with acute leukemia and fever of unknown origin where CMV could be proven; most patients were infected through blood transfusions. The prevention of the disease is not possible. It is advisable to avoid with fresh blood, and to use red blood cells at least 3

days old. Interferon has been tried as a therapy [56], but with no great success.

The hepatitis A, B and non A, non B should be kept in mind when icterus and disturbed serum transaminases are present. Adaption of the main-tainance therapy is necessary when the patient is infected with the disease.

Vaccinations with live attenuated vaccine are not permitted since the immune compromised patients might develop a subsequent infection. Killed measles vaccine is contraindicated as well; it could lead to pulmonary problems. DTP injection may be given, although the immune response is not optimal during the cytostatic therapy and shortly after its cessation [28].

All patients in a poor clinical condition with or without an impaired cellular immune system and granulocyte function disturbances are susceptible to *Pneumocystis carinii* [57]. A pneumonia-like picture with rapid breathing, a few rhonchi and an interstitial inflammation must give suspicion of *Pneumocystis carinii*. The diagnosis is sometimes difficult on clinical grounds. Laryngeal swabs and at times, open lung biopsies or needle biopsies, are required to establish the diagnosis. As therapy trimethoprim sulfamethoxazole is the drug of choice [58]. In unresponsive cases pentamidine isothionate i.v. for 5–7 days could be used [59]. Cytostatic therapy is suspended during the infection episode.

The prevention of *Pneumocystis carinii* becomes routine in oncology departments. Trimethoprim-sulfamethoxazole is administered [60]. A potential danger of this prophylaxis is resistance to enterobacteriaceae strains [61].

When granulocytopenia of less than $0.5 \times 10^9/l$ persists, with a continuing fever and negative blood cultures, candida albicans and aspergillus may be the source. Both organisms tend to infiltrate organs. Lung inflammations, aspergilloma, cerebral complications, infiltrations of the kidneys, liver and oesophagus occur, and even spleen abcess can occur [62]. Treatment consists of amphotericin in a dosage of 0.6 mg/kg body weight; often the disease heals only at the time of disappearance of the granulocytopenia. Sometimes fungaemias are severe and fatal. Meunier *et al.* [63] observed 79% death in 110 fungaemias. When the necessary dos-

age of amphotericine B is more than 200 mg, 71% of the patients die despite treatment. Aspergillosis has a similarly poor prognosis. Pennington *et al.* [64] described eight cases of aspergillosis in the lungs. Only two patients were cured with amphotericin B. When agranulocytosis persists for some weeks, strict reverse isolation in life island or laminar flow systems has to be considered [65, 66]. However, these expensive methods of isolation are only available in some specialized centres. The effect of the isolation methods has been studied in bone marrow transplantation procedures; however, in the treatment of leukemia, selective decontamination seems equally effective [67].

Viral infections may be fatal at times in patients with leukemia during cytostatic treatment, even during a complete remission. In childhood ALL and AML the most severe viral infections are varicella and measles, and in adults CMV and generalized herpes zoster. Chickenpox during cytostatic treatment might be life-threatening. Instead of a self-limiting disease, within 5–8 day the varicellae spread everywhere in the body, e.g. in the cerebrum and the lungs, ultimately leading to death. Herpes zoster tends to be generalized over the skin and then infiltrate the organs in immunosuppressed patients leading to the same dangers. Intravenously acyclovir® has improved the prognosis of both diseases dramatically [68]. This treatment has superseded Ara-A despite the beneficial effect of this drug, as shown in children with varicella and herpes zoster during cytostatic therapy [69]. The spreading of herpes zoster may be restricted by human leucocyte interferon [70].

Nutrition during leukemia treatment

The clinical condition is generally poor at diagnosis. Leukemic infiltration of different organs, anaemia and septicaemia affect the patient. Intensive cytostatic treatment is complicated by anorexia and vomiting. The unpleasant taste of the antibiotics and antimycotics aggravates the anorexia. Cytostatic therapy has an influence on the resorption [71]. In children with ALL, prophylactic irradiation of the brain leads also to

anorexia and vomiting. These lead to a severe catabolic state during the induction therapy, CNS prophylaxis and intensification courses.

A decrease of cellular immunity and decreased tolerance to cytostatic therapy may arise due to starvation. In children it is hardly ever possible to give enough nutrients; they cannot be made to eat since this may lead to increased vomiting. Adults, although more motivated, have no appetite either and become emaciated. Even the appetite promoting effect of prednisone in ALL is not enough to cause an adequate food intake in the first weeks. During the remission anorexia may persist in young children. With nausea and vomiting a hypercaloric diet is only possible via a nasogastric tube. This can be applied for weeks, even months. The nasogastric tube can be used either continuously or only during the night when the patient can still use oral food. Replacement of the nasogastric tube is necessary each week with the use of alternate nostrils. Either pasteurized home-made formulas or home-made formulas or commercial preparations like Nutrison® could be used. Adults require 2000–3000 calories/24 hours; for children the amount is adapted for age and ideal weight. In the case of chemotherapy induced lactose malabsorption [71] elementary tube feeding is applied, solutions containing aminoacids (Nutri 2000®) or peptides (Pepti 2000®) are in use. Regulation of the feeding can be managed with an Ivac Pump®. With tube feeding the serum albumen levels are decreased less than those of orally fed patients [8]. Patients receiving aggressive chemotherapy for AML do not always tolerate tube feeding. If, in addition, there is a danger of infection of the oesophagus during agranulocytic episodes, intravenous alimentation is given.

A risk of intravenous feeding is of sepsis via the indwelling venous catheter. Intracath® needles may be used for one or two days. In wards with an equipped nursing staff, deep lying subclavian or jugular vein catheters can be used. With aseptic measures they can be left *in situ* for weeks [72]. Septicaemia is possible via the connections of the catheter [73].

When complete remission persists, a combination of cytostatics is given for at least two years.

During these two years one has to guarantee an adequate food intake. In children there are smaller reserves than in adults and they need more calories because of their growth. With small, more frequent portions and pedagogic advice, a good calory intake can be achieved. In the majority of cases no tube feeding is necessary. 6 MP and Lanvis lead to a 'horror carnis'; in the presence of this complaint other protein supplies such as milk and soja are advised. A normal supply of vitamins has to be guaranteed.

Pain relief and anti-emetics

Pain may be present at the moment of diagnosis. If the pain is localised in the bones, it disappears during the induction treatment. Paracetamol or Glifanan® relieves the pains. Severe pains may accompany septicaemia; morphine derivatives are then necessary. Analgetics in high dosage may be indicated during the terminal stage of the diseases when the infiltrating masses of leukemic cells may be growing. Infusions with morphine 14 mg/kg/24 h relieve the complaints. Local pain in the mouth due to methotrexate ulcers is relieved with 1% lidocain and the ulcers healed with Ledervorin in saline solution or orabase. Localized pain from leukemic infiltrations is controlled with local X-ray irradiation. Pain due to local necrosis in the enlarged spleen in CML is relieved by means of X-ray irradiation of the spleen; splenectomy has the same beneficial effect. Anti-emetics should be given orally and not rectally. Phenothiazine, Primperan, neuroleptics alone or in combination have a good effect. Motilium® intravenously 0.1–0.2 mg/kg or orally 3 × 10 mg for adults is a potent anti-emetic.

Psychosocial support

Psychosocial support is different for adults and for younger people. In adults one has mainly to deal with the patient and his family; in youngsters with the child, the parents and with the siblings. General advice cannot be given but some rules can be kept in mind. One should take ample time to explain the

facts of the disease and treatment and should know the facts oneself.

Patients feel immediately uncertainty in the doctor, nurse or social worker. During the explanation honest answers to questions asked by the patient, young and old, make far less worrying since the disease and treatment are fully understood [74]. Interventions with bone marrow punctures should be in expert hands; a patient with a newly diagnosed leukemia should not be a teaching model.

School or work should be resumed at an optimal level after the remission induction. Schools can be asked for special training programs. Social and financial problems arising during and due to the disease should be relieved by an expert hospital social worker. The family, parents and siblings have to be taken into the psychosocial care; thus, later emotional problems may be prevented. The psychosocial support should point at an expectancy of cure, as long as there is a reasonable prognosis. A patient with leukemia is a man, woman or child, not ill all the time, with a disease which is not inevitably fatal. When the prognosis is poor, the patient and his family should be informed in order to make a new attitude to life and death possible.

References

1. Pinkel D, Simone J, Hustu HO, Aur RJA: Nine years experience with total therapy of childhood lymphocytic leukemia. *Pediatrics*, 1972; 50: 246-51.
2. Aur RJA, Simone JV, Venzosa MS, Hustu HO, Basker LF, Pinkel DP, Revira G, Hahl GV, Wood A, Stanger A, Mason C: Childhood acute lymphocytic leukemia. *Cancer*, 1978; 42: 2123-34.
3. Chessells JM: Acute lymphoblastic leukemia. *Semin Hematol*, 1982; 19: 155-71.
4. Haas RJ, Janka G, Netzel B, Helmig M: Munich ALL 77-01 study of treatment of acute lymphoblastic leukemia in childhood. *Klin Pediatr*, 1981; 193: 137-44.
5. Simone JV, Verzosa MS, Rudy JA: Initial features and prognosis in 363 children with acute lymphocytic leukemia. *Cancer*, 1975; 36: 2009-2108.
6. Simone JV: Leukemia remission and survival. *Lancet*, 1981; ii: 531.
7. Vogler LB, Crist WM, Sariff AM, Pullen DJ, Bartolucci AA, Falletta JM, Dowell B, Humphrey GB, Blackstock R, Eys vG, Metzgar G, Cooper MD: An analysis of clinical and laboratory features of acute lymphocytic leukemias with emphasis on 35 children with pre B leukemia. *Blood*, 1981; 58: 135-40.
8. Vries EGE de: Clinical and laboratory aspects of leukemia. Academic Thesis, University of Groningen, 1982; 26-40. Ed van Denderen, Groningen.
9. Evans AE, Gilbert ES, Zandstra R: The increasing incidence of central nervous system leukemia in children. *Cancer*, 1970; 26: 404-9.
10. Green DM, Freeman AI, Sather HS, Sallan SE, Nesbit ME, Cassady JR, Sinks LF, Hammond D, Frei E: Comparison of three methods of central nervous system prophylaxis in childhood acute lymphoblastic leukemia. *Lancet*, 1980; i: 1398-1402.
11. Nesbit ME, Sather HN, Robinson LL, Ortega J, Littman PS, d'Angio GJ, Hammond GD: Pre-symptomatic central nervous system therapy in previously untreated childhood acute lymphoblastic leukemia comparison of 1800 and 2400 Rad. A report for Children's Cancer Study Group. *Lancet*, 1981; i: 461-65.
12. Lister TA, Rohatiner AZ: Treatment of acute myelogenous leukemia in adults. *Semin Hematol*, 1982; 19: 172-92.
13. Mayer RJ, Weinstein HJ, Rosenthal DS, Coral FS, Nathan DG, Frei E: VAPA; A treatment program for acute myelocytic leukemia. *Haematol Blood Transf*, 1981; 26: 45-52.
14. Gale RP, Kay HE, Rimm AA, Bortin MM: Bone marrow transplantation for acute leukaemia in first remission. *Lancet*, 1982; ii: 1006-9.
15. Barrett J, de Koning J: Supportive care in bone marrow transplantation. In: *Supportive Therapy in Haematology*, Das PC, Smit Sibinga CTh, Halie MR (eds). Martinus Nijhoff, Boston, 1985.
16. Thomas ED: The role of marrow transplantation in the eradication of malignant disease. *Cancer*, 1982; 49: 1963-69.
17. Spiers ASD, Booth AE, Frith JL: Subcutaneous cerebro-spinal reservoirs in patients with acute leukemia. *Scand Haematol*, 1978; 20: 289-96.
18. Klose HJ, Kelson S, Schwarzbach K, Janka G, Netzel B, Haas R, Betke K: Initial treatment of acute childhood leukemia with extreme leucocytosis by blood exchange transfusion: rheological aspects. *Klin Paediatr*, 1981; 193: 172-76.
19. Drapkin RL, Gee TS, Dowling MD, Arlin Z, McKenzie S, Kempin S, Clarkson B: Prophylactic heparinotherapy in acute promyelocytic leukemia. *Cancer*, 1978; 41: 2484-90.
20. Bauer TW, Haskins GE, Armitage JD: Splenic rupture in patients with haematological malignancies. *Cancer*, 1981; 48: 2729-33.
21. Cervantes F: Tumour lysis syndrom with hypocalcemia in accelerated chronic granulocytic leukaemia. *Acta Haemat*, 1982; 68: 157-59.
22. Young RC, Ozolo RF, Myers CE: The anthracycline anti-neoplastic drugs. *N Engl J Med*, 1981; 305: 139-53.
23. Billingham ME, Mason JW, Bristow MR, Daniels JR: Anthracycline cardiomyopathy monitored by morphological

- changes. *Cancer Treat Rep*, 1978; 62: 865-72.
24. Steinherz PG, Miller LP, Ghavimi F, Allen JC, Miller DR: Dural sinus thrombosis in children with acute lymphoblastic leukemia. *JAMA*, 1981; 246: 2837-39.
 25. Simone J: Acute lymphoblastic leukemia in childhood. *Semin Haematol*, 1974; 11: 25-39.
 26. Komp DM, Fernandes CH, Falletta JM, Ragab AH, Humphrey GB, Pullen J, Moon T, Shuster J: CNS prophylaxis in acute lymphoblastic leukemia. Comparison of two methods. South West Oncology Group Study. *Cancer*, 1982; 50: 1031-36.
 27. Freeman AJ, Weinberg V, Brecher ML, Jones B, Gliksman AS, Sinks LF, Weil M, Plüss HJ, Hananian J, Burgert DE, Gilchrist S, Necheles T, Harris M, Kung F, Patterson RB, Maurer H, Leventhal B, Chevalier L, Forman E, Holland JF: Comparison of intermediate methotrexate with cranial irradiation for the post-induction treatment of acute lymphocytic leukemia in children. *N Engl J Med*, 1983; 308: 477-84.
 28. Does vd- Berg vd A, Hermans J, Nagel J, Steenis v G: Immunity of diphtheria, pertussus, tetanus and poliomyelitis in children with acute lymphocytic leukemia after cessation of chemotherapy. *Pediatrics*, 1981; 67: 222-29.
 29. Sallan SE, Weinstein HJ, Nathan DG: The childhood leukemia. *Pediatr*, 1981; 99: 676-88.
 30. Dutcher JP, Schiffer CA, Aisner J, Wiernik PH: Longterm follow up of patients with leukemia receiving platelet transfusions: Identification of a large group of patients who do not become alloimmunized. *Blood*, 1981; 58: 1007-11.
 31. Koning J de: Diagnostische en therapeutische aspecten van idiopathische thrombocytopenie. Academic Thesis, University of Leiden, Pasmans, The Hague, 1975: 52-53.
 32. Koning J de: Platelet transfusion in bone marrow transplantation in children. In: Paediatrics and transfusion, Smit Sibinga CTh, Das PC, Forfar JO (eds). Martinus Nijhoff, The Hague, 1982: 228-36.
 33. Schmitz N, Kayser W, Gassmann W, Huhn A, Kruger G, Sachs V, Loffler H: Two cases of graft versus host disease following transfusion of non irradiated blood products. *Blut*, 1982; 44: 83-88.
 34. Lowenthal RM, Menon C, Challis DR: Graft versus host disease in consecutive patients with acute myeloid leukemia treated with blood cells from normal donors. *Aust NZ J Med*, 1981; 11: 179-83.
 35. Kulpa J, Zaroulis CG, Good RA, Kutt J: Altered platelet function and circulation induced by amphotericin B in leukemic patients after platelet transfusion. *Transfusion*, 1981; 21: 74-76.
 36. Winston DJ, Ho WG, Gale RP: Prophylactic granulocyte transfusions during chemotherapy of acute nonlymphocytic leukemia. *Ann Intern Med*, 1981; 94: 616-22.
 37. Pflieger H, Arnold R, Bhaduri S, Dietrich M, Heimpel H, Kubick B, Rasche H, Wiesneth M: Granulocyte transfusion in acute leukemia, regeneration of granulopoiesis as determining factor of survival. *Scand J Haematol*, 1981; 26: 215-20.
 38. Ford JM, Cullen MH, Roberts MM, Brown M, Oliver RT, Lister T: Prophylactic granulocyte transfusions. Results of a randomized controlled trial in patients with acute myelogenous leukemia. *Transfusion*, 1982; 22: 311-16.
 39. Pochedley C: Infections in childhood leukemia. *Clin Med*, 1973; 80: 23-27.
 40. Tobias JC, Wrigley PF, O'Grady G: Bacterial infection and acute myeloblastic leukemia, an analysis of two hundred patients undergoing intensive remission induction therapy. *Eur J Cancer*, 1978; 14: 383-91.
 41. Siegel SE, Lipin S, Clerck de Y: Importance of gram positive bacterial infections during treatment for childhood leukemia (Meeting abstract). *Clin Res*, 1980; 28: 113.
 42. Novak R, Feldman S: Salmonellosis in children with cancer. *Am J Dis Child*, 1979; 133: 298-300.
 43. Newman KA, Schimpff SC, Young VM, Wiernik PH: Lessons learned from surveillance cultures in patients with acute nonlymphocytic leukemia, usefulness for epidemiologic, preventive and therapeutic research. *Am J Med*, 1981; 70: 423-31.
 44. Fainstein V, Rodriguez V, Turuck M, Hermann G, Rosenbaum B, Bodey GP: Patterns of oropharyngeal and fecal flora in patients with acute leukemia. *J Infect Dis*, 1981; 144: 10-18.
 45. Chessells JM, Leiper AD: Infection during remission induction in childhood leukemia. *Arch Dis Child*, 1980; 55: 118-23.
 46. Sleyfer DT, Mulder NH, Vries de-Hospers HG, Fidler V, Nieweg HO, Waay vdd, Saene vHK: Infection prevention in granulocytopenic patients by selective decontamination of the digestive tract. *Eur J Cancer*, 1980; 16: 859-69.
 47. Starke ID, Catovsky D, Johnson SA, Donelly P, Darrell J, Goldman JM, Galton DA: Co-trimoxazole alone for prevention of bacterial infection in patients with acute leukemia. *Lancet*, 1982; i: 5-6.
 48. Watson JG, Powles RL, Lawson DN, Morgenstern GR, Jameson B, McElwain TJ, Judson I, Lumley H, Kay HE: Co-trimoxazole versus non-absorbable antibiotics in acute leukemia. *Lancet*, 1982; ii: 6-9.
 49. Enno A, Darrell J, Hows J, Catovsky D, Goldman JM, Galton DA: Co-trimoxazole for prevention of infection in acute leukemia. *Lancet*, 1978; ii: 395-97.
 50. Vries de-Hospers HG, Sleyfer DT, Mulder NH, Waay vd D, Nieweg HO, Saene vHK: Bacteriological aspects of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients. *Antimicrobial Agents Chemother*, 1981; 19: 813-20.
 51. Kurrle E: Prevention of infection during induction therapy for acute leukemia. *Klin Wochenschr*, 1981; 59: 1075-79.
 52. Smith IE, Powles R, Clink MD, Jameson B, Kay HE, McElwain TJ: Early deaths in acute myelogenous leukemia. *Cancer*, 1977; 39: 1710-14.
 53. Bodey GP, Rodriguez V, Chang HY, Narboni G: Fever and infection in leukemic patients, a study of 494 consecutive patients. *Cancer*, 1978; 41: 1610-22.
 54. Ninane J, Chessells JM: Serious infections during con-

- tinuing treatment of acute lymphoblastic leukaemia. *Arch Dis Child*, 1981; 56: 841-44.
55. Bussel A, Danon F, Ferchal F, Perol Y: Cytomegalovirus infection in malignant blood diseases: clinical and laboratory data in 29 patients. *Nouv Rev Fr Hematol*, 1978; 20: 67-76.
 56. Cheeseman SH, Rinaldo CR, Hirsch MS: Use of interferon in cytomegalovirus infections in man. *Tex Rep Biol Med*, 1977; 35: 523-27.
 57. Ruebush TK, Weinstein RA, Baehner RL, Wolff D, Barlett M, Gonzalez-Crussi F, Sulzer AJ, Schultz MG: An outbreak of *Pneumocystis pneumonia* in children with acute lymphocytic leukemia. *Am J Dis Child*, 1978; 132: 143-48.
 58. Young LS: Trimethoprim-sulfamethoxazole in the treatment of adults with pneumonia due to *Pneumocystis carinii*. *Rev Infect Dis*, 1984; 4: 608-13.
 59. Ball F, Ulmer EM, Schuster W: Interstitial pneumonia in children with malignant diseases during intensive combined cytostatic therapy. Radiological manifestation and patho-anatomical findings. *Klin Paed*, 1978; 190: 83-93.
 60. Morgan E: Decreased incidence of nonspecific interstitial pneumonitis in children with acute lymphocytic leukemia treated prophylactically with trimethoprim-sulfamethoxazole. *J Pediatr*, 1981; 99: 807-10.
 61. Wilson JM, Guiney DG: Failure of oral trimethoprim-sulfamethoxazole in acute leukemia. Isolation of resistant plasmids from strains of enterobacteriaceae causing bacteremia. *N Engl J Med*, 1982; 306: 16-20.
 62. Page CP, Coltman CA, Robertson HD, Nelson EA: Candidal abscess of the spleen in patients with acute leukemia. *Surg Gynecol Obstet*, 1980; 151: 604-8.
 63. Menier-Carpentier F, Kiehn TE, Armstrong D: Fungemia in the immunocompromised host. Changing patterns, anti-ginemia, high mortality. *Am J Med*, 1981; 71: 363-70.
 64. Pennington JE: Aspergillus pneumonia in hematologic malignancy: improvements in diagnosis and therapy. *Arch Intern Med*, 1977; 137: 769-71.
 65. Rodriguez V, Bodey GP, Freireich EJ, McCredie KB, Guterman JU, Keating MJ, Smith TL, Gehan EA: Randomized trial of protected environment-prophylactic antibiotics in 145 adults with acute leukemia. *Med (Baltimore)*, 1978; 57: 253-66.
 66. Vial R, Ryan D, Williams W, Bickers J: The value of protected environment (PE) in the management of acute leukemia (AL) in adults (meeting abstract). *Clin Res*, 1979; 27: 393.
 67. Bhaduri S, Kurrle E, Krieger D, Plieger H, Arnold R, Kubanek B, Heimpel H: Infection prophylaxis in acute leukemia patients: Comparison of selective and total decontamination of the gastrointestinal tract. *Folia Haematol (Leipz)*, 1982; 109: 377-89.
 68. Weel v.-Sipman MH, Meer vd JW, Koning de J, Versteeg J: Severe atypical recurrent varicella in childhood leukemia. *Lancet*, 1981; i: 147-48.
 69. Lopes A, Bianchi A, Gabriel Neto M, Franco PC, Morias VL: Varicella and herpes zoster associated with neoplasia of infancy. The use of cytosine arabinoside-C and gamma-globulin. *Acta Oncol Bras*, 1977; 1: 14-23.
 70. Merigan TC, Rand KH, Pollard RB, Abdallah PS, Jordan GW, Fried RP: Human leucocyte interferon for the treatment of herpes zoster in patients with cancer. *N Engl J Med*, 1978; 298: 981-87.
 71. Haas RJ, Janka G, Netzel B, Helwig M: Munich ALL 77-01 study of treatment of acute lymphocytic leukemia in childhood. *Klin Paediatr*, 1981; 193: 137-44.
 72. Kubel M, Emmrich K, Helbig W, Schultze W, Ezold R, Delleman K, Schwenke H: Zentral venösel langzeitkatheter in der behandlung akuter leukämien: einführung einer modifikation der Hickman technik. *Z Gesammte Inn Med*, 1982; 37: 245-43.
 73. Wade JC, Newman KA, Schimpff SC, Echo vDA, Gelber RA, Reed WP, Wiernik PH: Two methods for improved venous access in acute leukemia patients. *JAMA*, 1981; 246: 140-44.
 74. Mulhern RK, Crisco JJ, Camitta BM: Patterns of communication among pediatric patients with leukemia, parents and physicians: Prognostic disagreements and misunderstandings. *J Pediatr*, 1981; 99: 480-83.

9. Immunosuppression and oncology

E.M. RANKIN and J.G. McVIE

Introduction

The study of the interaction between the host and the neoplastic cell is of fundamental importance in oncology. The topic has been frequently reviewed, so that the present chapter need only review the reviews, highlighting conclusions which are clinically relevant.

The idea that the immunological system might influence the behaviour of malignant cells in the host was first developed from studies in rodent tumours. Preimmunisation of normal rats with carcinogen-induced sarcoma, the cells of which had been pretreated so as to restrict their progressive growth *in vivo*, led to the development of specific tumour immunity. A subsequent challenge with tumour cells from the same sarcoma was rejected by the animals. Such studies have not been possible in humans, but extrapolation from animal models has led to the belief that manipulation of the immune system in man might be beneficial, and even therapeutic.

The early hopes of immunotherapy have not been fulfilled with the passage of time, indicating the need for a clearer understanding of the complex tumour-host relationship. The effect of the tumour on the host may depend on the type of malignancy, the tumour bulk, the level of host immunocompetence, and the therapeutic manoeuvres instituted to eradicate the tumour.

Any approach to rational planning of immunotherapy demands an intimate knowledge of the immune status of the patient.

Table 1 lists some of the battery of available

immune function tests. Immunomodulators can affect a number of these, including production, differentiation, migration and function of T, B and null lymphocytes, as well as monocytes and tissue macrophages. Immunorestoratives, for instance, act to normalise certain of these features, viz. *Corynebacterium parvum* and *Bacille Calmette-Guérin* vaccine (BCG) augment natural killer activity and the antitumour activity of macrophages. Informed use of such selective agents may provide a useful adjunct to conventional surgery, radiotherapy or chemotherapy by enhancing the response of the host to the tumour, leading to successful eradication. Their use until now has tended to be haphazard, taking little heed of functional assessment and even less regard of dose, schedule, and route of administration.

A. Immunodeficiency and neoplasms

1. Congenital immunodeficiency

In spontaneous immunodeficiency diseases in man there is an increased susceptibility to malignancy, especially in the lymphoid system. This risk has been estimated at between 4–10% [1, 2]. Humoral immunity is impaired in Bruton's type agamma globulinaemia in which there is a 10,000 fold increase in the incidence of leukaemia compared to the normal population. In those diseases in which cell mediated immunity is affected e.g. Wiskott-Aldrich and ataxia-telangiectasia the risk of malignancy, especially lymphoma, is also increased

Table 1. Tests useful in the evaluation of host defence.

1. Phagocyte associated
Granulocyte associated
Peripheral blood granulocyte levels
Phagocytosis
Intracellular killing
Acute inflammatory response
Monocyte associated
Peripheral blood monocyte counts
Phagocytosis and intracellular killing
Macrophage cytotoxicity to target cells
Chronic inflammatory response
Clearance from peripheral blood by reticulo-endothelial system
2. Lymphocyte associated
T cell associated
T cell levels in peripheral blood
Proliferative response to mitogens and antigens
Lymphocyte mediated target cell killing
Delayed hypersensitivity to recall and neoantigens
B cell associated
B cell levels in peripheral blood
Serum immunoglobulins
Antibody dependent cell mediated cytotoxicity
Proliferative response to mitogens and antigens
Antibody response to mitogens and antigens
Antibody responses primary and secondary
3. Other factors:
Serum complement
Macrophage function

10,000 fold. Common variable immunodeficiency is associated with an increased incidence of lymphoma and adenocarcinoma of the gastrointestinal tract [3].

2. Acquired immunodeficiency syndrome

The recent epidemic of the acquired immune deficiency syndrome (AIDS), (7,000 cases have been reported in the United States by the end of 1984), is arousing considerable interest. A study of the epidemiology of AIDS may yield some clues to the relationship between the predisposition for malignancy associated with the alterations in the immune status and the aetiological agent. The syndrome is characterised by severe opportunistic infections, altered immunological parameters, generalised lymphadenopathy and an increased incidence of Kaposi's sarcoma and undifferentiated

non-Hodgkin lymphoma in previously healthy individuals [4].

The disease is most prominent in the male homosexual and bisexual population of New York, San Francisco and Los Angeles but is also described in heterosexuals. Other affected groups include intravenous drug abusers, Haitian immigrants to the U.S. and haemophiliacs using commercially prepared lyophilised Factor VIII concentrates. The epidemiology is suggestive of a bloodborn pathogen, and reminiscent of hepatitis B virus-transmission by blood or body fluids with a prolonged incubation period and carrier state. The effects on the immune system include lymphopenia, cutaneous anergy, reduced helper T lymphocytes (T4), increased suppressor T lymphocytes (T8), inverted T4/T8 ratio, abnormal lymphocyte response to mitogenic stimulation, decreased natural killer activity, and hypergammaglobulinaemia. The relationship between this array of odd reports and the emergence of sarcomas and lymphomas is far from clear, and quite obviously requires meticulous dissection. AIDS seems to be related to a lymphotropic virus (LAV/HTLV III) infection.

B. Immune status in untreated patients with malignant disease

1. Hodgkin's disease and lymphomas

1.1. Hodgkin's disease

The defects in immunological function associated with Hodgkin's disease (HD) were first documented at the beginning of this century by Reed, who noted negative delayed hypersensitivity skin test responses to tuberculin in 5 of 8 patients [5]. The numerous studies since then show that, even in untreated patients, there is a defect in cell mediated immunity (Table 2).

Defects in the response to recall antigens (secondary response) such as tuberculin, candida, streptokinase - streptodornase and mumps have been demonstrated in various series in 34-60% of patients [6, 7]. Responsiveness in these tests decreases appreciably with the increasing anatomical mass and disease [8], but does not relate to the

presence of constitutional symptoms [7]. Conversely, intradermal injection with a neo-antigen 2,4-dinitrochlorobenzene (DNCB) showed that a response was produced more often in asymptomatic patients [7]. The ability to reject grafts of foreign tissue, which is also dependent on cell mediated immunity, has been shown to be adversely affected in Hodgkin's disease [9].

Studies of the *in vitro* function of lymphocytes show defects in the majority of untreated patients (Review: 22). The proliferative response to phytohaemagglutinin (PHA), pokeweed mitogen (PWM) and concanavalin A (conA) [10] to antigens such as tuberculin and to alloantigens are all impaired. There are also alterations in the surface membranes of lymphocytes as shown by enhanced lectin agglutinability and diminished cap formation. Another abnormality in surface membrane properties, the ability to bind sheep red cells, 'rosette formation', is impaired and seems to be due to the blocking of the Fc receptor. This defect can be repaired by incubation of cells overnight in fetal calf serum, and restored by incubation in the serum of patients with HD [12]. The nature of the factor, if it can be so named, is not yet established. Dysfunction of macrophages as shown by impaired chemotactic responses, lysozyme production and phagocytosis heralds advanced 'metastatic' disease.

Lymphopenia in untreated patients has been found in some series but there are conflicting reports on the relationship between lymphocyte counts, histology and stage of disease. The availability of highly specific monoclonal antibodies has helped to clarify this area. Posner *et al.* studied 20 patients with untreated HD, who had undergone extensive diagnostic evaluation for staging pur-

poses [13]. A reduction in the absolute lymphocyte count was seen in those patients with more advanced disease, but the proportions of T cells with inducer, and cytotoxic suppressor phenotype was preserved. There was no evidence of an activated T suppressor population. De Sousa and colleagues have suggested that sequestration of T cells or specific subsets thereof in the spleen, was one of the causes of the immune abnormalities in this disease [14]. However, Posner's study of six patients subjected to splenectomy failed to support this theory [13]. Thus there is still no satisfactory explanation for the alterations in cell-mediated immunity which are seen in untreated patients, even those with disease in its earliest stages. It should be noted that subcutaneous anergy does not influence prognosis as measured by response to treatment, relapse rate or survival for patients with a given stage of disease at presentation [6].

In striking contrast to the selective impairment of cell-mediated responses, untreated patients with Hodgkin's disease have normal B cell function and humoral immunity.

1.2. Non-Hodgkin lymphoma

There are surprisingly few data on immune parameters in untreated patients with non-Hodgkin lymphoma (NHL), a heterogenous neoplasm in which T and B cells occur in different proportions in different histologies (Table 3). The need for adequate staging and pathological classification makes comparison between studies difficult.

Jones *et al.* in 1977 studied a group of patients with untreated NHL of varying pathologies and reported impaired delayed hypersensitivity reactions to recall antigens, lymphopenia and reduced levels of serum IgA [15]. Other authors have detected hypogammaglobulinaemia in up to 15% patients. Immunological function is less well preserved in patients with high grade pathology. Advani and colleagues studied 101 untreated patients and confirmed these data [16]. Clearance by the reticuloendothelial system, bactericidal activity and macrophage activity remain normal. In patients with mycosis fungoides there may be a deficiency in suppressor T cells and diminished T-cell mediated responses. A monoclonal gammopathy

Table 2. Immunological abnormalities in Hodgkin's disease.

Impairment primary delayed type hypersensitivity response
Impairment of delayed hypersensitivity to recall antigens
Delayed homograft and heterograft rejection
Impaired <i>in vitro</i> lymphoproliferative responses to mitogens, antigens and allogenic cells
Impaired lymphocyte protein synthesis
Altered macrophage function with impaired chemotactic responses, lysozyme production and phagocytosis.

Table 3. Lukes and Collins' classification of lymphoid neoplasms.

B cell diseases
Small lymphocyte lymphoma (chronic lymphocytic leukaemia)
Plasmacytoid lymphocyte disease
Follicular centre cell lymphomas
Small cleaved cell lymphoma
Large cleaved cell lymphoma
Small transformed cell lymphoma
Large transformed cell lymphoma
Burkitt's lymphoma
Immunoblastic sarcoma of B cells
B cell acute lymphoblastic leukaemia
T cell disease
T cell variant of chronic lymphocytic leukaemia
Mycosis fungoides
Sézary syndrome
Convoluted lymphoblastic lymphoma
Immunoblastic sarcoma of T cells
T cell acute lymphoblastic leukaemia
Undefined (null) cell disease, acute lymphoblastic leukaemia

may occur in up to 8% of patients with diffuse pathology, but in less than 1% patients with nodular histology. The paraprotein is usually of the IgM class and its presence is related to a low level of serum IgM.

2. Immunocompetence in haematological malignancies

2.1. The leukaemias

Acute lymphoblastic leukaemia (ALL) may be subdivided according to the predominant cell surface phenotype. Approximately 20% exhibit thymic differentiation antigens (T cell ALL); the common ALL antigen can be detected on another 70%, and the remainder possess surface membrane immunoglobulin (B cell ALL) or lack all of these markers (undifferentiated ALL). Immunocompetence has yet to be specifically studied in these separate groups.

The overgrowth of leukaemia cells in the marrow decreases the production of polymorphonuclear leukocytes and interferes with their function. Response to chemotactic stimuli, bactericidal activity, phagocytic capacity and leukocyte metabolism may all be abnormal [17]. Immunoglobulin

levels are either normal or decreased. Cell-mediated and humoral immunity are usually normal.

Delayed cutaneous hypersensitivity may be reduced in up to 30% of patients with acute myeloid leukaemia. Although neutrophil chemotactic and bactericidal activity may be impaired, immunocompetence is not usually markedly affected in this patient group before treatment. Chemotherapy brings about a profound change in the immune status, causing a deterioration in T and B cell immunity, but this will be further discussed in detail later.

Chronic lymphocytic leukaemia (CLL), multiple myeloma and Waldenström's macroglobulinaemia are all associated with major disturbances in B cell function. CLL is associated with the accumulation of a monoclonal malignant B cell population. In this disease there is decreased production of immunoglobulin and a diminished response to antigenic and mitogenic stimulation. Delayed hypersensitivity phagocytosis and the bactericidal activity of granulocytes may all be preserved.

There is an imbalance in the T cell subsets in CLL. $T\gamma$ cells bear Fc receptors for IgG and directly suppress; $T\mu$ cells are characterised by an Fc receptor for IgM and have a 'helper' function. In CLL there is a marked increase in the number of $T\gamma$, and a reduction in $T\mu$. This abnormality is present early in the disease and is unaffected by treatment. Removal of the infiltrated CLL spleen results in a dramatic decrease in the proportion of $T\gamma$ cells [18]. These abnormalities may reflect an attempt by the host's immune system to deal with the monoclonal B cell population, perhaps by means of a cytotoxic response.

2.2. Multiple myeloma

The marked abnormalities in humoral immunity in myeloma are related to altered immunoglobulin levels. The neoplastic cells in 95% of cases manufacture a non-functioning immunoglobulin which is usually an intact IgG but may be IgA or IgD. Functioning polyclonal immunoglobulin is decreased in 80–90% patients due to a lower rate of synthesis as well as increased metabolism. There is a quantitative relationship between the accumulation of monoclonal plasma cells in the marrow,

elevation of monoclonal protein in the serum, the decline in antibody response and the production of functional immunoglobulin. Cell-mediated activity and T cell function are usually preserved before treatment. Depressed chemotactic, phagocytic and bactericidal activities of granulocytes are often found in patients with an IgA paraprotein. Bacterial infections especially of the lung and kidney are of course notoriously common presenting features in myeloma patients.

3. Immunocompetence in solid tumours

Solid tumours, both sarcomas and carcinomas, arising in different organs may produce similar defects in immune function despite their widely differing patterns of behaviour and histology. The degree of immunodeficiency depends on the extent of metastatic growth, cellular immunity in particular becoming defective in disseminated disease.

Reactivity to new antigens is more affected than recall of established immunity. Ability to react to neo-antigens e.g. DNCB has been correlated with early recurrence and poor prognosis, or inoperability at presentation in a variety of cancers [19, 20]. Two hundred and forty-five untreated patients with breast cancer of various stages were studied by Adler *et al.* for *in vivo* response to tuberculin and DNCB, *in vitro* lymphocyte response to PHA and for the proportion of E rosetting cells [21]. Fifty-one percent of patients with early operable tumours showed some immunosuppression compared with 11% of 107 normal controls: 68% of the inoperable and 89% of metastatic patients showed defects.

T cell subsets are altered in patients with untreated disseminated malignancy. There is an increase in the T_y (suppressor) and a diminution in the T_μ (helper) populations compared with age-matched controls [22]. T_y cells are known to mediate antibody-dependent cellular cytotoxicity *in vitro*, but the significance of this finding in the tumour-host relationship *in vivo* is not known.

Ovarian cancer has been shown, unusually, to be associated with a reduction in cells bearing immunoglobulin on their surface. The proliferative response to PWM and primary response to neoantigens are also adversely affected [23].

Krishnan *et al.* claimed that the number of macrophages maturing from monocytes is lower in cancer patients than normal individuals, and that these macrophages have a definite maturation defect demonstrable *in vitro* [24].

Monocyte-chemotactic responsiveness *in vitro* in untreated patients with breast cancer of various stages was also abnormal [25]. There are a number of other factors which may influence the immunological responsiveness of patients including age, genetic make-up, stress and nutritional status; to what extent, however, remains to be unravelled.

C. Effect of treatment modalities on immune status

It is difficult to define the extent of interaction between individual forms of treatment and the immune system. Statistical analysis requires that relatively large numbers of patients be accrued in order to detect small differences. So many of the above variables are relevant in cancer, rendering a seemingly large group meaningless due to many sub-groupings. Patients for instance can only be compared if their malignancies are at the same stage of development since tumour stage has, as already outlined, an effect *per se* on the immune response. Further stratification should be made for age, histology, coexistence of infection and so on.

In addition, standardised test procedures have rarely been used. For example the results of investigations into the effects on T and B cells of irradiation post mastectomy differ widely because one investigator removed the monocytes from the blood before the test and another did not [26, 27]. PHA stimulation tests using H^3 -leucine incorporation as an index of mitogenic response depend on the dose of PHA, the number of cells in culture, the type of microtitre plate used, the presence of monocytes, the length of incubation, the length of exposure to tritiated thymidine, activity of the radionuclide, culture medium and the type of serum used. In few of the reports scrutinised for the present review was sufficient attention paid to such detail. Further, much of the work done to date has been in animals, so extrapolation to humans is not always valid. The *in vitro* model systems mirror

poorly the *in vivo* situation. The very rapid recent advances in our knowledge of the innate controls of the immune system have invalidated much earlier work and warrant revision of basic concepts in this area.

1. Surgery

Surgery has only immuno-suppressive effects, resolving usually within one month or so [28]. The anaesthetic, the stress of the operation, blood loss and tissue necrosis are all factors contributing to the perturbation of the immune status. Malnutrition resulting from anorexia, gastrointestinal obstruction and alterations in intermediary metabolism may adversely affect immunocompetence. Other drugs such as antibiotics, analgesics, tranquillizers, anti-inflammatory agents, H₂-receptor antagonists, and β adrenergic receptor antagonists may be given to the patient around the time of surgery, and so dissection of the precise role of the operation itself in this context is difficult. In general, there is a transient immunodepression affecting both T and B dependant systems. The effects of splenectomy (which may be done as part of the staging for HD, or to treat hypersplenism) are discussed in more detail later.

2. Radiotherapy

Radiation damages the ability of lymphocytes to replicate DNA. Uncommitted lymphocytes are more susceptible than established effector cells which do not require further cell division to synthesise antibody or execute cell mediated cytotoxicity. The type of irradiation, the dose, the scheduling and the area treated have independent bearing on the degree of damage to the immune system. T and B cells are both affected but T cells are more sensitive because they repair damaged DNA less efficiently [29]. Although B cells are depleted in number, the primary and secondary humoral responses are not impaired nor are plasma antibody levels. *In vitro* tests show that mature granulocytes and monocytes retain chemotactic, phagocytic and bactericidal functions; monocyte numbers stay unaltered but granulocyte precursors are depleted by

radiation. Defects tend to be repaired in a matter of months, particularly if the patient achieves a remission from his cancer.

3. Hormones

Glucocorticoids alter the distribution and function of leukocytes leading to an apparent lymphopenia which is due to relocation of recirculating lymphocytes in the extravascular compartment. T cells are more influenced than B cells but numbers of natural killer (NK) cells may also alter. Steroids decrease the release of monocytes from the bone marrow and cause sequestration of circulating cells. Increased susceptibility to infection by intracellular organisms (characteristic of steroid exposure) may be due to decreased activation of macrophages, defective bactericidal function or diminished T cells resulting in insufficient quantities of lymphokines to attract, arrest and activate macrophages [30].

Oestrogens, antioestrogens, progesterones and androgens are included in the oncologist's armamentarium. Oestrogen will normalise PHA activity in post menopausal women, whereas androgens will do the same in premenopausal women [31]. In animals oestrogens produce thymic involution, delay graft rejection, decrease resistance to tumour growth, inhibit the response to mitogens, diminish T-cell dependent humoral activity and augment macrophage activity. Oestrogens also stimulate the function of the reticuloendothelial system as shown by brisker phagocytosis, an enhanced proliferative response to macrophage growth factor, and an increased cytotoxic activity for tumour cells. Progesterones also produce some stimulation of the reticulo endothelial system and suppress T cell mediated immunity, again in animal models.

4. Cytotoxic drugs

Cytotoxic drugs may be classified according to their effects on cell kinetics. Cell cycle specific agents act only on proliferating cells, but cell cycle independent agents will damage DNA in both the resting and the proliferating cell. Modern drug regimens are usually comprised of several different drugs

which are given intermittently in high doses, allowing pauses for recovery of normal tissues e.g. the haemopoietic system. Those schedules of drug administration which preserve the cellular immune response have the greatest impact on tumour growth [31]. Short intensive courses of chemotherapy suppress immune function for a short time; the recovery of responsiveness is followed by a rebound 'overshoot' before parameters settle down to normal [32]. In solid tumour patients the overshoot is due to a reduction in monocyte suppressor function [33] due to either a reduction in the relative numbers of macrophages during treatment and/or a reduction in suppressor cell function following treatment.

Primary cellular immunity is the most sensitive immune function, established cell immunity is rarely affected by cytotoxic agents. Primary and secondary antibody synthesis can also be impaired, primary synthesis being the more sensitive.

It is difficult to distinguish between interference with those mechanisms which regulate effector cell development and those which direct inhibition of specific effector cell functions. Caution is needed in interpreting *in vitro* work since any drug may alter target cell susceptibility as well as, or instead of, effector cells. T cell function may be variously affected depending on whether *in vitro* or *in vivo* systems are used. Cyclophosphamide will depress cell mediated cytotoxicity against allogeneic tumour cells *in vitro*, without affecting the rejection of these grafts *in vivo* [34]. The results in animal studies and *in vitro* systems also vary depending on the type of animal used, the lymphoid tissue used, the test antigen, the time interval, order of antigen and drug administration, the dose of drug and the drug schedule.

Altered T cell mediated immunity may result from inhibition of T cell proliferation, redistribution of circulating T cells, inhibition of T cell function or depletion of monocytes which are required to effect the T cell reaction. Elimination of suppressor T cells may be an important contributing factor to the success of some chemotherapy schedules. Studies on B cell dependant functions also need to consider the T-cell dependence of the B cells in question, the role of regulatory mechanisms

in the response and the nature of the response including the antibodies produced and their specific biological activities. Granulocyte function is affected only when circulating granulocyte numbers fall below $1 \times 10^9/l$. Monocyte function, however, may be impaired even if the total leukocyte count is within normal limits, as illustrated by measuring the chemotactic response to simple lymphokine preparations.

D. Combined effect of disease and treatment on immune system

Rather than list long and often inadequately described reports of treatment-related immune deficiency, a specific disease will be highlighted to illustrate the considerable problems created by the interplay of the disease and its treatment.

1. Hodgkin's disease

Patients with Hodgkin's disease are often subjected to splenectomy during laparotomy or to radiotherapy to the spleen as treatment which may result effectively in splenectomy due to splenic atrophy. The main function of the spleen is phagocytosis and the removal of particulate matter from the blood stream; it also influences phagocytosis by the liver and other parts of the reticuloendothelial system. Asplenic patients have impaired immunity to blood-borne bacterial and protozoal infections and are particularly prone to fulminant sepsis due to encapsulated bacteria, e.g. *Haemophilus influenzae* or *Streptococcus pneumoniae*. Since the reticulo endothelial system is important for the clearance of coagulation end-products, disseminated intravascular coagulation may occur and may be associated with Waterhouse-Friederichsen syndrome.

Splenectomy in otherwise normal patients is associated with subnormal levels of IgM and with a failure to switch from IgM to IgG synthesis after intravenous immunisation [35]. Non-immunosuppressed patients have shown an increased risk of severe cytomegalovirus infection [36]. An increase in the mortality of viral hepatitis has been associ-

ated with splenectomy [36]. The effect of splenectomy on the cutaneous dissemination of herpes zoster remains controversial [38, 39, 40].

The risk of bacteraemia is not in the early post-operative period [41] nor in the first year when sepsis correlates best with granulocytopenia and recurrence of disease [42]. Weitzman *et al.* found that aggressive treatment especially total nodal irradiation combined with chemotherapy was most likely to impair humoral defence against encapsulated organisms and to enhance the risk of post-splenectomy septicaemia [43]. Data from Stanford on 179 children with Hodgkin's disease, 146 of whom had had splenectomy, followed for up to 20 years, confirm that the risk of bacterial, fungal and viral infection is not related to splenic function but to the aggressiveness of treatment [44].

It is important to realise that patients are at risk of severe infection for a long time after splenectomy, even as long as 13 years [44]. Prophylactic antibiotic cover should be given to children although this may not prevent bacterial infection entirely. Asplenic patients undergoing surgery are at particular risk and should receive antibiotic prophylaxis and subcutaneous heparin to help prevent disseminated intravascular coagulation. Pneumococcal vaccination should be given after removal of the spleen before therapy begins, but cover is incomplete with the presently-available vaccines.

2. Chemotherapy and radiotherapy for Hodgkin's disease

Treatment of Hodgkin's disease is associated with a persistent defect in cell mediated immunity and with alterations in humoral immunity. Fuks *et al.* demonstrated abnormalities in cellular immunity which persisted for up to ten years after megavoltage radiotherapy [45]. Chemotherapy with MOPP (mustine, vincristine, prednisolone and procarbazine) in HD has been associated with persistent abnormalities in the *in vitro* tests of T cell function and with a lower percentage of rosettes in the peripheral blood [46], as well as with an altered delayed hypersensitivity response to neoantigens [47]. There was failure of restoration to normal pretreatment levels even after 12 years. A group of

patients with diffuse histiocytic lymphoma who received the same chemotherapy showed normal T cell proliferation in response to mitogenic stimulation, and levels of E rosettes, although not normal, were considerably higher than in the Hodgkin's patients. This argues against the chemotherapy per se being responsible for the deficits and rather that the defect in HD is inherent to the disease itself. Fisher *et al.* showed that B cell numbers and serum immunoglobulins in HD patients did not differ significantly from normal [46]. Hancock *et al.* showed in a small group of patients that low levels of immunoglobulin persisted for at least 5 years in patients treated with splenectomy and a modified MOPP regimen [48]. That there is a defect in humoral immunity after treatment was shown by Weitzman *et al.*, who demonstrated impaired specific antibody response to *Haemophilus influenzae* and also IgM deficiency: these effects were most marked in patients who had received splenectomy, radiotherapy and chemotherapy [43].

The observations on the alterations in the immune status of patients with Hodgkin's disease have profound clinical implications. There is an increased susceptibility to a wide variety of infections caused by pyogenic bacteria, to organisms which normally evoke a cell mediated response, e.g. mycobacteria, fungi, *Toxoplasma* and *Pneumocystis* and viruses, e.g. *Varicella zoster*, disseminated herpes simplex and cytomegalovirus. Infections now account for over half of all deaths in patients with HD [49]. Whilst immune status does not correlate with outcome in patients receiving modern treatment, it is to be noted that restoration of immune responsiveness after treatment appears to be associated with sustained remission, and adversely that failure of immune recovery appears to be associated with an increased risk of relapse.

The increased incidence of second malignancies, especially acute leukaemia and non-Hodgkin's lymphoma seen in patients treated for HD, 10% at 10 years [50, 51], may be related to impaired immunological surveillance arising from the defects in cell-mediated immunity, the altered humoral immunity and antibody response after therapy, and to the oncogenic and mutagenic potential of the therapeutic regimens. It is noteworthy that the

highest incidence of second malignancy is in those patients who have received the most aggressive therapy – radiotherapy and chemotherapy including MOPP, although it is claimed that other combinations of drugs (doxorubicin, dacarbazine, bleomycin and vincristine) do not appear to be carcinogenic.

3. Effects of treatment in patients with leukaemia

Patients with acute leukaemia remain susceptible to infection during induction treatment, remission and relapse. In spite of many advances in management and treatment infection remains a major cause of death. 161 of 378 patients with AML at the M.D. Anderson Hospital failed to achieve remission, 90 of these patients died of infectious complications; 37% of these were fungal infections [52]. Neutropenia is the most important factor responsible for the increased frequency of infection. There is an inverse correlation between the number of circulating neutrophils and lymphocytes and the frequency of infection. The longer the neutropenia the greater is the risk of infection. In the presence of a neutrophil count below $1 \times 10^9/l$ there were half as many major infections when the lymphocyte counts were more than $1 \times 10^9/l$ than when they were less than this [53]. Monocytopenia and defective monocyte migration may account for the increased susceptibility to infections seen in hairy cell leukaemia in which neutropenia occurs less commonly than in the acute leukaemias.

Defects in cell mediated and humoral immunity in patients with leukaemia are usually due to the effects of treatment. Craniospinal irradiation impairs the bactericidal activity of phagocytes. Corticosteroids cause lymphocytopenia and monocytopenia. Many of the antitumour drugs used cause immunosuppression and interfere with the function of the immune system. The side effects arising from these drugs, e.g. oral ulceration and diarrhoea, may further compound the problems of the host in defence against infection. When treatment finishes, the B cell numbers rebound rapidly whereas T cells recover more gradually, reaching normal levels within 8 to 12 months.

E. Manipulation of host response to tumour

Treatments for cancer fail because of the growth of metastases at sites distant from the primary tumour. Therapy is most effective when tumour bulk is minimal. At the time of primary diagnosis metastases may be already present but be too small to be detected by currently available methods. A single tumour contains cells of widely diverse phenotypes so that cells in metastases may differ in their response to treatment from those in the primary tumour, and individual metastases themselves may vary in their response to treatment. In addition many metastases may be located in sites which prevent effective treatment without causing excessive harm to surrounding normal tissues or to the host. All of these factors contribute to the lack of success experienced with currently available anti-tumour therapy.

Manipulation of the immune response of the host by methods which work principally through cell-mediated or humoral routes offer hope for an improvement in the near future in the lot of the patient with cancer.

1. Antibodies to tumour antigens

The tumour must express an antigen which is immunogenic in the host if therapeutic manoeuvres to enhance or modify the immune response of the host to the tumour are contemplated. Many experimental animal tumours induced by chemical carcinogens or oncogenic viruses are immunogenic but most spontaneously arising animal tumours are not. Thus there is a heterogeneity among tumours with variability in their immunogenicity.

There is some evidence for the presence of tumour antigens in humans. In the 1960's it was found that lymphocytes from cancer patients could inhibit the *in vitro* growth of cultured tumour cells and that sera of some patients contained antibodies that bound to tumour cell-surface antigens [54, 55]. The explosion in hybridoma technology following the work of Kohler and Milstein [56] has led to the production of monoclonal antibodies which react specifically with cell surface antigens [57].

One of the best characterised tumour associated

antigens is p97 which is found in melanoma tissues and in comparatively very much smaller quantities in virtually all adult tissues [57]. Monoclonal antibodies raised against a variety of other tumours including breast, lung, ovary, sarcoma, neuroblastoma, lymphoma and leukaemia all show some degree of cross reactivity, although antigenic expression is quantitatively greater on tumour cells. Antigens with the greatest specificity are expressed only on tumour cells and the clone of normal cells from which they arise. This means that the antibody, which may be considered 'tumour specific', will only react with the single clone of cells expressing the antigen.

Not all antibodies must be tumour specific in order to be useful. The density of antigen expression, and the affinity of the antibody for the antigen need to be considered. The antibody may be used as a targeting agent, linked to immunomodulators, drugs or toxins. A mouse monoclonal antibody reactive with several human osteogenic sarcoma cell lines was linked by Baldwin and colleagues to purified human lymphoblastoid interferon [58]. The conjugate was shown to localise in osteogenic sarcoma xenografts: it also augmented the activity of natural killer cells in an *in vitro* model.

There are many complex interactions between antigen and antibody which may act to enhance or subvert tumour rejection. The precise pathways of tumour antigen recognition are unclear. It is known that suppressor T lymphocytes inhibit the generation of effective tumour immunity and that the mode of antigen presentation is crucial, soluble antigen favouring the generation of suppressor cells [59]. It may be necessary to eliminate the suppressor cells, or alternatively block the shedding of antigen from the cells themselves as they grow to generate tumour immunity. Cyclophosphamide is known to eliminate suppressor T cell precursors and so could be usefully incorporated into an immunotherapy programme.

Active specific immunotherapy has yet to be shown to be effective in man. However, the therapeutic usefulness of tumour specific antibody was demonstrated by Miller and colleagues [60]. The idiotypic determinants of a B cell lymphoma were used as an antigen. Monoclonal antibody

against this antigen was used on its own to produce complete clinical remission of disease in a patient with non-Hodgkin's lymphoma.

2. Activation of macrophages

Another way of altering cell mediated tumour immunity apart from eliminating suppressor T cells and augmenting NK cell activity is to activate macrophages. Activated macrophages are tumoricidal and will recognise and selectively destroy neoplastic cells whilst leaving non-neoplastic cells unharmed. The exact mechanism by which macrophages recognise and kill tumour cells is not known, but seems to be related to an inherent property of the cell in the neoplastic state and is independent of antigenicity, invasiveness, metastatic potential and drug sensitivity of the tumour cell. The method of distinction between neoplastic and non-neoplastic is independent of transplantation antigens, species specific antigens, phenotypic expression or cell cycle [Review 61, 62].

There are two main ways by which macrophages can be activated *in vivo*. Substances in the cell walls of bacteria, e.g. Corynebacteria and Mycobacteria, are able to interact directly with macrophages and render them tumoricidal. T cells stimulated by antigen will release a lymphokine, the 'macrophage activating factor'. Lymphokines only have a short half-life *in vivo* because they are rapidly bound to serum proteins [62]. *In vivo* experiments have shown that injections of activated macrophages will reduce the metastatic tumour burden in syngeneic mice. Conversely agents which are toxic for macrophages eg silica and carageen will cause an increase in the incidence of both spontaneous and experimental metastases in animal tumour models. The degree of tumoricidal activity, and not the absolute number of the macrophages determines whether the tumour will progress or regress [63]. Progressive growth of metastases results from an excess tumour burden; tumour cell variants resistant to the killing activity of macrophages have not been found.

Immunological adjuvants, e.g. *Corynebacterium parvum* and BCG are known to stimulate NK cell activity and to activate macrophages. For

these cells to exert their effect they must travel to and localise within the tumour. Non-immune factors governing localisation within the tumour tissue include the presence of inflammation or necrosis within the tumour, the tumour blood flow and the vascular permeability. Numbers of NK cells may be low in the tumour [64] and their cytotoxic ability may be abnormal [65].

Regional immunotherapy may modify the host response to tumour by increasing the host cell infiltration into the tumour, or by targeting biological response modifiers to the tumour sited macrophages. An experiment by Niitsuma *et al.* neatly demonstrates this approach. Human lung tumours *in situ* were percutaneously injected with BCG 14 days before surgery; lymphocytes derived from these showed NK activity whereas no NK activity was discernible in cells from non-BCG injected lesions in the same patient [66]. These results suggest that NK cells infiltrate the tumour in response to BCG-mediated effects.

Macrophages are only able to respond to activation during a 3–4 day period after they emerge into the blood stream, and only remain tumoricidal for another 3–4 days [67]. Therefore only a small fraction of the total macrophage population can respond to injected lymphokines at any one time. Animal studies have shown that lymphocytes in large progressive tumours are deficient in their ability to release those lymphokines which recruit and activate macrophages. Any therapeutic activation of the macrophages *in vivo* will need to be capable of activating the refractory macrophages.

In a series of elegant studies Fidler *et al.* have used liposome encapsulated activating agents to passively target to macrophages *in vivo* [61, 62]. Liposomes are cleared from the circulation after injection by the reticulo endothelial system and by monocytes. Activation of tumoricidal macrophages *in vivo*, thought to be due to an interaction between the activator and an intracellular target, was demonstrated by liposomes containing either natural (lymphokines) or synthetic (N-acetyl L alanyl D isoglutamine – MDP) agents. Encapsulating of the agents has the advantages that refractory macrophages can be activated, the risk of undesirable immune sensitisation is reduced, and tumour

cell resistance to killing is not induced as it is with cytotoxic agents. The tumour bulk should be low if such therapy is to have optimum effect, so prior debulking of the tumour may be necessary.

Conclusion

Clearly, new approaches to the treatment of cancer are required. Patients usually die of either uncontrolled disease or infection, both of which are associated with immune system failure. A tumour-specific treatment which is able to eradicate all malignant cells while causing minimal damage to the host defence mechanisms remains the ideal. It is possible that current research along the lines outlined in the last sections of this chapter will lead to the development of such therapy.

References

1. Spector BD, Perry GS III, Kersey JH: Genetically determined immunodeficiency diseases and malignancy: Report for the immunodeficiency – Cancer Registry. *Clin Immunol Immunopathol*, 1978; 11: 12–29.
2. Melief CJM, Schwartz RS: Immunocompetence and malignancy. In *Cancer: A comprehensive treatise*. Becker FF (ed). New York, Plenum Press, 1975: 121–60.
3. Kersey JH, Spector BD, Good RA: Immunodeficiency and cancer. *Advances Cancer Res*, 1973; 18: 211–30.
4. Congress of the United States: Review of the public health service's response to AIDS. A technical memorandum. Office of Technology Assessment, Washington D.C., 1985.
5. Reed DM: On the pathological changes in Hodgkin's disease with especial reference to its relation to tuberculosis. *Johns Hopkins Hosp Rev*, 1902; 10: 133–96.
6. Young RC, Corder MP, Haynes MA, DeVita VT: Delayed hypersensitivity in Hodgkin's disease. *Am J Med*, 1972; 52: 63–72.
7. Eltringham JR, Kaplan HS: Impaired delayed hypersensitivity responses in 154 patients with untreated Hodgkin's disease. *Natl Cancer Inst Monograph*, 1973; 36: 107–15.
8. Brown RS, Haynes HA, Foley HT, Goodwin HA, Berard CW, Carbone PP: Hodgkin's disease. Immunologic clinical and histologic features of 50 untreated patients. *Ann Intern Med*, 1967; 67: 291–302.
9. Kelly WD, Good RA, Varco RL, Levitt M: The altered response to skin monografts and to delayed allergens in Hodgkin's disease. *Surg Forum*, 1958; 9: 785–89.
10. Levy R, Kaplan HS: Impaired lymphocyte function in untreated Hodgkin's disease. *N Engl J Med*, 1974; 290: 181–86.

11. Kaplan HS: *Hodgkin's Disease*. 2nd edn. Cambridge, Mass, Harvard University Press, 1980.
12. Fuks Z, Strober S, Kaplan HS: Interaction between serum factors and T lymphocytes in Hodgkin's disease: use as a diagnostic test. *N Engl J Med*, 1976; **295**: 1273-78.
13. Posner MR, Reinherz EL, Breard J, Nadler LM, Rosenthal DS, Schlossman SF: Lymphoid subpopulations of peripheral blood and spleen in untreated Hodgkin's disease. *Cancer*, 1978; **48**: 1170-76.
14. De Sousa M, Smithyan A, Tan C: Suggested models of ecotaxopathy in lymphoreticular malignancy. A role for non-binding proteins in the control of lymphoid cell migration. *Am J Pathol*, 1978; **90**: 497-520.
15. Jones SE, Griffith K, Dombrowski P, Gaines JA: Immunodeficiency in patients with non-Hodgkin's lymphomas. *Blood*, 1977; **49**: 335-44.
16. Advani SH, Dinshaw KA, Nair CN, Gopal R, Talwalkar GV, Iyler YS, Bhatia HM, Desai PB: Immune dysfunction in non-Hodgkin's lymphoma. *Cancer*, 1980; **45**: 2843-48.
17. Nedelkova M, Bacalova S, Georgiera B: Infectious complications and host immune defence in acute leukaemia. *Eur J Cancer*, 1981; **17**: 617-22.
18. Kay NE, Howe RB, Douglas SD: Effect of therapy on T cell subpopulations in patients with chronic lymphocytic leukaemia. *Leuk Res*, 1982; **6**: 345-48.
19. Eilber FR, Morton DL: Impaired immunological reactivity and recurrence following cancer surgery. *Cancer*, 1970; **25**: 362-67.
20. Oldham RK, Weese JL, Herberman RB, Perlin E, Mills M, Heims W, Blom J, Green D, Reid J, Bellinger S, Law I, McCoy JK, Dean JH, Cannon GB, Dieu I: Immunological monitoring and immunotherapy of carcinoma of the lung. *Int J Cancer*, 1976; **18**: 739-49.
21. Adler A, Stein JA, Ben-Efraim S: Immunocompetence, immunosuppression and human breast cancer. *Cancer*, 1980; **45**: 2061-73.
22. Cobleigh MA, Braun DP, Harris JE: Quantitation of lymphocytes and T cell subsets in patients with disseminated cancer. *J Natl Cancer Inst*, 1980; **64**: 1041-45.
23. Mandell GL, Fisher RI, Bostick F, Young RC: Ovarian cancer: a solid tumour with evidence of normal cellular immune function but abnormal B cell function. *Am J Med*, 1979; **66**: 621-24.
24. Krishnan EC, Menon CD, Krishnan L, Jewell WR: Deficiency in maturation process of macrophages in human cancer. *J Natl Cancer Inst*, 1980; **65**: 273-76.
25. Snyderman R, Meadows L, Holder W, Wells S Jr: Abnormal monocyte chemotaxis in patients with breast cancer: Evidence for a tumour-mediated effect. *J Natl Cancer Inst*, 1978; **60**: 737-40.
26. Blomgren H, Berg R, Wasserman J, Glas U: Effect of radiotherapy on blood lymphocyte population in mammary carcinoma. *Int J Oncol Biol Phys*, 1976; **1**: 177-88.
27. Stjernsward J, Jondal M, Vanký F, Wigzell H, Sealy R: Lymphopenia and change in distribution of human B - T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma. *Lancet*, 1972; **1**: 1152-56.
28. Slade MS, Simmons RL, Yunis E, Greenberg LJ: Immuno-depression after major surgery in normal patients. *Surgery*, 1975; **78**: 363-72.
29. Yew FH, Johnson RT: Human B and T lymphocytes differ in UV induced repair capacity. *Exp Cell Res*, 1978; **113**: 227-31.
30. Fauci AS: Glucocorticoid effects on circulating human mononuclear cells. *J Reticuloendothelia Soc*, 1979; **26**: 727-38.
31. Baum M: Effect of administered hormones on the immune reaction. In: *Host Defence in Breast Cancer*, Stoll BA (ed). London, Heineman, 1975: 130-46.
32. Heppner GH, Calabresi P: Effect of sequence of administration of methotrexate, leucovorin and 5 fluorouracil on mammary tumour growth and survival in syngeneic C3H mice. *Cancer Res*, 1977; **37**: 4580-83.
33. Braun DP, Cobleigh MA, Harris JE: The 'rebound overshoot' phenomenon. A consequence of selective cytotoxic drug effect on immunoregulatory suppression mechanisms. *Proc Am Soc Clin Oncol*, 1980; **21**: 373.
34. Garber M, Andresi D, Pioch Y, Radal M, Serou B: Effect of cyclophosphamide and methylprednisolone on in vitro cellular immune response to allogeneic tumour cells. Correlation with in vivo rejection. *Transplantation*, 1978; **26**: 142-49.
35. Hosea SW, Burch GG, Broun EJ, Berg RA, Frank MM: Impaired immune response of splenectomised patients in polyvalent pneumococcal vaccine. *Lancet*, 1981; **1**: 804-7.
36. Baumgartner JD, Glauser MP, Burgo-Black AL, Black RD, Pyndiah N, Chiolero R: Severe cytomegalovirus infection in multiply transfused splenectomised trauma patients. *Lancet*, 1982; **ii**: 63-65.
37. Stone HH, Stanley DG, DeJarness HH: Post-splenectomy viral hepatitis. *JAMA*, 1967; **199**: 851-53.
38. Goffinet DR, Glazstein EJ, Merigan TC: Herpes zoster-varicella infections and lymphoma. *Ann Intern Med*, 1972; **76**: 235-40.
39. Monfardini S, Bajetta E, Arnold CA, Kenda R, Bonadonna G: Herpes zoster-varicella infection in malignant lymphomas. Influence of splenectomy and intensive treatment. *Eur J Cancer*, 1975; **11**: 51-57.
40. Rebout F, Donaldson SS, Kaplan HS: Herpes zoster and varicella infection in children with Hodgkin's disease. *Cancer*, 1978; **41**: 95-99.
41. Donaldson SS, Moore MR, Rosenberg SA, Voski KL: Characterization of post splenectomy bacteraemia among patients with and without lymphoma. *N Engl J Med*, 1972; **287**: 69-71.
42. Schimpff SC, O'Connell MJ, Greene WH, Wiernik PH: Infections in 92 splenectomised patients with Hodgkin's disease. *Am J Med*, 1975; **59**: 695-701.
43. Weitzman SA, Aisenberg AC, Siber GR, Smith DH: Impaired humoral immunity in treated Hodgkin's disease. *N Engl J Med*, 1977; **297**: 245-48.
44. Donaldson SS, Kaplan HS: Complications of treatment of Hodgkin's disease in children. *Cancer Treat Rep*, 1982; **66**: 977-89.

45. Fuks Z, Stroker S, Bobrove AM, Sasazuki T, McMichael A, Kaplan HS: Long term effects of radiation on T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. *J Clin Invest*, 1976; 58: 803-14.
46. Fisher RI, DeVita VT, Bostick F, Vanhaelen C, Houser DM, Hubbard SM, Young RC: Persistent immunologic abnormalities in long term survivors of advanced Hodgkin's disease. *Ann Intern Med*, 1980; 92: 595-99.
47. King GW, Grozea PC, Eyre HJ, LoBuglio AF: Neoantigen response in patients successfully treated for lymphoma: A Southwest Oncology Group Study. *Ann Intern Med*, 1979; 90: 892-95.
48. Hancock BW, Bruce L, Whitham MD, Dunsmore IR, Ward AM, Richmond J: Immunity in Hodgkin's disease: status after 5 years remission. *Br J Cancer*, 1982; 46: 593-600.
49. Colby TV, Hoppe RT, Warnke RA: Hodgkin's disease at autopsy: 1972-1977. *Cancer*, 1981; 47: 1852-62.
50. Coltman CA Jr, Dixon DO: Malignancies complicating Hodgkin's disease: A Southwest Oncology Group 10-year follow-up. *Cancer Treat Rep*, 1982; 66: 1023-33.
51. Unger PF, Auderc G, Weil M, Jacquillat C: Tumeurs solides après traitement pour maladie de Hodgkin. *Nouv Presse med*, 1981; 10: 1463-67.
52. Bodey GP, Bolivar R, Feinstein V: Infectious complications in leukaemic patients. *Semin Hematol*, 1982; 19: 193-226.
53. Bodey GP, Buckley M, Sathe YS, Freireich EF: Quantitative relationships between circulating leukocytes and infection in patients with acute leukaemia. *Ann Intern Med*, 1966; 64: 328-40.
54. Baldwin RW, Embleton MJ: Assessment of cell-mediated immunity to human tumour-associated antigens. *Int Rev Exp Pathol*, 1977; 17: 49-95.
55. Hellström KE, Hellström I: Lymphocyte mediated cytotoxicity and blocking serum activity to tumour antigens. *Adv Immunol*, 1974; 18: 209-77.
56. Kohler G, Milstein C: Continuous cultures of fused cells secreting antibody of pre-defined specificity. *Nature*, 1975; 256: 495-97.
57. Hellström KE, Hellström I, Braun JP: Human tumour-associated antigens identified by monoclonal antibodies. *Springer Semin Immunopathol*, 1982; 5: 127-46.
58. Baldwin RW, Flannery GR, Pelham JM, Dixon Gray J: Immunomodulation by IFN-conjugated monoclonal antibody to human osteogenic sarcoma. *Proc Am Soc Clin Oncol*, 1982; 23: 1003.
59. Kahan BD, Pellis NR, LeGrue SJ, Tanaka T: Immunotherapeutic effects of tumour specific transplantation antigens released by 1-butanol. *Cancer*, 1982; 49: 1163-73.
60. Miller RA, Maloney DG, Warnke R, Levy R: Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med*, 1981; 306: 517-22.
61. Fidler IJ, Poste G: Macrophage mediated destruction of malignant tumour cells and new strategies for the therapy of metastatic disease. *Springer Semin Immunopathol*, 1982; 5: 161-74.
62. Fidler IJ, Raz A: The induction of tumoricidal capacities in mouse and rat macrophages by lymphokines. In: *Lymphokines*, Pick E (ed). New York, Academic Press, 1981: 345.
63. Russell SW, Gillespie GY, Pace JL: Evidence for mono-nuclear phagocytes in solid neoplasms and appraisal of their non-specific cytotoxic capacities. *Contemp Top Immunobiol*, 1980; 10: 143-66.
64. Eremin O, Coombs RR, Ashby J: Lymphocytes infiltrating human breast cancers lack NK cell activity and show low levels of NK cell activity. *Br J Cancer*, 1981; 44: 166-76.
65. Moore M, Vose BM: Extravascular natural cytotoxicity in man: anti-K562 activity of lymph node and tumour infiltrating lymphocytes. *Int J Cancer*, 1981; 27: 265-72.
66. Niitsuma M, Golub SH, Edelstein R, Carmack Holmes E: Lymphoid cells infiltrating human pulmonary tumours: effect of intralesional BCG injection. *J Natl Cancer Inst*, 1981; 67: 997-1004.
67. Poste , Kirsh R: Rapid decay of tumoricidal activity and loss of responsiveness to lymphokines in inflammatory macrophages. *Cancer Res*, 1979; 39: 2582-89.

10. Use of immunoglobulins

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1. Introduction

In general, passive immunization with immunoglobulins (Ig) of the IgG class is appropriate in situations where an individual at risk does not have sufficient antibodies to prevent or to overcome a serious infectious disease. For this purpose, two different types of antibody concentrates are available: (i) Standard human immune serum globulin, which is extracted from large pools of human plasma including several thousand healthy donors, contains all relevant antibodies normally occurring in the donor population. Such preparations are also termed polyvalent Ig. (ii) Special human immune serum globulins or specific Ig preparations which are derived from plasma pools of a limited number of convalescent or actively immunized subjects contain high concentrations of a specified antibody. Since the 1950s, both types of Ig preparations have been widely used, mainly for the prophylaxis of viral diseases and of tetanus. In addition, polyvalent Ig as a continuous replacement therapy was soon found to be effective in agamma- or hypogammaglobulinemic patients. Until recently, administration was strictly limited to the intramuscular (i.m.) route, since severe anaphylactoid reactions were noticed when Ig was infused intravenously (i.v.), particularly in immunodeficient patients. Considerations on pharmacokinetics and on dose-effectiveness relationships of IgG antibodies, however, have spurred the development of preparations which are suitable for i.v. application. Today, the use of these Ig preparations is rapidly spreading. It is the purpose of this chapter to review the

indications for passive prophylaxis and therapy with Ig including some new developments in this field.

2. Indications for intramuscular Ig preparations

The prophylactic value of i.m. injections of human Ig preparations is well established in a number of infectious diseases and in Rhesus sensitization [1]. Evidently, the relatively small amounts of antibodies which can be given by this route are sufficient for neutralization and immune elimination of viruses and bacterial exotoxins. As a result, infection is either prevented or clinical manifestations of the disease are suppressed or mitigated. In the prophylaxis of Rhesus (Rh) hemolytic disease of the newborn, it is assumed that the injected anti-D antibodies effectively opsonize any Rh-positive fetal red cells in the maternal circulation thereby inducing their elimination before the maternal immune system is stimulated [2].

The most important indications for polyvalent and for specific Ig preparations are compiled in Table 1.

2.1. Hepatitis A

The effectiveness of passive protection with polyvalent Ig in reducing the attack rate of icteric disease is high, i.e. between 80 and 90%, particularly when Ig is given before or early after exposure [3]. Ig administration up to several weeks after exposure, but not after the onset of symptoms, may

also provide some protection. There is evidence that the rate of infection is not affected by Ig prophylaxis. Thus mild anicteric hepatitis occurs which in turn confers passive-active immunity and long lasting protection. Pre-exposure prophylaxis is most often applied in travellers to some highly endemic Third World countries. Indications for postexposure prophylaxis are household contacts of hepatitis A patients including newborns of mothers with hepatitis A. The recommended dose is

0.2 ml per kg body weight of a polyvalent Ig preparation. If exposure continues, passive immunization should be repeated every 2 months.

2.2. *Hepatitis B*

Passive prophylaxis to viral hepatitis B (HB) depends on the presence of sufficient antibodies to HB surface (HBs) antigen in the Ig preparation [4]. High-titered specific anti-HBs is still in short sup-

Table 1. Indications and dosage of Ig preparations for intramuscular use.

Illness	Type and dose of Ig preparations ¹	Comments
Hepatitis A	Polyvalent Ig	0.2 ml per kg body weight.
Hepatitis B	Specific anti-hepatitis B Ig	0.06 ml per kg body weight or a total dose of 2 to 4 ml, as specified by producer. Repeat after 4 weeks.
Varicella	Specific anti-varicella, anti-zoster Ig	0.2 ml per kg body weight or a total dose of 5 to 10 ml, as specified by producer.
Rabies	Specific anti-rabies Ig ³	20 IU per kg body weight.
Measles	Polyvalent Ig	0.25 ml per kg body weight. 0.5 ml per kg body weight.
Tetanus	Specific anti-tetanus Ig (TIG) ³	250–500 IU total dose. 5000–10,000 IU initial dose, 3000 IU on following days.
Rh hemolytic disease of newborns	Specific anti-D Ig	2 ml containing 200 µg anti-D or as specified by producer.

¹ Polyvalent Ig and most specific Ig preparations contain approximately 160 mg IgG per ml (16% solution).

² High risk persons: Immunocompromised subjects, patients who are on cytostatic and immunosuppressive therapy because of malignant disease or transplantation.

³ Preparations of human origin. Details on equine specific Ig preparations are mentioned in the text.

* Combined vaccination and HB immunoglobulin is recommended.

ply and expensive. It is hoped, however, that this situation will change in the near future. The recent introduction of potent HB vaccines has made possible active immunization of persons at high risk for infection [5]. Thus, the use of anti-HBs will decrease and at the same time plasma from hyperimmunized donors will become more readily available. Passive prophylaxis is indicated in non-immunized hospital and laboratory personnel with needle stick or mucous membrane exposure to infective blood. This passive protection has to be combined with active immunization. Since anti-HBs serum titers are known to rise rather slowly after vaccination, i.m. injection of anti-HBs be repeated after four weeks. Prophylaxis with anti-HBs Ig is furthermore indicated in spouses or other intimate contacts of patients with acute or persistent HB infection, in newborns of HBs antigen positive symptomless mothers and in newborns of mothers who develop acute clinical HB between the second trimester of pregnancy and the third month of puerperium. Although passive immunization with immunoglobulin provides protection, infants born to hepatitis B antigen carrier mothers (especially HBe positive) should immediately be given hepatitis B vaccine together with HB-immunoglobulin [38].

Finally, passive-active immunization against HB is indicated in other high risk persons such as hemodialysis patients, hemophiliacs, promiscuous homosexuals, drug addicts and patients undergoing cytostatic and immunosuppressive therapy. The usual i.m. dose is 4 ml of specific anti-HBs Ig for adults and 2 ml for children. Optimal suppression of clinical symptoms is achieved when the anti-HBs Ig is given within 24 hours after exposure to HB virus. Some protection or at least prolongation of the incubation period can be expected when the Ig is applied up to 6 days after exposure. Later, and particularly when clinical disease is manifest, anti-HBs Ig is useless.

2.3. *Varicella-zoster*

Varicella is a relatively mild disease and in most persons there is no need for prophylaxis after exposure to a patient with chickenpox. In immu-

nocompromised individuals, however, the clinical course of varicella and herpes zoster often poses severe problems. Accordingly, the most important indications for passive protection with Ig are primary or secondary immunodeficiency states, including patients who are on cytostatic and immunosuppressive therapy for malignancies and for bone marrow or kidney transplantation [6]. Other indications are newborns of mothers with varicella and exposed pregnant women near term. Usually, specific anti-zoster Ig (ZIG) is given i.m. in a dosage of 0.2 ml per kg body weight or up to a total volume of 5 to 10 ml [1]. Immunoprophylaxis is most effective when ZIG is given within 72 h after exposure. But even under optimal conditions, protection is not absolute and failure rates of up to 30% have been reported [7]. However, by prophylaxis with ZIG in high doses the course of the disease is favourably modified in most of these patients [8,9]. Since polyvalent Ig preparations also contain relatively high concentrations of antibodies to varicella-zoster virus, they also appear to be appropriate for prevention and modification of the disease. Encouraging results have been obtained with polyvalent Ig preparations for i.v. use (see section 3).

2.4. *Rabies*

Post-exposure prophylaxis of rabies, including local wound care, vaccination and passive immunization, is indicated for all bites by animals in which rabies cannot be excluded. Passive and active immunization is furthermore recommended for nonbite direct contact to animals proved or suspected to be rabid. Regardless of the interval but as early as possible, human specific anti-rabies Ig should be used in a dosage of 20 international units (IU) per kg body weight. Generally, half of this dose is infiltrated locally at the wound site after extensive débridement has been performed and the rest is given i.m. If no human Ig is available, equine anti-rabies Ig should be administered in a dosage of 40 IU per kg body weight. One has to consider the possibility that the injected antibodies may suppress or delay the response to the vaccine. Thus if the animal proved to be rabid, antibody levels in

the serum of the patient should be monitored and booster doses should be given until sufficient active antibody synthesis occurs and serum neutralization titers of greater than 1:20 are reached [10].

2.5. Measles

In most individuals effective prevention of measles today is accomplished in the second year of life by administration of vaccine. This active immunization with live measles vaccine, however, is contraindicated in a group of persons who are known to be particularly of risk to developing severe or even fatal measles, i.e. children with cellular or severe combined immunodeficiencies and patients with malignant disease or transplants who are on cytostatic and immunosuppressive therapy. In this group of immunocompromised subjects, passive post-exposure prophylaxis with polyvalent Ig is indicated [11]. In addition, this preventive measure is recommended in normal infants who are also at considerable risk after exposure to measles and in non-immune pregnant women. In these healthy individuals, an i.m. dose of 0.25 ml of polyvalent Ig per kg body weight is sufficient. For immunocompromised patients, a larger i.m. dose of 0.5 ml per kg body weight is recommended. Passive prophylaxis is effective when given within 72 hours after exposure. Later, the illness is not prevented but its clinical course may be attenuated.

2.6. Tetanus

The prophylaxis of tetanus includes both active and passive immunization. Active immunization by a booster injection of tetanus toxoid or by the full vaccination schedule according to the history of immunization is sufficient in persons with fresh clean wounds where tissue damage is negligible. However, in patients with serious wounds or with particularly 'tetanus-prone' injuries yielding anaerobic conditions, as well as in patients with wounds neglected for more than 24 hours, additional passive prophylaxis is required [12]. This is accomplished by i.m. administration of human specific anti-tetanus Ig (TIG) in a dosage of 250 IU. In cases with severe tissue injuries 500 IU should be

given as soon as proper wound toilet has been performed. In countries where TIG is not available equine specific tetanus antitoxin (TAT) has to be given i.m. in doses of 3000 to 5000 IU [1]. Specific anti-tetanus Ig is also used for the treatment of tetanus. In such situations, initial TIG doses between 5000 and 10,000 IU are recommended, followed by 3000 IU on consecutive days. Equine TAT has to be used in high doses of 50,000 to 100,000 IU. Part of these human or heterologous Ig preparations is infiltrated locally at the wound site, the rest should be given i.m. Recently, the intrathecal administration of human anti-tetanus Ig has yielded encouraging results [13].

2.7. Rhesus hemolytic disease of the newborn

Rh hemolytic anemia in newborns is due to an accelerated destruction of red cells mediated by maternal IgG antibodies to Rh antigens. Such antibodies, directed predominantly against D, the most potent Rh antigen, are formed in Rh-negative women after exposure to Rh-positive fetal erythrocytes during pregnancy, at birth or abortion, and at the occasion of obstetric interventions including Cesarean section [14]. This maternal immune response does not take place when IgG antibodies to D are passively administered at the time of exposure or shortly, i.e. within 48 h, thereafter [15]. The introduction of this treatment with anti-D antibodies obtained from hyperimmunized volunteers has dramatically decreased the infant death rate from Rh hemolytic disease [2]. Today, the recommended doses vary from 100 to 300 µg of specific anti-D given i.m. [16, 17]. Evidently, to avoid sensitization and immune response, the contact of the maternal immune system with 'naked' fetal Rh-positive erythrocytes should be kept as short as possible. Since resorption of the injected Ig from muscular deposits is rather slow, anti-D Ig preparations for i.v. use have recently been manufactured and are administered with increasing frequency [18].

2.8. Prevention of other diseases

Both polyvalent and specific Ig preparations may

be used for passive prophylaxis of other miscellaneous infections which are not mentioned in Table 1. For diphtheria, poliomyelitis and for the prevention of complications after smallpox vaccinations, the efficacy of i.m. Ig application is well established [1]. But since world-wide vaccination programs have largely reduced the incidence of these diseases, passive prophylaxis appears to be of minor importance today. In some other infectious diseases, the value of Ig administration is questionable. One of these situations concerns Ig prophylaxis of congenital rubella in pregnant women with no or uncertain active immunity: there is evidence that viremia and hence fetal damage cannot reliably be prevented by Ig treatment [19, 20]. Nevertheless, in women who refuse to consider therapeutic abortion injections of 40 ml of specific anti-rubella Ig corresponding to about 200,000 IU distributed over several i.m. sites may be given as soon as possible after exposure. Since time is critical, even an i.v. infusion of a 1% solution of specific anti-rubella Ig, diluted in saline, may be considered. Prior to Ig injections and one month afterwards, blood samples have to be drawn in order to assess the actual immune situation by antibody determinations. Another controversial indication is prevention of mumps orchitis: specific anti-mumps Ig preparations do not reliably reduce the incidence or the severity of mumps orchitis [1]. However, passive prophylaxis with i.m. doses of 0.5 to 1 ml per kg body weight may be tried in unvaccinated exposed male adolescents. Finally, passive immunization against pertussis using specific anti-pertussis Ig may still be appropriate today in exposed young infants. For prevention, a single i.m. dose of 2.5 ml is recommended. For modification of severe pertussis, this dose may be given daily for three days, together with antibiotic treatment.

3. Indications of intravenous Ig preparations

Most of the Ig preparations for i.v. use which are currently licensed and marketed in various countries are polyvalent and have a similar range of antibody activities. They differ widely in their

chemical and biological properties, according to the procedures utilized for their production. In some preparations the source material is subjected to enzymatic digestion by pepsin or plasmin. They consist entirely or to a great part of antibody fragments and thus lack certain biological properties which are related to the Fc portion of IgG. In other products, the antibody molecules are chemically modified by reducing and alkylating agents or otherwise, or are stabilized after separation from the plasma by the addition of albumin, sugars or other compounds. Today, the trend is in favour of unfragmented Ig preparations with little or no modification of the molecules, which in addition to appropriate antigen-binding and neutralizing activity have intact Fc portion-mediated functions such as complement activation after antigen contact, interaction with phagocytes and a biological half-life of at least 14 days. These requirements have recently been formulated in a WHO meeting on i.v. Ig [21].

3.1. Adverse reactions to i.v. Ig preparations

With Ig preparations developed for i.v. use, untoward reactions are occasionally seen, particularly in patients with humoral immunodeficiencies at the beginning of continuous replacement therapy [22]. Two different types of adverse reactions to i.v. infusions are generally recognized. (i) The dangerous *anaphylactoid reactions* start immediately after the beginning of the infusion with flushing of the face, chest oppression and dyspnoea, followed by chills, lumbar pain, fever, nausea and vomiting. In severe cases there may be shock and loss of consciousness. Today, these side effects are rare. They could be observed with i.m. Ig preparations and with some earlier i.v. Ig products which contained aggregates of IgG molecules that spontaneously activated the complement cascade. Occasionally, similar reactions are seen in patients with selective IgA deficiency or common variable immunodeficiency who have circulating antibodies to IgA. They are assumed to be due to complement activation by immune complexes formed between these antibodies and IgA since most of the currently available Ig preparations are contaminated

with some IgA. (ii) The majority of adverse reactions are of the *inflammatory type* ('phlogistic reactions') and have a delayed onset. They start one or several hours after the beginning of the i.v. administration of Ig with headache and malaise, followed by shivering, occasionally by chills and by fever. Concomitant increases of blood leucocyte counts and acute-phase reactants are often seen. Symptoms regularly disappear within one hour after the infusion is stopped. These reactions are probably caused by the rapid supply of antibodies to immunodeficient patients who in the course of their disease have accumulated an 'antigen overload': after immune complex formation between infused antibodies and bacterial antigens in the tissues, the complement system is activated and inflammatory processes are triggered or intensified.

3.2. Polyvalent i.v. Ig in patients with primary humoral immunodeficiency

The classical indications for i.v. Ig are primary immunodeficiencies, i.e. patients with primary a-gamma- or hypogammaglobulinemia. Comparative studies have shown that high dose i.v. is clearly superior to i.m. replacement therapy in reducing the frequency of acute bacterial infections and improving the general status of these patients [22, 23]. The effect on already firmly established chronic upper respiratory tract infections is less pronounced. It is hoped, however, that by early and consequent i.v. treatment, development of chronic infections and concomitant fibrotic organ lesions

can be prevented. The doses required to keep the patients free of acute infections may vary individually. In an initial phase of accumulation, the serum IgG level should be raised to at least 300 to 400 mg/100 ml. This can be accomplished by approximately four infusions each of 0.2 g Ig per kg body weight in adults or 0.3 g per kg in children, given at intervals of 4 weeks as indicated in Table 2. In newly detected agammaglobulinemic patients, it is advisable to administer the first infusion at a slow rate of approximately 0.5 g per hour. By this precaution, inflammatory reactions may be avoided. This build-up of a serum IgG level has to be followed by a continuous life-long maintenance phase. Doses of about 3 to 6 g in children and 9 to 12 g in adults at intervals of four weeks are usually sufficient to keep the serum IgG above a critical level of 300 mg/100 ml. Monitoring of serum IgG concentrations has to be performed in samples taken immediately before infusions are given. If the serum Ig decreases to values below 300 mg/100 ml, intervals may be shortened to two weeks or doses have to be increased [24].

Passive immunization with polyvalent i.v. Ig has been reported to be of clinical benefit also in other primary immunodeficiency disorders, such as transient hypogammaglobulinemia of infancy, hypogammaglobulinemia in premature infants, cell-mediated immunodeficiency with associated humoral defects and others [21]. Doses and intervals depend on the clinical situation and require individual adaptation. Recently, it was shown that patients with IgA deficiency and severe recurrent viral or bacte-

Table 2. Polyvalent intravenous Ig preparations in patients with primary humoral immunodeficiency.

Mode of treatment	Single dose (g per kg body weight)	Intervals between infusions	Number of infusions
Slow accumulation			
Children	0.3 g	4 weeks	4
Adults	0.2 g	4 weeks	4
Maintenance			
Children	0.3 g	4 weeks ¹	life-long Ig
Adults	0.2 g	4 weeks ¹	replacement

¹ In some patients, maintenance of a minimal serum IgG level of 300 mg per 100 ml requires shortening of intervals or increase of doses.

rial respiratory infections could be improved by regular Ig infusions. Since IgA deficiency may be associated with IgG subclass defects, Ig replacement appears appropriate. However, in some of these patients, preexisting or induced antibodies against IgA represent a danger, as stated previously. Thus, before i.v. Ig replacement treatment is started in patients with known IgA defect, the presence of anti-IgA antibodies in the serum should be excluded in passive hemagglutination tests.

3.3. Polyvalent i.v. Ig preparations in patients without humoral immunodeficiency

The application of high dose Ig in patients with severe generalized infections but without demonstrable hypogammaglobulinemia is indicated under the assumption that in such patients a selective transient antibody deficiency develops, due to consumption of circulating antibodies in the course of antigenemia. Both, prophylactic and therapeutic

applications are known: some indications are relatively well established by controlled clinical trials whereas others are based on case reports and are still preliminary.

3.3.1. Prophylaxis of infections with polyvalent i.v. Ig in polytraumatized and in surgical patients

Recently, a prospective randomized and placebo-controlled trial was performed in polytraumatized adult traffic victims who needed intensive medical care including intubation. Patients of the study group received three infusions of 12 g Ig each at days 1, 5 and 7 after admission. They developed significantly less bacterial infections than the patients of the control group who received infusions of a placebo [25]. In another controlled study on patients undergoing thoracal or abdominal surgery, two i.v. applications of 10 g Ig at the first and at the second postoperative day could significantly reduce the frequency of local infections and of septic complications [26]. Dose recommendations for these and similar indications are given in Table 3.

Table 3. Prophylaxis and therapy of infections with polyvalent intravenous Ig preparations.

Mode of treatment	Single dose	Timing and intervals	Duration
Prophylaxis in polytraumatized and surgical patients	0.2 g per kg body wt	on day of admission or surgery followed by intervals of 1–3 days	3 infusions
Prophylaxis in patients with lymphoproliferative or related disorders and secondary hypogammaglobulinemia	0.1–0.2 g per kg body wt	on intervals of 4 weeks	long-term replacement
Prophylaxis in patients under cytostatic and immunosuppressive therapy (neoplasms, transplantations)	0.2–0.3 g per kg body wt	during induction and cycles of cytostatic therapy at intervals of 1–3 days, later of 4 weeks	undetermined
Supportive therapy of severe infections in children and adults	0.4 g per kg body wt	on consecutive days	5 infusions
Supportive therapy of neonatal sepsis in mature neonates	1 g total single dose	on consecutive days	6 infusions
in preterm neonates	0.5 g total single dose	on consecutive days	6 infusions
Therapy of infections in immunocompromised patients (neoplasms, transplantations, protein-loss due to gastro-enteropathy or severe skin injuries)	0.2–0.4 g per kg body wt	on consecutive days	2–6 infusions

3.3.2. Therapy of severe bacterial and viral infections in adults and in newborns with polyvalent i.v. Ig

As mentioned above, in these patients antibodies specific for the invading agent are consumed faster than they can be replenished by the immune system. In a given patient such a consumptive antibody deficiency is laborious to prove. Since protective antibodies which are needed to overcome a generalized infection represent only a small fraction of the polyvalent Ig, very high doses of polyvalent Ig have to be administered. There is anecdotal evidence that polyvalent Ig in fact is effective in such situations. Evaluation, however, is complicated by the simultaneous antibiotic treatment and other therapeutic measures [27]. If supportive Ig treatment is considered in an adult patient, high doses of up to 2 g per kg body weight should be infused within 3 to 6 days. In neonatal sepsis, data obtained in a prospective study provide good evidence for efficacy of Ig therapy [28]: A lower mortality was observed in infants who in addition to antibiotics obtained Ig infusions than in infants who were treated with antibiotics alone. The difference was most pronounced in premature infants. Doses of 1 g i.v. Ig on six consecutive days were given to neonates born at term, and 0.5 g Ig to preterm infants (Table 3).

3.4. Polyvalent i.v. Ig preparations in patients with secondary immunodeficiencies

Several diseases are known to be associated with an impairment of specific and non-specific defence mechanisms against infections. The resulting susceptibility to infections is further increased by cytostatic and immunosuppressive treatment in neoplastic disorders and in some other conditions. Clearly, i.v. supply of polyvalent Ig can only replace one out of many missing factors. Nevertheless, it can be helpful for the prophylaxis and for the therapy of bacterial or viral infections in immunocompromised patients.

3.4.1. Prophylaxis with i.v. Ig in secondary immunodeficiencies

In the majority of the patients with chronic lym-

phocytic leukemia and in some patients with non-Hodgkin lymphoma or with other lymphoproliferative disorders, hypogammaglobulinemia develops during the course of the disease. By regular i.v. Ig infusions of 0.1 to 0.2 g per kg body weight at intervals of 4 weeks, particularly during the cold season, the frequency of acute bacterial and viral infections may be reduced in these patients. Hypogammaglobulinemia develops in about 10% of the patients with thymoma, often years after surgical excision of the tumor. Symptoms of antibody deficiency like relapsing bacterial respiratory tract infections with severe pneumonias may be complicated by neutropenia and aplastic anemia. Adequate management of this disorder includes regular i.v. Ig replacement using doses of 0.2 g per kg body weight every four weeks.

Prophylaxis with Ig also helps patients with secondary antibody deficiency states induced or aggravated by cytostatic and immunosuppressive drugs and radiotherapy. A favourable influence on the frequency or on the severity of infections has been observed in patients with solid tumors who were on aggressive chemotherapy schedules [29]. In addition, Ig prophylaxis has recently been applied in patients undergoing bone marrow transplantation for acute leukemia and aplastic anemia. Clinical trials are still running and a definitive evaluation of this important indication is not yet possible. Of particular importance is the question whether polyvalent Ig in high doses can reduce the incidence or modify the clinical course of cytomegalovirus (CMV) infections in these patients. Preliminary data suggest that in fact the severity of CMV infection is modified and interstitial pneumonia is prevented [30]. Since data on optimal doses are scarce, a guideline is given in Table 3.

3.4.2. Therapy with i.v. Ig in secondary immunodeficiencies

There is evidence that severe varicella-zoster virus infections in immunocompromised patients can be effectively treated with infusions of polyvalent Ig. In adult tumor patients who had developed generalized herpes zoster during cytostatic and radiation therapy, infusions of polyvalent Ig resulted in a rapid relief of neuralgia and in disappearance of

vesicles and inflammatory reactions within a few days. No bacterial superinfections and no encephalitic complications were noticed. This effect was seen after infusions of 10 g each on two consecutive days [31]. An impressive improvement by i.v. Ig could also be observed in immunosuppressed children who suffered from disseminated and hemorrhagic varicella-zoster virus infections [32]. Fever and pain subsided within 24 hours and no new skin lesions appeared after 3 to 5 days of Ig therapy consisting of 0.4 g per kg body weight daily for at least 3 days. Furthermore, in renal transplant patients with life-threatening CMV infections, high doses of polyvalent Ig tended to reduce mortality, if given early in the course of the infection [33]. Finally, polyvalent Ig is assumed to be helpful in the treatment of acute bacterial or viral infections in children with protein-losing gastroenteropathy and infection-related exacerbation of the disease (Table 3).

3.5. Polyvalent i.v. Ig in idiopathic thrombocytopenic purpura

In children and adults with acute or chronic idiopathic thrombocytopenic purpura (ITP), i.v. Ig preparations consisting of intact IgG molecules were found to dramatically increase the levels of blood platelets within a few days [34, 35]. Correction of platelet counts was permanent in most cases with acute ITP. In chronic ITP, the duration of remission was variable. This new mode of ITP treatment is still under investigation. It may be indicated particularly in patients who are at special risk for cerebral hemorrhage or in patients who need surgery and have to be covered for a critical period. So far, the highest success rates have been obtained with doses of 0.4 g per kg body weight given on 5 consecutive days, thus with a total dose of 2 g per kg body weight.

3.6. Specific i.v. Ig preparations

In many clinical situations it would be desirable to have i.v. Ig preparations at hand with especially high antibody titers against certain infectious or other noxious agents. As already mentioned, such

a product is available for the prevention of Rhesus hemolytic disease. Other specific i.v. Ig preparations with high antibody titers against HBs antigen, varicella-zoster and cytomegalovirus have recently been announced by some manufacturers and appear on the market. It remains to be seen to what extent they are superior to the specific i.m. and to the polyvalent i.v. preparations. New and interesting developments include preparations which are aimed at the prophylaxis and therapy of infections with Gram-negative bacteria. They are produced by immunization of volunteers with vaccines of *Pseudomonas aeruginosa* or of the mutant *Escherichia coli* J5. So far, there is evidence that these antibacterial Ig preparations could be helpful in patients with burns and with gram-negative sepsis [36, 37].

Acknowledgement

This work was supported by grants of the Swiss National Science Foundation.

References

1. Stiehm ER: Standard and special human immune serum globulins as therapeutic agents. *Pediatrics*, 1979; 63: 301-19.
2. Clarke C: Prevention of Rh hemolytic disease by immunoglobulin anti-D. *Vox Sang*, 1983; 44: 396-99.
3. Prince AM: Prevention of viral hepatitis with immunoglobulins. In: *Immunohemotherapy. A guide to immunoglobulin prophylaxis and therapy*, Nydegger UE (ed). London, Academic Press, 1981: 325-35.
4. Prince AM, Szmuness W, Mann MK, Vyas GN, Grady GF, Shapiro FL, Suki WN, Friedman EA, Avram MM, Stenzel KH: Hepatitis B immune globulins. Final report of a controlled, multicenter trial of efficacy in prevention of dialysis-associated hepatitis. *J Infect Dis*, 1978; 137: 131-33.
5. Szmuness W, Olesko WR, Stevens CE, Goodmann A: Passive-active immunization against hepatitis B: immunogenicity studies in adult Americans. *Lancet*, 1981; i: 575-77.
6. Brunell PA, Gershon AA, Hughes WT, Riley H, Smith J: Prevention of varicella in high risk children: a collaborative study. *Pediatrics*, 1972; 50: 718-22.
7. Orenstein WA, Heymann DL, Ellis RJ, Rosenberg RL, Nakano J, Halsey NA, Overturf GD, Hayden GF, Witte JJ: Prophylaxis of varicella in high risk children: Dose-response effect of Zoster immune globulin. *J Pediatr*, 1981; 98: 368-73.

8. Ross AH: Modification of chickenpox in family contacts by administration of gamma globulin. *N Engl J Med*, 1961; 267: 369–76.
9. Brunnell PA, Gershon A: Review. Passive immunization against varicella-zoster infections and other modes of therapy. *J Infect Dis*, 1973; 127: 415–23.
10. Hattwick MAW: Rabies virus. In: *Principles and Practice of Infectious Diseases*, Mandell GL, Douglas RG, Bennett JE (eds). New York, John Wiley & Sons, 1979; 2: 1217–28.
11. Gershon AA: Measles (rubeola) virus. In: *Principles and Practice of Infectious Diseases*, Mandell GL, Douglas RG, Bennett JE (eds). New York, John Wiley & Sons, 1979; 2: 1203–11.
12. McComb JA, Dwyer RC: Passive-active immunization with tetanus immune globulin (human). *N Engl J Med*, 1963; 268: 857–63.
13. Gupta PS, Goyal S, Kapoor P, Batra VK: Intrathecal human tetanus immunoglobulin in early tetanus. *Lancet*, 1980; ii: 439–40.
14. Medical Research Council working party. Controlled trial of various anti-D dosages in suppression of Rh sensitization following pregnancy: Report. *Br Med J*, 1974; 2: 75–83.
15. Mollison PL: Annotation: Suppression of Rh-immunization by passively administered anti-Rh. *Br J Hematol*, 1968; 14: 1–4.
16. Report of a WHO-scientific group: Prevention of RH sensitization. *Wld Hlth Org tech Rep Ser*, No 468, 1971.
17. Freda VJ, Gorman JG, Pollack W, Bowe E: Prevention of Rh hemolytic disease – ten years clinical experience with Rh immune globulin. *N Engl J Med*, 1975; 292: 1014–16.
18. Smith GN, Griffith B, Mollison D, Mollison PL: Uptake of IgG after intramuscular and subcutaneous injection. *Lancet*, 1972; i: 1208–12.
19. Krugman S, Ward R: Demonstration of neutralizing antibody in gamma globulin and re-evaluation of the rubella problem. *N Engl J Med*, 1958; 259: 16–24.
20. Green RH, Balsamo MR, Giles JP: Studies of the natural history and prevention of rubella. *Am J Dis Child*, 1965; 110: 348–56.
21. World Health Organization, Expert Committee on Biological Standardization: Report of an Informal Meeting on Intravenous Immunoglobulins (Human), Geneva, 29 November–1 December, 1982.
22. Barandun S, Morell A, Skvaril F: Clinical experiences with immunoglobulin for intravenous use. In: *Immunoglobulins. Characteristics and use of intravenous preparations*, Alving BM, Finlayson JS (eds). US Department of Health, Human Services (FDA), 1980; 80–9005: 31–35.
23. Ammann AJ, Ashman RF, Buckley RH, Hardie WR, Krantmann HJ, Nelson J, Ochs H, Stiehm ER, Tiller T, Wara DW, Wedgwood R: Use of intravenous gamma globulin in antibody immunodeficiency: Results of a multi-center controlled trial. *Clin Immunol Immunopathol*, 1982; 22: 69–67.
24. Morell A, Schnoz M, Barandun S: Build-up and maintenance of IgG serum concentrations with intravenous immunoglobulin in patients with primary humoral immunodeficiency. *Vox Sang*, 1982; 43: 212–19.
25. Glinz W: personal communication.
26. Duswald KH, Müller K, Seifert J, Ring J: Zur Frage der Wirksamkeit von i.v. Gammaglobulin gegen bakterielle Infektionen chirurgischer Patienten. Ergebnisse einer kontrollierten, randomisierten klinischen Studie. *Muench Med Wochenschr*, 1980; 122: 832–36.
27. Barandun S, Imbach P, Morell A, Wagner HP: Clinical indications for immunoglobulin infusions. In: *Immunotherapy. A guide to immunoglobulin prophylaxis and therapy*, Nydegger UE (ed). London, Academic Press, 1981: 275–82.
28. Sidiropoulos D, Böhme U, von Muralt G, Morell A, Barandun S: Immunglobulinsubstitution bei der Behandlung der neonatalen Sepsis. *Schweiz med Wschr*, 1981; 111: 1649–55.
29. Schmidt RE, Lüttkenhorst M, Hartlapp JH, Illiger HJ, Stroehmann I: Prophylaktische Gabe von Immunglobulinen bei aggressiv chemotherapierten Patienten mit Bronchialkarzinom. In: *Beiträge zu Infusionstherapie und klinische Ernährung*, Bässler KH, Grünert A, Kleinberger G, Reissigl H (eds), Basel, Karger S, 1983; 11: 59–68.
30. Winston DJ, Winston G, Liñ CH, Budinger MD, Champlin RD, Gale RP: Intravenous Immunoglobulin for Modification of Cytomegalovirus Infections associated with Bone Marrow Transplantation. *Am J Med*, 1984; 76: 128–133.
31. Mondorf AW, Duswald KH: Intravenous immunoglobulin in high risk patients: Efficacy and tolerance of long term administration. In: *Immunoglobulins: Characteristics and Uses of Intravenous Preparations*, Alving BM, Finlayson JS (eds), US Department of Health, Human Services (FDS), 1980; 80–9005: 37–40.
32. Sulliger JM, Imbach P, Barandun S, Gugler E, Hirt A, Lüthy A, et al. Varicella and herpes zoster in immunosuppressive children: preliminary results of treatment with intravenous immunoglobulin. *Helv Paediatr Acta*, 1984; 39: 63–70.
33. Condie RM, Hall BL, Howard RJ, Fryd D, Simmons RL, Najarian JS: Treatment of life-threatening infections in renal-transplant recipients with high-dose intravenous human IgG. *Transplant Proc*, 1979; 11: 66–68.
34. Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schöni M, Vest M, Wagner HP: High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet*, 1981; i: 1228–31.
35. Newland AC, Treleavan JG, Minchinton RM, Waters AH: High-dose intravenous IgG in adults with autoimmune thrombocytopenia. *Lancet*, 1983; i: 84–87.
36. Jones RJ, Roe EA, Gupta JL: Controlled trial of pseudomonas immunoglobulin and vaccine in burn patients. *Lancet*, 1980; ii: 1263–65.
37. Ziegler EJ, McCutchan AJ, Fierer J, Glauser MP, Sadoff JC, Douglas H, Braude AI: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med*, 1982; 307: 1225–30.
38. Editorial: Prevention of perinatally transmitted hepatitis B infection. *Lancet*, 1984; i: 939–41.

11. Bone marrow transplantation (BMT)

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The concept of bone marrow transplantation dates back to the 1950's when Lorenz and Jacobson first showed that injections of marrow cells into irradiated mice could protect them against the effects of lethal irradiation [1]. They have subsequently shown that the protective effect was due to viable bone marrow precursor cells engrafting in the irradiated animal and reconstituting new bone marrow.

This led in the 1960's to the clinical application of marrow auto-grafting, whereby patient's marrow, harvested prior to treatment and cryopreserved, was reinfused following high dose of chemotherapy to patients with solid tumours. The limitations to success at this time were largely due to failure to eradicate the underlying malignant disease, and inability to demonstrate the contribution of the grafted marrow to the haemopoietic recovery of the patient.

With the rare exception of successful transplantation of patients in aplastic anaemia from identical twins, allografting – using marrow from normal donors – was not seriously considered until after the recognition and description of the HLA tissue antigens. Early clinical experiments with random bone marrow donors produced the clinical condition described variously as secondary syndrome of graft versus host disease (GVHD) which was predicted previously in animal models.

Allogeneic marrow transplantation progressed rapidly in the 1970's, largely due to pioneering work by Thomas, Mathé, Santos and Storb [2] amongst others. Initial clinical studies were performed on patients who were extremely ill and who

had failed all other treatments, and not surprisingly results were disappointing, particularly in end-stage leukaemia, where of the first 100 patients transplanted by the Seattle group, only 10% survived [2].

However, such studies demonstrated that BMT could be a curative treatment for patients with aplastic anaemia, leukaemia and the rare and otherwise fatal condition of severe combined immune deficiency disease. The last decade has seen tremendous advances in the overall success of BMT due to the development of better techniques of preventing graft rejection and GVHD, and advances in supportive care and clinical expertise. BMT now has an established place in the treatment of a variety of haematological and immunological disorders, and is being applied increasingly to the correction of a large variety of inborn errors of metabolism, such as the mucopolysaccharidoses.

Indications for bone marrow transplantation

Table 1 lists the conditions currently being considered for bone marrow transplantation. Since the engrafted bone marrow stem cell is capable of giving rise not only to a new lymphoid system, but also to osteoclasts and long lived tissue macrophages, the technique of BMT is applicable not only to the treatment of congenital and acquired bone marrow disorders and immune deficiency diseases, but also to a number of conditions where a missing enzyme produces a metabolic disorder [3]. In this case the bone marrow graft and the macrophages it gener-

ates serve as a source of the missing enzyme which is transported in the circulation to other parts of the body. It has still to be determined whether the provision of an enzyme by marrow transplantation is effective in arresting or reversing damage from the accumulation of metabolites in the central nervous system, and the table shows a large group of diseases where BMT should still be considered an experimental procedure.

Severe Combined Immune Deficiency Disease

The diagnosis of Severe Combined Immune Deficiency Disease (SCID) may be suspected when

Table 1. Conditions where allogeneic bone marrow transplantation has been used.

LEUKAEMIA AND LYMPHOMA

- Acute myeloblastic leukaemia
- Acute lymphoblastic leukaemia
- Chronic granulocytic/myeloid leukaemia
- Acute myelofibrosis
- Non-Hodgkin's lymphoma

APLASTIC ANAEMIA

- Idiopathic viral induced
- Drug induced, congenital
- Paroxysmal Nocturnal Haemoglobinuria

CONGENITAL BONE MARROW DISORDERS

- Congenital neutropenia
- Red cell aplasia
- Wiskott Aldrich Disease
- Chédiak-Higashi disease
- Osteopetrosis (Albers Schönberg disease)
- Thalassaemia major
- Chronic granulomatous disease

IMMUNE DEFICIENCY DISEASES

- Severe combined immune deficiency (SCID) (including ADA deficiency, Nucleoside phosphorylase deficiency and Reticular dysgenesis)
- Ataxia Telangiectasia
- Hypogammaglobinaemia

INBORN ERRORS OF METABOLISM

- Mucopolysaccharidoses (Hurler, Hunter, San Fillipo A disease, San Fillipo B disease)
- Morquio's disease
- Gauchers disease
- Metachromatic leucodystrophy
- G1 gangliosidosis

there is a family history of death in infancy due to infection. Lymphopenia, absence of the thymus, low values of immunoglobulins and demonstration of impaired cellular immunity confirm the diagnosis. In SCID, bone marrow transplantation should be carried out as soon as possible after diagnosis. Without treatment, this disease leads to early death from overwhelming bacterial and viral infection. Before the era of BMT, thymic transplants were attempted without any success and the ideal treatment is a bone marrow transplant from an HLA identical mixed leucocyte culture compatible donor. In patients without a compatible donor some successes are reported with foetal liver and thymic epithelium transplants [4]. If SCID is suspected during pregnancy, a sterile delivery, immediate reverse isolation procedures of the newborn infant or laminarflow isolator is necessary in order to avoid fatal infection [5]. Selective or total decontamination of the gut during the whole procedure of bone marrow transplantation may also improve the ultimate outcome [6].

Aplastic anaemia

In aplastic anaemia, the fundamental defect is a partial or complete absence of haemopoietic stem cells resulting in failure of production of erythrocytes, platelets and neutrophils. The prognosis is extremely poor when the patient's platelet count is less than $10 \times 10^9/l$ and granulocytes count less than $0.01 \times 10^9/l$, requiring blood and platelet transfusions [7]. The degree of infiltration of the patient's bone marrow with lymphocytes measured in trephine biopsies of the bone marrow may also correlate with prognosis with the more inflammatory signs, indicating a poorer prognosis. In some cases laboratory studies indicate the possible involvement of an immunological mechanism in the course of the aplasia, and many such patients respond to immunosuppressive treatment [8]. Most groups therefore consider only severe forms of aplastic anaemia for bone marrow transplantation. Preferably they should be patients without a long history of blood transfusion who are uninfected at the time of transplant with a matched sibling bone marrow donor. The congenital Fanconi type

aplasia is slowly progressive but ultimately fatal, and until recently results of BMT in this condition were very poor. The improvements in the conditioning regime used now encourage selection of these patients for BMT [9].

Leukaemia

It is now possible by using appropriate chemotherapy to obtain remissions in 70–80% of patients presenting with acute myeloblastic leukaemia. However, despite some promising improvements in chemotherapy, the majority of patients with this disease relapse and subsequently die of leukaemia. In patients under the age of 45 with matched sibling donors, bone marrow transplantation in the first remission offers a cure rate in the region of 65%. Second and subsequent remission with chemotherapy are more difficult to obtain, and marrow transplantation here or in the next relapse is not associated with as much success. Despite considerable success in the treatment of acute lymphoblastic leukaemia (ALL), particularly in childhood, overall cure rate is no more than 50% in children and even lower in adults.

Because prognostic factors determining the possibility of a cure can be defined in ALL, it is possible to select certain poor risk patients for bone marrow transplantation in first remission. Patients who relapse and are subsequently put into second remission are considered for BMT, but successes become fewer and the ability to eradicate the disease less the later in the patient's disease they are selected for transplantation.

Although chronic granulocytic leukaemia (CGL) has been considered a benign disease, it is now recognised that nearly all patients develop an accelerated terminal phase within a median of 3.5 to 4 years after diagnosis. Patients with CGL are selected for transplantation either after acceleration or blastic transformation for autotransplantation or allografting, or in the chronic phase if a matched donor is available.

BMT has also been used successfully in a variety of rarer leukaemic and lymphoproliferative disorders, such as hairy cell leukaemia, non-Hodgkin's

lymphomas, acute myelofibrosis and juvenile CGL.

Solid tumours

There is a current interest in bone marrow auto-transplantation in lymphomas, small cell carcinoma of the lung and neuroblastoma. Selection of older patients with malignant disease for transplantation presents considerable problems and the place of BMT in these conditions is still under evaluation.

Strategy of choice of donor

Bone marrow donors should be healthy volunteers, preferably HLA identical, mixed lymphocyte culture compatible siblings. Pretransplant investigations are required to prove the health of the donor and avoid complications due to general anaesthesia, and to exclude the possibility of chromosome aberrations or pre-aplasia in the donor. Hospitalisation of the donor for two to three days is necessary because bone marrow aspiration takes place under general anaesthesia in an operating theatre. Bone marrow cells are obtained from the anterior and posterior iliac crests and the sternum by multiple aspiration. Some days before transplantation, it is useful to collect half a litre of blood from the donor to be returned during or after the marrow harvest [10]. By using plasma expanders and the donor's own blood, transfusion from random donors can be avoided. In younger children, marrow cellularity is greater, and it is easier to obtain the number of cells required for transplantation. As the donor red cells which contaminate the marrow aspirate are not necessary for the recipient, these can be returned to the donor. Iron treatment may be necessary for several weeks after donation. In our knowledge, no fatal donor accidents have been described. In aplastic anaemia and leukaemia, transplantation with cells from an identical twin has the greatest chance of success because of the absence of GVHD. However, in twin transplants the possible benefit of a graft versus leukaemia effect cannot be achieved [11]. Donors are

selected by HLA A and B typing, together with serotyping of the HLA D locus, or identification of D locus compatibility in mixed lymphocyte culture or by cell mediated lympholysis techniques. The best chance of success in transplantation is with an HLA A, B and D identical sibling, but successes have been described with HLA A, B and D identical parents or other family members, and also between HLA A and B mismatched but D matched siblings [12]. In matched donor-recipient pairs, the chance of significant GVHD is over 50%. In the absence of an HLA identical sibling in many patients, transplants of haplo-identical cells from father or mother have been attempted either in acute leukaemia or in the treatment of inborn error of metabolism. Severe GVHD can occur but increasing successes are being reported [13]. Complete mismatching on all loci is generally avoided because of the increased risk of severe, fatal GVHD. Attempts have therefore been made to use unrelated HLA identical donors. Preliminary results show that graft take without significant GVHD can be obtained, but results are too few to provide accurate comparison with matched family donors [14].

When harvesting bone marrow, a total nucleated cell count of $1-3 \times 10^8$ cells/kg is required to transfuse into the recipient. Excessive cell loads may lead to an increased risk of fatal GVHD while too few cells may be associated with failure of graft take. Because mature donor lymphocytes are responsible for generating acute GVHD, there have been a variety of attempts to try and eliminate these lymphocytes from bone marrow infusion [15, 16, 17]. Such treatments may be necessary before BMT from less than fully matched donors can become a safe procedure.

The bone marrow aspirates from the donor are transfused into the patient and some transplant teams prefer to transplant only the stem cell rich buffy coat after filtering the marrow to break down particles before intravenous infusion.

Conditioning regimes for BMT

With the rare exception of patients with severe

combined immune deficiency disease who have no ability to reject a bone marrow transplant and BMT between identical twins where the recipient does not recognise the engrafted marrow as foreign, immunosuppressive conditioning is necessary to permit permanent engraftment of the donor marrow. The aim of immunosuppression is to ablate the recipient's immune system. In patients with leukaemia and malignant disease, the aim of conditioning is also to eradicate residual malignant cells with high dose chemotherapy and whole body irradiation. Table 2 shows some of the schedules currently adopted for conditioning in a variety of disorders.

Acute leukaemia

Most patients transplanted for leukaemia receive the Seattle schedule of cyclophosphamide and total body irradiation (TBI). The combination is both immunosuppressive and anti-leukaemic. The TBI

Table 2. Conditioning regimes

Aplastic anaemia	Cy4 PAPA Cy4 (Procarbazine + ATG + Cyclophosphamide $\times 4$) Cy4TNI (Cyclophosphamide $\times 4$ Total Nodal Irradiation) Cy4TBI Cyclophosphamide $\times 4$ + Total Body Irradiation Cy4TAI (Cyclophosphamide $\times 4$ + Thoraco-abdominal Irradiation)
Acute leukaemia	Cy2 TBI (Cyclophosphamide $\times 2$ + Total Body Irradiation) Cy2 TBI fractionated dose TBI + other agents Bu Cy4
Inborn errors bone marrow disorders in infants	Bu Cy4 (Busulphan or Dimethylbusulphan) Cy2 TBI
Cy4	= Cyclophosphamide 50 mg/kg $\times 4$ days
Cy2	= Cyclophosphamide 60 mg/kg/day $\times 2$ days
TBI	= Total Body Irradiation to 10 Gy dose + dose rate vary
Bu	= Busulphan, 3 mg/kg $\times 4$ days

technique most commonly used has been to give 8–10 Gy from a conventional cobalt source or a linear accelerator, or in some instances a purpose built cobalt irradiator is used. Careful measurements and attention to obtain an even dose distribution across the patient is important in order to achieve optimum results from TBI. Lung toxicity with subsequent development of pneumonitis is a major problem in some series but appears to be largely avoidable when the irradiation dose is delivered at a rate of 0.3 Gy per minute [18]. In order to increase the antitumour effect, some centres have used fractionated TBI given over several days to achieve a higher total dose but the optimum schedule producing an adequate anti-leukaemic effect with minimum tissues damage is not yet defined. Because of the problems associated with TBI, the Baltimore group have explored the use of busulphan and cyclophosphamide regimes but more data are required before the relative merits of this technique are known [19]. Since relapse after high-dose cyclophosphamide is a major problem, particularly in ALL transplants, some groups have attempted to increase the anti-leukaemic effect by intensifying the chemotherapy given before irradiation [29].

Severe aplastic anaemia (SAA)

Patients transplanted for SAA face two problems – failure of the graft to take or its subsequent rejection, and GVHD. To a certain extent, the outcome is determined by the conditioning regime: inadequate immunosuppression gives rise to graft failure, whereas excessive immunosuppression using high dose total body irradiation leads to death from severe acute GVHD. A variety of conditioning schedules have now been employed for SAA. Initial results from Seattle with cyclophosphamide as a sole conditioning agent were complicated by a high incidence of graft rejection.

Subsequently, with the identification of prognostic factors for rejection, this complication was reduced by the use of cyclophosphamide with procarbazine and ATG, high cell dose marrow infusions and transfusion of donor buffy coat after BMT. Other groups investigated combinations of

cyclophosphamide with TBI and lung shielding to prevent pneumonitis [21], cyclophosphamide with lymph node irradiation [22], or cyclophosphamide with low total dose total body irradiation [23]. All these schedules appear more effective in preventing graft rejection than cyclophosphamide alone, and schedules employing low dose TBI or total lymph node irradiation do not appear to be associated with an increased severity of GVHD. Good results with very occasional late rejections have been reported by the Hammersmith Hospital Group, using cyclophosphamide as conditioning agent and cyclosporin A to prevent GVHD. Cyclosporin A may be effective in preventing graft rejection by the residual host immunity [24].

Inborn errors of metabolism

A special problem in this group of younger patients who have a normally functioning immune and haemopoietic system is the difficulty of obtaining sufficient suppression of the patient's immune system and bone marrow. Recourse to total body irradiation carries certain unknown risks of possible damage to bone and brain development in infants below the age of two years, and cyclophosphamide conditioning alone is associated with a high rate of graft rejection and autologous regeneration. The most effective combination appears to be busulphan and cyclophosphamides given in high doses [25].

Graft versus host disease

Acute GVHD occurs between one week to 100 days after BMT, but most commonly in the first two weeks. The first signs are of a fever and rash, characteristically on the face, palms and soles, but also extending to the rest of the body, in severe cases progressing to exfoliation. The process may also affect the gastro-intestinal tract with abdominal cramps, diarrhoea and fluid and blood loss leading to severe malabsorption syndromes. The liver may also be damaged with rising liver enzymes and hyperbilirubinaemia. This may progress to fatal liver failure. About 80% of patients develop



Fig. 1. Graft versus host reaction of the skin.

some features of acute GVHD, but in only about 5% is the disease progressive and fatal. The diagnosis is made on clinical grounds and confirmed on skin and rectal biopsy. Chronic GVHD occurs later. It is usually preceded by acute GVHD and manifests itself in a variety of ways:

- (a) Lichen-planus-like eruptions of the skin and oral mucosa sometimes associated with gastro-intestinal malabsorption and weight loss (Fig. 1).
 - (b) Scleroderma with progressive fibrosis, sometimes severely affecting mobility.
 - (c) Chronic liver damage with progressive destruction of bile canaliculi leading to liver failure.
 - (d) Chronic exfoliative dermatitis with alopecia and erythroderma.
 - (f) Prolonged immune deficiency with increased susceptibility to viral, bacterial and fungal infection.
- Many attempts have been made to prevent acute GVHD. The most commonly used agent is methotrexate, but recently cyclosporin A has been

shown to be at least as effective as methotrexate as a prophylactic agent [26]. Because donor T lymphocytes are responsible for initiating the GVHD reaction, a number of methods to eliminate T lymphocytes from the bone marrow graft infusion are under evaluation. Several techniques have been used; removal of T lymphocytes by anti-lymphocyte globulin [15], or OKT3 monoclonal antibody [16], positive selection of stem cells by soy bean agglutinin and depletion of T cells by rosetting [17]. No technique produces complete abolition of GVHD and treatment of the acute and chronic form is still necessary. One of the most effective agents in treating acute GVHD is methyl prednisolone given in high doses of $1\text{ g}/\text{m}^2$. Some success has also been achieved with combinations of methotrexate, ALG and steroid treatment [27]. Chronic GVHD responds best to combinations of azathioprine and low dose prednisolone. There is little evidence that cyclosporin A is effective in the treatment of most patients with chronic GVHD. The optimum mode of administration of cyclosporin A is not determined. Excessive fluctuations in blood levels may be the cause of some treatment failures, and consequently attempts have been made to ensure a sustained blood level by the use of intravenous cyclosporin A or by starting the drug 7–10 days before the transplant [28]. Cyclosporin A has important toxicity which limits the dose used and it appears to have a low therapeutic index: renal damage, liver failure, hypertension, fits and adult respiratory distress syndrome have been reported [29] and dangerous interactions with nephrotoxic agents and ketoconazole can occur.

Other problems associated with bone marrow transplantation

Infection

Patients undergoing bone marrow transplantation are immune deficient either because of the conditioning regime or because of the underlying reason for the transplant. In the first few weeks after BMT there is a phase of neutropenia when the risk from bacterial infection is highest, but sub-

sequently there is a prolonged and slow recovery of antibody production and T lymphocyte cell mediated immunity. Normal immune function may not be achieved until six months to one year after the bone marrow transplant, and GVHD in its chronic form interferes with immune recovery causing persisting hypogammaglobulinaemia, lymphopenia, and increased risk of infection. Considerable efforts are therefore made to prevent infection in BMT patients and this takes the form of reverse barrier isolation of the patient, either in conventional cubicles, laminar airflow rooms, or isolator tents. In conjunction with the patient's isolation, decontaminating antibiotic schedules are used to reduce gastro-intestinal flora, either partially (leaving anaerobes behind), or totally. Topical disinfection with skin antiseptics is also carried out to prevent the risk of septicaemia from skin pathogens and sterile or clean food is provided. Much emphasis has been placed on the protective treatment of BMT patients, but there is no agreement on the relative importance of these procedures and the necessity for elaborate isolation. Some BMT teams have employed prophylactic granulocyte transfusions during the period of neutropenia but these confer a risk of cytomegalovirus infection and lung complications. Fever in the early post-graft period presents a diagnostic problem: bacterial, viral or fungal infection may be the cause but GVHD also has to be considered. The initial approach of blood culture followed by the use of broad spectrum antibiotics prevents the risk of death from bacterial septicaemia. If no bacterial cause is identified, and the patient remains febrile and ill, therapy with either amphotericin, if fungus is suspected, Acyclovir is given if herpes virus is suspected; methyl prednisolone is given if GVHD is suspected.

Pneumonitis is a particular problem encountered in BMT patients. The aetiology may be varied and multifactorial with GVHD and total body irradiation contributing to the lung damage, and complicating superadded infections by cytomegalovirus, bacterial or fungal agents. Because the patient may deteriorate rapidly with pneumonitis, either early lung biopsy and specific therapy or urgent empirical treatment is necessary. Neverthe-

less, pneumonitis carries a mortality in the region of 30%. In general the risk from infection diminishes the longer the time that has elapsed from the BMT, but late problems from streptococcal and pneumococcal septicaemia associated with hypotension and GVHD have been reported.

Alimentation

Intravenous feeding, preferably via a deep venous catheter, is indicated in many patients, especially those with severe combined immune deficiency disease, in whom diarrhoea and intestinal malabsorption is a common problem. As soon as the cellular and humeral immunity recovers, the infant gains in weight and is able to take in normal dietary formulae. In children and adults with aplastic anaemia, leukaemia or with storage diseases, special attention to alimentation is necessary during the conditioning phase when vomiting and electrolyte disturbance occur, and after bone marrow transplantation during strict isolation when patients suffer from long-lasting anorexia. In children particularly, continued intravenous feeding limits the catabolic state.

Major alimentation problems arise during GVHD of the gut. Profuse watery diarrhoea leads to an enormous loss of fluid, electrolytes, proteins and blood. Intravenous administration of up to 5 litres a day of full parenteral nutrition, fresh plasma and albumin solution is necessary. In extensive intestinal GVHD, the ileum and jejunum may be totally denuded, and sometimes subtotal ileojejunectomy has been life-saving. Patients can subsequently recover from the short bowel syndrome following surgery [30].

Transfusion support

Patients with severe aplastic anaemia produce no red cells and lose them due to overt or occult bleeding. In these patients regular transfusions of red cells are necessary at approximately monthly intervals. In patients with leukaemia the need for red cells before BMT is less frequent and usually associated with induction chemotherapy. Patients

with severe combined immune deficiency disease and inborn errors (excluding haemoglobinopathies) seldom require transfusion before BMT. During BMT, red cells are required in order to keep the haemoglobin level at at least 6 g%. After the BMT it takes between 3 and 6 weeks for the graft to function adequately. In this period weekly red cell transfusions may be necessary. In the case of severe GVHD of the gut, and haemorrhagic cystitis following cyclophosphamide induction treatment, blood loss has to be replaced. By washing red cells free of leucocytes, the sensitisation due to major and minor HLA antigens not present on the surface of red cells but abundantly found on the surface of granulocytes and leucocytes, can be avoided. It is important to limit the granulocyte and platelet transfusions before BMT because of the risk of HLA sensitisation which makes adequate platelet prophylaxis impossible or difficult. In the weeks following BMT procedure, granulocyte transfusions are seldom necessary if reverse isolation and decontamination is used.

Platelet transfusions are given during induction treatment for leukaemia and for transplantation in patients with aplastic anaemia. There is a regular need for platelets which may start during conditioning and continue at least until the graft takes and platelet counts are sustained. Platelet transfusions are also necessary during treatment with anti-lymphocyte serum, since some ALG's have an anti-thrombocyte activity. When GVHD develops, the need for platelet transfusion often increases and platelet loss via bleeding in the gut occurs. Platelet transfusion should ideally be prepared primarily from random donors, depleted from contaminating lymphocytes and granulocytes to avoid HLA sensitisation. When alloimmunisation to platelets exists or develops, platelets from HLA identical random donors can be used as support. To avoid the risk of graft rejection it is essential to avoid giving blood products from the bone marrow donor or the immediate family before the bone marrow transplantation procedure. After BMT all blood products must be irradiated with 1.2 Gy before transfusion to avoid the risk of blood products lymphocyte engraftment and consequent GVHD. It is particularly important to be aware of this risk in SCID

where fatal GHVD can occur before the BMT is attempted.

Late effects

Late effects of the BMT procedure due to the conditioning regimes may occur. Cyclophosphamide is known to lead to sterility in reproductive man. Total body irradiation may lead to delayed or absent pubertal development, growth retardation, and sterility. Cataracts eventually occur in every patient. So far, secondary tumours do not appear to be a problem. The relative effects of chemotherapy and total body irradiation on late damaged normal tissues have still to be determined, and co-operative studies to identify late effects of radiation have been set up [31]. In addition, chronic sclerosing GVHD can cause delayed late effects which have long lasting consequences for the patient.

Clinical results of bone marrow transplantation

Acute leukaemia

The initial study of BMT in 100 relapsed end stage patients with acute leukaemia by the Seattle transplant group showed that about 10% of patients became long-term survivors and were apparently cured of their disease [32]. Subsequently much more promising BMT results have been reported in acute myeloblastic leukaemia (AML) when the transplant is performed in first remission. Success reported in these patients range from 50 to 75% long-term survivors [33, 34]. Relapse of the disease occurs in up to 20% of patients in some series and GVHD and pneumonitis are the major contributing factors to death from the transplant procedure. While it is clear that BMT is superior to current chemotherapy in terms of preventing relapse, the overall survival from BMT is not unequivocally better than the results obtained by chemotherapy. In particular, the results of BMT and chemotherapy are also age-dependent. Careful comparison of BMT and chemotherapy with controlled trials is going to be necessary before a definitive statement

can be made about the relative value of BMT or chemotherapy in specific sub-group of patients.

BMT has been used in acute lymphoblastic leukaemia (ALL) in two situations: (a) patients in first remission who can be defined at diagnosis as having poor risk disease associated with a high chance of chemotherapy treatment failure (e.g. B cell ALL, T cell ALL, presenting blast count greater than $100 \times 10^9/l$, patients over the age of 15 years and patients with lymphoma type presentation); (b) Patients who have relapsed after adequate chemotherapy either during transplant or after maintenance treatment is stopped. Patients relapsing on treatment are seldom, if ever, cured of their disease, while those relapsing off treatment have less than a 20% chance of long survival. The results of BMT for ALL in first remission patients are extremely encouraging with long-term disease-free survivals of 85% in the best series [35]. In second and third remission or in relapse the major problem is relapse of the disease and disease-free survival of 30–40% can be anticipated [36, 37]. The future for BMT in ALL lies in devising better techniques of leukaemia eradication and in the identification of poor risk patients destined to relapse in order to transplant them in first remission (Fig. 2).

Chronic granulocytic leukaemia

Two initial studies of BMT have defined more clearly the optimal approach, in this disease. Attempts at allogeneic and autologous BMT in the blastic or transformed stage of the disease have met with almost universal failure, death occurring from failure to control leukaemia, pneumonitis and infection [38]. In 1978, Fefer reported a small series of identical twin transplants for young patients in the chronic phase of their disease [39] in which most patients became long-term disease-free survivors without evidence of recurrence of the Philadelphia chromosome. These results have encouraged BMT groups to carry out allogeneic transplants in young people with CGL in the chronic or accelerated phase. Results are encouraging with long-term disease-free survivors in the region of 80% [40, 41]. The main problem for BMT in this condition is the difficulty of recommending a po-

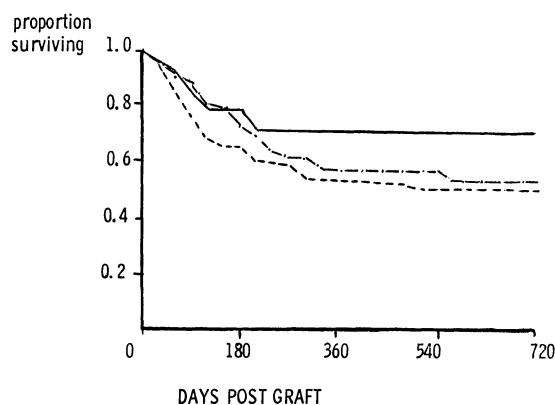


Fig. 2. Recent results of bone marrow transplantation in leukaemia. Data from European Co-operative group for Bone Marrow Transplantation, 144 patients with AML (----); 92 with ALL (-----); 28 with CGL (—).

tentially lethal but curative treatment for a disease where an excellent quality of life can be achieved with chemotherapy for four years or more; and the fact that most patients with CGL are older adults who have a comparatively poorer prognosis for BMT.

Severe aplastic anaemia (SAA)

In the last decade the overall success rate for BMT in SAA was in the region of 50%. This nevertheless represented an improvement over the best available supportive treatment for severely affected patients with blood counts of less than $20 \times 10^9/l$, platelets $0.2 \times 10^9/l$, neutrophils and $20 \times 10^9/l$ reticulocytes [42]. The most common problem in these initial studies was death from graft failure or rejection. Subsequently improvements in conditioning regimes, with the addition of irradiation, or cyclosporin A, or donor buffy coat have made BMT rejection an uncommon event; but GVHD, pneumonitis and infection remain important obstacles. Recently reported results indicate an overall success rate between 60 and 85% [43, 44].

A development which makes the decision to use BMT harder to make is the possibility of achieving substantial haemopoietic recovery but not complete cures with antilymphocyte globulin and high dose steroid immunosuppressive treatment [45]. The best results indicate an 80% survival from this

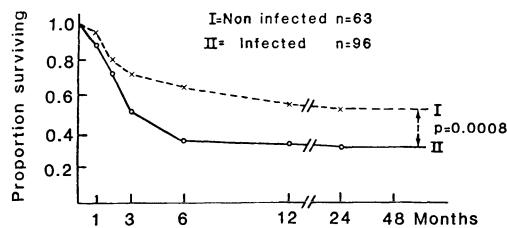


Fig. 3. Results of bone marrow transplantation in aplastic anaemia in infected and non-infected patients. Data from European Co-operative Group for bone marrow transplantation.

form of treatment. Nevertheless, BMT offers the best chance of permanent correction of the disease, but both BMT and immunosuppressive treatment continue to be evaluated side by side (Fig. 3).

Immune deficiency diseases

One of the first conditions to be successfully transplanted using allogeneic marrow was the rare severe combined immune deficiency disease. Since that time over 50 patients transplanted from matched sibling donors have been reported to the international bone marrow transplant registry [46]. The overall success rate is 65%. Because few of these infants have matched donors, BMT with foetal liver, foetal liver and thymus, and with bone marrow from haplotype identical parents have been attempted. The chance of success in mismatched patients is less than 50%, but long-term success rate of foetal liver and thymus transplants have been reported [47] and the use of marrow fractionated to remove T lymphocytes has resulted in some success without chronic GVHD [16]. Other immune defects such as Wiskott Aldrich disease, congenital neutropenia and Chédiak Higashi disease have been successfully transplanted. These patients require immunosuppression with busulphan and cyclophosphamide or irradiation plus cyclophosphamide in order to ensure engraftment.

Inborn errors of metabolism

With improvements in results from BMT it has now become possible to consider the use of the technique in conditions that are disabling but not immediately life threatening. Thus BMT has been

used in mucopolysaccharidosis such as Hurlers Disease and related conditions to provide the Alpha-iduronidase enzyme necessary for the degradation of tissues and brain mucopolysaccharides. Preliminary results show that BMT can cause biochemical correction of the disease with clinical improvement [48]. The ability of the procedure to permit normal brain development has yet to be fully assessed.

BMT has also been used in other storage diseases such as metachromatic leucodystrophy, Gauchers Disease and in osteopetrosis (Albers-Schönberg Disease); where defective osteoclasts are replaced by bone marrow graft derived cells.

An exciting new development is the application of BMT in thalassaemia major [49]. The graft can correct the life-long anaemia and provides the patient a better quality of life. However, the dilemma in using a potentially lethal but curative procedure in a condition compatible with a very long survival makes cautious selection of patients necessary.

References

1. Uphoff DE: Egm Lorenz – 30 years ago. In: Experimental Haematology Today, Baum SJ, Ledney GD, Thierfelder S (eds). Karger, Basel, 1982: 17–19.
2. Thomas ED: Marrow transplantation for patients with leukaemia. In: Experimental Haematology Today, Baum SJ, Ledney GD, Thierfelder S (eds). Karger, Basel, 1982: 137–46.
3. Hobbs JR: Bone marrow transplantation for inborn errors. Lancet, 1981; 2: 735–36.
4. Lucarelli G, Fliedner TM, Gale RP: Fetal liver transplantation: Current concepts and future directions. Proceedings of the first international symposium on foetal liver transplantation. Pisero, Italy. September, 1979. Elsevier/Excerpta Medica, Amsterdam, 1980.
5. de Koning J, v.d. Waay D, Vossen JM, Versprille A, Dooren LJ: Barrier nursing of an infant in a laminar cross-flow bench. Maandschrift voor Kindergeneeskunde, 1970; 38: 1–13.
6. v.d. Waay D, Vossen JM, Hartgrinh CA, Nieweg HO: In: New Criteria for Antimicrobial Therapy, v.d. Waay D, Verhoeft J (eds). Amsterdam, Excerpta Medica, 1979.
7. Camitta BM, Thomas ED, Nathan DG, Santos G, Gordon-Smith EC, Gale RP, Rappaport JM, Storb R: Severe aplastic anaemia prospective study of the effect of early marrow transplantation on acute mortality. Blood, 1976; 48: 63–70.
8. Gluckman E, Devergie A, Poros A, Degoulet P: Results of immunosuppression in 170 cases of severe aplastic anaemia. Br J Haematol, 1982; 51: 541–50.

9. Gluckman E, Devergie A, Schaison G, Bussel A, Berger R, Sohier J, Bernard J: Bone marrow transplantation in Fanconi anaemia. *Br J Haematol*, 1980; 45: 557-64.
10. Storb R: In: *Immunobiology of Bone Marrow Transplantation*, Thierfelder S, Rodt H, Kolb HJ (eds). Berlin, Springer Verlag, 1980: 367.
11. Weiden PL, Sillivan KM, Flournoy N, *et al.*: Antileukaemic effect of chronic graft versus host disease contribution to improved survival after allogeneic marrow transplantation. *N Eng J Med*, 1981; 304: 1529-33.
12. Clift RA, Hanson JA, Thomas ED, Buckner CD, Saunders JE, Mickelson EM, Storb R, Johnson FL, Singer JW, Goodell BW: Marrow transplantation from donors other than HLA identical siblings. *Transplantation*, 1979; 28: 235-42.
13. Powles RL, Morgenstern GR: Allogeneic bone marrow transplantation using mismatched family donors in Cyclosporin A, White D, Powles RL, Masters P (eds). Elsevier, Amsterdam, 1982: 539-44.
14. Gordon-Smith EC, Fairhead SM, Chipping PM, Howes J, James DCO, Dodi A, Batchelor JR: Bone marrow transplantation for severe aplastic anaemia using histocompatible unrelated volunteer donors. *Br Med J*, 1982; 835-37.
15. Rodt H, Netzel B, Kolb HJ, Haas RJ, Wilms K, Link H, Bender Gotze C, Niethammer D, Wernet P, Janka G, Thierfelder S: Suppression of graft versus host disease by antisera-clinical results in 20 patients. In: *Experimental Haematology Today*, Baum SJ, Ledney GD, Thierfelder S (eds). Kager, Basel, 1982: 147-53.
16. Fillipovitch AH, McGlave PB, Ramsay MK, Goldstein G, Warkentin PL, Kerssy JH: Pretreatment of donor bone marrow with monoclonal antibody OKT3 for prevention of acute graft versus host disease in allogeneic histocompatible bone marrow transplantation. *Lancet*, 1982; 1: 327-31.
17. Reisner Y, Kapoor N, Kirkpatrick D, *et al.*: Transplantation for acute leukaemia with HLA A and B nonidentical parental marrow cells fractionated with soy bean agglutinin and sheep red blood cells. *Lancet*, 1981; 2: 327-31.
18. Saunders JE: Effect of cyclophosphamide and total body irradiation on ovarian and testicular function. *Exp Haematol*, 1982; 10 suppl. 11: 49.
19. Tutschka PJ, Santos GW, Elfenbein GJ: Marrow transplantation in acute leukaemia following Busulphan and Cyclophosphamide. In *Immunobiology of bone marrow transplantation*, Thierfelder S, Rodt H, Kolb H (eds). Springer Verlag, Berlin, 1980: 375-80.
20. U.C.L.A. Bone Marrow Transplantation Group: Bone marrow transplantation with intensive combination chemotherapy radiotherapy (SCARI) in acute leukaemia. *An Int Med*, 1977; 86: 155-61.
21. Gluckman E, Devergie A, Dutreix A, Dutreix J, Boiron M, Bernard J: Bone marrow grafting in aplastic anaemia after conditioning with cyclophosphamide and total body irradiation with lung shielding. In: *Recent Trends in the Immunobiology of Bone Marrow Transplantation*. Bergmann, Munich, 1980: 339-47.
22. Ramsay MKC, Kim T, Nesbitt ME, Krivit W, Coccia PF, Levitt SH, Woods WG, Kerssy JH: Total lymphoid irradiation and cyclophosphamide as preparation for bone marrow transplantation in severe aplastic anaemia. *Blood*, 1980; 55: 344-46.
23. Gale RP, Ho W, Feig S, Champlin R, Tesler A, Arranson E, Cadish S, *et al.*: Prevention of graft rejection following bone marrow transplantation. *Blood*, 1981; 57, 49: 165-75.
24. Powles RL, Clink H, Sloane J, Barrett AJ, Kay HEM, McElwain TJ: Cyclosporin A for the treatment of graft versus host disease in man. *Lancet*, 1978; 2: 1327-31.
25. Joss V, Rogers TR, Hugh-Jones K, *et al.*: A bone marrow transplant for metachromatic leucodystrophy. *Exp Haematol Suppl*, 1982; 10: 52-53.
26. Powles RL, Clink HM, Spence D, Barrett AJ, Jameson B, Kay HEM: Cyclosporin A to prevent graft versus host disease in man after allogeneic marrow transplantation. *Lancet*, 1980; 1: 327-29.
27. Ramsay MK, Kersey JH, Robson L, McGlave PB, Woods WG, Krivit W, Kim HT, Goldman A, Nesbitt M: A randomised study of the prevention of acute graft versus host disease. *N Eng J Med*, 1982; 306: 392-97.
28. Barrett AJ, Kendra JR, Lucas CL, Joss V, Joshi R, Desai M, Pendharker P, Hugh-Jones K: Cyclosporin A as prophylaxis against graft versus host disease in 36 transplanted patients. *Br Med J*, 1982; 285: 162-66.
29. Howes JM, Palmer S, Want S, Dearden D, Gordon-Smith EC: Serum levels of Cyclosporin A and nephrotoxicity in bone marrow transplant patients. *Lancet*, 1981; 2: 144-55.
30. Valman MB: Growth and fat absorption after resection of ileum in childhood. *J Pediatr*, 1976; 88: 41-45.
31. Fliedner TM, Gossner W, Patrick G. (eds): *Late effects after therapeutic whole body irradiation. Report of the Commission of the European Countries (1982)*. EUCEP Symposium, August 1981. Munich.
32. Thomas ED, Buckner CD, Banaji M, *et al.*: One hundred patients with acute leukaemia treated by chemotherapy, total body irradiation and allogeneic marrow transplantation. *Blood*, 1977; 49: 511-33.
33. Powles RL, Morgenstern GR, Clink H, *et al.*: The place of bone marrow transplantation in acute myeloid leukaemia. *Lancet*, 1980; 1: 1047-50.
34. Thomas ED, Buckner CD, Clift RA, Fefer A, Johnson FL, Neiman PE, *et al.*: Marrow transplantation for acute non lymphoblastic leukemia in first remission. *N Eng J Med*, 1979; 301: 597-99.
35. Zwaan FE, Hermans J: For the European Bone Marrow Transplant group leukaemia working party: Allogeneic bone marrow transplantation for leukaemia - European results in 264 cases. *Exp Haematol Rev*, 1982; 12: 96-106.
36. Thomas ED, Saunders JE, Flournoy M, Johnson FL, Buckner CD, Clift RA, Fefer L, Goodell BW, Storb R, Weiden PL: Marrow transplantation for patients with acute lymphoblastic leukaemia in remission. *Blood*, 1979; 54: 465-78.
37. Barrett AJ, Kendra JR, Lucas CG, Joss DV, Joshi R, Desai M, Hugh-Jones K, Philips R, Rogers TR, Tabara Z,

- Williamson S, Hobbs JR: Bone marrow transplantation for acute lymphoblastic leukaemia. *Br J Haematol*, 1982; 52: 181-88.
38. Goldman JM: Modern approaches to the management of chronic granulocytic leukaemia. *Semin Haematol*, 1978; 15: 420-30.
39. Clift RA, Buckner CD, Thomas ED, Doney K, Fefer A, Neiman P, Singer J, Saunders J, Stewart P, Sullivan KM, Deeg A, Storb R: Treatment of chronic granulocytic leukaemia in chronic phase by allogeneic marrow transplantation. *Lancet*, 1982; 2: 612-25.
40. Goldman JM, Baugham ASJ, McCarthy DM, Worsley AM, Howes JM, Gordon-Smith EC, Catovski D, Batchelor MD, Goulden AWG, Galton DAG: Marrow transplantation for patients in the chronic phase of chronic granulocytic leukemia. *Lancet*, 1982; 2: 623-25.
41. Clift RA, Buckner CD, Thomas ED, Doney K, Fefer A, Neiman P, Singer J, Saunders J, Stewart P, Sullivan KM, Deeg A, Storb R: Treatment of chronic granulocytic leukaemia in chronic phase by allogeneic marrow transplantation. *Lancet*, 1982; 2: 621-22.
42. Storb R, Thomas ED, Weiden PL, *et al.*: One hundred patients with aplastic anaemia (AA) treated by marrow transplantation in Seattle. *Transplant Proc*, 1978; 10: 135-40.
43. Gluckman E, Barrett AJ, Arcese W, Devergie A, Degoulet P: Bone marrow transplantation in severe aplastic anaemia: a survey of the European group for bone marrow transplantation (EG-BMT). *Br J Haematol*, 1981; 49: 165-73.
44. Ramsay NKC, Kim TH, McGlave PB, Nesbitt ME, Golman A, Kravit W, Woods WG, Kersey JH: Bone marrow transplantation for severe aplastic anaemia using the conditioning regimen of cyclophosphamide and total lymphoid irradiation. *Exp Haematol Rev*, 1982; 12: 139-42.
45. Speck B, Gratwohl A, Nissen C, *et al.*: Treatment of severe aplastic anaemia with antilymphocyte globulin or bone marrow transplantation. *Br Med J*, 1981; 282: 860-63.
46. Bortin MM, Rimm AA: Severe combined immunodeficiency disease. *JAMA*, 1977; 238: 591-600.
47. Touraine JL: Foetal liver transplantation for severe combined immunodeficiency in Europe. *Exp Haematol*, 1982; 10: 40-45.
48. Hobbs JR, Hugh-Jones K, Barret AJ, Chambers D, James DCO, Byrom N, Henry K, *et al.*: Reversal of clinical features of Hurler's Disease and biochemical improvement after treatment by bone marrow transplantation. *Lancet*, 1981; 2: 709-12.
49. Thomas ED, Buckner CD, Saunders J, Papyannopoulou T, Borgna CE, Stefano P, Sullivan KM, Clift RA, Storb R: Marrow transplantation for thalassaemia. *Lancet*, 1982; 2: 227-29.

12. Transfusion support and obstetrical haemorrhage

J. BENNEBROEK GRAVENHORST and D.G. WOODFIELD

I. Blood transfusion support for the obstetric patient

The Blood Transfusion Service plays a key role in the adequate management of the obstetric patient. Firstly, obstetric units require an optimally accessible range of blood and blood products. Not only should these be of high quality but clinical advice on their appropriate usage must be available. Some obstetric problems, and notably those associated with acquired or congenital coagulation syndromes, can be of sudden onset and unless adequate consultation and advice is available to the clinician, blood and blood products may be ineffectively utilised. Platelet concentrates, fresh plasma, coagulation factor concentrates and red cells, all have an important role to play in such patients but their judicious use still requires skill.

Secondly, the immunohaematology department of the Transfusion Service is usually the area to which ante- and postnatal blood group antibody testing and identification is directed. Considerable care must be taken to ensure that techniques and procedures are developed to a high level of accuracy to enable consistent detection and full identification of significant blood group antibodies. Expert advice to clinicians on the significance of antibodies should be available from transfusion service staff and close collaboration is required in the management of highly immunised patients.

Thirdly, obstetric units commonly need a wide range of other activities to be carried out by the Transfusion Service. Platelet, leucocyte and lymphocyte tests may be required as well as coagula-

tion investigations (see Chapter 3) and plasma exchange facilities. Integration of all these activities in a Transfusion Service can help to provide an optimal service to seriously ill obstetric patients.

II. Dangers of blood transfusion in pregnancy

Blood transfusion is to be avoided, wherever possible, in potentially reproductive females. The theoretical risk of red cell iso-immunisation is 1% blood unit transfused and thus the average patient receiving 2–4 units of blood has a 2–4% chance of being immunised. In addition, immunisation to platelet, leucocyte or serum components may occur. The degree of immunisation will increase in frequency depending on the number of transfusions administered. Immunisation of a mother to platelet antigens may occasionally result in post-transfusion purpura or neonatal thrombocytopenic purpura particularly if she is PLA-1 antigen negative (see Chapter 5). Leucocyte antibodies may also be stimulated by transfusion but, except in rare cases of alloimmune neonatal neutropenia, do not appear to be significant in causing problems in the infant. However, as with transfusion in the non-pregnant patient, the formation of potent leucocyte antibodies is a frequent cause of non-haemolytic febrile transfusion reactions and occasionally are involved in patients who develop the rare but serious non cardiogenic pulmonary oedema. Immunisation to Gm immunoglobulin markers can also occur but the relationship of these to transfusion reactions is still unclear.

In addition, all the other problems of transfusion are present when an obstetric patient is transfused. Although the risks of hepatitis B infection transmission have been reduced by sensitive screening of all donor units, non A non B hepatitis still remains a potential problem (see Chapter 18). Transfusion can result in the transmission of cytomegalovirus infections, causing fevers (see Chapter 18) wrongly attributable to other infectious agents [1]. It is recommended that, when transfusion is considered necessary, packed or resuspended red cells should be requested, thus reducing the risks of immunisation to cellular and plasma antigens. Whole blood is usually only necessary in massive haemorrhage such as occurs in PPH. Furthermore, the plasma volume is increased in pregnancy and additional care is required in avoiding rapid transfusion in the non-urgent patient. Some clinicians advocate the routine administration of diuretics during transfusion in the pregnant patient but this procedure is unnecessary.

III. Haemorrhage in pregnancy

Antepartum haemorrhage in the second and third trimester occurs in about 3% of the pregnancies [2]. In the majority of cases haemorrhage results from the presence of placenta praevia, abruptio placentae or marginal sinus rupture; the remainder are due to circumvallate placenta, bloody show, vasa praevia, gynaecological causes and bleeding of undetermined causes.

1. Placenta praevia

Placenta praevia is defined as a placenta implanted in the lower uterine segment with varying degrees of encroachment on the internal os. The incidence of placenta praevia varies from 0.4 to 0.8% and appears to be related to age rather than to parity, being three times as high in women over 35 years as in those under 35 [3, 4].

The most important sign is painless vaginal bleeding in the third trimester. At least one significant haemorrhage occurs in about 90% of the patients and 10–25% will develop hypovolaemic

shock [5]. In the second trimester the diagnosis of placenta praevia should be regarded with caution, because in many cases the placenta is known to migrate towards the fundus uteri during the course of pregnancy [6].

Before 36 weeks of pregnancy conservative management is advocated when blood loss is not life threatening to the mother and can be managed by blood transfusion.

In most cases of placenta praevia Caesarean section will be necessary. In some cases of marginal placenta praevia with small or moderate blood loss it is justifiable to rupture the membranes and anticipate vaginal delivery under careful fetal and maternal monitoring. For a small percentage of women serious bleeding at the birth of the child is caused by placenta accreta. Control of this threatening haemorrhage can be extremely difficult but with optimal antenatal and obstetrical care, maternal mortality is small.

2. *Abruptio placentae*

The incidence of abruptio placentae, defined as the separation of a normally implanted placenta after the 20th week of pregnancy, varies from 0.4 to 1.3% of all pregnancies [7, 8, 9]. It is increased with high parity but not with age. More than 50% of the cases occur prior to 36 weeks of gestation. The signs and symptoms of abruptio placentae depend on the site and degree of placental separation. Some cases proceed subclinically and the diagnosis is not made before delivery of the placenta. In mild forms of separation, the blood loss is small and the symptoms consist of local pain, slight increase in uterine tone and signs of fetal distress. The bleeding may remain concealed and retroplacental or appear through the vagina. In some cases the placenta is entirely separated from the uterus. Concealed blood loss can be considerable and may result in hypovolaemic shock. The mother suffers a sudden onset of severe abdominal pain. The uterus is extremely hard and tender and does not relax. Concealed blood loss causes enlargement of the life-uterus, fetal palpation is no longer possible and, in most cases, fetal heart sounds tend to disappear. If maternal shock is not treated promptly and effec-

tively the patient may die, develop renal failure, or pituitary necrosis. Labour usually occurs spontaneously and progresses rapidly. Coagulation failure is encountered in 20–38% of cases of abruptio [10, 11]. Thromboplastin release from the site of placental separation is believed to trigger intravascular coagulation. The result is hypofibrinogenaemia, platelet deficiency and a decrease in factor V and VII. The fibrinolytic system is activated and fibrinogen-fibrin degradation products (F/FDP) appear in the maternal circulation. Mild forms of placental separation can be managed conservatively. Other causes of vaginal blood loss should be ruled out.

In severe abruptio placentae, management should be directed towards maintaining adequate circulatory volume. Blood replacement with whole blood is mandatory. In severe cases, central venous pressure monitoring is valuable. An accurate fluid balance should be maintained. With the aid of these measures, coagulation failure can be confined within safe limits. It should be seldom necessary to administer cryoprecipitate to replace both fibrinogen and factor VIII. Labour often commences spontaneously and can be accomplished vaginally.

Amniotomy and oxytocin infusion, when judiciously used, are recommended to induce or stimulate labour. Oxytocin should be used conservatively and with careful monitoring since uterine rupture has been associated with the use of oxytocin in cases of abruptio placentae. Caesarean section should only be contemplated when the fetal heart is audible and the coagulation status of the patient permits an operation without the danger of uncontrollable haemorrhage. With optimal obstetric management, the maternal mortality is estimated between 1 and 5 per thousand. Perinatal mortality is still very high and occurs in 30% or more of the cases [9, 12].

3. Marginal sinus rupture

Marginal sinus rupture is defined as a defect in the marginal placental sinus usually combined with a separation of the placental edge over a distance of 2–3 cm. Vaginal blood loss can be up to 700–800 ml

but is usually less severe. Symptoms of a small placental separation are sometimes present. Coagulation defects are not present. In most cases conservative management offers an excellent prognosis for mother and child.

IV. Post partum haemorrhage

The large post partum haemorrhage is a recognised serious problem in obstetrics. The obstetrician's judgment must be sure and swift to prevent calamities. Post partum haemorrhage is defined as a blood loss in the third and fourth stage of labour of 500 ml or more blood [13]. The incidence varies from 5 to 7% of births [14]. The three main causes of haemorrhage are:

- hypotonia of the uterus,
- lacerations of the birth tract,
- blood coagulation disorders.

1. Hypotonia of the uterus

Hypotonia of the uterus is encountered in over-distension of the uterus (polyhydramnios, twins, large fetus), in the 'grand' multiparous woman after long labour, and after oxytocin stimulation. A history of previous post partum haemorrhage should alert the obstetrician, for the recurrence rate is high. In the management of post partum uterine hypotonia one should differentiate between the third and the fourth period of labour. In the third period (when the placenta is not yet delivered) intravenous infusion of oxytocin and emptying of the bladder are recommended to promote contraction of the uterus. If the placenta is not delivered spontaneously or by active management, manual removal under general anesthesia should be expedited. A blood loss of up to 1000 ml in a healthy non-anaemic women is usually well taken and can be replaced by plasma expanders. When blood loss exceeds 1000 ml then transfusions with whole blood are often indicated. In the fourth stage, haemorrhage can be treated by gentle massage of the uterine fundus, catheterization of the bladder and i.v. administration of ergot preparations or oxytocin (10 U in 50 ml, 5% glucose or

Ringer's lactate). When these supportive measures fail, inspection of the birth tract for lacerations and examination of uterine cavity for retained placental fragments is necessary. In severe cases of post partum haemorrhage, prostaglandins $F_2\alpha$ or E_2 administered locally in the myometrium or cervix or intravenously, can be life saving.

2. Laceration of the vagina and cervix

Lacerations are generally the result of operative deliveries, but also can occur with spontaneous birth. Inspection of the vaginal tract is best done under general or local anesthesia. All lacerations should be conscientiously mended with continuous, running, locked or separate sutures.

3. Coagulation disorders

Post partum haemorrhage in patients with blood coagulation disorders (factor VIII deficiency, thrombocytopenia) require appropriate treatment with the missing elements. Haematological studies of coagulation factors in the blood of normal patients during labour and delivery demonstrate that, even in normal delivery, there is some degree of transitory intra vascular coagulation. Pathological intra vascular coagulation seems to be but an extension of this physiological process.

4. Uterine rupture

Uterine rupture is usually not characterized by heavy vaginal blood loss. The bleeding is primarily intra-abdominal. The post partum diagnosis is made by digital exploration of the uterus. Previous uterine surgery should be suggestive of the diagnosis. Hysterectomy is sometimes the treatment of choice. Often it is possible to suture the defect.

5. Inversion of the uterus

Inversion of the uterus is often accompanied by severe neurogenic and hypovolemic shock. If the placenta is still attached to the uterus it is removed and repositioning of the uterus should be attempted immediately. To do this, general anesthe-

sia is required. When manual repositioning is not possible operative correction of the inversion is necessary. Correction of hypovolemia with plasma expanders, frozen plasma and blood is crucial. Packing of the uterus for 24 hours is recommended to prevent repeat inversion.

V. Disseminated intra vascular coagulation in obstetrics

Since De Lee in 1901 described a temporary blood coagulation failure in one patient with abruptio placentae and in another with a macerated fetus, a considerable number of publications, pertaining to consumption coagulopathy, have appeared [15]. It is now apparent that disseminated intravascular coagulation (DIC) is associated with a variety of obstetric complications. DIC has been reported in combination with toxemia of pregnancy, abruptio placentae, retained dead fetus, amniotic fluid embolism, the obstetric acute yellow atrophy or fatty metamorphosis of the liver, saline induced abortion and sepsis.

1. Disseminated intra vascular coagulation

To obtain a clear understanding of the changes in coagulation mechanism under pathological conditions, it is opportune to review the normal clotting mechanisms [16].

In DIC there is an excess production of activated factor X and thrombin in the circulation, and DIC may occur as a result of activation of the extrinsic, the intrinsic or of both clotting systems. The activation of the extrinsic system is probably responsible for the development of DIC in patients with abruptio placentae, retained dead fetus syndrome and hydatidiform mole.

The intrinsic clotting system is activated by injury to endothelial cells exposing plasma to underlying collagen leading to activation of factor XII. Activated factor XII promotes conversion of plasminogen to plasmin resulting in the appearance of F/FDP (Fibrin Degradation Products) in the circulation and an increased destruction of factor VIII and V by an activated fibrinolytic system. This is

probably the pathological mechanism which occurs in severe sepsis. The mechanism of disseminated intravascular coagulation in amniotic fluid embolism still remains an unresolved issue. During DIC sufficient amounts of thrombin are generated to decrease the ATIII levels.

The uncontrolled activation (without inhibition) of the coagulation system along with the activation of the fibrinolytic system leads to depletion of clotting factors and platelets with subsequent bleeding. Useful tests for the detection of DIC are: blood fibrinogen and thrombin time determination, screening for F/FDP and platelet count (see Chapter 3).

2. Treatment of DIC

DIC always occurs in association with another condition. It is important to treat this underlying cause. With low grade or moderate coagulation disorder no other specific treatment is necessary. In severe DIC, replacement of deficient blood clotting factors, AT III and sometimes platelets seems to be the therapy of choice. Fresh plasma contains all factors except blood platelets. More specifically cryoprecipitate is rich in fibrinogen as well as factor VIII and may be administered to attain quickly a level of fibrinogen greater than 100 mg/100 ml. Whole blood contains 0.5–1 g of fibrinogen per bag (unit of blood) and should be used where clinically replacement is indicated with blood loss. Heparin has an anticoagulant effect mediated by AT III. Heparin should be reserved for patients with retained dead fetus or amniotic fluid embolism. It should be clear that heparin can sustain bleeding even when DIC improves.

3. Amniotic fluid embolism

This syndrome is rare and occurs in approximately 1 in 40,000 live births. Characteristics are nausea, vomiting, sudden onset of respiratory distress, cyanosis and severe circulatory shock during the intrapartum or early post partum period [17]. Twenty-five percent of the patients die within one or two hours of onset with manifest symptoms of acute lung embolism.

The syndrome is often seen concomitant with overstimulation of the uterus. Amniotic fluid is thought to enter the maternal circulation by the uterine veins and the placental site. The exact pathological mechanism responsible for these severe symptoms is not clear. When the patient survives the initial period, excessive haemorrhage occurs in 40% of these cases. Severe hypofibrinogenemia and acute overt haemolysis are the most common findings in these patients. Because of the rarity of this syndrome, assessment of the effectiveness of all the various suggested therapies is difficult but some supportive measures seem successful. Oxygen therapy, mechanical respiration, digitalization and adequate replacement of blood and clotting factors (cryoprecipitate, platelets, preferably after systemic heparinization) are well established means on combatting this rare but often lethal syndrome. In some patients up to 20 l blood may be needed to maintain haemostasis.

References

1. Brian CS, Foster CM, Edwards GW: Post partum cytomegalovirus infection. A hazard of multiple transfusions. *Obstet Gynaec*, 1978; 52: 685–95.
2. Hellman LM, Pritchard JA, Wynn RM: Placenta praevia and abruptio placentae. In: Williams Obstetrics, Eastman NJ, Hellman LM (eds). New York, Appleton-Century-Crofts, 1971: 612–38.
3. Naeyer RL: Placenta praevia. *Obstet Gynaec*, 1978; 52: 521–25.
4. Davis ME, Campbell A: The management of placenta praevia in the Chicago Lying-in Hospital. *Surg Gynaec Obstet*, 1946; 83: 777–88.
5. Hibbard LT: Placenta praevia. *Am J Obstet Gynaec*, 1969; 104: 172–84.
6. Comean J, Shaw L, Marcell CC, Lavery P: Early placenta praevia and delivery outcome. *Obstet Gynaec*, 1983; 61: 577–580.
7. Pritchard JA, Mason R, Corley M, Pritchard S: Genesis of severe placental abruption. *Am J Obstet Gynaec*, 1970; 108: 22–27.
8. Naeyer R, Harkness W, Litts J: Abruptio placentae and prenatal death: a prospective study. *Am J Obstet Gynaec*, 1977; 128: 740–46.
9. Hurd WW, Miodovnik M, Hertzberg , Lavin JP: Selective management of abruptio placentae: a prospective study. *Obstet Gynaec*, 1983; 61: 467–73.
10. Beller FK, Uszynski M: Disseminated intravascular coag-

- ulation in pregnancy. *Clin Obstet Gynaec*, 1974; 17: 250-78.
11. Pritchard JA, Brekken AL: Clinical and laboratory studies on severe abruptio placentae. *Am J Obstet Gynaec*, 1967; 97: 681-700.
 12. Lunan CB: The management of abruptio placentae. *J Obstet Gynaec Br Comm*, 1973; 80: 120-24.
 13. Greenhill JP: Pathology of the third stage. In: *Obstetrics*, Greenhill JP (ed). Philadelphia-London, WB Saunders Co, 1966; 946-71.
 14. Quigley JK: Third stage of labor. *Am J Obstet Gynaec*, 1947; 53: 271-74.
 15. Bennett B, Ratnoff OD: The normal coagulation mechanism. *Med Clin North Am*, 1972; 56: 95-104.
 16. Resnik R, Swartz WH, Plumer MH, Benirschke K, Stratthaus ME: Amniotic fluid embolism with survival. *Obstet Gynaec*, 1976; 47: 295-98.

13. Cardiac operations and transfusion requirements

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Introduction

The technique of cardiopulmonary bypass (CPB) to support the patient during cardiac surgery can be performed safely nowadays.

Although Jehovah's Witnesses have been operated upon successfully without the use of any donor blood, in most centers an average of five donor units of blood are needed for each heart operation [1-3]. A marked reduction of the need of donor blood was noted after the introduction of the non-blood priming technique of the extracorporeal circuit (ECC).

When blood lost during CPB is recovered by cardiotomy suction system and re-transfused, donor blood is only needed for the blood lost after CPB. Generally, there is a loss of about 1 to 1.5 l [5]. But in 4% of all heart operations, there is severe post-operative bleeding, half of the cases due to surgical causes [6]. However, surgical bleeding is enhanced by coagulation defects, although haemostatic defects can occur without severe bleeding.

In this chapter the first part describes the coagulation and platelet defects as causes of bleeding in cardiac surgery, while the second deals with the improvement of blood transfusion requirement by using membrane instead of bubble oxygenator. In the third part of the chapter the Kansas City Blood Center experience with the transfusion requirements in cardiac surgery is given.

Coagulation defect

Despite inhibition of the clotting cascade by heparin and subsequent neutralization of the heparin, major disorders of coagulation are observed in about 5-6% of the patients undergoing cardiac surgery. The most common abnormalities are low prothrombin times (PT) and impaired whole blood clot retraction. A discrepancy between low PTs and normal or only slightly depressed factor II, V, VII and IX activities has been explained by an inhibitor of the extrinsic system. The decline in factor V is often most pronounced. About 1-2% of the patients exhibit a heparin rebound phenomenon. Hepatic dysfunction or severe depression of vitamin K dependent factors due to oral anticoagulation occurs in about 1% of the patients [7, 8]. Disseminated intravascular coagulation occurs in less than 1%. The incidence of increased fibrinolysis varies with the mode of assessment, but is believed to be a rare cause of excessive bleeding, which does not warrant the regular use of epsilon-aminocaproic acid [2].

Platelet defects

Many of the reports related to the increased bleeding tendency in CPB patients are due to platelet damage. During CPB the platelet count drops to between 30 to 50% of the prebypass levels with usually a slight recovery in the first hours following bypass. The platelet count remains at this low level for 2-3 days. Thereafter it increases and returns to the preoperative level on the 6th or 7th day [9-11].

Only limited information is available on the course of platelet function. Platelet adhesiveness falls precipitously at the onset of CPB, remains low during perfusion and then sometimes returns to the normal range shortly thereafter, but usually not before the third postoperative day. Platelet aggregation with ADP, adrenalin and collagen decreases during CPB and increases again after the third postoperative day, reaching preoperative levels on the 6th day and maximum values on the 13th day, after which it declines to normal [8, 9, 11]. Platelet factor 4 (PF₄) levels in plasma increase during CPB and on the following day. The postoperative bleeding time is usually markedly prolonged.

Factors which contribute to the thrombocytopenia and platelet dysfunction include haemodilution, shear stresses, platelet surface interface, platelet gas interface, heparin and protamin.

The decrease in platelet number by *haemodilution* depends on the volume of clear prime used in the extracorporeal circuit. Priming volumes range from 1.5 to 3 l. The decrease of about 30–50% is attributable to haemodilution. The measured decrease, however, is 50–70%, but the actual platelet loss may be even greater because there is a rapid release of platelets from the splenic pool, which harbours about 40% of the total platelet population. It is known that during CPB platelet aggregates are sequestered in various sites, the major site being the liver. The rebound of the platelet count immediately after CPB has been attributed to the deaggregation of platelets and the release from their sites of sequestration [12]. Some priming solutions such as dextran have an inhibiting effect on platelet function. The platelet loss by haemodilution can be reduced by using devices with small priming volumes and by using platelet compatible priming solutions [13].

Shear stresses are generated by the cardiotomy suction, pumps, connectors, geometry of the oxygenators and high blood flow rates. These factors induce turbulence of the laminar blood stream which causes destruction of platelets. Shear stresses and resultant platelet damage can be reduced by the use of controlled cardiotomy suction, non-occlusive pumps and well designed oxygenators which are efficient at low flow rates [14, 15].

The platelet surface interface is actually an interaction of platelets with plasma proteins on the synthetic surface. The protein layer has a depth of approximately 20 μ M and is composed largely of fibrinogen, gammaglobulins, albumin and prothrombin. Adsorption of coagulation proteins, factor XII, prekallikrein and kininogen occurs with ensuing activation of the intrinsic pathway of coagulation. Platelet adhesion is primarily an enzymatic mechanism but some physical adhesion may be involved. The adherence of platelets results in a decrease in the platelet count proportional to the area of the synthetic surface.

A reduction of platelet loss has been attempted by the complete but immediately reversible pharmacological inhibition of platelet function. This requires a platelet function inhibitor which is intravenously effective, has an immediate action, a strong effect and which is directly reversible and without side effects. The platelet function inhibitor PGI₂ is up to now the substance which meets most of these requirements [16].

Since 1980, studies have been published on the effect of PGI₂ on the platelets during CPB in man (Table 1). Radegran *et al.* [17] obtained platelet preservation with dosages of 50 nanograms per kg per minute. Patients received PGI₂ only during the first 30 minutes of bypass. The inhibition of platelet function during this period was effective enough to result in higher platelet counts for the following period. These results emphasize that the events at the start of CPB determine, to a large extent, the degree of platelet loss. The postoperative blood loss was slightly less in these patients than in the non-treated control group. However, PGI₂ has an important hypotensive side-effect. This dose of PGI₂ resulted in a significant fall in arterial blood pressure to 26 mmHg. Such a perfusion pressure is generally considered to be unsafe.

Longmore *et al.* [18] treated 12 patients undergoing coronary artery bypass grafting (CABG) with PGI₂. They found preservation of platelet number and function, and a significant decrease in postoperative blood loss, as well as a reduction in postoperative blood requirements. PGI₂ also appeared to have a heparin sparing effect and the hypotension was reported not to be troublesome.

Walker *et al.* [19] and Faichney *et al.* [20] treated 28 patients with PGI₂. Platelet counts were better maintained and a decrease was found in beta-thromboglobulin levels and platelet factor 4 release in the PGI₂ treated group. Furthermore, a reduction of platelet and fibrin deposition on the arterial mesh filter was observed, which might benefit the microcirculation. However, a statistically significant reduction in the extent of bleeding after bypass of about 400 ml could only be found in the patients undergoing valve replacement, and hypotension was found not to be a problem. An interesting phenomenon in this study was the greatest fall in platelet count occurring in the first 15 minutes after CPB. This suggests a relation with the administration of protamine. Continuation of PGI₂ until protamine has been given might have preserved the platelets from the protamine induced damages. Fabiani *et al.* [21] treated 13 patients with PGI₂. This resulted in higher platelet numbers and a more depressed platelet function during CPB. After CPB significantly higher platelet number and function could be measured. The postoperative blood loss was slightly less in the PGI₂ treated group. The mean arterial blood pressure decreased significantly ($p<0.05$), requiring transfusion and resulting in decreased urine production. In this study also a significant improvement of platelet aggregation is documented after PGI₂ treatment.

Table 1. Studies of PGI₂ in clinical cardiopulmonary bypass

	n	Dose	Operation	Platelet count	B-TG	PF ₄	TXB ₂	HSE	Part	Platelet aggregation	Blood loss
Radegran	10	2-20		=					43		
	12	50		+*				+	26*		-
Longmore	12	20	CABG	+*					58	+	-*
Walker	24	20	CABG (14)	+*	-*	-*	=				-
			VR (7)	+	-*	-	=				-
Faichney	28	20	CABG/VR	+*	-*	-*	=		45		-
											(CABG)
											-*
											(VR)
Fabiani	2	50	CABG/VR								
	11	25		+*				+	55*	(+*)	-

n = number of patients; B-TG = beta-thromboglobulin; PF₄ = platelet factor 4; TXB₂ = thromboxane B₂; HSE = heparin sparing effect; Part = lowest mean arterial pressure; CABG = coronary artery bypass grafting; VR = valve replacement; + = increase; = = no change; - = decrease; * = change is statistically significant.

The discrepancies in vasodilating effect of PGI₂ in these studies are most likely due to differences in haemodynamic conditioning of the patients. In a clinical study with PGI₂ it was found that the degree of hypotension correlates with the systemic vascular resistance (SVR) at the start of the infusion.

Although PGI₂ can preserve platelet numbers and function during clinical CPB, no significant reduction of postoperative blood loss was obtained in most studies. However, hypotensive side-effects were often experienced. The instability of PGI₂ at room temperature is a further limitation for routine use. Recently a stable PGI₂ analogue, a carbacyclin derivate (ZK 36374 Schering, Berlin) has been developed. Experimental studies showed an appreciable dissociation between antiplatelet and blood pressure lowering activities of this compound. ZK 36374 might therefore be superior to PGI₂ for *in vivo* use [22]. Further studies are needed to show that this compound can be used to preserve platelets during CPB.

E.C.C. component analysis

It is well recognised that the bubble oxygenator and uncontrolled cardiotomy suction are the main sources of platelet damage [15]. The effects of both are dependent on the duration of bypass while the

amount of suction also varies widely among the different types of operations [23]. We evaluated the effects of these two factors on the transfusion requirements in a retrospective study over a 3 year period. In coronary artery bypass grafts (CABG) operations, transfusions of whole blood with a bubble oxygenator were about equal in the perfusion periods 1–2 and 2–3 h. However, the need for blood products, such as fresh plasma and platelet concentrates, increased in the 2–3 h perfusion period.

By replacing the bubble oxygenator (BO) with a membrane oxygenator (MO) to reduce platelet damage, there was significant reduction of whole blood transfusion (50%) and the need for blood products (80%) in the 1–2 h perfusion periods. An increase in the perfusion time employing a MO does significantly increase the blood transfusion requirements. Whole blood requirements almost doubled for each hour that the perfusion period was prolonged. The use of blood products increased considerably when the perfusion period exceeded three hours.

In a recent study [24] it has been identified that the increasing volume of cardiotomy suction during longer perfusion periods is the main source for platelet damage, and is responsible for the increase in blood loss, thus negating the beneficial effect of a membrane oxygenator. This major effect of conventional uncontrolled cardiotomy suction on platelet damage is apparent from the fact that the reduction in blood requirements using a membrane oxygenator is shown more in the shorter perfusion periods, rather than in the long perfusion requiring increased cardiotomy suction. This was demonstrated in a recent study comparing platelet damage using a bubble oxygenator and membrane oxygenator perfusion in CABG operations of over 3 h [25]. This study found whole blood requirements and the need of blood products were about the same in both groups while our results, with shorter perfusion periods, showed large differences.

The amount of suction may also play a role in explaining differences in blood transfusion requirements among the various types of operations. The same effects of perfusion times and BO and MO perfusion described for the CABG operations

were seen for the mitral valve replacements (MVR). A doubling of the donor units was required when the perfusion period was prolonged and there was considerable reduction of donor units if a MO was used. This was not the case in the aortic valve replacements (AVR). This might be explained by the fact that in the AVR operations the amount of cardiotomy suction varied with the quality of the aortic wall, which determined the bleeding to a large extent. The platelet damaging effect of large amounts of uncontrolled cardiotomy suction has already been demonstrated and might also explain the increased need for postoperative blood transfusion after short perfusion periods. In the longer perfusion periods there was in particular a greater need for blood products in the BO perfusion. Because of this a membrane oxygenator was preferred when perfusion periods of over 3 h were expected. The total amount of donor units required was about the same in the CABG and AVR when the perfusion time was over 2 h. Also in the AVR a further reduction of blood requirements can be expected if controlled cardiotomy suction is employed in conjunction with the use of a membrane oxygenator.

In the operations for congenital heart diseases minimal blood is required in the short perfusion period of less than one hour. In the perfusions between 1 and 2 h again a reduction of about half of the blood requirements were noted with the use of a membrane oxygenator. In comparable perfusion periods there were no significant differences in total blood requirements between the congenital and the acquired heart diseases except for AVR operations in the 1–2 h perfusion period, which exceeded all the others. Blood requirements seem to indicate an increased bleeding tendency post-operatively. The use of blood products is largely dependent on availability of blood products, indications and policy of treatment, which might differ from centre to centre.

The Kansas City Blood Center experience

Cardiac surgery patients in Kansas City have been transfused with an average of 3 to 4 units of whole

blood and/or packed cells since 1974 [26]. The trend, however, from 1981 through the first quarter of 1983, has been toward a slightly higher number of transfused units per case. In attempting to analyse this trend we have compared the blood usage of hospitals which have not changed their cardiac surgery staff since 1978 and which perform cardiac surgery on adults. Table 2 illustrates that the trend toward increased blood use has also occurred in these institutions, indicating that increased usage is not due to a turnover of surgeons. We attempted to determine if the increase in use in 1982 occurred because patients were being operated on with much shorter notice, such as would occur if hospitals were performing less elective cases and more semi-emergency cases. It was found that 46% of 1109 cases, analysed in a six month period in the same five hospitals, were operated on with 24 h or less notification. However, the lowest average use of blood occurred at the two hospitals that had the highest and lowest (71% and 34% respectively) number of cases with the shortest notification times. Thus, the increased usage could not be attributed to increasing number of semi-emergency cases. Another possibility was that the increased use of blood was due to increasing numbers of repeat cardiac surgery cases. Table 3 shows the blood usage in three hospitals in which the coronary artery bypass grafts (CABG) and other cases are subdivided into first time and repeat procedures. The blood usage in 1982 is compared to the usage in 1977. Blood use per patient in all CABG operations increased from 2.6 to 3.5 units and for all other operations, it increased from 4.7 to 5.5 units. The increase was not explained by the

likelihood of more repeat operations today than in the past as the repeat cases only made up 10% of the total.

The slight increase in average blood use might be explained by an increase in haemostasis problems for heart surgery patients. This is illustrated by the findings shown in Table 4. There is no significant difference between the percentage of patients receiving platelets or fresh frozen plasma in 1980, the year the least blood per patient was used, compared to 1977. The percentage of patients receiving platelets or fresh frozen plasma in 1982, however, rose dramatically and increased again in the first quarter of 1983. This trend was particularly noticeable in one institution, even though their surgical staff had not changed. The percentage of patients receiving fresh frozen plasma increased more than the percentage of patients receiving platelets. A six-fold increase in fresh frozen plasma use and a four-fold increase in platelet use has occurred in CABG surgery. An even greater increase of platelet use was noted in the repeat CABG cases.

We also investigated whether the increased use of fresh frozen plasma could be explained by a change in the age of blood used in these patients. Table 5 illustrates no difference in this parameter

Table 3. Comparison of blood and component transfusion

	Cases	Blood	Average FFP	Platelets
1982				
ALL CABG	922	3251	3.5	218
1st Cabg	828	2846	3.4	190
Repeat Cabg	94	405	4.3	28
ALL OTHER	323	1772	5.5	335
1st Other	299	1626	5.4	305
Repeat Other	24	146	6.1	28
1977				
ALL CABG	1259	3312	2.6	51
ALL OTHER	510	2397	4.7	122

Table 4. Percent of all heart surgery patients transfused platelets and fresh frozen plasma in 5 hospitals compared to 1977.

	1977	1980	1982
Platelets	2.4%	2.1%	6.6%
Fresh frozen plasma	3.8%	4.5%	12.1%

Table 2. Average blood use in 5 institutions since 1978

Hospital no.	1978	1979	1980	1981	1982	1983 (1/4)
1	3.7	2.4	1.7	2.0	2.6	2.2
2	4.0	2.9	3.1	3.5	4.4	4.3
3	3.5	3.4	3.1	3.5	4.2	4.5
4	4.1	3.9	4.2	4.9	4.7	4.5
5	2.6	2.9	2.7	3.3	2.6	2.9
Average	3.6	3.2	3.0	3.4	3.7	4.0
No. of cases	1474	1711	1777	1785	1829	564

between 1980 and 1982 to account for the increase in transfusions. The anticoagulant-preservative solution used for the collection was changed to CPDA-1 in September 1980. However, assays of the coagulant factors V and VIII were no different from that found in blood collected in ACD, offering no explanation for the increased transfusion of fresh frozen plasma. The percentage of blood transfusions given to these patients as packed red blood cells increased from 23% to 32% between 1980 and 1982, and this perhaps could partly explain some of the increase in fresh frozen plasma transfusions. It is not, however, likely to be the cause, but rather the result of a hemostasis problem since fresh frozen plasma transfusions were not increased in some institutions using an even greater percentage of blood as packed red blood cells. The increase in platelet transfusion is most likely explained by the increased use of aspirin and dipyridamole for patients with coronary artery disease and also because of the awareness that graft patency is presumably improved with the pre-operative administration of these two drugs.

The initial report on the beneficial effects of dipyridamole and aspirin described no difference in blood loss, red cell replacement, fresh frozen plasma and platelet use, or the incidence of re-operation in treated versus untreated patients [27]. No data was given for platelets or fresh frozen plasma transfusions but the total red cell transfusions averaged 4.7 units, an amount higher than the 3.5 units transfused to our CABG patients.

Dipyridamole and aspirin are given for their platelet inhibition effect. In the dosage given they should not affect plasma coagulation factors. The surgeons, however, have noted more patients oozing from the operative sites and we suspect that fresh frozen plasma, as well as platelets, have been

Table 5. Blood age time of transfusion in percentage.

Age of blood in days	0-2	3-5	6-8	9-10	>10
1977	7	20	24	9	40
1980	17	38	28	5	12
1982	20	40	24	4	12

transfused empirically because of this clinical observation. Interestingly, only one of the three surgical groups analysed, who switched to dipyridamole and aspirin perioperatively, and who had a slight increase in blood use for CABG, had an inordinate increase in FFP and platelet use.

The mortality rate remained unchanged from the 1-2% reported in 1977 and was no different in the groups receiving and not receiving platelets and FFP. Thus, postoperative bleeding was not excessive and did not need to be treated with additional platelet and FFP transfusions. A similar transfusion pattern occurred in these three hospitals for the non-coronary artery cases. Dipyridamole and aspirin were not used perioperatively in the non CABG patients. When comparing transfusion patterns in these surgical groups, it was observed that 11.3% of the patients received platelets and 11.8% received FFP in two institutions; 36.5% received platelets and 22.6% received FFP in the third hospital.

From the foregoing discussion it may be concluded that today in Kansas City, despite the use of platelet inhibition agents, more semi-emergency and repeat surgery, the use of whole blood and packed red cells has only slightly increased. The transfusion of platelets and frozen plasma to patients on dipyridamole and aspirin offers no real advantage and need not to be given.

Future research intended to reduce the demands for donor blood in cardiac surgery is most likely to be successful if it is directed to:

- the initial phase of blood circuit contact;
- the anticoagulants used, including inhibition of platelet function;
- and last but not least, proper surgical techniques.

References

1. Ott DA, Cooley DA: Cardiovascular surgery in Jehovah's Witnesses. *JAMA*, 1977; 238: 1256-58.
2. Lambert CJ, Marengo Rowe AJ, Leveson JE, Green RH, Thiele JP, Geisler JF, Adam M, Mitchell BF: The treatment of postperfusion bleedings using e-amino caproic acid, cryoprecipitate, fresh frozen plasma and protamine sulphate. *Ann Thor Surg*, 1979; 28: 440-44.

3. Hardin SA, Shakorr MA, Guidon AJ: Platelet support for cardiopulmonary bypass surgery. *J Thorac Cardiovasc Surg*, 1975; 70: 350-53.
4. Gomes MMR, McGoon DC: Bleeding patterns after open heart surgery. *J Thorac Cardiovasc Surg*, 1970; 60: 87-97.
5. Geralnick HR, Fisher DR: The haemostatic response to open heart operations. *J Thorac Cardiovasc Surg*, 1971; 61: 909-15.
6. Bachman F, McKenna R, Cole ER, Njafi: The haemostatic mechanism after open heart surgery. *J Thorac Cardiovasc Surg*, 1975; 70: 76-85.
7. Moriau M, Masure R, Huslet A, Deteys C, Chalant C, Ponlot R, Jaumain P: Haemostasis disorders in open heart surgery with extra corporeal circulation. *Vox Sang*, 1977; 32: 41-51.
8. Kalter RD, Saul ChM, Wetstein L, Sorians C, Reiss RF: Cardiopulmonary bypass associated haemostatic abnormalities. *J Thorac Cardiovasc Surg*, 1977; 77: 427-35.
9. McKenna R, Bachmann F, Whittaker B, Gilson JR, Weinberg M: The haemostatic mechanism after open heart surgery. *J Thorac Cardiovasc Surg*, 1975; 70: 298-308.
10. McKenzie FN, Dhall DP, Arfors KE, Nordlund S, Matheson NE: Blood platelet behaviour during and after open heart surgery. *Br Med J*, 1969; 2: 795-98.
11. Kennedy PS, Soles RTh, Storey SS, Viancos JG, Debakey ME: Cardiopulmonary bypass surgery. *Arch Surg*, 1978; 113: 1429-32.
12. De Leval MR, Hill JD, Mielke JD, Macur MF, Gerbode F: Blood platelets and extracorporeal circulation. *J Thorac Cardiovasc Surg*, 1975; 69: 144-51.
13. Smith B, Al Omeri M, Melrose DG, Bentall HH: Blood loss after cardiopulmonary bypass. *Lancet*, 1964; ii: 273-79.
14. Ten Duis HJ: Intraoperative autotransfusion. Thesis, University of Groningen, 1982.
15. Van den Dungen JJAM, Karliczek GF, Brenken U, Homan van der Heide JN, Wildevuur ChRH: Clinical study of blood trauma during perfusion with membrane and bubble oxygenators. *J Thorac Cardiovasc Surg*, 1982; 83: 108-16.
16. Moncade S, Vane JR: Arachidonic acid metabolites and the interactions between platelets and blood vessel walls. *N Eng J Med*, 1979; 300: 1142-47.
17. Radegran K, Papaconstantinou C: Prostacyclin infusion during cardiopulmonary bypass in man. *Thromb Res*, 1980; 19: 267-70.
18. Longmore DB, Greame Bennett J, Koyle RM, Smith MA, Gregory A, Orivand T, Gones WA: Prostacyclin administration during cardiopulmonary bypass in man. *Lancet*, 1981; i: 800-3.
19. Walker ID, Davidson JF, Faichney A, Wheathley DJ, Davidson KC: A double blind study of prostacyclin in cardiopulmonary bypass surgery. *Br J Haematol*, 1981; 49: 415-23.
20. Faichney A, Davidson KC, Wheathly DJ, Davidson JF, Walker ID: Prostacyclin in cardiopulmonary bypass operation. *J Thorac Cardiovasc Surg*, 1982; 84: 601-8.
21. Fabiani JN, Bunting S, Ferrier E, O'Grady J, Fontane B, Prigent C, Carpenterier A, Vane J, Dubost C: Etude clinique de prostacycline dans les circulations extra corporelles; effects sur l'hemodynamique et la coagulation. *Arch Mal Coeur*, 1982; 75: 241-48.
22. Schröder K, Dauris H, Matzky R, Ohlendorf R: The anti-platelet and cardiovascular actions of a new carbacyclin derivative (ZK 36374) equipotent to PGI₂ in vitro. *Arch Pharmacol*, 1981; 316: 252-55.
23. Boonstra PW, Karliczek GF, Homan van der Heide JN, Wildevuur ChRH: Cardiotomy suction related to type of operation and perfusion time. Submitted for publication.
24. Boonstra PW, van Imhoff GW, Eijssen L, Kootstra GJ, Homan van der Heide JN, Karliczek GF, Wildevuur ChRH: Reduced platelet damage and improved haemostasis after controlled cardiotomy suction during clinical membrane oxygenator perfusions. Submitted for publication.
25. Boonstra PW, Vermeulen FEE, Leusink JA, De Nooy EH, van Zalk A, Soons JBJ, Wildevuur ChRH: Clinical evaluation of the haematological advantage of a membrane oxygenator over a bubble oxygenator in cardiopulmonary bypasses of long duration (3 hrs.). Submitted for publication.
26. Bayer WL, Coenen WM, Jenkins DC, Zucker ML: The use of blood and blood components in 1769 patients undergoing open-heart surgery. *Ann Thorac Surg*, 1980; 29: 117-22.
27. Chesbro JH, Clements IP, Fuster V: A platelet inhibitor drug trial in coronary artery bypass operations; benefit of preoperative dipyridamole and aspirin therapy on early post-operative vein graft potency. *N Engl J Med*, 1982; 307: 73-78.

14. Supportive therapy for liver transplantation

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Introduction

Transplantation of the liver in man has been a practical possibility for over fifteen years. Problems of tissue matching and rejection are significantly less than those found in renal transplantation [1]. The principal stumbling block is the technical complexity of the operation. Not only is the surgical procedure difficult and time consuming [2], but the perioperative management of the patients by the anaesthetic team requires considerable resources of manpower, equipment and laboratory facilities.

There is no artificial liver as there is a renal dialysis machine, and therefore the patients cannot be maintained by supportive therapy in a relatively satisfactory state awaiting surgery. The selection of patients for transplantation depends on the ability to predict the onset of end-stage liver disease and a life expectancy of no more than three to six months [3]. Inevitably some patients deteriorate and succumb during that time, and there is a considerable variability in the condition of patients at operation. This variability significantly affects the perioperative prognosis and means that there has to be a built-in flexibility in the treatment protocol to correct insofar as possible, haematological and biochemical abnormalities. The organizational back-up required for liver transplantation is considerable, and requires not only a team of some twenty medical specialists, but also a complete round-the-clock laboratory service, and extensive on site blood preparation facilities [4].

Preoperative preparation of the patient

The majority of patients presenting for liver transplantation fall into three groups depending on diagnosis: primary biliary cirrhosis, chronic active hepatitis, and primary hepatocellular carcinoma. The rest present with a variety of congenital anatomical defects, or as a result of inborn errors of metabolism. The hepatocellular carcinoma patients present with minimal changes in hepatic function, and are usually preoperatively clinically fit. Blood loss and coagulation problems during operation are therefore limited, and these patients are among the easiest to transplant. Unfortunately the rate of undetected metastasis is high [5] and significantly reduces the long-term survival rate. Primary biliary cirrhosis yields the largest number of patients where the liver is small and contracted, the operation becomes technically easier. The earlier the operation, the less problem with coagulation function postoperatively. The greatest risk is with chronic active hepatitis patients who have not only liver dysfunctions related to the cirrhosis, but also have severe portal hypertension. This gives rise to bleeding from varices in the preoperative period, with concomitant loss of coagulation factors, and also operative bleeding from the many huge collateral vessels.

Upon these diagnosis and the assessment of their severity rests the choice of the scheme to be used to maintain blood volume and function during the procedure. Severe bleeding can, however, occur unexpectedly during any liver transplantation, either from the incision or the vascular ana-

stomoses, and some flexibility of response must be available.

The general condition of the patients before operation is very variable and can change from day to day in the individual. The patients must be free from cardiovascular and renal defects, and vitamin supplements and blood components are given as required to maintain coagulation function. On the day of operation a full baseline review of blood biochemistry, haematological and coagulation status is necessary for the planned supplementation therapy to begin. Hypokalaemia and hypocalcaemia are corrected as far as possible before the operation is begun. Table 1 shows the principal laboratory data freshly available to the anaesthetist before operation.

Liver transplantation, the operation

To understand the problems encountered, it is necessary to describe briefly the procedure of orthotopic liver transplantation [2, 6]. When a donor liver becomes available, a team of surgeons goes to the donor hospital to remove it. At the same time the recipient is prepared for surgery. Blood is taken for base line investigations, and, where necessary, corrective therapy is begun. Approximately ninety minutes before the expected arrival time of the donor liver, the patient is anaesthetized and monitoring is set up according to a

scheme similar to that set out in Table 2. Initial preparative surgery is begun up to, but not beyond the point of no return in the resection of the diseased liver. At this stage, while waiting for the arrival of the donor team, the patient can be assessed particularly with regard to the blood loss up to this point, and to clinical evidence of *in vivo* clotting. On arrival of the donor liver the free preparation of the old liver is completed and the resection performed.

During the pre-anhepatic phase (Table 3), the anaesthetic team can get a good idea of the scale of bleeding and clotting problems they are likely to expect, and are able to assess the patient's cardiovascular response to the stress of operation. When the liver is resected, portal and systemic blood flow from the lower half of the body are stopped, and there is a massive fall in cardiac filling, with an additional loss of blood via the descending aorta to the lower extremities. Rapid transfusion at this time may help to prevent serious hypotension, but it is not possible to return the cardiac output to normal until the circulation is

Table 2. Patient monitoring during liver transplantation.

Cardiovascular

ECG, Peripheral pulse, Beat-to-beat rate, Radial artery pressure, Central venous pressure, Pulmonary artery pressure (flow directed catheter), Cardiac output (thermodilution).

Respiratory

Respiratory pressure, Central temperature, Expired volume, Peripheral temperature, End expiratory CO₂, Urine production, Inspired gas temperature.

Table 1. Preoperative laboratory investigations.

Biochemistry	Haematology
Na, K, Cl,	Hb, HCt, Diff.,
Urea, Creat., Urate,	Leucos, Thrombocytes
Alk. Phos., LDH, SGOT,	
SGPT, Bilirubin, Prot/Alb.,	
Glucose, Ca, P.	
Coagulation	Acid-base
PTT (Quick)	pO ₂
APTT (cef)	pCO ₂
Reptilase	SO ₂
Fibrinogen	pH
FDP (Latex)	Bicarbonate
Antithrombin	Base excess

Table 3. The stages of liver transplantation.

1. Anaesthetic preparation	1 h
2. Pre-anhepatic phase (dissection of old liver and haemostasis)	1½-2½ h
3. Anhepatic phase (implantation of major liver vessels)	1-2 h
4. Recirculation	10 min
5. Hepatic artery and biliary reconstruction and haemostasis	2-3 h
6. Transport to ITU	

fully re-established. At the end of the anhepatic phase, the liver is progressively reconnected to the circulation with a rapid inflow of acid metabolites to the circulation, both from the donor liver and the lower body. It may also not always be possible to vent completely the donor liver in order to flush out all the preservation fluid, and a large bolus of potassium rich blood can contribute to a temporary rise in the serum level. Biochemically, therefore, the recirculation places a severe strain on the cardiovascular system, and it is least able to cope with the additional problems of massive blood loss that can also occur at that time. Measures have to be taken to keep optimum cardiac filling pressures, to maintain the temperature of the infused blood at about 32°C (preventing hypothermic arrhythmias [7], and to prevent unnecessary oozing by restoring clotting function as quickly as possible.

During the post-anhepatic phase, when the hepatic artery and biliary tract have to be constructed, considerable blood loss may occur, due to both leakage from the many vascular anastomoses, and from inadequate coagulation. The anaesthetist is required to be able to cope during operation with a blood loss that in surviving patients may vary between one and over twenty litres. To do this the anaesthetic team must work hand in hand with the blood bank staff, and the laboratory haematologist, who are an integral part of the transplant team.

Preparation of blood and blood products

In placing a patient on the waiting list for liver transplantation, a preliminary assessment of the patient is made by the anaesthetist and the hepatologist as to the extent of possible bleeding and clotting problems, and a list of requirements can be drawn up according to prearranged formulae such as those seen in Tables 4 and 5. At any time not more than a limited number of patients can be held ready for transplantation because from the moment that the patient is put on 'green light' for transplantation, blood and components have to be prepared and held for the patient in agreed quantities. It had been hoped initially that the pre-

operative screening of patients would allow prediction of the patient's needs during operations, at least for blood components. The large variability in blood loss that in fact occurs has prevented this and every centre has had to adopt an empirical approach. For this scheme A (Table 5) has been used in Groningen for patients in a reasonable condition, with normal or corrected *in vitro* tests before operation, and scheme B was used for all chronic active hepatitis patients, patients who had been bleeding in the preoperative period, and patients in whom correction of clotting was still underway at the start of operation. Flexibility allows switching from one scheme to another under defined circumstances which will be discussed. With the improved keeping properties of CPD-A blood

Table 4. The preparation of blood components for liver transplantation.

Blood	15 units whole blood (recent date)
	15 units whole blood (any date)
	10 units red cell concentrate
Coagulation factors	(minimal clotting disturbance)
Fresh frozen plasma	16 donor units
Cryoprecipitate	8 packs of 4 donor units
Thrombocytes	3 packs of 6 donor units
Coagulation factors	(severe clotting disturbance)
The above quantities of FFP, Cryo and Thrombocytes	are doubled.

Table 5. Correction Schemes for Coagulation during liver transplantation.

Scheme A	
THROMBOCYTES:	
Stage 2	only when indicated
Stage 4	6 donor units
Stage 5	6 donor units or more ordered if necessary
Fresh frozen plasma	5 ml · kg ⁻¹ · h ⁻¹
Cryoprecipitate:	1 ml · kg ⁻¹ · h ⁻¹

Scheme B	
THROMBOCYTES:	
Starting with induction of anaesthesia	6 donor units per hour, running through the procedure.
Fresh frozen plasma:	
10 ml · kg ⁻¹ · h ⁻¹	
Cryoprecipitate:	2 ml · kg ⁻¹ · h ⁻¹

(8), and the availability of fresh frozen plasma (FFP) and cryoprecipitate, the use of fresh blood for liver transplantation is of questionable value, although still practiced in several centres, for clotting correction on recirculation.

One measure which can be adopted is to save the later dated blood until after the risk of severe bleeding is over in the hope to optimalize its oxygen carrying capacity in the postoperative period. Centres in both Europe and America [9] have adopted methods of infusion that allow the rapid infusion of either plasma reduced whole blood, or red cell concentrate diluted with saline or an albumin solution to overcome resistance.

The organizational problems of supplying the quantities of blood necessary for liver transplantation are immense [10]. Not only must the blood bank preferably be on site, and large enough to accommodate the blood (up to fifty units) and components, but it must also be able to prepare short life components on a priority basis 24 hours a day. There must, in addition, be enough over-capacity to fulfil all the other needs of the hospital, otherwise the necessary good-will needed to operate an organ transplant service will quickly evaporate. In most circumstances this means that a blood transfusion laboratory of regional size must participate, and that a senior member of its staff be a member of the liver transplant team.

Techniques of infusion

Blood loss during liver transplantation can vary between 1.5 and over twenty litres. Typical peaks are met at times of initial incision and recirculation, and, in order to maintain cardiac output, it is necessary to infuse quickly, in our experience at least up to 500 ml/min. At such rapid rates, measures have to be taken to ensure that red cells are not damaged, and that the blood is brought quickly up to room temperature. Strunin [9] has suggested that an auto transfuser pump can be used to facilitate administration and modifications of this technique have been used with success in London, Groningen and Pittsburg.

The present model which we use is shown in

Fig. 1. Blood runs through filters into a cardiotomy reservoir is infused through a standard cardiopulmonary bypass roller pump. In order to prevent sludging when the patient is not being transfused, blood can be slowly recirculated back to the reservoir. Warming is quickly and efficiently accomplished using a countercurrent heat exchanger, also borrowed from a heart lung machine. The weakest link in such a system must be the connection to the patient. Fortunately the large majority of liver transplant patients have large and accessible peripheral veins. In place of conventional intravenous cannulas it is more convenient to use catheter introducers which are available in sizes up to 8 FG and can be introduced by the Seldinger technique into any accessible vein. Using such large access routes has two advantages; firstly, flow resistance is minimized, limiting pump pressure, and secondly inflow turbulence at the cannula is considerably reduced. These factors help to reduce erythrocyte damage.

Figure 2 shows a typical transfusion record from a liver transplant patient. With the high infusion rates that frequently have to be used, peripheral veins have the advantage that there is less risk of inadvertently missed extravascular infusion a known and possibly lethal complication of internal jugular or subclavian cannulation [11].

Coagulation factors and other fluids can be given either through additional (smaller) intravenous lines, or T-ed into the infusion system at a point near the patient.

Peroperative coagulation problems

Coagulation problems can arise at any time during the procedure, either as a continuation of the existing uncorrected preoperative state, or may arise as a result of excessive transfusion and loss or dilution of clotting factors. Monitoring of coagulation takes place in two equally important ways.

Clinical observation plays a critically important role in the management of intraoperative coagulation; constant and continuous monitoring of the rate of loss of blood gives an idea of the rate of loss of clotting factors and the need to replace them, on

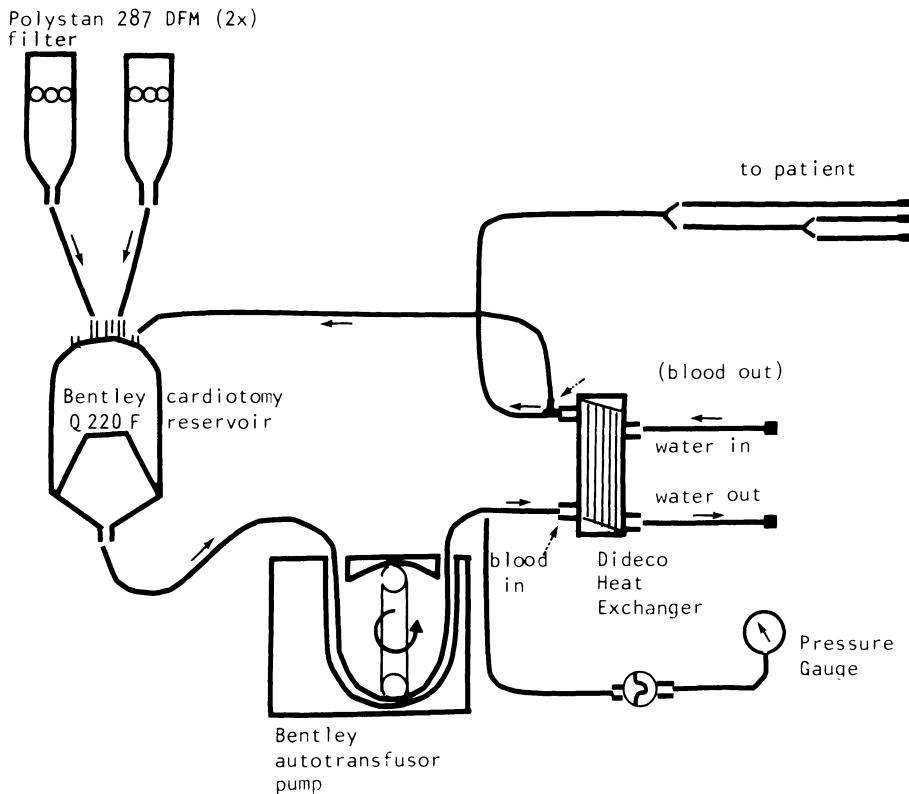


Fig 1. Blood infusion system for liver transplantation.

a purely empirical basis. Such action can only later be confirmed and checked by laboratory investigation. Observation of bleeding and clot formation in the wound is also an essential diagnostic procedure; whatever the laboratory findings, the blood *in vivo* may not clot, and this may be due to disorders of acid-base, ionised calcium, temperature, or oxygen transport, outside the scope of laboratory coagulation testing. As a functional test in the operating room, the thromboelastogram [12] has been used as a predictor while awaiting restoration of clotting function towards the end of operation.

Coagulation protocols, when used, have to be based initially on the preoperative findings and later modified as a result of clinical findings. What is therefore the role of the laboratory investigation? Experience has shown that clotting investigations conducted during operation show trends, and provide a check that the correct scheme is being followed. In addition, trends in the thrombocyte level can only be followed by regular investigation.

Table 6 lists the sort and frequency of tests that can be performed in relation to the stage of the operations. With a latent period of around 30 minutes between sample and result the risk of serious deficit or over-supplementation ought not to be great. Finally the accumulated experience of the results of coagulation tests has enabled the formulation of simple schemes (Table 5) in subsequent patients.

The findings in a series of eleven consecutive

Table 6. Coagulation investigation during operation

PTT (Quick), APTT (cef), Reptilase (time), Fibrinogen, FDP (Latex, AT III, Thrombocytes).

AT:

1. Induction of anaesthesia
2. Incision
3. 5 min before anhepatic phase
4. 5 min before recirculation
5. 1 min after recirculation
6. After surgical haemostasis, before substitution, if necessary
7. 30 min after substitutive therapy
8. On indication

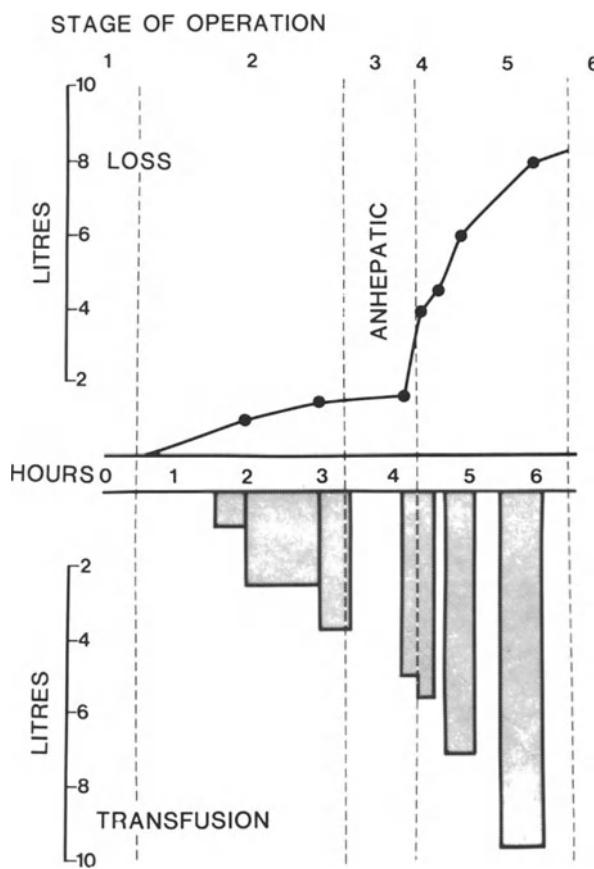


Fig. 2. Cumulative blood loss and transfusion during liver transplantation related to the stages of operation (Table 3).

perioperatively successful transplants are set out in Table 7 [13]. In these patients about half presented before operation with low thrombocyte levels, but this had no correlation with subsequent blood loss. It remains to be seen whether the temporary clamping of the splenic vessels as advocated in some centres will keep thrombocytes in the circulation. APTT and reptilase times remained high in a different half of the patients studied, but again there was no correlation with blood losses. Antithrombin III remained the only consistent parameter of prognostic value. Despite adequate provision of replacement clotting components, many patients never showed improvement in AT III levels, at least during operations, and there is little doubt that a combination of a poor preoperative state, a malfunctioning liver and the massive trauma of operation produces a state of severe disseminated intravascular coagulation, confirmed by high titres of fibrin degradation products.

Following operation the rate of blood loss is drastically diminished, and further correction of coagulation can take place at more leisurely pace. By far the major factor in the management at this stage is not the administration of coagulation factors, but more efficient oxygen transport, giving rise to a general important improvement in the patient's condition and therefore in thrombocyte and vessel wall function and permeability. The most dramatic effect is the coming 'on-line' of the

Table 7. Blood loss and clotting disturbances during liver transplantation

Transplant number	Duration (hours)	Blood loss (litres)	Thrombocytes pre-op ($\times 10^3/\mu\text{l}$)	AT-III pre-op	Other clotting tests		
					pre-op	during	post-op
11	7	2.5	> 60	119	C ↑ Q ↑	Rep ↑	norm
12	7	2.6	> 60	113	norm	norm	norm
13	8½	14.0	< 60	40	C ↑ Q ↑	AT III	C ↑
15	7	5.5	> 60	50	norm	norm	norm
16	7½	3.6	> 100	65	C ↑ Rep ↑	norm	norm
17	7	5.1	< 60	43	C ↑ Rep ↑	C ↑ Rep ↑	norm
18	11	42.0	< 60	47	norm	AT III	AT III
19	8	2.6	< 60	40	C	C ↑	C ↑
22	5	2.4	< 50	78	norm	norm	norm
23	9	9.8	< 60	45	norm	norm	norm
24	6½	2.8	> 60	83	norm	Rep ↑	norm

Q = PTT; C = APTT; Rep = Reptilase time.

transplanted liver itself. In some patients this is seen biochemically during the operation, with a rapid metabolism of citrate, uptake of potassium and glucose, signs that the new liver will shortly be taking on a synthetic function. The extent and speed to which this occurs determines the specific haematological therapy during the immediate postoperative period.

Correction of coagulation disturbances

The use of the schemes set out in Table 5 relies considerably on common sense. The patients are assessed throughout the operation (Table 6), but the main therapeutic activity takes place at the beginning of the operation, after recirculation of the donor liver, and following bouts of severe bleeding. It is important to know when not to waste valuable coagulation factors when unavoidable major blood loss is occurring, and this can only happen by the close attention of the anaesthetic team to the operation, and co-operation with the surgeon. Conversely when definitive large vessel haemostasis has been achieved, the anaesthetist should have anticipated and ordered a full 'packet' of components, in addition to red cells and be able to transfuse rapidly. To appreciate this urgent need it is helpful if the blood bank haematologist has had some operating room experience of the problems involved.

The use of plasma volume expanders

Replacement of blood volume with component therapy entails a continuous monitoring of haemoglobin, haematocrit, and serum protein levels. Patients with advanced liver disease frequently have severely impaired plasma protein production, with consequent ascites, and a reduced plasma osmolarity. In these patients human albumin preparations are used freely to raise the plasma levels to normal. Albumin preparations can also be used to dilute red cell concentrates as a prime in the infusion apparatus. Apart from the temporary correction of the transient changes that occur in the cir-

culating blood volume during induction of anaesthesia, synthetic plasma volume expanders such as dextran and gelatine are rarely used except in emergency.

Crystalloids are given as glucose, glucose/salt, and compound Ringer's lactate to maintain urine output and match evaporative losses from the vast operative exposure.

Future developments in liver transplantation depend on a solution to the problems of severe operative bleeding, particularly in patients with chronic active hepatitis.

Recent studies have indicated [14] that there is a close correlation between severe bleeding and minimal signs of renal impairment (impending hepatorenal syndrome) possibly due to pressure changes in the abdominal vasculature.

New techniques in blood autocollection, and the use of cell saver centrifuges [15] may help to limit the demand for donor blood. Blood can be collected and stored, using specially designed suction filters (Sorensen Inc.), anticoagulated, and stored. The advantage of this system is that a choice can be made later, to discard the blood, or wash and separate the red cells, or even in emergency to transfuse directly.

Various forms of vascular bypass either to return venous blood from the legs [16] or to maintain partial cardiopulmonary bypass [17] have met with limited success, but may be developed in the future to support the weakest patients.

The purpose of this chapter has been to illustrate a clinical situation in which blood and blood products have to be given in combination with a wide range of other therapeutic activities on a minute by minute basis.

Liver transplantation serves as a useful model for supportive therapy in other forms of major surgery, particularly trauma. The opportunity to study the effects of severe blood loss and massive transfusion with full laboratory investigation facilities has shown that the limit to the amount of blood that can be acutely transfused has yet to be defined, and secondly, how it is possible by component therapy to maintain coagulation function in the most adverse circumstances. On the other hand there remains the moral, ethical, and practical problem

with all massive transfusion situations of when to stop.

References

1. Calne RY (ed): *Liver Transplantation*. London, Grune & Stratton, 1983.
2. Starzl TE, Iwatsuki S, Van Thiel DH, Gartner JG, Zittelli BJ, Malatack J, Schade RR, Shaw BW, Hakala TR, Roseenthal JT, Ported KA: Evolution of liver transplantation. *Hepatology*, 1982; 2: 614-34.
3. Krom RAF, Gips CH, Houthoff HJ, Newton DEF, van de Waaij D, Beelen J, Haagsma EB, Slooff MJH: Orthotopic liver transplantation in Groningen (The Netherlands). *Hepatology*, 1984; 4: 616-656.
4. Sherlock S: Hepatic transplantation: The state of play. *Lancet*, 1983; 2: 778-9.
5. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, WB Saunders, 1969: 350.
6. Starzl TE, Marchioro TL, Von Kaulla HN, Hermann B, Brittain RS, Waddell WR: Homotransplantation of the liver in humans. *Surg Gynec Obstet*, 1963; 117: 695-76.
7. Boyan CP, Howland WS: Blood temperature: A critical factor in massive transfusion. *Anesthesiology*, 1961; 22: 559-63.
8. American Association of Blood Banks and American Red Cross: Circular of Information for the Use of Human Blood and Blood Components by Physicians. Washington, DC., Revised, 1978.
9. Strunin L: *The Liver and Anaesthesia*. London, WB Saunders, 1977: 121.
10. Goldsmith MF: Liver transplants: Big business in blood. *JAMA*, 1983; 250: 2904-5.
11. Van Berge Henegouwen DP: Infraclavicular subclavia-catheterisatie. Academic Thesis, University of Amsterdam, 1979.
12. Howland WS, Castro EB, Fortner JB, Gould P: Hypercoagulability, thrombelastographic monitoring during extensive hepatic surgery. *Arch Surg*, 1974; 108: 605-8.
13. Imhoff GV van, Wesenhausen H, Haagsma E, Snit Sibinga CTh, Krom RAF, Gips CH: Bleeding during orthotopic liver transplantation in man. In: *Haemostatic Failure in Liver Disease*. Amsterdam, Martinus Nijhoff Publishers, 1983.
14. Haagsma EB, Wesenhausen H, Imhoff GW van, Krom RAF, Gips CH: Predictive factors concerning haemostasis during orthotopic liver transplantation. *Eur Society for Organ Transplantation Proceedings*. Zürich, 1983; 40.
15. Ansell J, Parrilla N, King M, Fournier L, Symanski I, Doherty P, der Salm T, Cutler B: Survival of autotransfused blood cells recovered from the surgical field during cardiovascular operations. *J Thorac Cardiovasc Surg*, 1982; 4: 387-91.
16. Griffith BP, Shaw BW, Hardesty RL, Iwatsuki S, Bahnsen HT, Starzl TE: Veno-venous bypass without systemic auto-coagulation for human liver transplantation. *Surg Forum*, 1983; 34: 380-82.
17. Calne RY McMaster P, Smith DP, Craddock GN, Rolles K, Farman JV, Lindop M, Bethune DW, Wheeldon D, Gill R, Williams R: Use of partial cardiopulmonary bypass during the anhepatic phase of orthotopic liver grafting. *Lancet*, 1979; 2: 612-14.

15. Supportive therapy in the new born

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Supportive therapy in the newborn

Over the past thirty years paediatrics has seen enormous change and progress and nowhere are these better exemplified than in neonatal care. It is with the role of transfusion and the use of blood products in the newborn that this chapter is concerned.

Within the last decade greater success in the care of extremely preterm and low birth weight (LBW) infants has added a new dimension to paediatrics. Coincidentally the mortality from rhesus haemolytic disease of the newborn has diminished as a result of improvements in both obstetric and neonatal care, and the incidence of rhesus disease has fallen with the introduction of anti-D preventative programmes [1]. Much of the successful management of both premature and rhesus affected infants stems from a greater willingness to use blood products more extensively and logically, although many aspects of such care remain still to be explored. It is a subject requiring continuing clinical and laboratory collaboration.

Exchange transfusion

The credit for the introduction of exchange transfusion probably belongs to Wallerstein [2], who in 1946 described the removal of blood from the sagittal sinus and its simultaneous replacement through a peripheral vein. Improved techniques developed rapidly thereafter and there are now several well-established methods of exchange. The simplest and

most suitable for robust well grown infants involves a manual 'push-pull' method through an umbilical venous catheter. A second method involves simultaneous infusion of blood through a catheter in the umbilical vein and removal through a catheter in the umbilical artery. This method requires two operators and is usually indicated in the case of LBW, 'sick' or hydropic infants. A modification of this method involves the simultaneous infusion of blood via the umbilical vein by syringe-pump and withdrawal from the arterial catheter by an operator using a syringe. Meticulous recording of volumes throughout any of these techniques is essential. It is desirable to use blood pre-warmed to 37°C. In severe rhesus disease and hydrops fetalis, where the initial exchange is performed for severe anaemia ($Hb < 10 \text{ g/dl}$) a single blood volume exchange (80–90 ml/kg) is adequate. Further exchanges for hyperbilirubinaemia for whatever reason, usually require the exchange of twice that volume, namely 160–180 ml/kg. Such twice-volume exchanges result in an 80–90% removal of recipient cells. All exchanges should be performed slowly over $1\frac{1}{2}$ –2 h (1.5–2.0 ml/kg/min) using aliquots no greater than 10% of the estimated blood volume, particularly in small stressed infants. Umbilical catheters should be removed after exchange transfusion unless a clear indication exists for their retention. Prophylactic antibiotics may be indicated according to the practice of the individual neonatal unit. Our recommendations for exchange transfusion in hyperbilirubinaemia and haemolytic disease are indicated in Fig. 1 and Table 1.

The incidence of exchange transfusion for rhesus

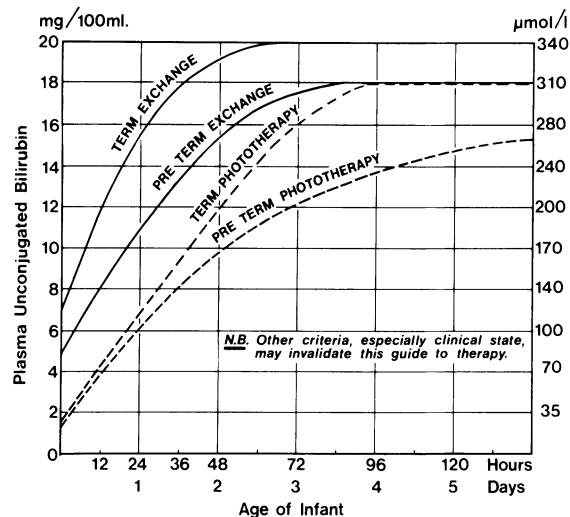


Fig. 1. Criteria for exchange transfusion and phototherapy.

disease has now fallen dramatically and has been overtaken by other indications (Fig. 2). Hyperbilirubinaemia due to ABO incompatibility has increased relatively. Glucose-6-phosphate dehydrogenase deficiency in our Chinese and Mediterranean population has become a more recent indication.

Controversy exists over the type of blood to be used for exchange transfusion. Most agree that citrated type blood should be fresh (less than 48 h old), ABO compatible or O negative with low anti A and anti B titres, and partially packed to a haematocrit of 55–60%. The contention surrounds the best anticoagulant to be used. Acid citrate dextrose (ACD) blood is unsuitable and the choice is between citrate phosphate dextrose (CPD) or heparinised blood. Exchange transfusion produces considerable stresses in the sick infant. These are exacerbated by the metabolic and plasma volume changes which occur although, in the main, they are well tolerated by term infants. Roberton has discussed this aspect of exchange transfusion in a recent review [3]. At term, changes in the plasma sodium and potassium concentrations are transient

Table 1. Indications for exchange transfusion for rhesus disease.

1. Cord haemoglobin concentration <10 g/dl
2. Cord serum bilirubin concentration >120 mmol/l
3. Hyperbilirubinaemia (See Fig. 1)

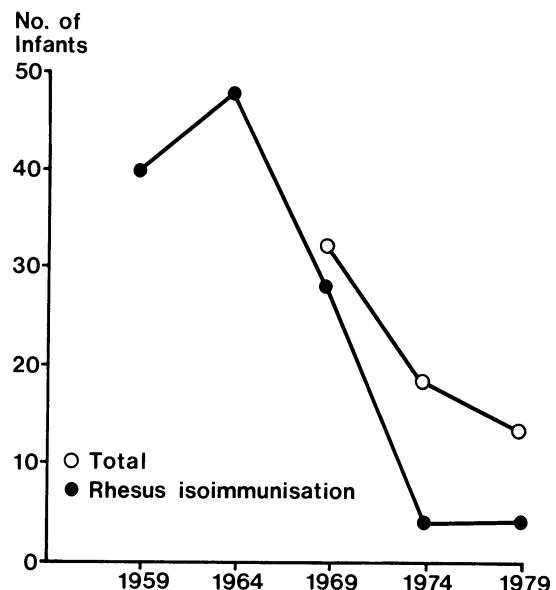


Fig. 2. Exchange transfusion in Simson Memorial Maternity Pavilion Hospital during 1959–1979.

and well tolerated provided the blood is less than 48 h old. The citrate load of CPD blood is important in lowering plasma ionised calcium concentrations, particularly in preterm infants, yet few develop symptomatic or electrocardiographic evidence. The common practice of adding 1 ml of 10% calcium gluconate slowly at each 100 ml interval during exchange appears effective in preventing hypocalcaemia [4]. We routinely use calcium gluconate during second and subsequent exchanges. In a study of 21 second exchanges we found a mean rise of 0.14 mmol/l between the pre and post transfusion plasma calcium concentrations when calcium gluconate was used. If heparinised blood is used there should be no alteration in the calcium status.

CPD blood less than 48 h old has a 7.1 pH. This does not appear to affect adversely term infants although they frequently develop a mild metabolic acidosis towards the end of the exchange. This is usually followed by a citrate induced mild metabolic alkalosis. Clearly, infants who already are acidotic should have this corrected before exchange if possible, or consideration should be given to the use of heparinised blood.

Alterations in blood glucose concentrations also occur during and following CPD exchanges. Hy-

hyperglycaemia occurs, but seldom exceeds the renal threshold [5]. Where there is hyperinsulinism, as in rhesus disease, rebound hypoglycaemia can be troublesome, but is easily detected and treated. Hypoglycaemia can occur during heparinised exchange and frequent blood glucose monitoring is indicated.

Heparinised blood also causes release of free fatty acids which compete with bilirubin binding sites. This seems to be more of a theoretical than a practical danger. The anticoagulant effect of heparinised blood is minimal unless there is coexisting thrombocytopenia which may occur in severe rhesus disease. Table 2 summarises the various risks.

Increasingly exchange transfusion is being used in preterm infants with severe idiopathic respiratory distress syndrome (IRDS), sepsis, severe metabolic disturbances and coagulopathies.

In IRDS the principal benefit accrues from the improvement in the P50. 2,3 Diphosphoglycerate (2,3 DPG) concentration in banked red cells is maintained both in fresh CPD and heparinised blood. Exchange transfusion therefore allows improved oxygen release to the tissues by substituting adult for fetal haemoglobin. Adult haemoglobin has a lower oxygen affinity and sustains 2,3 DPG concentration. Other possible benefits, such as improvement in carbonic anhydrase concentrations and plasma protein concentrations, are more difficult to evaluate. At present most centres reserve exchange transfusion in IRDS for several affected infants not responding to standard therapy. Concern has been expressed over the risk of promoting retrosternal fibroplasia (RLF) in these small preterm infants by the use of adult haemoglobin con-

taining blood and this has recently been reviewed by Silverman [6]. Conspicuous improvement has been reported in preterm infants with IRDS >1000 g who are treated with exchange transfusion [7], yet caution must be advised when the arterial oxygen tension is <40 mm Hg [13].

Occasionally, exchange transfusion may be required to eliminate some toxic substance (e.g. drugs) and in these circumstances a routine twice-volume CPD blood exchange should suffice. Where sepsis is clearly overwhelming (e.g. severe early group B Streptococcus infections) exchange transfusion can be life-saving. It must be performed by an experienced team.

There is also contention in respect of the use of exchange transfusion in disseminated intravascular coagulation (DIC). Several workers have suggested that in severe DIC where platelet counts and fibrinogen concentration continue to fall, and prothrombin and partial thromboplastin times to lengthen despite attempts to remove the trigger factors, exchange may be of value. Both CPD and heparinised blood have been used with success when supported by active blood factor and platelet replacement therapy. Our own experiences with exchange transfusion to date have not been encouraging and further exploration of these techniques is required. As yet no convincing data are available to determine any advantage between heparinised and CPD blood in the treatment of DIC [8].

Exchange transfusion can be attended by important complications. In addition to the metabolic effects previously discussed, vessel damage, thrombotic episodes, peripheral embolisation, thrombocytopenia, bacteraemia, hypothermia and cardiac arrhythmias have all been described. An association with necrotising enterocolitis has also been described and this may be due to interference with the intestinal blood supply. Many of these complications can be avoided by experienced medical, nursing and laboratory teamwork, but the procedure should never be undertaken lightly because even in experienced hands difficulties can arise.

Simple (top-up) transfusion

Unlike the reduction in the use of exchange trans-

Table 2. Heparinised blood vs citrated blood.

Pro	Con
Better preservation of platelets and granulocytes	Has to be very freshly obtained
No citrate effect	Heparin anticoagulant effect
No acidotic effect	Clots tend to occur
No hyperglycaemic effect	Raises free fatty acids
No sodium load	Higher rate of infection
	Waste and expense

fusion for rhesus disease there has been a steady increase in simple transfusion in the newborn.

The well established indications for transfusion remain: acute and chronic prenatal blood loss and acute revealed or occult postnatal haemorrhage. The initial infusion volume is usually 15–20 ml/kg, followed by a clinical assessment of further needs supported by central venous pressure (CVP) or blood pressure measurements. The following conditions are instances where acute losses may occur and these episodes are best managed by rapid replacement with fresh whole or semi packed CPD blood.

- Feto-placental transfusion (recent)
- Feto-maternal transfusion
- Vasa-prævia haemorrhage/placenta prævia
- Massive subgaleal or subaponeurotic haemorrhage
- Ruptured viscus (e.g. spleen, liver) / retroperitoneal haemorrhage
- Massive gluteal haemorrhage (e.g. post-breech)
- Umbilical cord haemorrhage
- Gastrointestinal haemorrhage – haemorrhagic disease of the newborn or acute gastric ulcer
- Haemoglobin concentrations lag behind clinical events, so therapeutic decisions based on clinical assessment along with central venous or systemic blood pressure measurements are the best guides to the need for transfusion.

The number of term infants who require transfusion in the neonatal period is unlikely to be greater than 1–2%, including those with a low haemoglobin concentration resulting from haemolytic disease of the newborn. With increasing prematurity the need for transfusion increases exponentially. In our own experience 70% of 28–30 week gestation infants require blood transfusion. This is often due to the blood sampling required for investigative studies. Assuming a blood volume of 80–90 ml/kg, investigating a sick preterm newborn will rapidly lead to a 10% depletion in blood volume, at which level we recommend top-up transfusion. It is imperative to record and consider the blood volumes withdrawn and to limit investigations to those which have clinical relevance. Another index of transfusion requirements in the first few days of life in preterm infants can be obtained from the packed

cell volume (PCV) which should seldom be allowed to fall below 45%, especially if there are coincidental respiratory or septic problems. In some intensive care nurseries almost 60% of all infants admitted require top-up transfusion [9] at some period during their admission. Apart from the obvious advantage of improvement in cardiac output, blood pressure and blood volume, tissue oxygenation improves when adult haemoglobin with its improved oxygen release is transfused. A guide to the volumes of blood required can be gauged from the recorded blood loss or the use of the equation, 4 ml packed cells or 6 ml whole blood, raises the Hb of a 1 kg infant by 1 g/dl. Thus using packed cells to raise the Hb of a 1500 g infant from 8 g–12 g/dl requires $(4 \text{ ml} \times 1.5 \times 4) = 24 \text{ ml}$ packed cells.

Robinson *et al.* [10] suggested that top-up transfusion with fresh blood might be effective in improving hypoxic, acidaemic infants. This has not been our experience nor that of others [11]. This is probably due to the fact that when arterial oxygen tension is less than 45 mm Hg the uptake of oxygen by capillary blood in the lung is limited by the decreased oxygen affinity of adult haemoglobin. Lactic acidaemia also occurs when arterial oxygen tension falls below 40 mm Hg [12], and hence top-up or exchange transfusion in these circumstances may well be to the detriment of the infant.

Anaemia of prematurity, a descriptive term which encompasses a number of aetiologies including 'physiological' anaemia and iatrogenic causes, is common, yet its effect varies between infants. The point at which transfusion becomes necessary depends not on the haemoglobin concentration alone but also on the clinical effects which the infant exhibits. The latter include pallor, lethargy, apnoea, tachypnoea and tachycardia, and less obviously but equally pertinent, failure to gain weight and sluggish feeding. This anaemia usually declares itself between four to six weeks post delivery and can be usefully confirmed by calculating available oxygen [13]. This ideally should be determined from knowledge of the arterial and venous oxygen tensions, P_{50} and haemoglobin concentration, but it is simpler to use the formula: Available oxygen = (post conceptual age in wks \times 0.005) + 0.54 \times

Hb g/dl (ml/dl blood) [normal range – 10–12 ml/dl blood]. A value of less than 7 ml/dl [14] represents the point at which blood transfusion is indicated. A simple transfusion of CPD concentrated red cells quickly alleviates symptoms in most infants. The concept of available oxygen means that established ranges of haemoglobin concentration [14] should not be adhered to slavishly, as this may be to the detriment of tissue oxygenation.

Throughout this section we have indicated a preference for CPD blood either whole or packed. However, it may well be that further research will demonstrate that in blood transfusion graft-vs-host (GVH) disease is more common than supposed and that irradiation of blood immediately before use may be necessary for prevention.

A system of 'walking donors' has been advocated by some. This has not been popular in the United Kingdom because of the potential risks of haemolytic reactions occurring if time is not taken to fully cross match the donor and recipient. If full cross match is performed no real time advantage appears to accrue as between the 'walking donor' and 'off-the-shelf' fresh blood. Other hazards inherent in this system include lack of full screening for hepatitis, cytomegalovirus (CMV) and syphilis. Advantages of the system are the reduced blood wastage and continuity of donor/recipient for further transfusion. Paediatric (small volume) packs or similar multiple subunit packs are used in some centres with a view to economising in blood usage.

Top-up transfusion also has its hazards. Complications include haemolytic reaction due to mismatch, transmission of infection (e.g., hepatitis, CMV, EB virus and toxoplasmosis) and sensitisation to transfused HLA antigens and reactions to them including late thrombocytopenia. Metabolic complications are less likely than in exchange transfusion but there is an undoubted association in the newborn with necrotising enterocolitis which may be related to the plasticiser di-2-ethyl hexyl phthalate (DEHP) used in the manufacture of polyvinyl tubing. Cold blood may produce arrhythmias. The rate and method of infusion used (e.g., avoiding peristaltic pumps) require careful attention.

Platelet and factor replacement therapy

The sick newborn infant commonly exhibits alterations in his haemostatic mechanisms. It is therefore imperative to have a basic knowledge of the techniques of specimen collection and the normal coagulation parameters (Tables 3 and 4). Preterm infants in association with and by the very nature of their shortened gestation, are more prone to coagulation abnormalities. Term infants rarely produce such abnormalities unless hypoxic although they occasionally develop platelet disorders and vitamin K₁ deficiency - haemorrhagic disease of the newborn.

The majority of plasma procoagulant concentrations in term and preterm infants are lower than the adult values with the exception of factors V, VIII and XIII (Table 3). As a consequence, the screening tests usually used to assess coagulation status reflect this with a progressive drift from adult val-

Table 3. Procoagulant concentrations related to gestation.

Factor % of normal adult values	Preterm (30–36 wks)	Term
II	30– 65	40– 65
V	50–100	50–150
VII	20– 50	40–70
VIII	60–120	70–150
IX	10– 30	15– 55
X	10– 45	20– 55
XI	10– 50	15– 70
XII	-	25– 70
XIII	50–100	50–100

Table 4. Commonly used coagulation screening tests.

Screening test	Preterm (30–36 wks)	Term	Adult
Prothrombin time (PT)	13–23	13–17	13–16
Thrombotest (TT) %	15–50	15–60	80–100
Partial thromboplastin time (PTTK)	35–100	35–70	35–45
Thrombin time/s	12–24	12–18	10–14
Reptilase time/s	18–30	18–24	18–22
Platelet count $\times 10^9/l$	100–350	150–400	150–400
Fibrinogen concentration g/l	1.2–3.5	1.5–3.5	1.5–3.5

ues (Table 4). Sampling techniques are critical and if specimens are taken from peripheral veins, a new syringe, needle and site must be used for each attempt. In sick newborn infant, despite the risks involved, many samples will be taken from indwelling umbilical venous or arterial catheters. Heparin is frequently used to maintain the patency of these catheters, and to avoid contamination of samples with this they must be well flushed before sampling. This flushing is best achieved by withdrawing 3–4 ml of blood through the catheter, sampling, and then replacing the original 3–4 ml. More recently, capillary sampling has been used more frequently [15]. It is important in this method to ensure that there is adequate capillary flow by prewarming the foot to 42°C for at least five minutes. Our own experiences with this technique have been encouraging. It is also imperative that good liaison is maintained between laboratory and clinician when these investigations are contemplated.

The common bleeding disorders in the newborn and their management will now be discussed.

Thrombocytopenia

Thrombocytopenia is uncommon as an isolated disorder and does not usually give rise to clinical problems unless platelet counts are under $100 \times 10^9/l$. Even then, bleeding is unlikely, although petechia, especially in the flexures, and scalp or breech bruising are common. When $<40 \times 10^9/l$ there is an increased risk of spontaneous haemorrhage, and if platelet counts fall below $10 \times 10^9/l$ there is a high risk of gastrointestinal and intracranial haemorrhage.

The principal causes of isolated thrombocytopenia are iso-immune or auto-immune reactions. In the former, platelet antigen specific IgG antibody crosses transplacentally from a platelet antigen negative mother to destroy fetal and neonatal platelets. The usual antigen involved is Zw^a or Zw^b, but occasionally may be from the HLA system. The thrombocytopenia induced is usually severe and lasts up to one month. The greatest risk of severe bleeding, often intracranial, occurs during delivery and Caesarian section is usually recom-

mended when the diagnosis is anticipated antenatally. If the platelet count in the infant remains $>40 \times 10^9/l$, observation alone and avoidance of intramuscular injections are sufficient. When the count is less than $10 \times 10^9/l$ or if bleeding occurs, we recommend exchange transfusion with fresh (less than 24 h old) CPD blood from a platelet antigen negative donor if possible, followed by infusion of concentrated washed maternal or known antigen negative platelets (10 ml/kg). The survival of these platelets must be carefully monitored and further donations anticipated. In situations of extreme emergency, random platelet donations may be given but will have only a brief effect. Where the platelet count lies in the intermediate zone, prednisolone 2 mg/kg/day may be given, but its value has never been established. We seldom use corticosteroids although theoretically they may well be protective to blood vessel integrity.

In auto-immune thrombocytopenia a maternal history of thrombocytopenia is usually obtained. Although the mother may herself have responded to corticosteroids or splenectomy, this does not prevent the IgG non-specific antibodies passing transplacentally. Where the maternal platelet count is $>100 \times 10^9/l$, the risk of neonatal effect is diminished. Unless overt bleeding occurs we recommend no treatment as platelet transfusions are rapidly destroyed and corticosteroids are of unproven value. Nonetheless, many employ corticosteroid therapy as the thrombocytopenia, although moderate is often prolonged.

Other causes of thrombocytopenia only rarely require platelet transfusions. These include drug reaction (e.g. to tolbutamide), congenital viral infections, and amegakaryocytic thrombocytopenia. In our experience a conservative approach is indicated in these conditions.

Procoagulant deficiencies

In the apparently healthy term infant procoagulant deficiencies seldom occur. A few are due to specific inherited deficiencies, the majority to vitamin K₁ deficiency states. The sick preterm infant is particularly prone to the effects of delayed synthesis of coagulation factors and to disseminated intravascular coagulation.

The inherited factor deficiencies do not usually present in the newborn with major organ bleeding. Rarer varieties which may do so are dealt with in Table 5. Congenital factor VIII and IX deficiencies can produce bleeding from the cord, from fetal monitoring sites or following neonatal surgery. Cryoprecipitate is usually used in treatment and assuming that it contains 80 units of factor VIII per donor unit, is prescribed on the basis that one unit factor VIII/kg body weight will raise the plasma concentration by 2%. One unit of specific factor VIII concentrate will raise the plasma concentration to a similar degree. In factor IX deficiency specific concentrate is available, but fresh frozen plasma (FFP) provides sufficient quantities. In von Willebrand's disease, although this is not a true coagulation factor deficiency, cryoprecipitate usually provides adequate replacement.

Factor XIII deficiency merits special mention. It can cause persistent umbilical cord oozing and there is increased risk of intracranial haemorrhage. Replacement therapy on a monthly basis throughout childhood with FFP, cryoprecipitate or factor XIII concentrate is indicated [16].

Deficiency of vitamin K₁ dependent factors is usually experienced in the form of haemorrhagic disease of the newborn (see below). Other causes include maternal anticonvulsant and maternal oral anticoagulant therapy. Breast feeding whilst on warfarin therapy is safe whilst other coumarin anti-coagulants such as phenindione are contra-indicated. Where parenteral nutrition is used for prolonged periods or malabsorptive disorders occur in the neonatal period, vitamin K₁ supplementation should prevent the deficiency state.

The classical form of vitamin K₁ deficiency occurs in breast fed infants in whom, as well as reduced vitamin K₁ intake, low vitamin levels have been present from birth [17]. The bleeding is often from the gastro-intestinal tract and occurs between the second and sixth days of life. The prothrombin time is prolonged and the infant usually responds rapidly to parenteral vitamin K₁ (1 mg) and blood loss replacement with fresh CPD blood. Alternatively FFP or prothrombin concentrate can be given. Prevention with prophylactic vitamin K₁ at birth intramuscularly or orally (1mg) is indicated.

In the very low birth weight (VLBW) preterm infant, the available evidence [18] suggests that daily evaluation of their coagulation status during the first three days of life should be performed and when this is abnormal, correction attempted even though the infant is asymptomatic.

Disseminated intravascular coagulation

Disseminated intravascular coagulation is a common consequence of sepsis, hypoxia and shock in both term and preterm sick infants. We would agree with the definition and criteria of Preston [19] who describes it as a 'process characterised by widespread activation of the coagulation system with formation of soluble or insoluble fibrin and in which clotting factors and platelets are consumed with secondary activation of fibrinolysis'. The resultant tissue disturbance can be extensive and multisystem as DIC is often generalised. A localised variety of DIC is seen in the giant haemangioma syndrome (Kasabach - Merritt) and responds to corticosteroids [20]. The clinical diag-

Table 5. Inherited factor deficiencies.

	Treatment	Notes
Afibrinogenaemia	FFP	15 ml/kg
Hypofibrinogenaemia	Cryoprecipitate	1 unit = 0.2 g fibrinogen
Dysfibrinogenaemia	Fibrinogen concentrate	seldom used
Prothrombin deficiency	FFP	15 ml/kg
	Prothrombin concentrate	1-2 ml/kg
Factors V, VII, XI (V rare)	FFP	15 ml/kg
Factor X (rare)	FFP	
	Prothrombin concentrate	

nosis of DIC in the newborn is often suggested by renewed bleeding from old puncture sites, haematuria or spontaneous gastro-intestinal haemorrhage. There is prolongation of the prothrombin and partial thromboplastin times, reduced plasma fibrinogen concentration ($<1\text{ g/l}$) and thrombocytopenia ($<50 \times 10^9/\text{l}$). The peripheral film reveals burr and fragmented red cells; the haemoglobin concentration falls and fibrin degradation products appear in the plasma.

Therapy is controversial. Trigger factors should be removed where possible with correction of hypoxia and acidaemia, treatment of infection and careful management of fluid balance. If despite these measures the process appears to be continuing, exchange transfusion with fresh CPD blood followed by platelet and appropriate coagulation factor replacement should be carried out. Heparin is seldom required except where major blood vessel thrombosis has occurred (e.g. renal vein thrombosis or major arterial vessel occlusion). Where heparinisation is indicated a regimen of intravenous heparin 100 units/kg/4 hourly or 25–35 units/kg stat followed by 10–15 units/kg/h continuously has been effective. Trials with anti-thrombin III (AT III) are currently taking place in the newborn and AT III may find a place in the management and prevention of DIC in infants who normally have significantly diminished levels compared to adults.

References

1. Forfar JO: Haemolytic disease of the newborn, An overview of the principles of therapy. In: Paediatrics and Blood Transfusion, Smit Sibinga CTh, Das PC, Forfar JO (eds). The Hague, Martinus Nijhoff, 1982.
2. Wallerstein H: Treatment of severe erythroblastosis by simultaneous removal and replacement of the blood of the newborn infant. *Science*, 1946; 103: 583.
3. Robertson NCR: Metabolic effects of exchange transfusion. In: Paediatrics and Blood Transfusion, Smit Sibinga CTh, Das PC, Forfar JO (eds). The Hague, Martinus Nijhoff, 1982.
4. Maisels MJ, Li TK, Piechocki JT, Werthman MV: The effect of exchange transfusion on serum ionised calcium. *Paediatrics*, 1974; 53: 683.
5. Milner RDG: Neonatal metabolism and endocrinology studied by exchange transfusion. In: *Clinics in Endocrinology and Metabolism*, Forfar JO (ed). Philadelphia, WB Saunders, 1976: 221.
6. Silverman WA: Retinopathy of prematurity: oxygen dogma challenged, *Arch Dis Child*, 1982; 57: 731.
7. Delavoria-Papadopoulos M, Miller LD, Brancia PA, et al.: Exchange transfusion in low-weight infants I. Initial observations. *J Paediatr*, 1976; 89: 273.
8. Woods GW, Corman Luban NL, Hilgartner MW, Miller DR: DIC in the newborn. *Am J Dis Child*, 1979; 133: 44.
9. Hill RM, Cobrera-Meya GA, Tennyson L: Utilisation of blood components in neonatal medicine. *Paediatric Res*, 1979; 13: 477.
10. Robinson RD, Fiyimura M, Howat P, Salisbury DM: Effect of blood transfusion in low birth weight infants. *Arch Dis Child*, 1977; 52: 696.
11. Holland BM, Wardrop CAP: Anaemia in premature babies. *Maternal and Child Health*, 1983; 8: 244.
12. Zoroulis CG, Pivacek LE, Lowrie GB, Valeri CR: Lactic acidaemia in baboons after transfusion of red blood cells with improved oxygen transport function and exposure to severe arterial hypoxaemia. *Transfusion*, 1979; 19: 420.
13. Jones JG, Holland BM, Veale KEA, Wardrop CAP: 'Available Oxygen' a realistic expression of the ability of the blood to supply oxygen to tissues. *Scand J Haematol*, 1979; 22: 77.
14. Williams ML, Shott RJ, O'Neal PL, Oski FA: Role of dietary iron and fat on vitamin E deficiency anaemia of infancy. *N Eng J Med*, 1975; 292: 887.
15. Stuart J, Breeze GR, Picken AM, Wood BSB: Capillary blood coagulation profile in the newborn. *Lancet*, 1973; ii: 1467.
16. Kitchens CS, Newcombe TF: Factor XIII, *Medicine*, 1979; 58: 413.
17. Shearer MJ, Barkham P, Raken S, Stummel L: Plasma vitamin K₁ in mothers and their newborn babies. *Lancet*, 1982; ii: 460.
18. Turner TL, Prowse CV, Prescott RJ, Cash JD: A clinical trial on the early detection and correction of haemostatic defects in selected high risk neonates. *Br J Haematol*, 1981; 47: 65.
19. Preston FE: Disseminated intravascular coagulation. *Br J Hosp Med*, 1982; 28/2: 129.
20. Evans J, Batchelor ADR, Stark G, Uttley WS: Haemangioma with coagulopathy. Sustained response to prednisone. *Arch Dis Child*, 1975; 50: 809.

16. Therapeutic plasma exchange

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Introduction

The terms 'plasma exchange' and 'plasmapheresis' have been used interchangeably in the literature. This is regrettable as the two words have different meanings and derivations. The word 'apheresis' is derived from the Greek word *aphairesis* which basically means to remove or withdraw. Therefore, the word 'plasmapheresis' refers to the removal of plasma strictly speaking without its replacement. In contrast, plasma exchange indicates that plasma is removed and replaced by another medium such as plasma. Therefore, in general terms, plasmapheresis is not a therapeutic procedure but one in which plasma is removed from normal donors. This practice takes place in many parts of the world using a variety of sophisticated equipment or it can be done manually. Plasma exchange, on the other hand, is a therapeutic procedure in which a patient is attached to one of many types of machines which remove plasma and at the same time replace it with another solution. Therefore, the two terms should not be used interchangeably but with precision [1].

Plasma exchange is basically a very unsophisticated form of treatment which has been applied to a wide variety of diseases. Unfortunately, it has only been shown to be beneficial in a handful of conditions among those for which it is used. The explosive increase in the use of plasma exchange in the last decade indicates that many practitioners feel that it offers benefit to patients with a wide variety of diseases: however, on close scrutiny, there is little or no scientific basis for this approach.

Such an attitude with respect to the use of plasma

exchange for a wide variety of diseases has very important implications for those physicians who are involved in the procurement of plasma and plasma products for use in plasma exchange programs. There is no question that if plasma exchange continues to expand for the treatment of a wide variety of diseases without documented evidence of benefit, we will soon be unable to meet the needs of plasma exchange programs with respect to some of the replacement solutions. To this end, several years ago the Canadian Aphesis Study Group (CASG) was formed to conduct controlled multi-centre clinical studies in the use of plasma exchange for some diseases. Currently, thrombotic thrombocytopenic purpura (TTP), immune thrombocytopenia (ITP) and rapidly progressive glomerulonephritis (RPGN) are being studied. Only by embarking upon such studies will we eventually have answers with respect to the usefulness of plasma exchange for certain diseases. A negative study in therapeutic terms will be just as valuable as a positive one for it will reduce the amount of plasma wasted.

Withdrawal of patient plasma for the purpose of treating a disease was described first in 1914 by Abel *et al.* [2] and has found widespread application since cell separators made large-volume exchanges possible. Plasma exchange has since been used in over 50 different disorders [3-7] which vary considerably. Unfortunately it is extremely difficult if not impossible to evaluate the results obtained with this expensive and potentially dangerous treatment.

In fact, for the large majority of applications

cited in the literature the efficacy is unproven, or at least doubtful. This is due to the lack of control groups in many study designs, the anecdotal nature of many studies and the lack of objective parameters. Often there is difficulty in discriminating between the possible effects of plasma exchange and of other concomitant, treatment modalities. Even a simple measure such as bed rest may affect study results.

Plasma exchange can be expected to be most effective when the purpose of the procedure is to remove large molecules, like IgM antibodies, or immune complexes, from the patient's blood. This is clearly illustrated by a much greater effectiveness in reducing plasma hyperviscosity in patients with macroglobulinemia, in whom the monoclonal IgM is largely confined to the intravascular space, than in patients with multiple myeloma, in whom the smaller IgG or IgA molecules are also distributed in the extracellular volume [8]. In assessing the effectiveness of plasma exchange the rate of production of the factors to be eliminated has to be taken into account, as well as the possibilities for repair of a damaged target organ. It cannot be assumed that totally obliterated renal glomeruli will recover to a normal physiological state after plasma exchange, although the primary indication for this procedure may have been justified.

Patient considerations

An issue of much debate in many clinical studies has been the possibility of a placebo effect of plasma exchange and whether sham procedures should be carried out. Many investigators argue against such sham procedures, since this may subject the patients to some of the potential dangers of plasma exchange without offering any of the benefits. From data that is now becoming available on the complications of plasma exchange [6, 9] it is clear that this procedure is not without hazards. Diseases transmissible by infusion of plasma or plasma products such as hepatitis in its different forms and possibly also acquired immune deficiency syndrome (AIDS) have been recognized as such hazards. More recently acute pulmonary

edema, which often is fatal despite positive end expiratory pressure (PEEP) ventilation has been described in a significant number of patients [5, 6]. As of January, 1983 over 40 deaths has been associated with the use of cell separators throughout the world, and the institution of an apheresis register to record such disastrous events has been proposed [10]. There is every reason to believe that this information represents only a small fraction of the actual number of fatalities.

Whether or not to perform plasma exchange, therefore, requires a decision in which the potential benefits have to be carefully reviewed against this background.

The condition of the patient, advanced age, prior major vascular problems like recent myocardial infarction, cerebrovascular ischemia, bleeding disorders, also influence such a decision. Venous access has to be assured, and sometimes arterio-venous shunt surgery will be necessary. Arterio-venous shunts or fistulas can both be used, though the former will be preferred in acute situations.

Volume and replacement solutions

The volume of plasma to be removed is not defined for any of the indications. It has to be recognized that with increasing volumes of plasma exchange the efficiency of the procedure will be drastically reduced due to increasing dilution by the infused normal plasma, albumin or other plasma expanders and therefore 'total plasma exchange' will not exceed 60–70% efficiency [11, 12]. Generally, in any one session it is recommended not to remove more plasma in litres than 5% of the patient's body weight in kilograms [11]. Plasma volume monograms have been established [13].

As replacement fluid 5% albumin solution in combination with 0.9% NaCl solution generally will suffice [11], although in repeated, large-volume plasmapheresis, often part of the replacement is given as fresh frozen plasma (FFP). It is well worth recognizing that this use of FFP places an extra burden on blood transfusion services for the procurement of this scarce product, which often is not necessary in plasma exchange. Also there is a grow-

ing concern regarding transmissible diseases, when using FFP. Such risks are reduced when fractionated solutions like 5% albumin are used, however, such solutions may be less effective than FFP for certain diseases such as TTP [14].

Methods available for plasma exchange

A wide variety of sophisticated machinery is available with which to carry out plasma exchange. Initially, large-volume plasma exchange was begun using the continuous-flow cell separators. These machines allowed the operator to remove plasma and at the same time replace the removed plasma with other solutions such as FFP, banked plasma, albumin, purified protein fraction (PPF), and other crystalloid or colloid solutions.

Later, the discontinuous-flow cell separators were used just as successfully. More recently, plasma has been removed using the newer membrane cell separators [15]. These machines have the advantage that there is virtually no alteration of the cellular components of the blood when the plasma is removed. Thus, a patient with a low platelet count does not suffer from a further decrement in his platelet count during a plasma exchange. Hollow membrane filtration is also available and basically employs the same concept of a very small pore size through which the plasma escapes, leaving the cellular components behind [16]. It should be remembered, however, that plasma exchange can be carried out in desperate circumstances in a manual fashion in which blood is removed in bags, spun in a large centrifuge and the red cells returned to the patient while the plasma is discarded. This can be done repetitively until the concentration of the offending substance has been lowered sufficiently.

All of the foregoing methods lack specificity and hopefully in the near future we will move from plasma exchange to the specific removal of the substance which is considered to be the cause of the patient's problem. Some initial work in this field was done by Dr. Terman of Baylor University in Texas using staphylococcal protein A immobilized in a column with which he was able to remove

specific proteins related to malignant tumors. More recently, he has been able to devise a method for removing the antibodies which are implicated in lupus nephritis using similar columns [17]. In Seattle, Dr. Bensinger has been able to immobilize A and B blood substances in columns and by passing plasma over these columns has achieved a significant decrement in anti A and anti B titers [18]. This technology has been useful in ABO incompatible bone marrow transplants. Thus, the technology is moving rapidly in the direction of specificity and hopefully, plasma exchange as it is now practiced will be a matter of history within the next decade.

Mechanism of action of plasma exchange

The exact method by which plasma exchange produces benefit is unknown in many instances and often reflects an incomplete knowledge about the disease being treated. Because of the lack of specificity with respect to plasma exchange, many things happen concurrently that may have a beneficial effect on the disease being treated. During a plasma exchange the patient is anti-coagulated; procoagulant levels, particularly fibrinogen are lowered, the levels of complement and immune complexes are also reduced, electrolyte concentrations are perturbed, and so on.

In some diseases we know that the putative pathogenetic substance is removed such as IgM in macroglobulinemia. In others, the antibody mediators of an immunological process may be removed such as Anti-D in hemolytic disease of the newborn, anti-GBM (glomerular basement membrane) antibody in Goodpasture's syndrome and anti-ACL (acetylcholine receptor) antibody in myasthenia gravis. The removal of immune complexes may benefit a number of diseases such as systemic lupus erythematosus, rapidly progressive glomerulonephritis and polyarteritis nodosa. The removal of antibodies which block the immune response to malignancies may produce temporarily amelioration of the disease [19].

Established indications for plasma exchange

It would be difficult to find unanimity between two practitioners in the field of plasma exchange with respect to the established indications for its use; therefore, the short list (Table 1) that follows with respect to diseases for which plasma exchange can be considered useful and established is a personal one. In the hematological field, macroglobulinaemia and multiple myeloma when associated with hyperviscosity of the blood clearly benefit from plasma exchange. Macroglobulinaemia benefits in the short term through rapid reduction of blood viscosity and can also be used as maintenance treatment (Fig. 1).

As most of the signs and symptoms of macroglobulinemia are due to the accumulated abnormal protein, any treatment which controls this protein concentration will control the disease manifestations. Hyperviscosity syndrome caused by macroglobulinemia responds rapidly, and this syndrome often remains controlled for prolonged periods with a single plasma exchange every 2–3 weeks [8]. Much less effective is plasma exchange in multiple myeloma for reasons mentioned above, and improvements in hyperviscosity by plasma exchange usually are of short duration.

It is quite clear now that TTP also benefits from plasma exchange when fresh frozen plasma is used. It does not appear to benefit this disease when an albumin solution is used. Furthermore, some pa-

tients will respond to the infusion of fresh frozen plasma without an exchange procedure and therefore, the CASG study is designed to show whether or not plasma exchange using fresh frozen plasma (FFP) is any more beneficial than a simple infusion of fresh frozen plasma [20].

Finally, plasma exchange has proven itself useful in bone marrow transplantation when the patient and the donor are ABO mismatched [18]. Basically, plasma exchange is designed to reduce the levels of anti A or anti B which may exist in the patient at the time he receives the marrow infusion.

These levels of anti A and anti B can be reduced drastically during the few days before transplant, thus reducing the possibility of serious transfusion reactions at the time of the infusion of the marrow. Since the patient's antibody in these cases usually will be mainly of IgM class, removal by plasma exchange can be achieved with relative ease, and since these patients will be severely immunosuppressed before, and immediately after, bone marrow grafting the synthesis rate of ABO antibodies will be slow. Specific adsorption of these antibodies by immobilized A and B antigens will reduce the time required for antibody removal, but is not necessary in general [18].

Nephrologists use plasma exchange on a routine basis for Goodpasture's syndrome and this is combined with immunosuppressive therapy [21]. Such management has altered the prognosis considerably for this disease which had a very high mortality rate prior to the use of plasma exchange. Unfortunately, once a patient becomes oliguric or anuric the prognosis is considerably worse and plasma exchange is less likely to be beneficial; however, it will control otherwise fatal pulmonary hemorrhage. In summary, therefore, plasma exchange must be used early in the course of Goodpasture's syndrome in order to preserve renal function. Plasma exchange has also been used in crescentic glomerulonephritis with variable success. There is now a CASG study for RPGN which hopefully will define the role of plasma exchange in this disease more clearly.

Finally, plasma exchange has been used in the haemolytic uremic syndrome (HUS) very successfully. This disease is very much like TTP but

Table 1. Established indications for plasma exchange.

A. Hematological
1. Macroglobulinemia
2. Multiple myeloma
3. Thrombotic thrombocytopenic purpura (TTP)
4. Bone marrow transplantation
B. Nephrological
1. Goodpasture's syndrome
2. Hemolytic uremic syndrome (HUS)
C. Neurological
1. Myasthenia gravis
D. Connective tissue disorders
1. Systemic lupus erythematosus (SLE)
E. Metabolic disorders
1. Congenital hyperlipidemias

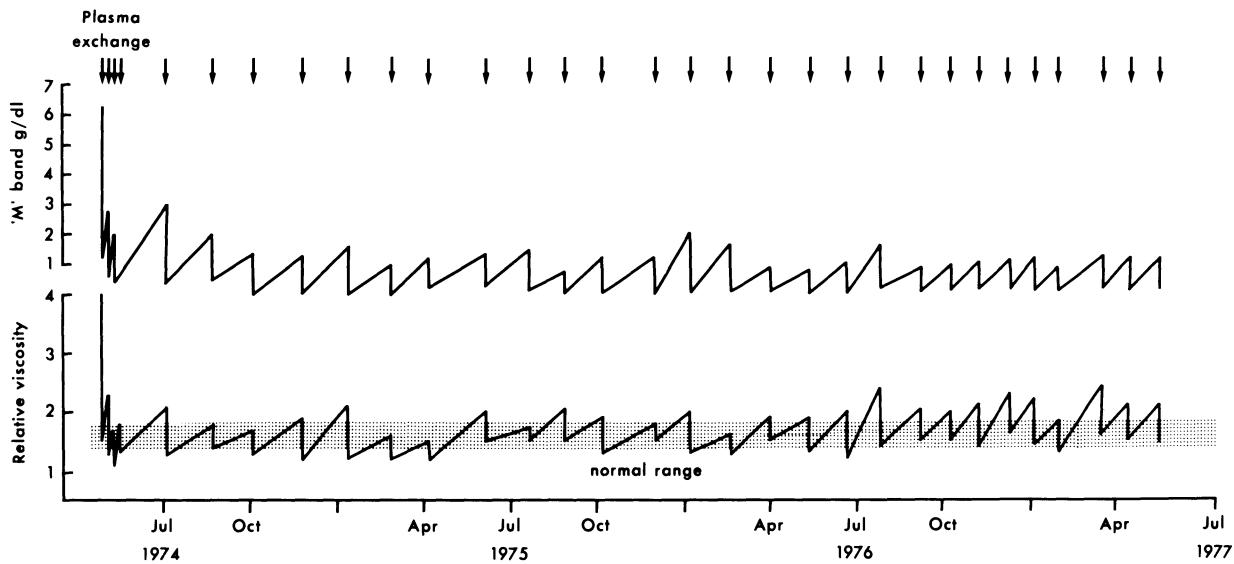


Fig. 1. Long-term plasma exchange therapy only for Waldenström's macroglobulinaemia in an 81-year-old woman

lacks the central nervous system manifestations.

Myasthenia gravis is the only neurological disease for which plasma exchange is clearly established. This has been both for the acute situation and for chronic management of the patient. It can be used for myasthenic crisis which may develop after thymectomy and in some centres, plasma exchange is used routinely before such surgery. Plasma exchange is usually reserved for the situation in a myasthenic patient in which other forms of therapy have failed and the patient is clearly going downhill. Most patients do not require this form of therapy and are managed very well by medical means only. In the very young patient, it allows one to provide a therapy that does not have severe immune suppression as one of its side effects [22].

A variety of other neurological diseases have been treated by plasma exchange but there is no clear evidence that this is beneficial [23, 24, 25]. The Guillain Barre Syndrome (GBS) is being studied now in the United States in a systematic fashion which may demonstrate some benefit [26]. Multiple sclerosis is also being studied in a controlled or uncontrolled fashion by several groups [27, 28].

Many connective tissue disorders have been treated with plasma exchange but at the present time there is little evidence that any of them benefit from it. Systemic lupus erythematosus (SLE) may

benefit from this form of therapy in certain circumstances. The cerebral vasculitis that sometimes is associated with this disease in a patient who presents with seizures often benefits from the rapid institution of plasma exchange therapy on an intensive basis. The other aspects of this disease may or may not benefit from plasma exchange and further study is required [29, 30, 31].

Finally, a group of physicians at Hammersmith Hospital has clearly shown that plasma exchange will benefit some patients with congenital hyperlipidemia. This is an intensive and long-term treatment for a highly fatal disease and involves a major commitment not only by the physician and his staff but also by the patient [32].

This is a short list of diseases for which we consider plasma exchange established therapy. Convincing anecdotal information is available on many other diseases discussed below (Table 2) for which plasma exchange has been used: however, scientific information is lacking and we are unable to draw firm conclusions at this time. Controlled studies therefore must be the order of the day before we proceed to treat patients with a wider variety of disorders with plasma exchange.

Table 2. Unestablished uses of plasma exchange.

A. Hematological	Scleroderma Behcet's syndrome Rheumatic fever Polymyositis Polyarteritis nodosa Wegener's granulomatosis Raynaud's syndrome Cryoglobulinemia Mixed connective tissue disease Sjogren's syndrome
Immune thrombocytopenia (ITP)	
Hemophilia with inhibitors	
Post transfusion purpura	
von Willebrand's disease	
Pruritus of myeloproliferative disease	
Hemolytic disease of the newborn	
Autoimmune hemolytic anemia	
Cold hemagglutination syndrome (CHAD)	
Paroxysmal cold haemoglobinuria	
Paroxysmal nocturnal hemoglobinuria	
Pure red cell aplasia	
Aplastic anemia	
B. Nephrological	F. Gastrointestinal
Crescentic glomerulonephritis	Hepatic coma Primary biliary cirrhosis Crohn's disease
Light chain nephritis	G. Endocrinological
Henoch Schonlein nephritis	Thyroid storm Exophthalmos and pretibial myxoedema Insulin resistant diabetes
Mesangiocapillary glomerulonephritis	H. Toxins and Drugs
Renal transplant rejection	Paraquat poisoning Ammanita phalloides poisoning Phenylbutazone overdose Digoxin overdose Methyl parathion poisoning
C. Neurological	I. Malignant Diseases
Eaton-Lambert Syndrome	A variety of malignancies have been treated
Amyotrophic lateral sclerosis	J. Respiratory Disease
Schizophrenia	Idiopathic pulmonary haemosiderosis Idiopathic pulmonary fibrosis
Guillain Barre syndrome	Asthma
Multiple sclerosis	K. Miscellaneous Disorders
Parkinson's disease	Uncontrolled hypertension Refsum's syndrome Fabry's disease Gaucher's disease Eclampsia Angioneurotic oedema HLA Antibodies
D. Dermatological	
Pemphigus	
Pemphigoid	
Herpes gestationis	
Porphyria cutanea tarda	
Necrotising vasculitis	
Psoriasis	
Autoerythrocyte sensitisation	
Scleromyxoedema	
E. Connective Tissue Disorders	
Rheumatoid arthritis	
Dermatomyositis	
Still's disease	

Unestablished indications for plasma exchange

Hematological indications form roughly $\frac{1}{3}$ of the procedures in therapeutic plasma exchange [12]. Cryoglobulinemia occasionally requires plasma exchange and generally responds well but briefly. Plasma exchange for alloimmunization to the Rhesus D antigen in pregnant women has been claimed to prevent to a major extent the development of erythroblastosis foetalis [33]. In the absence

of alternative treatment strategies it may be justified to continue with plasma exchange while monitoring anti-D titers. Plasma exchange has an equally unconfirmed but seemingly beneficial role in some patients with idiopathic thrombocytopenic purpura but some patients with acute ITP may benefit from the procedure [34] (Fig. 2). For a variety of other hematological disorders like aplastic anemia [35], angioimmunoblastic lymphadenopathy [36], hemolytic anemia [37] and hemophilia

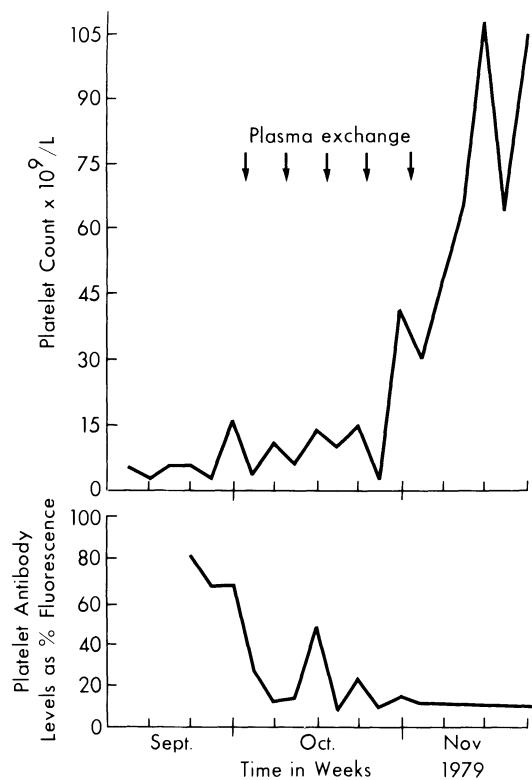


Fig. 2. Platelet and platelet antibody responses to plasma exchange with albumin in a 15 year old male with acute idiopathic thrombocytopenic purpura.

with anti-VIII antibodies [38], plasma exchange has been claimed to be beneficial, but the data are largely anecdotal. All of the above disorders require a properly constructed study to establish efficacy for plasma exchange.

Similarly, plasma exchange has been applied in a large variety of autoimmune disorders for obvious reasons: the elimination of the harmful auto antibody from the patient's circulation.

As discussed earlier, removal of anti-glomerular basement membrane antibodies in Goodpasture's syndrome is perhaps one of the exceptions [20]. Early plasma exchange, in combination with cytotoxic drugs, will prevent permanent damage to the glomeruli in this previously often fatal disease. Plasma exchange has been used extensively in the treatment of acute graft rejection in renal transplantation but the results are inconclusive. There is no evidence that chronic rejection is influenced by plasma exchange as an addition to the immune suppression used [39].

In SLE the conclusions are even more difficult to draw [29, 30]. This is due to the variable course of SLE, and the fact that it is not possible to measure activity of SLE with unequivocal parameters. The patients almost invariably are also treated with immunosuppressive or cytotoxic drugs, which complicates the evaluation of plasma exchange as a treatment modality. Although present results warrant cautious optimism, definitive studies have not yet been published, and it seems reasonable to restrict the use of plasma exchange in SLE to severe cases, not controlled by high doses of steroids [30, 31]. Plasma exchange without concurrent immune suppression is contra indicated in this disease as it may result in a serious increase of clinical activity after the termination of plasma exchange.

Plasma exchange in rheumatoid vasculitis [40], scleroderma, Wegener's granulomatosis [20] and primary biliary cirrhosis [41] has not yet been proven to have a therapeutic effect. Studies in these patients are often difficult to perform because of the 'soft' parameters that have to be used, or because of small numbers of patients as in Wegener's granulomatosis. Unless performed in a clinical investigation setting, we feel that plasma exchange should not be carried out for these indications. Rheumatoid arthritis (RA) may benefit from plasma exchange although the most rigorous study that has been published so far by Dwosh and colleagues clearly indicates that plasma exchange is no better than placebo therapy [42]. No other trial has been so well designed or executed. A negative result was also achieved in a study by Rothwell and colleagues [43]. In contrast, poorly designed studies by Wallace and Goldfinger have reported to show benefit with plasma exchange; however, they have not been as rigorously designed as the Dwosh endeavour [44].

The responses in a variety of neuromuscular disorders pose similar problems as mentioned above. Myasthenia gravis and GBS have been inconclusively studied in relatively large numbers of patients [22, 23, 24]. Especially in GBS with a known high spontaneous recovery rate this is important. Despite this restriction some preliminary conclusions can be drawn from the available data. In myasthenia gravis of relatively short duration (3

years) initial response to plasma exchange often is rapid and impressive. With prolonged treatment, however, this effect seems to wear off.

In chronic myasthenia the results are much less impressive, which perhaps reflects chronic damage of the motor neuron endplate, the supposed site of the disease. In GBS, plasma exchange seems to be accompanied by a more rapid recovery, and often improvement during or shortly after plasma exchange. However, the definitive place of plasma exchange in GBS treatment cannot yet be determined. In patients with multiple sclerosis and polyneuropathy (other than GBS) the role of plasma exchange is uncertain, and on the basis of the present literature doubtful [26, 27]. Some success has been reported in polymyositis [24], but in uncontrolled studies, with limited patient numbers.

Removal of plasma porphyrins in porphyria cutanea tarda has met with success [45], and although the number of patients studied is small, the parameters studied seem to indicate therapeutic benefit in this rare, and almost intractable disorder.

Recently, trials have been undertaken with plasma exchange in patients with advanced malignant disease [19], in an attempt to remove 'blocking' antibodies and to improve the patient's immune response against the tumor. The data are inconclusive and further studies are needed to evaluate the therapeutic benefit, which to date has been evanescent if at all.

Summary

In summary, it is clear that despite a large body of literature there is only scanty evidence that plasma exchange is therapeutically beneficial. Plasma exchange is largely a symptomatic treatment modality, and as such very expensive, potentially dangerous and often used without a solid justification. It is often continued for prolonged periods without clear indications, because of lack of an endpoint in many disorders, exposing the patient to unnecessary risks. Plasma exchange as a treatment modality should be restricted to an investigational type of setting for the majority of disorders for which it is presently used [46].

References

1. Buskard NA: Plasma exchange and plasmapheresis. *Can Med Ass J*, 1978; 119: 681-82.
2. Abel JJ, Rowntree LG, Turner BB: Plasma removal with return of corpuscles (plasmapheresis). *J Pharmacol Exp Ther*, 1914; 5: 625-41.
3. Mielke CH: Apheresis: Development, applications, and collection procedures: (Progress in clinical and biological research, Volume 65) Alan R. Liss, Inc, New York, 1981.
4. Vogler WR: Cytapheresis and plasma exchange clinical indications (Progress in clinical and biological research, Volume 88) Alan R. Liss, Inc, New York, 1982.
5. Beyer JH, Gottingen H, Borberg K, *et al.*: Plasmapheresis in immunology and oncology. *Contributions to Oncology*. Karger S, Basel, 1982.
6. Aufeuvre JP, Martin-Hartel F, Cohen-Solal M, Lefloch A, Baudelot J: Clinical tolerance and hazards of plasma exchanges: A study of 6200 plasma exchanges in 1033 patients. *Plasmapheresis in immunology and oncology*. Karger S, Basel, 1982.
7. Oda T (ed): Therapeutic plasmapheresis (I). Proceedings of the 1st symposium on therapeutic plasmapheresis, Tokyo, June 20th, 1981.
8. Buskard NA, Galton DAG, Goldman JM, *et al.*: Plasma exchange in the long-term management of Waldenstrom's macroglobulinemia. *Can Med Ass J*, 1977; 117: 135-137.
9. Heustis DW: Mortality in therapeutic hemapheresis. *Lancet*, 1983; ii: 1043.
10. Hamblin TJ: An apheresis register. *Lancet*, 1982; ii: 550.
11. Mielke CH, Mielke MR: Technical and therapeutic applications of plasma exchange. *Prog Clin Biol Res*, 1981; 65: 123-45.
12. Vogler WR: Therapeutic apheresis: Where we've been and where we are going. *Therapeutic apheresis and plasma perfusion*. Alan R Liss, Inc, 1982: 1-3.
13. Buffaloe GW, Heineken FG: Plasma volume nomograms for use in therapeutic plasma exchange. *Transfusion*, 1983; 23: 355-57.
14. Taft EG: Thrombotic thrombocytopenic purpura and dose of plasma exchange. *Blood*, 1979; 54: 842-49.
15. Buffaloe GW, Erickson RR, Dau PC: Evaluation of a parallel plate membrane plasma exchange system. *J Clin apheresis*, 1983; 1: 86-94.
16. Sieberth HG, Glockner WM: Separation of cells from plasma in man using hollow-fiber membranes of large pore size. In: *Plasma Exchange Therapy*, Borberg H, Reuther P (eds). Thieme-Stratton, New York, 1981: 20-25.
17. Terman DS, Garcia-Rinaldi R, Dannemann B, *et al.*: Specific suppression of antibody rebound after extra corporeal immunoabsorption. 1 Comparison of single versus combination chemotherapeutic agents. *Clin Exp Immun*, 1978; 34: 32-41.
18. Bensinger WI, Baker DA, Buckner CD, *et al.*: Immunoabsorption for removal of A and B blood-group antibodies. *N Eng J Med*, 1981; 304: 160-62.

19. Micksche M, Colot M, Kokoschka EM, Moser K, Rainer H: Plasmapheresis in patients with advanced malignant disease: A pilot study. *Oncology*, 1982; 39: 146-51.
20. Byrnes JJ, Lian ECY: Recent therapeutic advances in thrombotic thrombocytopenic purpura. *Seminars in thrombosis and hemostasis*, 1979; 5: 199-215.
21. Lockwood CM, Peters DK: The role of plasma exchange and immunosuppression in the treatment of Goodpasture's syndrome and glomerulonephritis. *Plasma Ther*, 1979; 1: 19-27.
22. Keesey J, Buffkin D, Kebo D, Ho N, Hermann C: Plasma exchange alone as therapy for myasthenia gravis. *Ann NY Acad Sci*, 1981; 377: 729-743.
23. Greenwood R, Newsom-Davis J, Hughes RAC, Aslan S, Stott RB, Bowden AN, Chadwick DW, McLellan DL, Millac P, Gordon NS, Armitage P: British multicentre trial of plasma exchange in acute inflammatory polyradiculoneuropathy (AIP). *Therapeutic Apheresis and Plasma Perfusion*. Alan R. Liss Inc, New York, 1982: 189-196.
24. Brettle RP, Gross M, Legg NJ, Lockwood M, Palles C: Treatment of acute polyneuropathy by plasma exchange. *Lancet*, 1978; ii: 1100.
25. Dau PC: Plasmapheresis in idiopathic inflammatory myopathy: Experience with 35 patients. *Arch Neurol*, 1981; 38: 544-52.
26. Shumak KH, Rock GA: Therapeutic plasma exchange. *N Engl J Med*, 1984; 310: 762-771.
27. Tindall RSA, Walker JE, Ehle AL, Near L, Rollins J, Becker D: Plasmapheresis in multiple sclerosis: Prospective trial of plpheresis and immunosuppression versus immunosuppression alone. *Neurology*, 1982; 32: 739-43.
28. Stefoski D, Schauf CL, McLeod BC, Haywood CP, Davis MA, Davis FA: Plasmapheresis decreases neuroelectric blocking activity in multiple sclerosis. *Neurology (NY)*, 1982; 32: 904-907.
29. Barnes CC, Linton AL: Monthly plasmapheresis for systemic lupus erythematosus with diffuse proliferative glomerulonephritis: A pilot study. *Can Med Ass J*, 1981; 125: 171-174.
30. Verrier Jones J: The application of plasmapheresis in systemic lupus erythematosus. *Therapeutic Apheresis and Plasma Perfusion*. Alan Liss Inc, 1982: 81-89.
31. Wei N, Huston DP, Lawley TJ, Steinberg AD, Klippen JH, Hall RP, Balow JE, Decker JL: Randomized trial of plasma exchange in mild systemic lupus erythematosus. *Lancet*, 1983; i: 17-21.
32. Thompson GR: Plasma exchange for hypercholesterolemia. *Lancet*, 1981; i: 1246-48.
33. Fraser ID, Bothamley JE, Bennet MO, et al.: Intensive antenatal plasmapheresis in severe rhesus immunization. *Lancet*, 1976; i: 6-8.
34. Marder VJ, Nusbacher J, Anderson FW: One-year follow-up of plasma exchange therapy in 14 patients with idiopathic thrombocytopenic purpura. *Transfusion*, 1981; 21: 291-98.
35. Abdou NI: Plasma exchange in the treatment of aplastic anemia. *Therapeutic Apheresis and Plasma Perfusion*. Alan Liss Inc, New York, 1982: 333-346.
36. Gordon BR, Suthanthiran M, Saal SD, Stenzel KH, Rubin AL: Plasmapheresis in a patient with angioimmunoblastic lymphadenopathy. *Cancer*, 1983; 51: 829-833.
37. Brooks BD, Steane EA, Sheehan RG, Frenkel EP: Therapeutic plasma exchange in the immune hemolytic anemias and immunologic thrombocytopenic purpura. *Therapeutic Apheresis and Plasma Perfusion*. Alan Liss Inc, New York, 1982: 317-29.
38. Sultan Y, Maisonneuve P, Bismuth A, et al.: Successful management of a patient with an acquired factor VIII inhibitor. *Transfusion*, 1983; 23: 62-64.
39. Cardella CJ, Sutton D, Udall PR, et al.: Intensive plasma exchange and renal-transplant rejection. *Lancet*, 1977; 1: 264.
40. Allen C, Elson CH, Wallington TB: Plasma exchange in rheumatoid vasculitis. *J Rheum*, 1981; 8: 433-439.
41. Kelling PWN, Bull J, Kingston P, Thompson RPH: Plasma exchange in primary biliary cirrhosis. *Postgrad Med J*, 1981; 57: 433-35.
42. Dwosh IL, Giles AR, Ford PM, et al.: Plasmapheresis therapy in rheumatoid arthritis: A controlled, double-blind and crossover trial. *N Engl J Med*, 1983; 388: 1124-29.
43. Rothwell RS, Davis P, Gordon PA, et al.: A controlled study of plasma exchange in the treatment of severe rheumatoid arthritis. *Arthritis Rheum*, 1980; 23: 785-90.
44. Wallace DJ, Goldfinger D, Thompson-Breton R, et al.: Advances in the use of therapeutic plpheresis for the management of rheumatic diseases. *Semin Arthritis Rheum*, 1980; 10: 81-91.
45. Spiva D: Erythrocytapheresis and plasma exchange in the management of the porphyrias. *Therapeutic apheresis and plasma perfusion*. Alan Liss Inc, New York, 1982; 331-36.
46. Berkman E.: Issues in therapeutic apheresis. *N Engl J Med.*, 1982; 307: 1418-20.

17. Transfusion related morbidity and mortality in the intensive care facility

M.D. O'CONNELL and D. GOLDFINGER

Introduction

The Intensive Care Unit (ICU) is one of the most common sites for transfusion requests, as well as an area at highest risk for transfusion related morbidity and mortality. Transfusion errors, resulting in serious injury to the patient, are identified most often in the ICU, the Emergency Room and the Surgical Suite.

Clerical error (i.e., misidentification of recipient or pretransfusion blood sample drawn for cross-matching) is cited in studies of hemolytic transfusion reactions at the most common cause of transfusion error. Technical errors within the blood bank occur less frequently. The emergency setting with a critically-ill patient receiving multiple transfusions is hazardous, because of the tendency to bypass standard transfusion practices.

Other life-threatening complications of blood transfusion include volume overload (particularly significant in elderly patients), anaphylactic reactions in IgA-deficient patients and non-cardiogenic pulmonary edema of leukoagglutinin etiology. Some types of transfusion reactions may be hazardous, due to the delay they cause in delivery of red blood cells (RBCs) while a transfusion reaction investigation proceeds (e.g., febrile non hemolytic transfusion reaction).

Transfusion complications, manifested several days to several months following receipt of blood products, include delayed hemolytic transfusion reactions, infectious disease transmission, graft-versus-host disease and probably the acquired immunodeficiency syndrome (AIDS).

Acute hemolytic transfusion reactions

Acute hemolytic transfusion reactions can result in serious morbidity or death. This most-feared complication of transfusion therapy occurs most commonly when ABO-incompatible RBCs are infused into a patient possessing antibodies to the corresponding RBC membrane antigen. Other RBC antibodies (Rh, Kell, Duffy, Kidd) may cause hemolytic reactions, but only in patients previously sensitized by transfusion or pregnancy. The sequence of events that follows the hemolysis of RBCs may include fever, chills, respiratory distress, shock, disseminated intravascular coagulation (DIC) and renal failure.

Epidemiology

Published studies of hemolytic transfusion reactions cite an incidence ranging from 1:6000 to 1:35,000 transfusions [1, 2]. These statistics probably underestimate the actual hemolytic complication rate, because of under-reporting or failure to identify a reaction to transfusion in seriously-ill patients with complicated, multisystem disease.

The mortality associated with hemolytic transfusion reactions has been estimated at one per 1,000,000 transfusions [3-5]. It is difficult to assess the contribution of the hemolytic reaction in many of these cases, because such reactions occur frequently in gravely-ill patients in emergency settings. We had the opportunity, recently, to review all reports submitted to the United States Food and Drug Administration (FDA) during the years 1978

to 1982. Despite scant information in many cases, an attempt was made to classify reactions as contributory or non-contributory to the patient's demise. Fifty-eight cases were judged to be contributory. Forty-five (78%) were due to ABO-incompatible blood, 13 cases were due to Rh, Kell, Duffy, Kidd or other antibodies. The majority occurred in three areas – the ICU, the Surgical Suite and the Emergency Room. Previous studies suggest that approximately two-thirds of the errors occurred during administration of the blood [4–6]. In our review, over 50 percent of the errors occurred in the collection of the pretransfusion blood specimen or in the blood bank itself (Table 1).

Pertinent data from these reports and other studies include:

1. ABO incompatibility accounts for the majority of serious transfusion reactions. Antibodies to other RBC membrane antigens may cause reactions in previously transfused or parous patients. It is significant to note that, in a center where strict control was maintained over blood products from the drawing of the pretransfusion blood specimen to infusion into the recipient, ABO-mediated reactions were less common than those due to other RBC antibodies [1].

2. Carelessness or 'human error' is the cause of

Table 1. Fatal hemolytic transfusion reactions reported to the U.S. Food and Drug Administration (1978–1982*).

Total reactions contributing to patient mortality	58
ABO Incompatible	45
Non-ABO	13
Location of error	
Ward	2
Surgery	8
Emergency Room	1
Intensive Care Unit	9
Not specified	38
Type of error	
Misidentification within blood bank	14
Misidentification in specimen collection	7
Technical error	1
Misidentification in surgery	8
Misidentification in Emergency Room	1

* Only partial data available for 1982.

the vast majority of hemolytic transfusion reactions. More than 95% of these reactions are caused by patient misidentification in drawing of the pretransfusion blood specimen, misidentification of the tube used for crossmatch by the blood bank technologist or misidentification of the patient into whom blood is transfused by the nurse or physician. Technical error within the blood bank is less common than misidentification.

3. Errors are more likely to occur in the emergency room, the intensive care facility or the surgical suite than in the general hospital ward. This probably is due to the crisis nature of the patients in these settings, as well as the multiplicity of caretakers, each of whom has many tasks to perform in addition to administering blood.

4. Women are at greater risk for hemolytic transfusion reactions, due to antibodies other than anti-A or anti-B.

5. Surgical patients are at highest risk, because anesthesia masks the early signs and symptoms of reactions and surgery personnel have numerous tasks to perform. The source of error often is the removal of the wrong unit of blood from a blood bank refrigerator located in the operating room and failure to identify the patient prior to initiating the transfusion.

6. Many transfusion reactions occur because of multiple errors by several people. Misidentification in the blood bank may be followed by failure to detect the error on the ward, and most situations involve failure by more than one person to follow standard hospital transfusion policy.

Prevention of transfusion reactions must be the primary goal and entails the following common sense policies:

1. The chain of identification, from drawing of the pretransfusion blood specimen by the phlebotomist to infusion of blood products into the recipient, must be defined clearly and understood by all persons potentially involved in administration of blood. Continuing education and actual observation of employees at work are mandatory. Extra training should be given to workers in the most dangerous areas, namely, the emergency room, the ICU and the surgical suite. Attention to such non-glamorous aspects of blood banking

could prevent more than 90 percent of all fatal hemolytic transfusion reactions.

2. Blood bank employees should be required to make use of previous records available on patients for whom blood is being crossmatched. This simple check would have prevented several of the transfusion-related mortalities reported to the FDA between the years 1978 and 1982.

3. Care in the handling of blood, following collection until the time of transfusion, is critical. Reported fatalities enumerate a potpourri of odd incidents resulting in the death of patients. They include overheating of blood in a blood warmer, overheating of blood while in transport from a regional blood donor center, bacterial contamination during pooling of platelet concentrates. Personnel transfusing blood should be trained to inspect the unit of blood for evidence of hemolysis or cloudiness suggesting bacterial contamination, before starting the transfusion.

4. A cardinal mistake is transfusing blood to the patient in 'Room 1213' rather than identifying the patient by means of a wrist identification band. It must be remembered that patients are moved frequently in a busy hospital and hazards are inevitable, if identification is made only by the room number where a transfusion has been ordered.

5. Observation of the patient for 15 minutes (longer if possible) after beginning transfusion is recommended. Generally, the severity of a reaction is proportional to the amount of incompatible blood infused. If the patient is observed for transfusion reaction, less blood will be infused, with concomitant decrease in morbidity and mortality.

Evaluation of a Transfusion Reaction

An acute hemolytic transfusion reaction presents non-specifically and is easily masked by the patient's underlying condition. Leukemic patients, for example, often have unpredictable fevers, sometimes coincidental with transfusion of blood products. It may be difficult or even impossible to determine whether the transfusion caused the fever.

Fever, with or without chills, is the most frequent presentation. Chest pain, respiratory dis-

stress, back or abdominal pain or hypotension also may occur. In the surgical patient, most of these signs and symptoms are masked by the anesthetized state, but unexplainable hypotension or bleeding may be a clue to a hemolytic reaction.

Initial assessment must be rapid to minimize the quantity of incompatible blood transfused and to allow quick reinitiation of transfusion (with a new unit of blood). A febrile non-hemolytic transfusion reaction is the most common problem in the differential diagnosis and is a diagnosis of exclusion. A carefully-planned investigation (Table 2) might proceed as follows:

(1) Clerical Check. Identification on the unit of blood should be verified against that on the patient's identification band. Most errors will be discovered at this point. The transfusion should be discontinued and the unit of blood, along with a post-transfusion patient blood specimen, sent to the blood bank.

(2) The responsible physician should be called to evaluate the patient, while the laboratory investigation is in progress.

(3) The post-transfusion blood specimen should be centrifuged and examined for evidence of hemolysis. The blood bank should perform a clerical check and repeat ABO testing on the post-transfusion specimen and the returned unit of blood. This will identify any ABO incompatibility not discovered during the clerical check. The direct anti-

Table 2. Transfusion reaction investigation

-
1. Discontinue transfusion.
 2. Doctor or nurse should evaluate patient clinically for signs and symptoms of a severe reaction.
 3. Perform clerical check at patient's bedside.
 4. Obtain post-transfusion blood specimen and send with discontinued unit of blood to blood bank.
 5. Perform clerical check in blood bank.
 6. Centrifuge post-transfusion blood specimen and examine for hemolysis (compare with pre-transfusion specimen, if available).
 7. Recheck ABO typing on post-transfusion specimen and unit of blood.
 8. Perform direct antiglobulin (Coombs) test on post-transfusion specimen.
 9. Optional tests: Serum bilirubin, haptoglobin, urine hemoglobin.
-

globulin (Coombs) test on the post-transfusion specimen will, as a rule, be positive in an acute hemolytic transfusion reaction, and a 'mixed field' microscopic appearance often is seen. If the investigation is negative to this point, a hemolytic transfusion reaction is unlikely. Attention should turn to providing another unit of compatible blood to the patient, without undue delay. Consideration should be given to using leukocyte-poor RBCs (e.g. filtered or saline washed RBCs).

(4) Completion of a hemolytic transfusion reaction investigation includes testing (often not feasible in a timely manner) which provides an estimate of the rate of RBC destruction, including elevated serum bilirubin, decreased serum haptoglobin, falling hemoglobin concentration and spherocytes on a peripheral blood smear.

Pathogenesis of the Hemolytic Transfusion Reaction

Intravascular hemolysis of RBCs constitutes an antigen-antibody reaction capable of activating the coagulation system and generating release of vasoactive compounds (bradykinin, serotonin, histamine, norepinephrine). Thus, complicated systems, usually subserving the patient in a protective manner (via mediation of inflammation and coagulation), are set loose in a sudden, devastating cascade capable of producing hypotension, shock, DIC and renal failure within hours [7]. Morbidity and mortality is high without early intervention, and the patient often is refractory to all forms of standard therapy, even when instituted early. Rapid institution of hemodynamic monitoring in the optimum setting (intensive care facility), with rapid evaluation and initiation of therapy by physicians expert in the treatment of shock and its complications, will provide the best opportunity for survival to the patient experiencing a hemolytic transfusion reaction.

Acute renal failure. Acute renal failure (ARF) is the product of complex interacting mechanisms, including DIC and alterations in vasomotor function resulting in renal tubular ischemia. The interrelationships between the coagulation system and

vasomotor mediators appear to act in concert to produce damage.

The end-product of coagulation system activation is desposition of fibrin thrombi in the microcirculation of the kidney, resulting in diminished intrarenal blood flow and ischemia. The action of the chemical mediators of inflammation (bradykinin, serotonin, histamine, norepinephrine) results in alterations of blood flow within the renal microcirculation (shunting of flow from the renal cortex to the renal medulla). This produces stasis of blood within the renal cortex, thereby causing fibrin to be deposited in the microvasculature.

Once initiated, the ARF can take on different clinical forms, such as (1) transient ARF, which follows infusion of small amounts of incompatible blood, (2) acute tubular necrosis resulting in ARF, most commonly seen following transfusion of larger quantities of blood (200 to 500 ml), or (3) bilateral renal cortical necrosis, which is both rare and irreversible. Previous theories of the pathogenesis of ARF proposed a direct toxic effect of hemoglobin or implicated renal tubular obstruction by pigment casts. Many lines of evidence speak against the toxic effect of hemoglobin, *per se*. Most important is the recent enthusiasm for use of hemoglobin solutions prepared to be free of RBC membrane constituents ('stroma-free hemoglobin'). Hemoglobin thus prepared can be infused into humans to provide oxygen-carrying capability without affecting renal function. Support for the obstructive theory has been based on microscopic observation of pigment casts in kidneys, following death from hemolytic transfusion reactions. For this theory, also, experimental verification is lacking. For example, detailed studies of battlefield casualties showed that pigment casts were never present early and were seen only one-to-three days after onset of renal failure. A crucial clarification on the question came when Schmidt and Holland showed that renal failure occurred after infusion of incompatible RBC stroma alone [8]. Indeed, infusion of compatible stroma had no ill effect. This meant that the antigen-antibody reaction itself, not the release of hemoglobin, was pivotal in the pathogenesis of ARF.

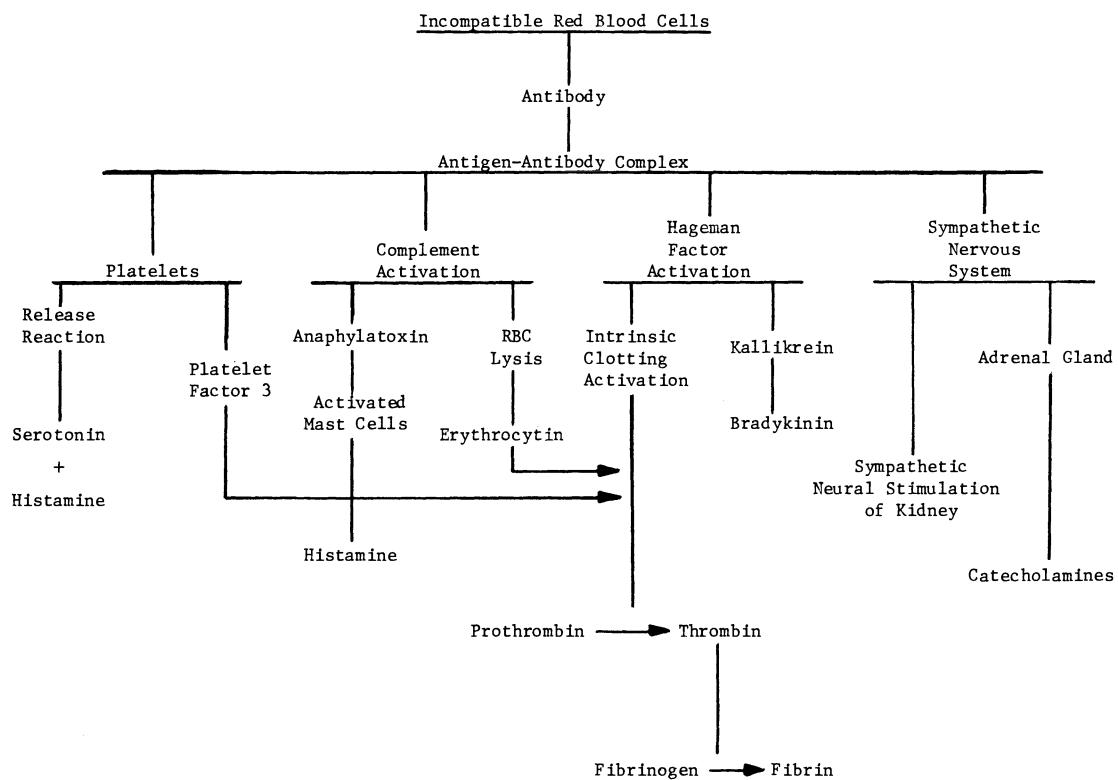


Fig. 1. Incompatible red blood cells.

Disseminated intravascular coagulation. Disseminated intravascular coagulation (DIC) can be precipitated by all forms of intravascular hemolysis. Hemolytic transfusion reactions of significant proportions frequently are complicated by DIC. In some patients, the DIC-induced bleeding may be the first sign of reaction.

DIC is produced by intravascular hemolysis through several proposed mechanisms. (1) Lysed erythrocytes release a procoagulant substance ('erythrocytin') which has platelet factor 3-like activity. Various studies indicate this factor alone will produce only mild degrees of DIC, but, when acting in conjunction with other factors, more severe degrees of intravascular coagulation may be seen. (2) Reticuloendothelial system blockade enhances intravascular coagulation. (3) Antigen-antibody complexes can activate the coagulation system alone, via interaction with Hageman Factor, platelets, leukocytes and complement.

Hypotension and shock. Hypotension and shock

were mentioned in most of the fatal hemolytic transfusion reactions reported to the FDA. The situation is analogous to endotoxin shock, with activation of multiple pathways leading to generation of vasoactive compounds. This is clearly a distributive type of shock and should be amenable to therapy aimed at reversing distribution abnormalities [9].

The multiple systems interacting during the course of a hemolytic transfusion reaction are summarized in Fig. 1. It is probable that such a complex sequence of events is explained inadequately by such a summary. It may, however, be useful for organizing an approach to therapy.

Therapy for hemolytic transfusion reactions

Treatment must be instituted rapidly, with knowledge of the serious complications likely to occur. A physician skilful in the management of shock should be involved immediately upon discovery of the untoward event. The following are guidelines

to therapy, which will, of necessity, be modified by the patient's underlying disease state and clinical factors:

(1) Fluid resuscitation should be initiated immediately with colloids or crystalloids.

(2) Cardiac and renal output must be supported, preferably with an agent such as dopamine, which possesses the pharmacologic attributes of stimulating cardiac output and increasing systemic blood pressure, while increasing blood flow in the critical renal cortical vasculature. Dopamine has a positive inotropic effect on the myocardium, similar to digoxin, yet acts as a vasodilator in renal and splanchnic vascular beds [10,11]. Previously, it was thought diuretic agents, such as furosemide and mannitol, were sufficient therapy for hypotension and renal failure following hemolytic transfusion reactions. Subsequent clinical studies have established that such modalities actually can worsen the clinical situation (e.g., when furosemide-induced hypovolemia aggravates renal failure) [11]. Since dopamine at standard dosage (1–5 mcg/kg) is both antihypotensive and renal protective, it appears to be the drug of choice for distributive shock of any etiology. Support for this choice of therapy is found in studies looking at outcome of large groups of patients in cardiogenic shock refractory to all prior therapy. Eighteen percent of these patients survived after addition of dopamine therapy [12].

(3) DIC must be anticipated in patients receiving large quantities of incompatible RBCs. Treatment should be aimed at prevention of DIC under these circumstances. By the time such signs as oozing from venipuncture or surgical incision sites become evident, it may be too late for successful intervention. When it is judged that a significant volume of incompatible RBC has been transfused, heparin therapy should be begun cautiously utilizing appropriate loading doses and continuous infusion [13]. The hemolytic transfusion reaction represents one of the rare clinical situations where DIC often can be expected. Heparin therapy will need to be continued for 6–24 hours, until the likelihood of DIC has passed.

(4) Pulmonary distress is managed with oxygen therapy and steroids for complement-mediated complications and prevention of adult respiratory distress syndrome [14].

(5) Timely administration of compatible RBCs may be of great importance, since this was the original reason for infusion of the ill-fated, incompatible blood.

It is an unusual event for a physician to manage a hemolytic transfusion reaction, but it is common to manage patients with the complications of shock, DIC and renal failure. For this reason, it is best to dispel the myths surrounding hemolytic transfusion reactions and to involve physicians skilled in the management of shock.

Volume overload

Volume overload is a complication of transfusion therapy which may be equally as deadly as a hemolytic transfusion reaction. The complication often is precipitated by failure to take into account fluids received prior to arrival in the emergency room (e.g., colloids administered by ambulance personnel). By the time the patient arrives in the ICU, he/she may have received several liters of fluid and may display early signs of pulmonary edema. Transfusion of blood products magnifies the volume problem. In this setting, the optimum strategy is to use packed RBCs, rather than whole blood, for the patient clearly in need of oxygen-carrying capacity. When signs of pulmonary edema are present, use of diuretics, limitation of fluids and augmentation of inspired oxygen may be indicated.

Patients with chronic anemia frequently develop complications of volume overload following too rapid infusion of blood products. Two practical rules to follow are never to give whole blood and never to give more than two units of packed RBCs in a 24-hour period to these non-bleeding patients.

Anaphylaxis

Patients with IgA deficiency, who have been transfused previously, are at risk of anaphylactic reactions manifested by wheezing, dyspnea, flushing, hypotension and shock. The pathogenesis involves anti-IgA antibodies reacting with IgA in donor plasma. Such reactions are rare, despite an incidence of IgA deficiency approaching 1:700 in normal individuals. Known IgA-deficient patients,

who have had previous reactions, can be managed effectively by the administration of IgA-deficient blood products (e.g., frozen or washed RBCs).

Non-cardiogenic pulmonary edema

This is a rare complication of blood transfusion, although more recent evidence indicates an incidence approaching 1:6000 [15]. Such reactions must be distinguished from pulmonary edema occurring in the elderly patient with compromised cardiac function. Non-cardiogenic pulmonary edema can be severe, even fatal, and is thought to be caused by antibodies to transfused leukocytes or by the presence of antibodies in donor plasma reacting with recipient leukocytes [16]. The mainstay of therapy is ventilatory support and possibly steroids for complement mediated vascular injury.

Febrile non-hemolytic transfusion reactions

Febrile, non-hemolytic transfusion reactions are the most commonly encountered transfusion complications. Their significance is due to the fact that (1) the febrile response elicits an investigation to rule out a hemolytic reaction, thus delaying appropriate hemotherapy, and (2) the fever may confuse the evaluation of a sick, questionably septic patient.

Febrile transfusion reactions are thought to be caused by immune reactions to leukocyte or platelet antigens (most common in multiply transfused or multiparous patients), or to protein constituents of plasma. It has been shown that saline washing of RBCs significantly reduces the number of leukocytes per unit (by approximately 90 percent) and virtually eliminates plasma. As a result, substitution of saline washed RBCs, in place of packed RBCs, produces an overall 50 percent reduction of febrile reactions, with only a 10–15% loss of RBC mass per unit of blood washed [17,18], and further information on this aspect is dealt in Chapter 6.

Management of febrile reactions should be aimed at rapid determination that a hemolytic transfusion reaction has not occurred. This allows for rapid reinstitution of transfusion with a new, compatible unit of appropriately processed RBCs.

Miscellaneous reactions

Bacterial contamination. Bacterial contamination is possible, whenever the integrity of the unit of blood is violated. Observance of standard blood bank practices has virtually eliminated this problem. Such complications, nonetheless, still are being reported, and they underscore the importance of taking meticulous care in collecting and processing units of blood, and examining the blood product prior to infusion.

Metabolic alterations. Metabolic changes in stored blood may result in elevated plasma concentrations of potassium, lactic acid and ammonia. Addition of citrate to blood during collection results in binding of ionized calcium. Therefore, infusion of blood may produce metabolic disturbances in the recipient. These, generally, are seen only in massively-transfused patients. Saline washing of RBCs, prior to transfusion, significantly reduces this problem.

Microaggregates. Microaggregates, formed from degenerated leukocytes and platelets, accumulate in banked blood during storage. Infusion of microaggregates may result in respiratory distress. Transfusion of washed RBCs, use of microaggregate blood filters or preferential infusion of fresher units of blood, is advisable for patients in whom transfusion of relatively massive quantities of blood can be anticipated.

Delayed hemolytic transfusion reactions

Hemolytic reactions, occurring days or weeks following blood transfusion, are referred to as delayed hemolytic transfusion reactions (DHTR) and represent an anamnestic immune response in a previously sensitized patient. The distinction from acute hemolytic transfusion reactions is not simply defined. Because of the reduced rate of destruction, the DHTR is usually more benign, with less morbidity and mortality.

The hallmark of the DHTR is a falling hemoglobin concentration in a non-bleeding, recently-transfused (3–21 days) patient. Fever, in the absence of obvious infection, may call attention to the

problem. Mild jaundice may be evident. The true incidence of DHTR is difficult to determine, because the diagnosis often is not entertained. Many patients have no symptoms, or their mild symptoms may be missed, due to the underlying medical condition.

Studies of DHTR, in large medical centers with excellent surveillance and reporting policies, suggest an incidence of approximately 1:7000 transfusions [2]. Prevention may be difficult, because, frequently, antibodies are present at undetectable levels in pre-transfusion specimens. Some authorities recommend increasing the sensitivity of pre-transfusion antibody detection tests. Detractors argue that evaluation of false positive tests, generated by more sensitive techniques, would delay delivery of blood to patients and create a more deleterious effect than that encountered by occasionally failing to detect an unexpected antibody prior to transfusion.

Diagnosis of a DHTR rests, first and foremost, on a high degree of clinical suspicion. Laboratory studies may reveal a falling hemoglobin concentration, decreased or absent serum haptoglobin and indirect hyperbilirubinemia. Treatment involves careful observation and avoidance of transfusion of additional units of incompatible RBCs. Serious complications are rare, but renal failure and death have been reported [19].

Prevention of transfusion reactions

Transfusion complications range from life-threatening, hemolytic reactions to troublesome, febrile reactions. Prevention, rather than treatment, is clearly the preferred course. Careful blood banking policies, with strict adherence to identification of blood components and their recipients, as well as technical competence in pre-transfusion testing, will prevent the majority of transfusion-related complications. Careful monitoring of the patient, during transfusion, will reduce the severity of most complications. Provision of only that portion of whole blood actually needed (i.e., component therapy) can reduce the overall risk of untoward reactions.

References

1. Pineda AA, Brzica SM Jr, Taswell HF: Hemolytic transfusion reaction. Recent experience in a large blood bank. *Mayo Clin Proc*, 1978; 53: 378-90.
2. Pineda AA, Taswell HF, Brzica SM Jr: Delayed hemolytic transfusion reaction. An immunologic hazard of blood transfusion. *Transfusion*, 1978; 18: 1-7.
3. Schmidt PJ: Transfusion mortality; with special reference to surgical and intensive care facilities. *J Florida Med Assoc*, 1980; 67: 151-53.
4. Honig CL, Bove JR: Transfusion-associated fatalities: Review of Bureau of Biologics reports 1976-1978. *Transfusion*, 1980; 20: 653-61.
5. Myhre BA: Fatalities from blood transfusion. *JAMA*, 1980; 244: 1333-35.
6. Schmidt PJ: The mortality from incompatible transfusion. In: *Immunobiology of the Erythrocyte*. New York, Alan R Liss, Inc., 1980: 251-61.
7. Goldfinger D: Acute hemolytic transfusion reactions - a fresh look at pathogenesis and considerations regarding therapy. *Transfusion*, 1977; 17: 85-98.
8. Schmidt PJ, Holland PV: Pathogenesis of the acute renal failure associated with incompatible transfusion. *Lancet*, 1967; ii: 1169-72.
9. Sobel BE: Cardiac and noncardiac forms of acute circulatory collapse (shock). In: *Heart Disease, A Textbook*, Braunwald E (ed). London, W.B. Saunders, 1980: 590-29.
10. Goldberg LI: Drug Therapy. Dopamine-clinical uses of an endogenous catecholamine. *N Engl J Med*, 1974; 291: 707-10.
11. Lucas CE, Zito JG, Carter KM, Cortez A, Stebner FC: Questionable value of furosemide in preventing renal failure. *Surgery*, 1977; 82: 314-20.
12. Thompson WL: Dopamine and other vaso-active agents in shock (edited summary). *Scot Med J*, 1979; 24: 89-93.
13. Rock RC, Bove JR, Nemerson Y: Heparin treatment of intravascular coagulation accompanying hemolytic transfusion reactions. *Transfusion*, 1969; 9: 57-61.
14. Popovsky MA, Abel MD, Moore SB: Adult respiratory distress syndrome associated with passive transfer of leukocyte antibodies. Abstract. American Association of Blood Banks, 35th Annual Meeting, Anaheim, California. *Transfusion*, 1982: 403.
15. Thompson J, Severson CD, Parmely MJ, Marmorstein BL, Simmons A: Pulmonary 'hypersensitivity' reactions induced by transfusion of non-HL-A leukoagglutinins. *N Engl J Med*, 1971; 284: 1120-25.
16. Dubois M, Lotze MT, Diamond WJ, Kim YD, Flye MW, Macnamara TE: Pulmonary shunting during leukoagglutin-induced noncardiac pulmonary edema. *JAMA*, 1980; 244: 2186-89.
17. Goldfinger D, Lowe C: Prevention of adverse reactions to blood transfusion by the administration of saline-washed red blood cells. *Transfusion*, 1981; 21: 277-80.

18. Goldfinger D, Lowe C: A critical analysis of saline washed red blood cells with special reference to their ability to prevent adverse reactions to blood transfusion. In: Safety in Transfusion Practices, Polesky HF, Walker RH (eds). Skokie, Illinois, College of American Pathologists, 1982: 205-22.
19. Meltz DG, David DS, Bertles JF, DeCutiis AC: Delayed haemolytic transfusion reaction with renal failure. *Lancet*, 1971; ii: 1348-49.

18. Infections transmitted by blood transfusion

E. TABOR

1. Introduction

Infectious complications of blood transfusion [1] are twentieth century diseases which occur as adverse reactions to a twentieth century form of therapy, blood transfusion. Many of these infections are caused by historically well-established microorganisms; others are caused by agents of known diseases which have only been identified in recent decades; still others are caused by yet unidentified agents.

2. Hepatitis B virus

Although the transmission of hepatitis by the inoculation of human blood or plasma has been recognized since the late nineteenth century, a means for detecting the hepatitis B virus (HBV) has only been available since 1964. A variety of well-defined serologic markers have been identified since then, permitting the accurate diagnosis of almost all cases of HBV infection.

Prior to the introduction of serologic testing, as many as 60% of post-transfusion hepatitis cases were due to hepatitis B [2]. As a result of federal regulations that donor blood be tested by sensitive techniques in all blood banks in the United States, the prevalence of hepatitis B was reduced to 11–13% of cases of post-transfusion hepatitis [3,4], occurring in about 1.7% of all blood recipients [3].

The reservoir for post-transfusion hepatitis B is the asymptomatic adult donor who is chronically infected with HBV at a level too low to be detected

by screening his or her serum for HBsAg. Although the exact number of such HBsAg-negative, HBV-infected blood donors is not known, their prevalence may be about 0.6%, based on a 3% infection rate of hepatitis B in recipients of 1–5 units of blood [3]. However, this is an estimate, and the prevalence varies among different cities in the United States [3].

Certain pooled plasma derivatives, anti-hemophilic factor (factor VIII, AHF) and factor IX complex (factor IX) have had a particularly high risk for the transmission of HBV, although recently developed means to inactivate HBV in these products may significantly reduce this risk in the future. The plasma pools used in the manufacture of AHF and factor IX include more than 1000 units of plasma in each pool and therefore have a statistical risk of including a unit of plasma with a level of HBV below the limit of detectability for HBsAg. Despite the fact that not all lots of AHF and factor IX at the present time transmit HBV, the continued transmission by a percentage of lots has been documented [5]. Patients with newly diagnosed hemophilia who receive these products for the first time are at a particularly high risk for acquiring HBV infection.

The recent development of methods to inactivate HBV in AHF and factor IX is expected to have a major impact on the transmission of HBV to hemophiliacs. Heimberger and co-workers developed a method of stabilizing AHF using glycine and saccharose to permit heating this otherwise thermo-labile product at 60°C for 10 h. The combination of the manufacturing process plus heating

was shown to inactivate 10^6 infectious doses of HBV per ml (based on a recalculation of the published data) [6, 7]. AHF stabilized by another method (an unspecified proprietary method) could be heated at 60°C for 10 h [8]; although this procedure resulted in prolongation of the incubation period of HBV, it is not clear whether this represented a reduction in infectivity. Other investigators have shown that the addition of high titer anti-HBs to factor IX can neutralize the infectivity of HBV [9].

Passive immunization is ineffective in the prevention of post-transfusion hepatitis B. In two prospective, double-blind controlled studies [10, 11], both pre-transfusion and post-transfusion administration of hepatitis B immune globulin (HBIG) to transfusion recipients have been shown to have no effect on the incidence of hepatitis B. HBIG is useful, however, for prophylaxis for those exposed to HBV through accidental needle stick exposure, mucocutaneous splash, or ingestion of materials containing HBV.

Active immunization, in the form of hepatitis B vaccines, may decrease the overall incidence of post-transfusion hepatitis B if an inexpensive vaccine can be made widely available so that the prevalence of infections among blood donors is reduced. In the absence of successful propagation of HBV *in vitro*, the currently available vaccines have been manufactured from 22-nm HBsAg spherical particles purified from the plasma of asymptomatic humans chronically infected with HBV; these particles are treated with pepsin, urea, and formalin. These vaccines have been shown to be safe and effective [12,13]. Recent studies have suggested that less expensive vaccines may be developed in the future from the immunogenic polypeptides of HBsAg synthesized de novo [14] or from HBsAg produced by cloning in yeast cells [15].

3. Hepatitis A virus

The infectivity of serum from patients with hepatitis A virus (HAV) infections has been shown in volunteer studies to begin sometime less than two weeks before the onset of symptoms and to extend

until four days after the onset of symptoms [16]. However, the rare transmission of HAV by transfusion of blood donated 11, 15, and 18 days before onset of symptoms in the donor suggests that HAV can be present in the blood of some infected persons throughout a substantial portion of the incubation period [17, 18, 19]. This limited period of infectivity of blood during acute HAV infection and the fact that no chronic carrier of HAV has ever been detected means that HAV is rarely transmitted by blood transfusion.

4. Non-A, non-B hepatitis

As many as 89% of cases of transfusion-associated hepatitis are due to the agent or agents of non-A, non-B hepatitis [20], unmasked by the elimination of HBsAg-positive blood from transfusion services. Between 5 and 15% of recipients of 1–5 units of blood transfused in the United States develop non-A, non-B hepatitis [3]. It appears that more than one agent may be responsible for non-A, non-B hepatitis, and the diagnosis remains based on the exclusion of other known causes of hepatitis because there has been no general acceptance of the experimental serologic tests.

Transmission of non-A, non-B hepatitis must occur by nonparenteral means as well as by transfusion, in order to account for the high prevalence of this infection among healthy blood donors, most of whom have had no exposure to potential sources of hepatitis. Although clinically apparent infection does not appear to be transmitted easily to close contacts [21], 27% of sporadic cases of clinically recognizable hepatitis have been shown to be non-A, non-B hepatitis [21].

Chronic hepatitis has been shown to result from up to 46% of cases of post-transfusion non-A, non-B hepatitis [22]. Biopsy evidence of chronic active or chronic persistent hepatitis was observed in 80–100% of these patients with chronic non-A, non-B hepatitis. Furthermore, the high prevalence of persistent elevation of aminotransferase levels in hemophiliacs has been thought to represent in part chronic non-A, non-B hepatitis. The prevalence of chronic hepatitis following sporadic cases is much

lower, usually around 7% [23], for reasons which have not been established.

Many, if not all, of these chronic infections, if present in an asymptomatic blood donor, would result in the transmission of non-A, non-B hepatitis to a susceptible transfusion recipient. The duration of infectivity may be many years, even in the presence of normal aminotransferase levels, as documented using the chimpanzee animal model for the study of this disease [24].

Non-A, non-B hepatitis is frequently transmitted by certain plasma derivatives. Antihemophilic factor (AHF; factor VIII), factor IX complex (factor IX), and fibrinogen have been shown to transmit one or more agents of non-A, non-B hepatitis to humans and chimpanzees. In fact, it appears that nearly every lot of unheated AHF or factor IX transmits non-A, non-B hepatitis [5]. The inactivation of an agent of non-A, non-B hepatitis by heating at 60°C for 10 h [25] makes it likely that the heating of stabilized AHF or factor IX at 60°C for 10 hours will result in safer products when such processes are widely applied [6,7,8]. Albumin, which routinely is subjected to this treatment, probably does not transmit non-A, non-B hepatitis. Immune globulin (γ -globulin;IG) manufactured by Cohn cold ethanol fractionation is thought not to transmit non-A, non-B hepatitis because of the concentration of antibodies from convalescent donors in this product, and no cases of transmission have been reported.

The diagnosis of non-A, non-B hepatitis is still based on the exclusion by serologic tests of known etiologic agents of hepatitis and the exclusion by history of toxic causes of hepatitis. Candidate serologic assays for non-A, non-B hepatitis have been the subject of at least 18 reports in the literature. Although relatively little is known at present concerning the nature of the antigen-antibody systems, it is clear that one or more antigens have been detected in the serum and liver of patients and experimentally infected chimpanzees with non-A, non-B hepatitis. Given the likelihood of more than one non-A, non-B hepatitis agent, the possibility of misdiagnosed cases of non-A, non-B hepatitis and the scarcity of collaborative studies to compare reagents and inocula, it is not surprising that there

is neither clarity nor agreement concerning these experimental assays.

The efficacy of immune globulin in ameliorating or preventing non-A, non-B hepatitis following transfusion cannot be evaluated adequately until a method is developed for measuring the titer of protective antibody in immune globulin preparations. Preliminary results, however, suggest that some lots of immune globulin can reduce the number of icteric cases [26] and the number of patients who develop chronic non-A, non-B hepatitis [27] following transfusions.

Inactivation of non-A, non-B hepatitis agent(s) by heating at 60°C for 10 hours [8, 25] and by formalin [28, 29] may provide the basis for development of vaccines against the agent(s) in the future. The introduction of clotting factor concentrates which have been heated or otherwise treated to inactivate viruses will ensure the absence of viable non-A, non-B hepatitis agents from these products.

Elevated levels of serum aminotransferase activity, particularly alanine aminotransferase (ALT; SGPT) in donor blood have been found to be associated with the transmission of non-A, non-B hepatitis by blood transfusion [30]. However, the problem of nonspecific false-positive results in donors would make ALT tests for the screening of blood donors difficult to interpret [31]. In fact, many cases of posttransfusion non-A, non-B hepatitis are transmitted by blood from donors with normal ALT levels [30, 31, 32]. Standardization of ALT tests to ensure uniform application, were they to be used for screening blood donors, is a technological difficulty which has not been resolved. Screening blood donors for ALT levels is not recommended at the present time.

5. Cytomegalovirus

Cytomegalovirus (CMV) was first recognized as a cause of transfusion-transmitted infections when its association with the postperfusion syndrome was first described [33]. This syndrome, characterized by fever, atypical lymphocytosis, and splenomegaly, is most frequently caused by CMV

transmitted by transfusions during open heart surgery. Results of numerous studies have shown that CMV can be transmitted by whole blood [1]. The risk of CMV infection, defined by seroconversion, is proportional to the amount of blood received, with a 7% risk among recipients of single-unit transfusions and 21% among recipients of multiple-unit transfusions in one study [34]. In some studies the percentage of patients infected has been even higher.

Since CMV is associated with the leukocyte fraction of blood, reducing the number of white cells in blood units has been found to reduce the incidence of post-transfusion infections. In general, the use of washed, packed red blood cells carries a lower risk than whole blood, and the use of frozen, deglycerolized red blood cells carries no risk. In contrast, leukocyte transfusions are associated with a very high incidence of CMV infections.

The transmission of CMV by blood products which do not contain leukocytes has not been described. One report of a high prevalence of antibodies to CMV among hemophiliacs may reflect the higher frequency of whole blood transfusions in these patients, rather than any association with AHF or factor IX [35]. CMV has not been successfully cultured from AHF [36].

It has not been established whether CMV infections transmitted by transfusions occur as a result of blood donations by actively infected persons, by reactivation of latent CMV infections in the donor leukocytes following transfusions, or by reactivation of latent infections in the recipients resulting from the transfusion. Probably all three mechanisms are operative in different clinical situations, with the first two being more common.

Prevention of transfusion-transmitted CMV infections is still a technological challenge. Efforts to develop a vaccine against CMV are still in the preliminary stages of clinical testing. Attempts to use interferon, immune globulin, or immune plasma have not produced satisfactory results. Storage of blood for 48–72 h prior to use did not significantly reduce the rate of CMV infections. Longer periods of storage have not yet been evaluated.

For selected patients at high risk for severe CMV

infections, particularly those who are immunocompromised, the use of more expensive techniques may be appropriate. The use of frozen-deglycerolized red blood cells which have no leukocytes is suitable [37]. Blood from donors selected for the absence of antibody to CMV has also been used [38] with elimination of CMV infections. However, the sensitivity and specificity of the currently available tests for antibody to CMV are not acceptable at the present time for general screening of blood donors.

The use of ultraviolet or gamma irradiation has been discussed for the elimination of CMV infectivity of blood without destruction of vital blood elements. However, concern resulting from the observation that radiation-induced defects in the CMV genome can confer on the virus the ability to cause cellular transformation *in vitro* has prevented its use. Furthermore, in one study, administration of gamma-irradiated leukocytes to immunosuppressed patients was associated with an increased incidence of CMV infections [39].

6. Epstein-Barr virus

Epstein-Barr virus (EBV) infections can be transmitted by blood transfusion [40]. Following transfusion of between two and 14 units of fresh blood, 8% of recipients may develop EBV infections, most of which are asymptomatic [41]. Some of these infections are actually reinfections of persons with pre-existing antibodies to EBV. New infections among totally susceptible patient populations may reach 33–46% of transfusion recipients [41, 42].

EBV is a highly prevalent virus in all donor populations. It may remain latent in a host for years after apparent recovery. All previously infected persons, are thought to have EBV in one of every 10^7 circulating lymphocytes [43].

Infectivity of blood from EBV-infected donors is known to precede their symptoms, probably by two to 17 days [44, 45]. EBV infectivity is known to survive storage at 4°C for three days, although the effect of longer storage is unknown. Transmission by plasma and plasma derivatives probably rarely

occurs, if ever, because the virus is predominantly intracellular, although this has not been extensively studied. However, EBV has apparently been transmitted by ingestion of infected serum [46] and also probably by infusion of infected single-donor platelet-rich plasma [45].

There is at present no practical means for preventing EBV infections transmitted by blood transfusions. The high prevalence of EBV, the persistence of latent EBV in circulating lymphocytes after recovery from acute infection, the usually self-limited nature of the disease, and the cost of testing make it unlikely that routine screening of blood donors will ever be instituted. However, the potential risks of EBV infection in immunocompromised patients indicate a possible benefit from screening blood by serologic methods for selected recipients; such screening is not routinely practiced at the present time.

7. Delta agent

Delta agent is a defective virus which requires helper functions from HBV. It is most common in Italy, but is also found in other countries, particularly among those who are repeatedly exposed to blood or plasma derivatives. For instance, antibodies to delta agent (anti-delta) may be found in 45–100% of HBsAg-positive hemophiliacs from Italy, Germany, and the United States [47]. HBsAg-positive drug addicts may also be found to have delta infections due to exposure to multiple sources of blood. Delta agent has also been shown to be transmitted from delta-infected HBsAg-positive mothers to their newborn infants.

Since delta agent infection appears to occur only in the presence of HBV infection, effective screening of blood donors for HBsAg will prevent most cases of transfusion-transmitted delta infection. Further technological advances in screening blood donors for HBV will at the same time eliminate all transfusion-transmitted delta infections.

8. *Treponema pallidum*

Transmission of *Treponema pallidum*, the agent of syphilis, by blood transfusion is of concern only where fresh blood or fresh blood components are used, because of its limited survival in stored blood. *T. pallidum* can be transmitted to blood recipients in the absence of symptoms in the donor, in the incubation period (as early as 20 days before the appearance of a chancre), or during primary syphilis, with or without a positive serologic test. Blood obtained from donors in the secondary and tertiary stages has not been documented to transmit the infection to recipients or to experimental animals [48], although donors with latent stage syphilis, following the symptomatic resolution of secondary syphilis, have been implicated [49].

T. pallidum is killed by storage of citrated human blood at 4°C for 48 to 72 h [50,51]. No case of *T. pallidum* infection has been reported following transfusion of stored blood, including in prospective studies of the transfusion of serologically reactive blood [52,53]. However, the increasing demand for fresh blood for open heart surgery and premature newborn infants with hemolytic disease has resulted in an increased risk of transmission of *T. pallidum* in recent years.

Screening blood by means of the serologic test for syphilis (STS), of which the VDRL is a variant, is only partially effective in preventing the transmission of the disease to blood recipients. Although there have been discussions about possible elimination of the requirement for such testing in the United States, the regulation has remained intact, in part for aesthetic reasons and in part because of the partial benefit for fresh blood. However, only 25% of symptomatic individuals have a positive STS at the time the chancre of syphilis first appears [54]. The STS may remain permanently negative in 1–2% of all cases because of a prozone phenomenon [54].

9. Malaria

Transfusion malaria is an occasional problem in developed countries because of international travel and the migrations of large numbers of refugees.

As few as 10 malarial parasites in a blood sample can transmit malaria, and many instances of transmission by accidental needle stick have occurred [55]. Transmission by red blood cell transfusion is well-documented. Transmission by plasma and by leukocytes has also occurred, presumably due to residual red blood cells. Transmission has also been documented to occur by platelet transfusion; the malarial parasite has in fact been observed inside platelets [56]. Frozen plasma appears not to transmit malaria [57]; frozen-deglycerolized red blood cells can transmit malaria, since the glycerolization contributes to preservation of the parasite [58].

The viability of malarial parasites in erythrocytes declines when blood is stored at refrigeration temperatures for more than seven days. However, transmission has occurred with blood stored longer than 10 days. The incubation period for transfusion malaria is generally longer than that for mosquito-transmitted malaria, but may range from one to 110 days.

Prevention of transfusion malaria depends entirely upon the history obtained from the prospective donors. Persons taking suppressive chemoprophylaxis and those who have some immunity from previous infections may currently have asymptomatic malaria infections and thus may not be successfully screened by asking for a history of having had malaria. Furthermore, a simple history of travel to endemic areas may miss a case which was contracted during a brief stopover in transit. Thus a good history should include the place of birth, places of residence during childhood and adulthood, immigration, a complete travel history for the past three years including seasonal travel, history of diagnosed malaria, bouts of fever, and prior chemoprophylaxis or treatment for malaria [1].

10. Microfilariae

The microfilariae of five species of filariae can be found in human blood and have been documented to be transmitted to blood transfusion recipients; microfilariae of the other three common filariae

have not been documented to be transmitted by blood. The transfusion-transmissible microfilariae are those of *Wuchereria bancrofti*, *Acanthocheilonema perstans*, *Mansonella ozzardi*, *Loa loa*, and *Brugia malayi*. Some microfilariae can survive in blood stored at refrigeration temperatures for at least 14 days [59]. Tourists to endemic regions may transmit these infections when they donate blood after their return to developed countries. However, these parasites never reach the adult filarial stage outside of their endemic regions, since the required insect vectors are not present.

The rarity of symptoms following transmission of microfilariae has led to a consensus that routine screening of donor blood for these organisms is not warranted. However, the recommendation of the American Association of Blood Banks for malarial prevention, that donors should not be accepted who have visited malarial countries within the preceding six months, should help prevent transmission of microfilariae by blood.

11. Trypanosomiasis

American trypanosomiasis, also called Chagas' disease, is caused by *Trypanosoma cruzi*, and it is one of the most important transfusion hazards in South America. The prevalence of chronic infection with *T. cruzi* in the general population in South and Central America and Mexico ranges from 1 to 33%. Although occasional sporadic cases have been reported from Texas, and chronic infections may reach a prevalence of 2.5% in isolated areas of the southern United States, symptomatic cases are rare in the United States; no explanation for this has been found [55]. From 12 to 24% of recipients of blood from donors with serologic evidence of Chagas' disease become infected [60, 61, 62]. Hemophiliacs in endemic regions may have a prevalence of Chagas' disease of 63% [63], believed to be due to frequent exposure to infected blood. It is not known whether clotting factor concentrates can transmit this disease, however. Potentially infectious *T. cruzi* can survive the preparation of plasma from whole blood [55].

T. cruzi can survive at 4°C for at least 17–21 days

in blood [63]. Thus, prevention of transfusion-transmitted infections in endemic areas has been proposed by the addition of sterilizing compounds to blood for transfusion, although none of the proposed methods has been generally accepted. It has also been proposed that blood collected in endemic areas be screened for *T. cruzi* using the Machado-Guerreiro test, a complement fixation assay for antibodies to the agent, although this too has not been generally applied, presumably because of the high prevalence of infections among blood donors.

12. Babesiosis

Babesia microti may occasionally be transmitted by blood transfusion. The best documented case was transmitted by platelet transfusion [64]. Since the distribution of this parasite is limited to Nantucket Island, Martha's Vineyard, Long Island, and Shelter Island in the northeastern United States, it could only be transmitted in association with blood transfusion if the donor lived in or had recently visited those areas. This malaria-like agent has a two-year life cycle which includes a rodent and a deer in those areas. The incubation period is one month, and the maximum period of parasitemia in humans is about six months.

Persons who have been immunosuppressed or have undergone splenectomy are particularly susceptible to being infected by *B. microti*; however, most infections occur in immunologically intact hosts. Although it has been suggested that blood recipients who are immunocompromised be transfused with blood obtained outside areas endemic for *B. microti*, this has been logistically impractical. However, the use of blood screened by examination of stained smears or by tests for antibodies to *B. microti* for transfusion of immunocompromised recipients or those who have had splenectomies is probably reasonable in endemic areas.

13. Bacteria

Bacterial infections transmitted by blood transfusion are a common occurrence in many parts of the

world. However, in industrialized countries such as the United States, they are now uncommon because of the introduction of the 'closed system' of blood collection, the use of sterile disposable plastic blood containers and tubing, and the universal storage of blood at refrigerator temperatures. An extensive review of the risks incurred when such techniques are not properly applied has been published [1].

14. Acquired immunodeficiency syndrome (AIDS)

The discovery of a small number of cases of Acquired Immunodeficiency Syndrome (AIDS) in heterosexual hemophiliacs, raised the question of whether an AIDS agent might be transmissible by blood transfusion. Additional evidence to support this theory included the fact that the distribution of AIDS cases among homosexuals, drug addicts, and Haitian immigrants resembles that of hepatitis B virus, a blood-borne agent. A total of about 7,000 cases of AIDS were reported in the United States by the end of 1984. The rapid accumulation of epidemiologic information about this disease led to the conclusion that an infectious agent is the cause.

AIDS is recognized by the occurrence of either a life-threatening opportunistic infection such as *Pneumocystis carinii* or Kaposi's sarcoma in a person under age 60 years who has no underlying immunosuppressive disease and has not received immunosuppressive therapy. Mortality from AIDS has ranged from 22% for those with Kaposi's sarcoma to 46% for those with *P. carinii* pneumonia [65], far higher than the mortality seen from these disorders in patients without AIDS. Many AIDS patients had a prodrome lasting weeks to months characterized by weight loss, lymphadenopathy, fever, and diarrhoea. Although this prodrome was more common in patients with *P. carinii*, it was by no means universal among AIDS patients.

A case of an AIDS-like syndrome has been observed in a 14-month old infant following the receipt of a platelet transfusion from a donor who later developed AIDS. The infant received the implicated platelets in the course of receiving transfusions of irradiated whole blood and platelets in

the first two months of life from 18 donors to treat hemolytic disease of the newborn [66]. Although this case appears to meet the criteria established for the diagnosis of AIDS, 17 other donors were involved and the infant appeared to handle infections poorly as early as four to seven months of age (and hence may never have had an intact immune system).

AIDS, or a syndrome indistinguishable from AIDS, had been diagnosed in 52 hemophiliacs in the USA (including 2 in hemophilia B and 2 patients with other clotting disorders) and 3 in the UK. So far, all hemophiliacs with AIDS have had either *Pneumocystis carinii* or, in a small number of cases, some other opportunistic infection; none has had Kaposi's sarcoma. Despite concern about the apparently rising rate of reporting of new cases in other high risk populations, the rate of new cases in hemophiliacs has remained quite constant throughout 1982 and 1983. Recent studies have revealed that in 1979, two of 101 hemophiliacs who died in the United States died of opportunistic infection [36]. This suggests that AIDS or an AIDS-like syndrome may have been present among hemophiliacs even before the current recognition of the syndrome.

Certain interim precautions have been taken, including the screening of potential plasma donors by history and by a physical examination for lymphadenopathy, and in the case of at least one manufacturer, the exclusion of plasma collected in the cities where AIDS cases have been concentrated [67]. Recommendations by the Centers for Disease Control, the Food and Drug Administration, and the National Institutes of Health include a recommendation that collection centers request that high-risk persons refrain voluntarily from donating blood or plasma [68]. Detailed recommendations were compiled by groups representing government and the blood collecting community and were distributed to all collection establishments in March 1983. These included, in addition to the recommendation for voluntary self-screening, alterations in donor history and physical examination to identify donors who might be in the incubation period of AIDS, instructions concerning labeling, and advice on the proper dispo-

sition of blood or plasma from donors who are found to have AIDS after having donated. Such interim procedures are expected to reduce the risk of AIDS transmission, if the agent is in blood, until technological advances make possible laboratory screening for LAV/HTLV III antibody [77].

15. Serum parvovirus-like virus (SPLV)

Serum parvovirus-like virus (SPLV) is a 20–23 nm virus found in serum and associated with aplastic crises in patients with sickle cell disease. Although SPLV is said to be associated in most cases with a transient viremia, the high association of the use of AHF and factor IX with evidence of SPLV infection [69] suggests that chronic viremia may occur in some plasma donors. The virus has been identified in the serum of asymptomatic blood donors [70]. Respiratory transmission is also believed to occur.

At least 22–30% of the normal population have antibodies to SPLV by the age of 16 years. The peak incidence of infection occurs between ages four to six years [71]. Most infections are probably asymptomatic. It has been suggested that the epidemics of aplastic crisis in sickle cell patients which have been observed every three to five years may be due to epidemics of SPLV.

The incubation period of SPLV following transfusion is nine to ten days. The incubation period for non-parenteral transmission to close contacts has been observed to be 17 days; shorter incubation periods of one to seven days have been ascribed to infection by a common source, although this remains to be confirmed.

Relatively high titers of antibodies to SPLV are found in immunoglobulin lots [69]. Although it has not been documented that these antibodies confer protection, the fact that most sickle cell patients have at most one aplastic crisis in a lifetime suggests that naturally acquired immunity does exist.

In addition to the suggested role of this virus in aplastic crisis in sickle cell disease, an association of this virus with aplastic crisis in thalassemia and pyruvate kinase deficiency has also been noted. SPLV has been closely associated with one outbreak of erythema infectiosum (fifth disease) as

well as with a mild febrile illness in other hematologically normal persons.

16. Human T-cell lymphotropic virus (HTLV)

Human T-cell lymphotropic virus (HTLV), a retrovirus associated with certain T-cell malignancies in humans, appears to be transmissible by blood transfusion. *In vitro*, HTLV can be transmitted from peripheral blood by co-cultivation with susceptible T-cells. The detection of antibodies to HTLV, indicating latent infection, in 11% and 16% of hemophiliacs from two cities in the United States strongly suggests transmission by blood or plasma derivatives [72]. The development of latent infections, thought to last for many years, may result in a reservoir for the transmission of HTLV by blood donors.

The highest prevalence of HTLV infection is found in patients with T-cell malignancies. HTLV infections are found in 88 to 100% of patients with adult T-cell leukemia and in 50 to 100% of patients with T-cell lymphoma [73, 74]. As a retrovirus, an RNA virus carrying an RNA-directed DNA polymerase (reverse transcriptase) which can cause transformation of a normal cell into a malignant cell, HTLV bears a conceptual resemblance to the retroviruses which are closely associated with certain animal leukemias and sarcomas [73].

HTLV is endemic in the southwestern Japanese islands of Kyushu and Shikoku, as well as in certain Caribbean islands; these areas also have an unusually high prevalence of T-cell leukemia. Among the general population of these endemic areas, HTLV infections may be present in 4 to 12% of normal blood donors. In one city on Kyushu, antibodies to HTLV were found in 37% of the general population [74]. In the southeastern United States, antibodies to HTLV were detected in 2% of normal blood donors [75]. Transmission of HTLV appears to occur commonly among family members [73, 76]. However, HTLV is not usually transmitted from parent to infant via the germ cell line, since nucleic acids and proteins associated with the presence of HTLV have not been found in cell types other than T-cells from infected persons.

References

1. Tabor E: Infectious complications of blood transfusion. Academic Press, New York, 1982.
2. Alter HJ, Holland PV, Purcell RH: The emerging pattern of post-transfusion hepatitis. *Am J Med Sci*, 1975; 270: 329-34.
3. Aach RD, Lander JJ, Sherman LA, Miller WV, Kahn RA, Gitnick GL, Hollinger FB, Werch J, Szmuness W, Stevens CE, Kellner A, Weiner JM, Mosley JW: Transfusion-transmitted viruses: interim analysis of hepatitis among transfused and nontransfused patients. In: *Viral Hepatitis*, Vyas GN, Cohen SN, Schmid R (eds). Franklin Inst. Press, Philadelphia, 1978: 383-96.
4. Alter HJ, Purcell RH, Holland PV, Feinstone SM, Morrow AG, Moritsugu Y: Clinical and serological analysis of transfusion-associated hepatitis. *Lancet*, 1975; ii: 838-41.
5. Tabor E: The three viruses of non-A, non-B hepatitis. *Lancet*, 1985; i: 743-45.
6. Heimburger N, Schwinn H, Gratz P, Luben G, Kumpe G, Herchenhan B: Factor VIII concentrate, highly purified and heated in solution. *Arzneim Forsch*, 1981; 31: 619-22.
7. Heimburger N, Schwinn H, Mauler R: Factor VIII concentrate, hepatitis-safe: progress in the treatment of hemophilia A. *Die Gelben Hefte*, 1980; 20: 165-74.
8. Hollinger FB, Dolana G, Thomas W, Gyorkey F, Kingdon H: Heat-inactivation of a non-A, non-B hepatitis agent and hepatitis B virus in human clotting factor concentrates. *Hepatology*, 1982; 2: 705 (abstract).
9. Tabor E, Aronson DL, Gerety RJ: Removal of hepatitis-B-virus infectivity from factor-IX complex by hepatitis-B immune-globulin. *Lancet*, 1980; 2: 68-70.
10. Knodel RG, Conrad ME, Ginsberg AL, Bell CJ, Flannery EP: Efficacy of prophylactic gamma-globulin in preventing non-A, non-B post-transfusion hepatitis. *Lancet*, 1976; i: 557-61.
11. Seeff LB, Wright EC, Zimmerman HJ, Hoofnagle JH, Dietz AA, Felsher BF, Garcia-Pont PH, Gerety RJ, Greenlee HB, Kiernan T, Leevy CM, Nath N, Schiff ER, Schwartz C, Tabor E, Tamburro C, Vlahcevic Z, Zemel R, Zimmon DS: Posttransfusion hepatitis, 1973-1975: a Veterans Administration cooperative study. In: *Viral Hepatitis*, Vyas GN, Cohen SN, Schmid R (eds). Franklin Inst. Press, Philadelphia, 1978: 371-81.
12. Szmuness W, Stevens CE, Harley EJ, Zang EA, Oleszko WR, William DC, Sadovsky R, Morrison JM, Kellner A: Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med*, 1980; 303: 833-41.
13. Crosnier J, Jungers P, Couroucé AM, Laplanche A, Benhamou E, Degos F, Lacour B, Prunet P, Cerisier Y, Guesy P: Randomised placebo-controlled trial of hepatitis B surface antigen vaccine in French haemodialysis units: I, Medical staff. *Lancet*, 1981; i: 455-59.
14. Lerner RA, Green N, Alexander H, Liu FT, Sutcliffe JG, Shinnick TM: Chemically synthesized peptides predicted

- from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles. *Proc Natl Acad Sci USA*, 1981; 78: 3403-7.
15. Valenzuela P, Medina A, Rutter WJ: Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature*, 1982; 298: 347-50.
 16. Havens WP: Period of infectivity of patients with experimentally induced infectious hepatitis. *J Exp Med*, 1946; 83: 251-58.
 17. Seeberg S, Brandberg A, Hermodsson S, Larsson P, Lundgren S: Hospital outbreak of hepatitis A secondary to blood exchange in a baby. *Lancet*, 1981; i: 1155-56.
 18. Skidmore SJ, Boxall EH, Ala F: A case report of post-transfusion hepatitis A. *J Med Virol*, 1982; 10: 223.
 19. Barbara JAJ, Howell DR, Briggs M, Parry JV: Post-transfusion hepatitis A. *Lancet*, 1982; i: 738 (letter).
 20. Alter HJ, Purcell RH, Holland PV, Feinstone SM, Morrow AG, Moritsugu Y: Clinical and serological analysis of transfusion-associated hepatitis. *Lancet*, 1975; ii: 838-41.
 21. Dienstag JL, Alaama A, Mosley JW, Redecker AG, Purcell RH: Etiology of sporadic hepatitis B surface antigen-negative hepatitis. *Ann Intern Med*, 1977; 87: 1-6.
 22. Berman M, Alter HJ, Ishak KG, Purcell RH, Jones EA: The chronic sequelae of non-A, non-B hepatitis. *Ann Intern Med*, 1979; 91: 1-6.
 23. Norkrans G, Frösner G, Hermodsson S, Iwarson S: Clinical, epidemiological and prognostic aspects of hepatitis 'non-A, non-B' - a comparison with hepatitis A and B. *Scand J Infect Dis*, 1979; 11: 259-64.
 24. Tabor E, Seeff LB, Gerety RJ: Chronic non-A, non-B hepatitis carrier state: transmissible agent documented in one patient over a six-year period. *N Engl J Med*, 1980; 303: 139-43.
 25. Tabor E, Gerety RJ: The chimpanzee animal model for non-A, non-B hepatitis: new applications. In: *Viral Hepatitis: 1981 International Symposium*, W Szmuness, HJ Alter, JE Maynard (eds). Franklin Inst Press, Philadelphia, 1982: 305-17.
 26. Seeff LB, Zimmerman HJ, Wright EC, Finkelstein JD, Garcia-Pont P, Greenlee HB, Dietz AA, Leevy CM, Tamburro CH, Schiff ER, Schimmel EM, Zemel R, Zimmon DS, McCollum RW: A randomized, double-blind controlled trial of the efficacy of immune serum globulin for the prevention of post-transfusion hepatitis. *Gastroenterology*, 1977; 72: 111-21.
 27. Knodel RG, Conrad ME, Ishak KG: Development of chronic liver disease after acute non-A, non-B post-transfusion hepatitis: role of gamma-globulin prophylaxis in its prevention. *Gastroenterology*, 1977; 72: 902-9.
 28. Tabor E, Gerety RJ: Inactivation of an agent of human non-A, non-B hepatitis by formalin. *J Infect Dis*, 1980; 142: 767-70.
 29. Yoshizawa H, Itoh Y, Iwakiri S, Kitajima K, Tanaka A, Tachibana T, Nakamura T, Miyakawa Y, Mayumi M: Non-A, non-B (type 1) hepatitis agent capable of inducing tubular ultrastructures in the hepatocyte cytoplasm of chimpanzees: inactivation by formalin and heat. *Gastroenterology*, 1982; 82: 502-6.
 30. Aach RD, Szmuness W, Mosley JW, Hollinger FB, Kahn, RA, Stevens CE, Edwards VM, Werch J: Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients: the Transfusion-Transmitted Viruses Study. *N Engl J Med*, 1981; 304: 989-94.
 31. Holland PV, Bancroft W, Zimmerman H: Post-transfusion viral hepatitis and the TTVS. *N Engl J Med*, 1981; 304: 1033-34.
 32. Tabor E, Hoofnagle JH, Smallwood LA, Drucker JA, Pineda-Tamondong GC, Ni LY, Greenwalt TJ, Barker LF, Gerety RJ: Studies of donors who transmit posttransfusion hepatitis. *Transfusion*, 1979; 19: 725-31.
 33. Kreel I, Zoroff LI, Cantes JW, Krasna I, Baronofsky ID: A syndrome following total body perfusion. *Surg Gynecol Obstet*, 1960; 111: 317-21.
 34. Prince AM, Szmuness W, Millian SJ, David DS: A serological study of cytomegalovirus infections associated with blood transfusions. *N Engl J Med*, 1971; 284: 1125-31.
 35. Enck RE, Betts RF, Brown MR, Miller G: Viral serology (hepatitis B virus, cytomegalovirus, Epstein-Barr virus) and abnormal liver function tests in transfused patients with hereditary hemorrhagic diseases. *Transfusion*, 1979; 19: 32-38.
 36. FDA. AIDS update. *FDA Drug Bulletin*, 1983; 13: 9-11.
 37. Tolkoff-Rubin NE, Rubin RH, Keller EE, Baher GP, Stewart JA, Hirsch MS: Cytomegalovirus infection in dialysis patients and personnel. *Ann Intern Med*, 1978; 89: 625-28.
 38. Yeager AS, Grumet FC, Hafleigh EB, Arvin AM, Bradley JS, Prober CG: Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J Pediatr*, 1981; 98: 281-87.
 39. Winston DJ, Ho WG, Howell CL, Miller MJ, Mickey R, Martin WJ, Lin CH, Gale RP: Cytomegalovirus infections associated with leukocyte transfusions. *Ann Intern Med*, 1980; 93: 671-75.
 40. Gerber P, Walsh JH, Rosenblum EN, Purcell RH: Association of EB-virus infection with the post-perfusion syndrome. *Lancet*, 1969; i: 593-96.
 41. Henle W, Henle G, Scriba M, Joyner CR, Harrison RS, von Essen R, Paloheimo J, Klemola E: Antibody responses to the Epstein-Barr virus and cytomegaloviruses after open-heart and other surgery. *N Engl J Med*, 1970; 282: 1068-74.
 42. Goetz O, Peller P, Weick B: Epstein-Barr virus antibodies after blood transfusion. *Dtsch Med Wochenschr*, 1974; 99: 448-50.
 43. Rocchi G, de Felici A, Ragona G, Heinz A: Quantitative evaluation of Epstein-Barr-virus-infected mononuclear peripheral blood leukocytes in infectious mononucleosis. *N Engl J Med*, 1977; 296: 132-34.
 44. Solem JH, Jørgensen W: Accidentally transmitted infectious mononucleosis: report of a case. *Acta Med Scand*, 1969; 186: 433-37.

45. Turner RA, MacDonald RN, Cooper BA: Transmission of infectious mononucleosis by transfusion of pre-illness plasma. *Ann Intern Med*, 1972; 77: 751-53.
46. Evans AS: Further experimental attempts to transmit infectious mononucleosis to man. *J Clin Invest*, 1950; 29: 508-12.
47. Rizzetto M, Purcell RH, Gerin JL: Epidemiology of HBV-associated delta agent: geographical distribution of anti-delta and prevalence in polytransfused HBsAg carriers. *Lancet*, 1980; i: 1215-19.
48. Morgan HJ: Factors conditioning the transmission of syphilis by blood transfusion. *Am J Med Sci*, 1935; 189: 808-13.
49. Klauder JV, Butterworth T: Accidental transmission of syphilis by blood transfusion. *Am J Syph, Gonorrhea, Vener Dis*, 1937; 21: 652-66.
50. Turner TB, Diseker TH: Duration of infectivity of *Treponema pallidum* in citrated blood stored under conditions obtaining in blood banks. *Bull Johns Hopkins Hosp*, 1941; 68: 269-79.
51. Kolmer JA: A note on the survival of *Treponema pallidum* in preserved citrated human blood and plasma. *Am J Syph, Gonorrhea, Vener Dis*, 1942; 26: 156-58.
52. Guthrie N: Failure of stored syphilitic blood to transmit syphilis: a case report. *J Vener Dis Inf*, 1951; 32: 246-47.
53. Walker RH: The disposition of STS reactive blood in a transfusion service. *Transfusion*, 1965; 5: 452-56.
54. Spangler AS, Jackson JH, Fiumara NJ, Warthin TA: Syphilis with a negative blood test reaction. *J Am Med Assoc*, 1964; 189: 87-90.
55. Bruce-Chwatt LJ: Blood transfusion and tropical disease. *Trop Dis Bull*, 1972; 69: 825-62.
56. Fajardo LF: The role of platelets in infections: I. Observations in human and murine malaria. *Arch Pathol Lab Med*, 1979; 103: 131-34.
57. Lozner EL, Newhouser LR: Studies on the transmissibility of malaria by plasma transfusions. *Am J Med Sci*, 1943; 206: 141-46.
58. Miller LH: Transfusion malaria. In: *Transmissible Disease and Blood Transfusion*, TJ Greenwalt, GA Jamieson (eds) Grune and Stratton, New York, 1975: 241-66.
59. Knott J: The periodicity of the microfilaria of *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg*, 1935; 29: 59-64.
60. Salazar HJ, Arends T, Maekelt GA: Documentation in Venezuela of the transmission of *Trypanosoma cruzi* by blood transfusion. *Arch Venez Med Trop Parasitol Med*, 1962; 4: 355-63.
61. Undiano C: Posttransfusion Chagas' disease. *Rev Fac Cienc Med Cordoba*, 1969; 27: 143-46.
62. Coura JR, Nogueira ES, da Silva JR: Indices of transmission of Chagas' disease by blood transfusion from donors in the chronic phase of the disease. *Hospital (Rio de Janeiro)*, 1966; 69: 991-98.
63. Cerisola JA, Rabinovich A, Alvarez M, DiCorleto CA, Pruneda J: Chagas' disease and blood transfusion. *Bol Of Sanit Panam*, 1972; 73: 203-21.
64. Jacoby GA, Hunt JV, Kosinski KS, Demirjian ZN, Higgins C, Etkind P, Marcus LC, Spielman A: Treatment of transfusion-transmitted babesiosis by exchange transfusion. *N Engl J Med*, 1980; 303: 1098-1100.
65. Jaffe HW, Bregman DJ, Selik RM: Acquired immune deficiency syndrome in the United States: the first 1,000 cases. *J Infect Dis*, 1983; 148: 339-45.
66. Ammann AJ, Cowan MJ, Wara DW, Weinruth P, Dritz S, Goldman H, Perkins HA: Acquired immunodeficiency in an infant: possible transmission by means of blood products. *Lancet*, 1983; i: 956-58.
67. Gury DJ: AIDS and the paid donor. *Lancet*, 1983; ii: 575.
68. Centers for Disease Control: Prevention of acquired immune deficiency syndrome (AIDS): report of inter-agency recommendations. *Morbid Mortal Week Rep*, 1983; 32: 101-3.
69. Mortimer PP, Luban NLC, Kelleher JF, Cohen BJ: Transmission of serum parvovirus-like virus by clotting-factor concentrates. *Lancet*, 1983; ii: 482-84.
70. Cossart YE, Field AM, Cant B, Widdows D: Parvovirus-like particles in human sera. *Lancet*, 1975; i: 72-73.
71. Anderson MJ: The emerging story of a human parvovirus-like agent. *J Hyg Camb*, 1982; 89: 1-8.
72. Evatt BL, Stein SF, Francis DP, Lawrence DN, McLane MF, McDougal JS, Lee TH, Spira TJ, Cabradilla C, Mullens JI, Essex M: Antibodies to human T cell leukaemia virus-associated membrane antigens in haemophiliacs: evidence for infection before 1980. *Lancet*, 1983; ii: 698-701.
73. Gallo RC, Wong-Staal F: Retroviruses as etiologic agents of some animal and human leukemias and lymphomas and as tools for elucidating the molecular mechanism of leukemogenesis. *Blood*, 1982; 60: 545-57.
74. Hinuma Y, Komoda H, Chosa T, Kondo T, Kohakura M, Takenaka T, Kikuchi M, Ichimaru M, Yunoki K, Sato I, Matsuo R, Takiuchi Y, Uchino H, Hanaoka M: Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide sero-epidemiologic study. *Int J Cancer*, 1982; 29: 631-35.
75. Blayney DW, Blattner WA, Robert-Guroff M, Jaffe ES, Fisher RI, Bunn PA, Patton MG, Rarick HR, Gallo RC: The human T-cell leukemia-lymphoma virus in the South-eastern United States. *J Am Med Assoc*, 1983; 250: 1048-52.
76. Miyoshi I, Taguchi H, Fujishita M, Niiya K, Kitagawa T, Ohtsuki Y, Akagi T: Asymptomatic type C virus carriers in the family of an adult T-cell leukemia patient. *Gann*, 1982; 73: 339-40.
77. Editorial: Blood transfusion, haemophilia, and AIDS. *Lancet*, 1984, ii: 1433-35.

Part Three

Future trends

1. Hepatitis B in pregnancy

D.G. WOODFIELD

In Western countries hepatitis occurs in 0.01–0.02% of pregnancies [1] but appears to be more common in Asia [2] and Africa [3]. However, mild cases of hepatitis in pregnancy are probably not always diagnosed, being attributed to 'morning sickness' or in the third trimester to 'cholestasis of pregnancy' [4]. In Third World countries, hepatitis in the third trimester of pregnancy has a higher maternal morbidity, possibly because of associated factors such as poor nutrition [5].

About 20% of the cases of pregnancy hepatitis in England and the U.S.A. are Hepatitis B antigen (HBsAg) positive [6]. The severity and prognosis of this disease in pregnancy does not appear to differ from that of non-pregnant patients. However, late third trimester clinical hepatitis B infections can have serious implications for the infant, with up to 50% of infants being infected with the hepatitis B virus [7]. It is probable that if the mother is Hepatitis B e antigen (HBeAg) positive, and if small transplacental leaks of blood occur, the infant can be infected *in utero*. However, most infections take place at delivery following accidental inoculation of the infant with maternal sera [8].

Mothers who have clinical hepatitis of any type are more prone to early delivery and with Hepatitis B there is a significantly increased perinatal mortality partly due to prematurity [9]. Overall foetal loss in hepatitis may not be greater than that observed for infants without hepatitis and the frequency of congenital abnormalities does not appear to be increased [10].

Diagnosis

Acute infections

Clinically it is not possible to distinguish the various forms of hepatitis, and only relatively recently has it been practical to make a clear serological diagnosis. The presence of HBsAg at the time of the acute infection is provisional evidence of a hepatitis B infection if it is known that the patient was not a chronic HBsAg carrier. Such carriers will not usually have high titre IgM hepatitis B core antibody (anti HBc-IgM) present, and this test may assist in diagnosis. If high titre anti HBc-IgM is present in a jaundiced patient, and HBsAg is absent, this is presumptive evidence of acute hepatitis B.

Sequential tests to detect seroconversion are useful. The appearance of hepatitis B antibody (anti HBs) 3–6 months after the primary infection confirms the diagnosis of Hepatitis B.

In the acute phase of infection, the HBeAg test may be positive. In most patients this reverts to negative, followed by the appearance of its antibody (anti HBe). The presence of HBeAg is correlated with a period of high infectivity for the hepatitis B virus (HBV).

Some pregnant women with hepatitis may have hepatitis A, which can be serologically diagnosed by the use of a test for IgM hepatitis A antibody. A diagnosis of cytomegalovirus or Epstein-Barr virus infection can sometimes be made by using the appropriate serial serological tests. Non A non B hepatitis can be reasonably reliably diagnosed if all the above tests are negative. This form of hepatitis

may account for as much as 25% of urban infections [11].

Chronic carriers

In some patients HBsAg may be present without detectable clinical symptoms or other biochemical liver abnormalities. Nearly all such persons will have IgG anti HBc present but will be negative for high titre IgM anti HBc. These chronic carriers of HBV may be HBeAg positive or negative. It is unusual for HBeAg negative mothers to infect their children.

The percentage of HBsAg carriers that are positive for the HBeAg marker varies greatly between countries from 3 to 5% in Caucasians to over 50% in Asians [8]. The infant born of an HBsAg positive HBeAg positive mother is at considerable risk to HBV infection and the subsequent development of the long-term carrier state.

Treatment

There is no specific treatment for clinical hepatitis B that can be recommended at the present time. Conservative symptomatic management is required and hepatitis B immunoglobulin (HBIG) is of no value.

For the infant born of an HBsAg/HBeAg positive mother current accepted practice is to administer HBIG at delivery followed by vaccination within 6 months. If HB vaccine is not available HBIG given at birth, 3 and 6 months will reduce the risk of the carrier state developing, but the infant is not then protected against the later development of HBV infections once the passive effect of HBIG has waned. It is probable that once results of vaccination studies are widely available, simultaneous HBIG and vaccine administration at birth may become standard practice for these infants [14].

The frequency of HBV infections in neonates born of mothers acutely ill with hepatitis B is higher than that for infants born of asymptomatic chronic HBsAg carriers and these babies should be also protected. The presence or absence of HBeAg in maternal blood is the most important prognostic factor.

Various studies have shown the effectiveness of both active and passive immunisation programs in selected populations [12, 13]. Further studies in different countries will be necessary to determine immunisation patterns as they relate to local factors. Ideally, active hepatitis B vaccination at birth of all children could result in a considerable reduction of the pool of hepatitis B carriers in the community. Such a procedure would be expensive, and perhaps the most reasonable compromise at the present stage is to develop programs to protect infants born of HBsAg positive mothers (HBeAg positive or negative) as well as children of families where there is a known risk of horizontal transmission of hepatitis B.

There is evidence of a causal relationship between the long-term carriage of HBV and the development of hepatocellular carcinoma [8]. Logistic and financial problems will probably preclude the widespread use of the HBV vaccine in the susceptible populations of the most populous countries, but the evidence is now sufficiently strong to use the vaccine as a means of preventing this type of cancer.

References

1. Sever J, White LR: Intrauterine viral infection. *Ann Rev Med*, 1968; 19: 471-86
2. Naidu SS, Viswasathan R: Infectious hepatitis in pregnancy during the Delhi epidemic. *Ind J Med Res*, 1957; 45 Suppl: 71-6
3. Morrow RH, Smetana HF, Sai FT, Edgcomb JH: Unusual features of viral hepatitis in Accra, Ghana. *Ann Intern Med*, 1968; 68: 1250-64
4. Bennett NMCK, Forbes JA, Lucas CR, Keegers A: Infective hepatitis and pregnancy; analysis of liver function test results. *Med J Aust*, 1967; 2: 974-6
5. Borhanmanesh I, Haghghi P, Hekmat K, Rezaizadeh K, Ghavami AG: Viral hepatitis during pregnancy: severity and effect on gestation. *Gastroenterology*, 1973; 64: 304-12
6. Keys TF, Sever JL, Hewitt WL, Gitnick GL: Hepatitis associated antigen in selected mothers and newborn infants. *J Pediat*, 1972; 80: 650-3
7. Schwirzter IL, Mosley JW, Ashcavai M, Edwards VM, Overby LB: Factors influencing neonatal infection by Hepatitis B. *Gastroenterology*, 1973; 65: 277-83
8. Technical Report Series 691 'Prevention of liver cancer', World Health Organization, Geneva, 1983
9. Smithwick EM, Pascual E, Go SC: Hepatitis associated

- antigen: A possible relationship to premature delivery. *J Paediatr*, 1972; 81: 537-9
10. Siegel M. Congenital malformations following chicken pox, measles, mumps and hepatitis. Results of a cohort study. *JAMA*, 1973; 226: 1521-4
 11. Goldwater PN, Woodfield DG, Anderson RA, Gill MB, Carpenter S: Acute sporadic Non A-Non B hepatitis in an urban community in New Zealand. *Aust NZ J Med*, 1982; 12: 268-71
 12. Reesink HW, Reerink-Brongers EE, Lafeber-Shut BJ, Ka-
lshoven-Benschop J, Brummelhuis HG: Prevention of chronic HBsAg carrier state in infants of HBsAg positive mothers by hepatitis B immunoglobulin. *Lancet*, 1979; ii: 436-8
 13. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY, Chen CL: Prevention of perinatally transmitted hepatitis B virus infections with Hepatitis B immune globulin and Hepatitis B vaccine. *Lancet*, 1983; ii: 1099-02
 14. Editorial: Prevention of perinatally transmitted hepatitis B infection. *Lancet*, 1984; i: 939-41

2. Interferons: past, present and future

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Introduction

In 1935, Magrassi [1] and Hoskins [2] reported that an organism infected with a particular virus cannot be superinfected with another type of virus. This phenomenon was called viral interference. Although it is possible to explain how one type of virus can restrict the replication of another one by direct interaction, the main mechanism underlying viral interference appeared to be a nonviral agent. While studying the interfering capacity of inactivated influenza virus preparations in chicken cells, Isaacs and Lindenmann in 1957 discovered, in contrast to their expectations, that the interfering capacity of their preparations increased instead of decreased [3].

They were able to show that this increase was caused by the excretion of an interfering agent by the chicken cells challenged with the inactivated influenza virus. They showed that chicken cells grown in culture medium containing this agent were protected against challenge with a live virus. They called this interfering agent interferon. It was soon discovered that not only chicken cells challenged with influenza virus produced interferon, but many types of viruses were capable of inducing interferon in many types of nearly all species studied. Interferon appeared to be an important part of the defence mechanism against viruses throughout nature. Even in plants, an interferon-like substance has been described [4]. The activity of interferon was shown to be species specific: it was especially active in cells of the same species in which it was induced. The antiviral activity, however, was

not virus specific. Interferon was active against many different types of viruses. Especially because of this broad spectrum of activity, several laboratories started to investigate interferon with the main objective being to make the agent applicable as an antiviral agent in man. However, before the first clinical studies on an acceptable scale could start, some great problems had to be solved.

Properties of interferon

For complete biological and physicochemical characterization of a substance, complete purification is a necessity. It proved to be very difficult, however, to completely purify interferon and it is only recently that human and mouse interferon were purified to homogeneity (see Section on Purification of interferon).

The lack of pure preparations has immensely influenced interferon research. For years, the properties of interferons could be studied and characterized only indirectly. The general properties that could be concluded from these studies are shown in Table 1. Forced by the absence of practical alternatives, interferon preparations had to be quantified by a bioassay and this is still the accepted method. Different assay methods are employed by different laboratories, but all of these are based on the same principle. Dilutions of the interferon preparations are made and the *in vitro* antiviral activity of the dilutions determined by assessing the effect on some viral function. The most widely used tests have protection against the viral cytopatho-

genic effect as the indicator for antiviral activity. The highest dilution that still shows 50% of the antiviral effect contains by definition one unit of interferon per milliliter. In a properly performed assay, an internal laboratory standard is included. This internal standard is regularly calibrated against the appropriate international standard. When these conditions are met, the activity can be expressed in international reference units.

Different types of interferon can be produced by one species. There are three types of human interferon (Table 2): alpha, beta and gamma (HuIFN α , β and γ). These types differ in antigenic, physicochemical and biological properties. HuIFN α is produced by virally stimulated leukocytes and is a mixture of at least 8 subtypes (α_1 , α_2 , ... α_8). These subtypes differ slightly in amino acid composition and have different biological properties. HuIFN β is a product of fibroblasts. There seems to be only one type and the protein is glycosylated. HuIFN γ is produced by sensitized T lymphocytes after antigenic stimulation or treatment with mitogens. There also seems to be a single species of HuIFN γ . Also, this type is a glycoprotein, but un-

like HuIFN α and HuIFN β , it is not stable at pH 2.0.

Purification of interferon

The first effort to purify interferon was started immediately after the discovery in 1957 that the mediator of viral interference was a protein. However, many years of research in a large number of laboratories were necessary to surmount the obstacles that were initially encountered during the purification steps. The high specific activity of interferon combined with the usual problem of trace amounts of protein in solution often resulted in the spontaneous disappearance of the activity. This effect might have been merely due to the adhesiveness of interferon to glassware and other equipment in the laboratory. Hence, the quantitative determinations of interferon, possible only by its biological activity, suffered generally from insufficient accuracy. Only recently has there been rapid progress in the purification and quantitation of interferons, and that for several reasons.

Firstly, it was eventually realized that the investigator was dealing with extremely small amounts of interferon in the cell culture. Secondly, methods which increased interferon production by the cells ('priming' and 'superproduction') were developed. Thirdly, the application of the technique of SDS polyacrylamide gel electrophoresis has greatly facilitated the isolation of minute amounts of interferon in pure form. Fourthly, a breakthrough in

Table 1. General characteristics of interferons.

Glycoproteins	
Molecular weight:	20,000
Isoelectric point:	6.5 to 7.5
Activity:	not virus specific species specific mediated by the cell

Table 2. Different types of human interferon.

	Conventional production methods	Constituents	Old nomenclature
HuIFN α	Buffy coat derived leukocytes or lymphoblastoid cells induced with NDV or Sendai virus	Protein, at least twelve genes which led to the production of eight subtypes with different biological properties	Leukocyte – lymphoblastoid – type I –, buffy coat derived, interferon acid stable
HuIFN β	Diploid fibroblast induced with poly(r-I).poly(r-C)	One gene which leads to production of one glycoprotein	Fibroblast, type I acid stable interferon
HuIFN γ	Buffy coat derived T lymphocytes induced by mitogens	One gene, but different glycoproteins with different properties produced	Type II, immunotype, acid labile interferon

interferon production occurred at the beginning of 1980 as a result of the development of recombinant DNA technology. This led to the greatly increased synthesis of biologically active interferon in *E. coli*. For example, in the case of human interferon alpha II, about 2.5×10^8 units of interferon were produced per liter of culture medium [5]. It is only very recently that the remarkable properties ascribed to interferon can be studied with pure interferon protein.

Of all the protein synthesizing systems studied, the murine one was the animal model of choice for a long time. Interferon was primarily produced by mouse L and C243 cells after induction with Newcastle disease virus. A high ratio of biological anti-viral activity to a unit weight of protein was achieved by a wide variety of purification procedures: conventional protein fractionation techniques including high performance liquid chromatography, SDS-polyacrylamide gel electrophoresis [1] and affinity chromatography with interferon antibodies, lectins, polynucleotides and small ligands [6]. Stewart *et al.* [7] showed that the interferon from C243 cells contained two molecular variants with molecular weights of 22,000 and 38,000 dalton. The authors designated them as MuIFN α and MuIFN β , respectively [8]. Both types of interferons appeared to be glycoproteins. The heterogeneity of both the 22,000 and 38,000 dalton components in SDS-polyacrylamide gels was ascribed to different degrees of glycosylation. No functional differences between partially, extensively or non-glycosylated proteins have been found up to now.

DeMaeyer-Guignard *et al.* [9] succeeded in a complete purification of interferon from mouse C243 cells by a two-step procedure using poly(U)-Sepharose chromatography combined with antibody affinity chromatography. The purity of both the alpha and beta forms was assessed by SDS polyacrylamide gel electrophoresis. The two bands visible on the gel corresponded exactly with the interferon activity found at the same positions. The ultimate output was about 50% of the amount originally present in the crude extract.

Later, the attention of many investigators shifted towards the human system, mainly because of the increasing amounts of human interferon which be-

came available. Until recently, a large portion of the human interferon was obtained from leukocytes. The leukocytes were derived from human blood and induced to synthesize interferon of the alpha type (Table 2). Human interferon of the beta type was produced in monolayer cultures of fibroblasts induced with poly I-C. The purification of both interferons clearly demonstrated that the two types of interferon differ in physicochemical properties [6].

Several groups have attempted to purify both leukocyte and fibroblast interferon on Sepharose-immobilized blue dextran, cibacron blue or triazine dyes. Purification of up to 1000 fold has been achieved with these procedures. Combined processes such as ammonium sulphate precipitation, ion-exchange chromatography, gel filtration and the techniques mentioned for the murine system were often necessary to purify both interferon types to apparent homogeneity. A two-step procedure for the purification of human fibroblast interferon has been recently described by Heine *et al.* [11]. The procedure consists of a combination of adsorption of interferon to controlled pore glass beads and Zn²⁺ chelate affinity chromatography.

It was shown that leukocyte interferon was a mixture of alpha (99%) and beta (1%) types, while the fibroblast interferon consisted mainly of the beta type with varying percentages of the alpha one. This variation depended largely on the inducer used [6]. A third type of interferon was described at the end of the sixties and designated as IFN gamma. Mainly based on studies with the murine species (MuIFN gamma), this type of interferon received much attention. IFN gamma has been shown to differ from IFN alpha and beta in its chemical properties. Its main physicochemical difference from the other two interferon species is its loss of activity at low pH. However, despite much effort, the purification methods described are far from ideal for both human and murine gamma interferon. Fortunately, at present, a 'turn of tide' is clearly visible for this protein. The genes coding for all three types of human and murine interferon have now been inserted into *E. coli* and/or yeast cells, allowing the synthesis of considerable amounts of different types of interferon, including

interferon gamma [12]. This enables the investigator to raise specific antibodies against this type of interferon and employ these antibodies for the purification of gamma interferon by means of antibody affinity chromatography. This effective and rapid procedure is certainly preferable to other methods. From the current experiments, we can expect a considerable amount of knowledge about the chemical and molecular basis of this interferon species in the near future.

Induction and mode of action

Under normal conditions, the interferon genes in a cell are suppressed and no interferon is produced. Interferon production has to be induced. The normal stimulus for a cell to produce the substance is a viral infection. Its production seems to be related to the appearance of viral nucleic acids in the cells. Not only viruses but other agents are also able to induce interferon. The other agents are called non-viral interferon inducers (Table 3). The applicability of inducers as alternatives for interferon itself in man is limited. Some of them are toxic, others are antigenic. The main obstacle to the clinical application of inducers is the hypersensitivity after repeated use. The amounts of interferon induced decrease substantially when the inducers are repeatedly used.

Table 3. Some nonviral inducers of interferon.

Polynucleotides	
Poly I-C	
Mycophages and bacteriophages	
Bacteria and other microorganisms	
Polymers	
Pyran, Polyvinyl	
Mitogens	
Phytohaemagglutinin	
Pokeweed mitogen	
Low molecular weight inducers	
Telorone	
Acridine	

The study of its mode of action has been greatly hampered by the lack of pure interferon preparations. The effects determined in cells after treatment with impure preparations cannot be unambiguously explained as being caused by interferon. This situation and the use of many different cell/virus combinations to study the mode of action have resulted in a plethora of hypotheses on how interferon inhibits the replication of viruses. During the last few years, a consensus seems to have been reached among the major investigators concerning the general features of interferon/cell interactions. Interferon does not have to enter the cell, but interacts with receptors on the cell surface. As a result of this interaction, the cyclic AMP system is activated and two enzymes come into action: an oligo(A)synthetase and a phosphokinase. The synthetase catalyses the production of oligonucleotides that activate RNase activity. The latter enzyme degrades the m-RNA of an infecting virus and viral replication is blocked. The phosphokinase activity induced by interferon results in inactivation by phosphorylation of an initiation factor that is necessary for the translation of viral m-RNA. A general outline of the induction and mode of action of interferon is given in Fig. 1.

For many years, it has been uncertain whether the antiviral effect was the only effect of interferon on cells. Early after its discovery, other biological effects were already reported, but because of the lack of pure interferon preparations in these studies, impure preparations were used and the other effects could be explained as being due to impurities. We now have pure interferon and it has become clear that it has multiple biological effects. The major activities of interferon are listed in Table 4. One of the first discovered and best stud-

Table 4. Some of the multiple biological effects of interferon.

- (1) Induction of an antiviral state in cells
- (2) Increase and decrease of interferon production by priming and blocking
- (3) Inhibition of mononuclear synthesis and cell division
- (4) Activation of nonspecific defence mechanisms such as macrophages and natural killer cells
- (5) Modulation of the specific immune system
- (6) Reversion of the transformed state of cells

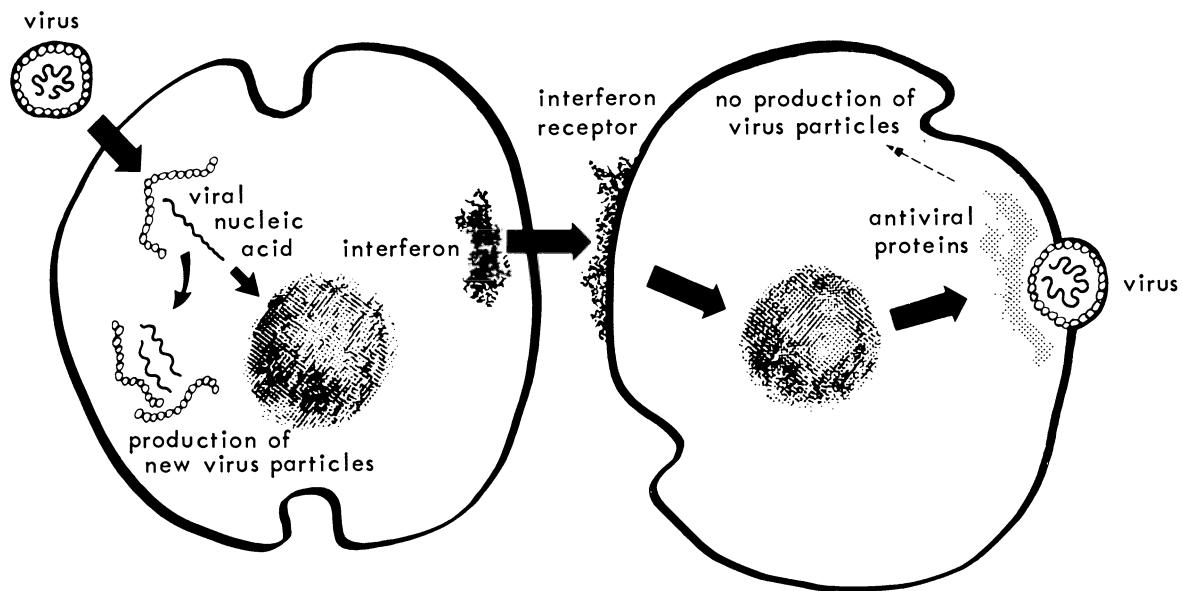


Fig. 1. A simplistic view on the induction mechanism and mode of action of the direct antiviral activity of interferon. The cell on the left is infected with a virus. The viral nucleic acid activates the interferon genes in the nucleus and interferon is produced. In the cell on the right the interferon molecules interact with

receptors on the cell membrane. Because of this interaction, enzymes are induced that inhibit the translation of viral m-RNA and thereby the production of viruses when this cell is infected with a virus.

ied effects of interferon, apart from the antiviral activity, is the inhibition of cell multiplication. This is quite possibly the main mechanism by which interferon inhibits tumour growth *in vivo*. Interferon regulates its own production. Low doses enhance and high doses inhibit production after induction. Important are the effects of interferon on the host defence mechanisms. Many of its effects *in vivo* are based on this activity. It is clear that the biological function of interferon is more than as illustrated in Fig. 1. It is not only induced by viral infections and it can do more than make cells resistant to viruses. It probably serves as a general protector against the processing of unfamiliar genetic information.

The biology of the interferon system

Interferon research over the years has been dominated by virological investigators. Therefore, the function of interferon during recovery from viral infections has best been studied. Virus infections

are the main causes of morbidity in man. An individual may suffer up to 6 virus infections a year. Nearly all such infections are self-limiting, suggesting that the human body has a very efficient manner of dealing with viruses. The major defence mechanisms operating during virus infections are listed in Table 5. The interferon system is probably the most important primary defence action against viruses. The symptoms of many virus infections are

Table 5. Major antiviral defence mechanisms

	Time needed to activate	Duration of activity	Antiviral spectrum
Interferons	hours	few weeks	broad
Local inflammatory reaction	-	-	-
Antibody production	days	years	specific
T-cell production	days	years	specific
General fever	hours	days	broad

Adapted from S. Baron. In: Interferons and Interferon Inducers, Finter (ed.). North-Holland, Amsterdam, 1973.

already diminished before the immune system is activated. A reduction in viral replication during many infections is related to the appearance of interferon and not of antibodies and T cells. When the interferon system is experimentally blocked by the use of specific antiserum, avirulent viruses can become lethal. The effect of exogenous interferon in experimental infections also shows important properties of the interferon system. In most studies, the best protection was achieved when the interferon was given before or early in the infection. It is one of the first host defences activated and is capable of suppressing the replication of viruses and keeping the infection localised until the immune system can clear the virus from the body. Interferon acts not only by making cells resistant to infection but also by activating other host defence mechanisms such as macrophages and natural killer cells.

Clinical evaluation of interferon

The scarcity of interferon was not only a problem for basic research but also hampered the clinical evaluation. In the years following the discovery, small-scale studies were performed on the effect of locally applied interferon produced in monkey cells. In the sixties a few studies were performed in the U.S.S.R., but it was expected then that interferon inducers would be alternatives to the use of interferon. When it became clear that inducers would not solve the problem, methods were devised for large-scale production of interferon from human cell cultures. With these interferons, clinical trials on a small scale could be initiated in the mid-seventies.

The main source of interferon for clinical evaluation up to now has been leukocytes from buffy coats produced from donated blood (Table 6).

For commercial production, the use of cell lines is preferred. Wellcome Laboratories has developed a method for the production of HuIFN α from lymphoblastoid cells. These cells are lymphocytes immortalized by Epstein Barr virus (EBV) infection that can be grown in any amount desired. Although EBV is implicated in oncogenesis in man

the rigorous purification methods used by Wellcome result in an interferon that can be safely used [21].

In the Rega Institute in Leuven, Belgium, a method was developed for the production of interferon for clinical use in diploid human fibroblasts [22]. The fibroblasts are stimulated by poly I-C. Because these cells are anchorage dependent and have to be grown on large surfaces, various procedures are employed to provide large surfaces in small volumes; however, this method of production still remains expensive. Also, the activity of this type of interferon is less than that of HuIFN α . For these reasons, it never became popular for clinical application.

Table 6. General outline of the production of HuIFN α from buffy coat according to the method of Cantell.

- (1) Blood is collected from healthy donors
- (2) Twenty-five buffy coats of 40 ml each are pooled and stored overnight at 4°C
- (3) Pooled buffy coats of 50 donors are suspended in 8 l of cold 0.83% NH₄Cl and kept at 20°C for 10 min
- (4) Three pools of 50 buffy coats are centrifuged successively in the same rotor at 1100–1400 g at 20°C
- (5) The cells are dispensed in cold phosphate buffered saline (PBS) with 25 mg neomycin per liter and distributed in 4 one-liter centrifuge bottles
- (6) Cold 0.83% NH₄Cl is added and after 10 min at 4°C the cells are spun down at 165–200 g for 25 min
- (7) Cells are resuspended in 100 ml cold incubation medium (Eagle's minimal essential medium supplemented with 3 g tricine, 2.4 g of human gamma globulin free serum and 25 mg neomycin per liter)
- (8) After 45 min incubation at 4°C on a magnetic stirrer, cold incubation medium is added to a volume of 750 ml. This suspension contains 2×10^8 leukocytes per ml
- (9) The cell suspension is added to prewarmed incubation medium containing 100–200 units of HuIFN α to prime the production to give 1×10^7 cells per ml in wide neck round bottom flasks
- (10) The flask is covered with aluminium foil and transferred to a water bath at 37.5°C
- (11) Two hours later, Sendai virus is added to a final concentration of 100–150 HA units per ml
- (12) After overnight incubation, cells are removed by centrifugation at 1300 g for 40 min

The supernatant is crude interferon with an activity of about 64,000 units per ml. This crude interferon can be stored at 4°C for more than a year without loss of activity. About 10^7 units of crude interferon are obtained per buffy coat.

Not only human interferon but also interferons for animal studies are difficult to produce in large quantities in cell cultures. Therefore, the number of animal studies with experimental viral infections and tumours that are essential for the design of the proper clinical trials is comparatively very small [23]. The few animal studies that have been performed have nearly all been done in mice. The number of studies and the number of species studied need to be extended.

The successful cloning and expression of mouse and rat interferon genes in microorganisms has opened up the possibility to do the animal studies on which the researchers can base their clinical evaluation [24, 25]. We will first outline here the method of recombinant DNA technology which has proven to be a powerful new instrument in the production of various types of interferons *in vitro*. This technique fulfilled a major goal of many laboratories to obtain large amounts of pure interferon for the determination of its clinical potential.

Application of recombinant DNA technology in interferon research

Recombinant DNA techniques have had a major impact on interferon research over the past two years. This new technology has now for the first time made it possible to produce single interferon species in substantial amounts in order to acquire detailed information on their biological properties in both human and animal models.

The structure and function of interferon genes have been investigated by using a wide variety of techniques that can be grouped into three categories.

The first category comprises a number of indirect techniques to obtain information on the interferon system as one for studying the control of gene expression. Interferon is formed by a process called gene activation in which the genes are already present in silent form not producing mRNA or protein but that can become activated upon addition of well defined inducers, leading to increased transcriptional activity and interferon protein synthesis.

The second category involves the detailed characterization of the expression products of the interferon genes. A first breakthrough came with the observation that injected interferon mRNA could be translated in oocytes of *Xenopus laevis* [13]. Biologically active interferon was not only produced but also exported into the incubation medium. From then on, this assay has been used for the characterization of partially purified interferon mRNA. A second observation of interest came from the purification of some interferon α and β proteins to homogeneity and their subsequent characterization by amino acid sequence analysis [14]. Here again, it was possible to show the existence of multiple interferons differing only slightly in structure.

The third category of techniques that has resulted in detailed information on the structure of interferon genes has included the molecular cloning of different interferon mRNA species [15, 16, 17], the characterization of the cDNA clones and the subsequent isolation of the corresponding interferon chromosomal genes [18, 19, 20]. The expression of human and mouse α , β and γ interferon genes has been recently described in a number of organisms (*Escherichia coli*, *Saccharomyces cerevisiae*, animal cells), leading to mg amounts of single interferon species and subspecies.

Gene cloning in principle involves the amplification of the genetic information of a particular gene as it is present in the mRNA or DNA of that gene. For many reasons, one takes the mRNA as the starting material for cloning a given gene: firstly an eucaryotic cell contains less mRNAs than there are genes present in the DNA, since not all genes are expressed under certain conditions. Secondly, there might exist a difference in the structural organization of the mRNA derived from a given gene and the organization of this gene.

If we wish to construct a clone containing sequences derived from an eucaryotic mRNA coding for interferon, we first have to construct the sequence in a DNA form. The most commonly used procedure for preparing such a complementary (cDNA) copy of the mRNA is outlined in Fig. 2. This procedure has been applied to prepare clone banks (cDNA libraries) from human leukocytes

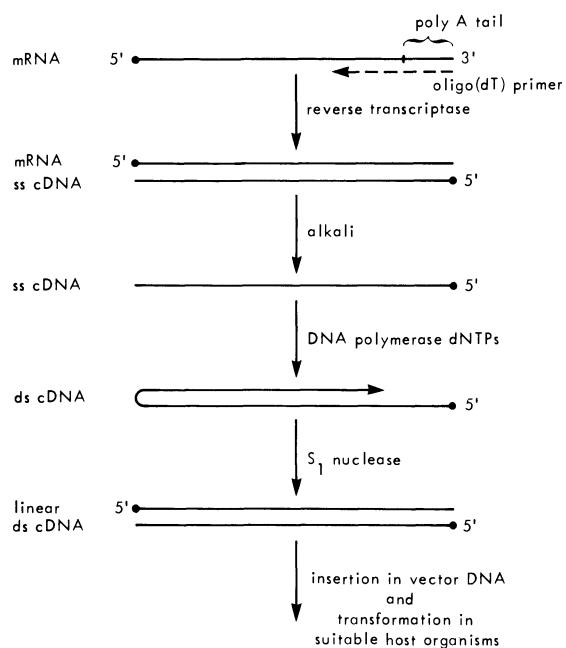


Fig. 2. Preparation of double-stranded c-DNA from RNA.

and the myeloblastoid kG-1 cell line (interferon α), human fibroblasts (interferon β) and human peripheral blood lymphocytes (interferon γ). These populations of cDNA molecules can then be inserted into vectors representing replicons that are stably inherited in an extrachromosomal state and that can be used to transform suitable hosts.

The most serious problem now encountered is the detection in a total population of that organism which contains a hybrid vector carrying a cDNA of an interferon mRNA. The first alpha interferon was originally detected by Nagata *et al.* [26] by use of laborious techniques involving hybridization translation assays on groups of transformed organisms; a positive group was then further divided until a single organism containing a hybrid vector including the interferon gene message was obtained.

A prerequisite for the production of an eucaryotic protein in prokaryotic hosts (e.g. *E. coli*) is the positioning of the gene in the vector DNA in such a way that prokaryotic control signals (promotor-operator systems; ribosome binding sites and initiation codons) will direct the expression of the eucaryotic gene. Prokaryotic expression vectors constructed in this way have been shown to direct

the synthesis of the different α , β and γ interferon species in *E. coli* in amounts up to 5×10^9 international units per liter of bacterial culture. This is equivalent to about 10–50 mg of interferon protein. However, *E. coli* produced interferons may vary in their biochemical characteristics from the naturally derived interferons, since prokaryotic organisms such as *E. coli* are not able to carry out the post-translational modifications (e.g., glycosylation, specific proteolytic cleavages, etc.) occurring in eucaryotic cells. Because of this the different interferon genes have also been manipulated in such a way that they can be expressed in organisms such as *Saccharomyces cerevisiae* (yeast) and other eucaryotic cells (monkey cells, Chinese hamster cells, etc.). This has resulted in interferon proteins more like their naturally derived homologues.

Antiviral activity in man

Prevention of virus infections

From the limited number of animal studies performed, it is clear that interferon is much more active when applied before or early in the virus infection than when this occurs during the acute phase. This preventive effect makes it attractive to evaluate the antiviral efficacy of interferon in man. An ideal group of patients in which to study this effect are kidney allograft recipients. These individuals receive immunosuppressive agents after transplantation, and because of this treatment there is a nearly 100% incidence of viral infections during the first three months after transplantation. Hirsch and his co-workers have studied the prophylactic effect of HuIFN α on virus infections in these patients in a number of double blind placebo controlled trials [27]. In their initial study in which low doses of interferon were administered, a significant effect on cytomegalovirus (CMV) excretion in the urine and CMV viremia was noted.

In a later study with higher doses, a significant effect on the clinical symptoms of CMV infection was achieved. In a double blind placebo controlled trial of HuIFN β in low doses in a small number of patients, Weimar and his colleagues failed to show

an effect [28]. HuIFN β , however, is less active than HuIFN α *in vivo*, and in the study of Weimar *et al.*, diagnosis was based only on serology. In the study of Hirsch, virus isolations were also made. Serological data are unreliable in immune suppressed patients.

Pazin and his co-workers studied the prophylactic effect of HuIFN α on Herpes virus reactivation in patients undergoing microsurgery for trigeminal neuralgia [29]. They reported that interferon treatment resulted in a significant reduction in the extensive *Herpes labialis* infections ('cold sores') that normally occur in the majority of these patients. The results of studies on the preventive effect of local interferon applications on the common cold have varied over the years. The problem seems to have been a technical one, i.e., how to get all nasal mucosal cells in contact with interferon for the desired period. This problem has been overcome by the use of highly active preparations, and consistent positive results with both buffy coat derived and recombinant DNA derived interferons have been reported by Tyrell and his co-workers from the Common Cold Unit, Salisbury, U.K. [30].

Treatment of acute infections

The efficacy of interferon therapy starting when the virus infection is well established has been studied by Merigan and his co-workers [31]. In a placebo controlled double-blind study, the effect of different doses of HuIFN α on *Herpes zoster* infections ('shingles') was monitored. The interferon therapy was started within 24 hours after the first blisters appeared. The highest dose administered significantly reduced pain and the vesicular complications.

Recurrent *Herpes keratitis* is the most common cause of blindness in the Western world. The effect of interferon eye drops in this infection has been extensively studied by several groups [32, 33]. Interferon preparations of high potency in combination with mechanical removal of infected cells ('debridement') have a significant effect on the healing time. Especially the combination of Trifluorothymidine (TFT) with interferon results in an impressive antiviral effect. Unfortunately, interferon

treatment seems to have no influence in the recurrences.

Treatment of chronic virus infection

The effect of interferon on chronic virus infection has been studied in chronic hepatitis B virus (HBV) infections. There are estimated to be 200,000,000 chronic carriers of this virus worldwide. Fifty percent of these patients show clinical signs of the infection, and chronic disease often leads to cirrhosis and liver cancer. Viral parameters can be easily determined in the serum of patients and the efficacy of a potential therapy regimen monitored. In 1976, Merigan and his co-workers observed permanent beneficial effects of interferon administration on chronic HBV infection in four patients [34]. In a double-blind placebo controlled trial performed by Weimar and his colleagues, a temporary effect on viral replication during the first week of the 6-week treatment period was shown [35]. No permanent clinical effect was achieved. It is possible that prolonged treatment for many months can lead to the cure of chronic HBV infections. However, the failure of interferon to reduce viral replication after the first week of treatment in the trial of Weimar *et al.* makes this quite unlikely.

Later, Merigan *et al.* reported the beneficial effect of the combination of interferon with vidarabine in HBV carriers [36]. A double-blind study has also been done in chronic CMV infections. Under normal conditions, CMV infections are harmless. In newborns and immune-compromised patients, they can cause serious problems. Interferon has been reported to inhibit viraemia during treatment, and permanent clinical effects were achieved in some cases [37].

Virus associated disease

Juvenile laryngeal papillomatosis (JLP) is a wart-like disease usually localized on the vocal chords. It is associated with a papova virus infection. In severe cases, the warts can spread to supra and subglottic areas. The severe cases often occur before the age of 4 years. When the disease spreads to the

lungs, it can become a life-threatening condition because of superimposed bacterial infections. There is no permanent treatment for this disease and frequent surgical excisions are often required. A tracheostomy is required in many cases. Several groups have reported on the dramatic effect which interferon treatment can have in this disease [38, 39]. In most cases, the papillomas disappear after a few months of treatment. They tend to reappear after interferon administration is stopped, but permanent cures are described in patients who have been free of papillomas for 1½ years during interferon treatment. Studies on the effect of interferon on laryngeal papillomas in adults are in progress.

There is a report indicating an effect of interferon administered intrathecally in multiple sclerosis patients [40]. In a double-blind trial, the interferon treated groups showed significantly fewer exacerbations than did the control group. The results need independent confirmation. Interferon has been used in several other virus-associated diseases such as juvenile onset diabetes, rheumatoid arthritis, schizophrenia and others. The results have been inconclusive. All of these studies have been open and, especially in the types of disease mentioned, only double-blind trials are decisive.

Interferon and tumors

Interferon not only inhibits replication and transformation by tumour viruses but it also inhibits cell division, regulates NK cell activity, activates macrophages, enhances macrophage activity, modulates the immune system and reverses the transformed state of cells *in vitro*. Thus, the antitumour effect of interferon can be easily explained. In experimental animals, it has been reported to increase survival and inhibit the development of spontaneous, viral and chemically induced and transformed tumours.

The prototype trial on the antitumour effect of interferon in man is the study of Strander and his co-workers who since 1971 have treated all new osteosarcoma patients at the Karolinska Hospital in Stockholm. They evaluated interferon as adjuvant therapy for the prevention of the lung meta-

stases which occur after surgical excision of the tumour in most cases. There are two control groups: an historical one from the Karolinska Hospital and a concurrent one consisting of patients treated elsewhere in Sweden. The patients of the interferon treated group are free of metastases for a longer time and have a significantly longer survival time than either of the control groups [41]. Neither control group is satisfactory and the comparison may be misleading. A prospective randomized trial is the only way to establish the value of interferon in osteosarcoma. Interferon has been reported to have an effect in phase I-II studies in non-Hodgkin lymphoma, melanoma, lung cancer, myeloma, breast cancer, leukemias and other types of malignancy. In general, the effects do not seem to be better than those of conventional therapy. Phase III and IV studies, however, may show a definite place of interferon in cancer therapy. The availability of recombinant DNA derived interferons now makes these types of studies possible.

Side effects and toxicity

The side effects of interferon treatment are summarized in Table 7. Most of them only occur after the first interferon injection and disappear on pro-

Table 7. Side effects of interferon treatment in man.

Frequent	fever pain and local inflammation at injection site
Sometimes	malaise chills fatigue muscle pain
Infrequent	lumbago arthritis anorexia vomiting tremors pale hands convulsion pain behind eyes neck stiffness
Abnormal laboratory values	leukopenia (frequent) transaminase increase (rare) antibodies to IFN (rare)

longed treatment. Experiments with highly purified interferons have shown that all side effects are caused by interferon itself and not by the impurities in the preparations [42]. Because the common side effects strongly resemble an influenza-like syndrome, it has been suggested that the symptoms of some viral infections are caused by the interferon induced by the infecting virus rather than by the virus itself. These side-effects do not permit the systemic use of interferon in minor diseases. The side effects of local application are minor. Intranasal treatment sometimes results in minor irritation that does not contraindicate the use of interferon for rhinovirus infections. Antibodies to interferon have been reported in patients treated with the substance [43]. These antibodies have also been found in tumour patients never treated with interferon and, therefore, could have pre-existed in the treated patients. It is attractive to speculate on whether the antibodies in the tumour patients were related to the development of the tumours.

The chronic toxicity of interferon has still to be established. There are patients who have been treated with low doses for more than 1½ years without any symptoms of deleterious effects. Newborn rats and mice treated with interferon have shown growth inhibition, severe kidney and liver damage and death [44]. This extreme sensitivity to the toxic effects of interferon is restricted to newborns. Treatment starting a week or later after birth was not toxic. We have treated newborn rhesus monkeys with high doses of human interferon daily for 28 days starting the day after birth. We have found no signs of toxicity. The absence of toxicity in this study should not lead to the conclusion that human interferon is devoid of toxicity for newborn babies. Adult rhesus monkeys are not sensitive to the common side effects of human interferon. We are able to reproduce those side effects only in chimpanzees [45]. However, because of the limited availability of this species and the ethical or practical problems involving some experiments, it is not possible to study all aspects of toxicity in chimpanzees.

The problem of toxicity testing can be partly solved by studying the chronic and acute toxicity of

their own interferons in rats and mice. The cloning and expression of rat and mouse interferon genes in microorganisms make these type of studies feasible.

The implication of interferon in certain chronic diseases makes it essential that its toxicity be carefully studied before it is applied on a wide scale in man.

The future of interferon

The interferons of choice for future clinical trials are the recombinant DNA derived ones. The quality and composition of these interferons can be better controlled than those of cell culture derived ones. They can be more easily produced in large quantities and can therefore be better purified. The recombinant DNA technique also makes it possible to produce the subtypes separately. The subtypes differ in biological activity and will have their specific applications. This does not mean that there is no longer a need for cell culture produced interferons. These natural products will continue to be important as reference material for studies both *in vitro* and *in vivo*. Much is expected from HuIFNγ. This interferon type differs quite extensively in physicochemical and biological properties from the other two types. The amino acid composition of HuIFNγ only shows 12% homology with HuIFNα and β, and HuIFNγ is the only acid labile interferon type. The most striking differences concern the biological properties. With the antiviral activities as reference, HuIFNγ inhibits cell multiplication 10–100 times more efficiently than either HuIFNα or β. It inhibits the division of cells resistant to other interferons. It has a pronounced effect on the immune system and is capable of enhancing the *in vitro* and *in vivo* effects of other interferon types.

Phase I studies with HuIFNγ derived from T lymphocytes have been initiated in the U.S.A. and its successful production by recombinant DNA techniques has been reported. The future results of application of this type of interferon in man are eagerly awaited.

The recombinant DNA techniques make it pos-

sible to produce artificial interferons by combining genes of different subtypes or by changing single genes. Therefore, it is possible to design the ideal interferon for any given condition once the biological significance of the different parts of the interferon molecules is discovered. This may provide us with important tools for manipulating the defence mechanism of the body. Perhaps, in the clinical area, interferon will not fulfill all expectations, but there are important lessons to be learned by exploiting the promises.

References

1. Magressi F: Studii sull'infezione e sull'immunità da virus erpetico. *Z Hyg Infektionskr Med Microbiol Immunol Virol*, 1935; 117: 573.
2. Hoskins M: A protective action of neutropic against viscerotropic yellow fever virus in Macacus rhesus. *Am J Trop Med Hyg*, 1935; 15: 675.
3. Isaacs A, Lindenmann J: Virus interference. I. The interferon. *Proc Roy Soc Ser B*, 1957; 147: 258.
4. Sela I, Applebaum SW: Occurrence of an antiviral factor in virus-infected plants. *Virology*, 1962; 17: 543.
5. Sloccombe P, Easton A, Boseley P, Burke DC: High-level expression of an interferon alpha₂ gene cloned in phage M13mp7 and subsequent purification with a monoclonal antibody. *Proc Natl Acad Sci USA*, 1982; 79: 5455-59.
6. Stewart II WE: The interferon system. 2nd edn. Springer-Verlag, New York-Wien, 1981.
7. Stewart II WE, LeGoff S, Wiranowska-Stewart M: Characterization of two distinct molecular populations of type I mouse interferon. *J Gen Virol*, 1977; 37: 277-84.
8. Stewart II WE, Blalock JE, Burke DC, Chany C, Dunnick JK, Falcoff E, Friedman RM, Galasso GJ, Joklik WK, Vilcek JT, Youngner JS, Zoon KC: Interferon nomenclature. *Nature (Lond.)*, 1980; 286: 110.
9. De Maeyer-Guignard J, Tovey MG, Gresser I, De Maeyer E: Purification of mouse interferon by sequential affinity chromatography on poly(u)- and antibody-agarose columns. *Nature (Lond.)*, 1978; 271: 622-25.
10. Cantell K, Hirvonen S, Kauppinen HL, Myöhälä G: Production of interferon in human leukocytes from normal donors with the use of Sendai virus. *Meth Enzymol*, 1981; 78: 29.
11. Heine JW, De Ley M, van Damme J, Billiau A, De Somer P: In: *Regulatory Functions of Interferons*, J Vilcek, I Gresser, TC Merigan (eds). *Ann NY Acad Sci*, 1980; 350: 364-73.
12. Gray PW, Leung DW, Pennica D, Yelverton E, Najarian R, Simonsen CC, Deryck R, Sherwood PJ, Wallace DM, Berger SL, Levinson AD, Goeddel DV: Expression of human immune interferon cDNA in *E. coli* and monkey cells. *Nature*, 1982; 295: 503-8.
13. Reynolds FJ Jr, Premkumar E, Pitha PM: Interferon activity produced by translation of human interferon messenger RNA in cell-free ribosomal systems in *Xenopus laevis* oocytes. *Proc Natl Acad Sci USA*, 1975; 72: 4881-87.
14. Knight E Jr: Interferon: purification and initial characterization from human diploid cells. *Proc Natl Acad Sci USA*, 1976; 73: 520-23.
15. Taniguchi T, Sakai M, Fujii-kariyama Y, Muramatsu M, Kobayashi S, Sudo T: Construction and identification of a bacterial plasmid containing the human fibroblast interferon gene structure. *Proc Japan Acad*, 1979; 55: 464-69 (Ser. B.).
16. Nagata S, Taira H, Hall A, Johnsrudd L, Streuli R, Escodi J, Boll W, Cantell K, Weissmann C: Synthesis in *E. coli* of a polypeptide with human leukocyte interferon activity. *Nature*, 1980; 284: 316-20.
17. Goeddel DV, Yelverton E, Ullrich A, Heyneker HL, Miozarti G, Holmes W, Seeburg PH, Dull T, May L, Stebbing N, Crea R, Maeda S, McCaudliss R, Slore A, Tabor JM, Gross M, Familletti PC, Pestka S: Human leukocyte interferon produced by *E. coli* is biologically active. *Nature*, 1980; 287: 411-16.
18. Nagata S, Brack C, Moenck K, Schamböck A, Weissmann C: Partial mapping of ten genes of the human interferon alpha family. *J Interferon Res*, 1981; 1: 333-36.
19. Goeddel DV, Lewry DW, Dull TJ, Gross M, Lawn RM, McCaudliss R, Seeburg PH, Ullrich A, Yelverton E, Gray P: The structure of eight distinct cloned human leukocyte interferon cDNAs. *Nature*, 1981; 290: 20-26.
20. Gray P, Goeddel DV: Structure of the human interferon gene. *Nature*, 1982; 298: 859-63.
21. Finter NB, Fantes KH: The purity and safety of interferons prepared for clinical use: the case for lymphoblastoid interferon. In: *Interferon 1980 I* Gresser (ed). Academic Press, London, 1980.
22. Billiau A, Van Damme J, Van Leuven F, Edy VG, De Ley M, Cassiman JJ, van den Berghe H, De Somer P: Human fibroblast interferon clinical trials: production, partial purification and characterization. *Antimicrob Agents Chemother*, 1979; 16: 49.
23. Schellekens H, de Reus A, Bolhuis R, Weimar W: The activity of human interferons in rhesus monkeys. In: *The Biology of the Interferon System*, E De Mayer, G Galasso, H Schellekens (eds). Elsevier/North-Holland Biomedical Press, 1981: 335.
24. Gray PW, Lee SH, Goeddel DV: Human and murine IFN gamma structure and expression. Abstract, The third annual congress for interferon research, Miami, 1982.
25. Dijkema R, Pouwels P, de Reus A, Schellekens H: Cloning and expression of rat IFN genes. Abstract, The third annual congress for interferon research, Miami, 1982.
26. Nagata S, Tana H, Hall A, Johnsrudd L, Streuli M, Ecsödi A, Boll W, Cantell K, Weissmann C: Synthesis in *E. coli* of a polypeptide with human leukocyte interferon activity. *Nature*, 1980; 284: 316.

27. Hirsch MS, Cheeseman SH, Ruben RH, Schooley RT, Henle W, Lenette ET, Andrews C, Shah KV, Cantell K: Prophylactic interferon alpha in renal transplant recipients. In: *The Biology of the Interferon System*, E De Mayer, G Galasso, H Schellekens (eds). Elsevier/North-Holland Biomedical Press, 1981: 339.
28. Weimar W, Schellekens H, Lameyer LDF, Edy VG, Biliau A, De Somer P: Double-blind study of interferon administration in renal transplant recipients. *Eur J Clin Invest*, 1978; 8: 255.
29. Pazin G, Armstrong JA, Lam MT, Tarr GC, Janetta PJ, Ho M: Prevention of reactivated herpes simplex infection by human leukocyte interferon after operation of the trigeminal root. *N Engl J Med*, 1979; 301: 226-30.
30. Scott GM, Phillipotts RJ, Wallace J, Secher DS, Cantell K, Tyrell DAJ: Purified interferon as protection against rhinovirus infection. *Br Med J*, 1982; 284: 1822.
31. Merigan TC, Rand KH, Pollard RB, Abdallah PS, Jordan GW, Fried RP: Human leukocyte interferon for the treatment of herpes zoster in patients with cancer. *N Engl J Med*, 1978; 298: 981-87.
32. Sundmacher E, Neumann-Haefelin D, Cantell K: Successful treatment of dendritic keratitis with human leukocyte interferon: a controlled study. *Albrecht v. Graefs Arch Klin Exp Ophthalmol*, 1976; 201: 39.
33. De Koning EWJ, van Bijsterveld GP, Cantell K: Human leukocyte interferon and trifluorothymidine versus albumin placebo and trifluorothymidine in the treatment of dendritic keratitis. In: *The Biology of the Interferon System*, E De Mayer, G Galasso, H Schellekens (eds). Elsevier/North-Holland Biomedical Press, 1981: 351.
34. Greenberg HB, Pollard RB, Lutwich LI, Gregory PB, Robinson WB, Merigan TC: Effect of human leukocyte interferon on hepatitis B virus infections in patients with chronic active hepatitis. *N Engl J Med*, 1976; 295: 517-22.
35. Weimar W, Heytink RA, Ten Kate FJP, Schalm SW, Schellekens H, Cantell K: Double-blind study of leukocyte interferon administration in chronic HBsAg-positive hepatitis. *Lancet*, 1980; i: 336-38.
36. Smith CI, Kitchen LW, Scullard GH, Robinson WS, Gregory PB, Merigan TC: Vidarabine monophosphate and human leukocyte interferon in chronic hepatitis B infection. *JAMA*, 1982; 247: 2261.
37. Arvin AM, Jaeger AS, Merigan TC: The effect of leukocyte interferon on urinary excretion of cytomegalovirus by infants. *J Infect Dis*, 1976; 133 (suppl. A): 205.
38. Haglund S, Lundquist PG, Cantell K, Strander H: Interferon therapy in juvenile laryngeal papillomatosis. *Arch Otolaryngol*, 1981; 107: 327.
39. Schouten TJ, Weimar W, Bos JH, Bos CE, Cremers CWRJ, Schellekens H: Treatment of juvenile laryngeal papillomatosis with two types of interferon. *Laryngoscope*, 1982; 92: 686.
40. Jacobs L, O'Malley J, Freeman A, Ekes R: Intrathecal interferon reduces exacerbations of multiple sclerosis. *Science*, 1982; 214: 1026.
41. Strander H: Interferons: antineoplastic drugs? *Blut*, 1977; 35: 288.
42. Scott GM, Secher DS, Flowers D, Bate J, Cantell K, Tyrell DAJ: Toxicity of interferon. *Br Med J*, 1981; 282: 1345-48.
43. Vallbracht A, Treuner J, Flehming B, Joester KE, Niethammer D: Interferon-neutralizing antibodies in a patient treated with human fibroblast interferon. *Nature*, 1981; 289: 496.
44. Gresser I, Tovey MG, Maury C, Choumoulinkers I: Lethality of interferon preparations for newborn mice. *Nature*, 1975; 258: 76.
45. Schellekens H: The toxicity of human interferons in non-human primates. In: *Interferons*, TC Merigan, RM Friedman (eds). Academic Press, 1982: 387.

3. The use of antibody in the malignant blood diseases

T.J. HAMBLIN

Introduction

Because of their exquisite specificity, antibodies have long been thought of as ideal reagents for the management of blood diseases and many attempts have been made to use them for this purpose [1]. These attempts have been for the most part ill-conceived, messy and unproductive. The main reason for this has been that they have lacked molecular precision. At the molecular level conventional antibodies are poor reagents because of their polyclonality; they react with several epitopes on the same antigen with variable avidity and cross react with the same epitopes on unrelated antigens.

Two recent developments have improved precision and allowed events at the cell surface to be dissected cleanly. The first is the discovery of how to engineer monoclonal antibodies [2] and the second the recognition of the idotypic determinants of surface immunoglobulin as an operationally tumour specific antigen in certain B cell lymphoid malignancies [3]. These discoveries have opened vistas of opportunity for the manipulation of blood cells and their precursors *in vitro* and the *in vivo* treatment of malignant blood diseases.

1. Monoclonal antibodies

Techniques for raising monoclonal antibodies are outside the scope of this review but it is important to note that success has really only attended those working with rodent systems. Although human-human hybridomas are easily formed, secretion

rates have been insufficient for useful amounts of antibody to accrue.

The raising of monoclonal antibodies to surface antigens of normal and malignant human blood cells is a lucrative and rapidly moving field. Our understanding of normal function and of pathologic processes has been much enhanced by the technique, but their use as therapeutic tools requires further research.

1.1. Specificity

Monoclonality does not imply monospecificity. The same epitope may be present on an unrelated antigen; the same antigen may be present on an unrelated tissue. In manipulating a specific blood cell subpopulation with monoclonal antibody it is important that the antigen is present on all cells of that subset; that it is confined to that subset; and that a precursor population lacking the antigen is not able to replenish the subset when the antibody is removed. In fact as study of Table 1 indicates many monoclonal antibodies that are considered for therapeutic manipulations have important cross reactivities. Just how important depends on the use to which the antibody is to be put. For example, the cross reaction of J5 with renal glomeruli [4] is unimportant for *in vitro* experiments but is potentially hazardous if the antibody is to be used *in vivo*. On the other hand when bone marrow is treated with antibody prior to transplantation it is important that the antibody does not cross react with either the pluripotential stem cells responsible for giving rise to all five blood cell lineages (T lymphocytes, B

lymphocytes, erythrocytes, megakaryocytes, and monocyte/granulocytes) or with the precursors of the endothelial cells that comprise the haematopoietic microenvironment. Although cells committed to the five lineages may be recognised by colony assays in semi-solid media, no satisfactory assays for stem cells or endothelial precursor cells exist.

The presence or absence of a precursor population of tumour cells that lacks the designated antigen may be even harder to assess; yet it is crucial to the success of a number of therapeutic strategies. This question will probably have to be put for each disease. Experiments with the mouse prolymphocytic leukaemia BCL1 [5] have demonstrated that affected bone marrow treated with monoclonal anti-surface idiotype is able to salvage lethally irradiated syngeneic mice without recurrence of the leukaemia. This experimental system is able to detect the presence of between 1 and 10 tumour cells, and the result effectively means that no antigen negative precursor population exists for this tumour. However, this result cannot be extrapolated to other tumours. In chronic granulocytic leukaemia, for example, the detection of the Ph δ chro-

mosome in B cells [6] implies a clonogenetic role for a very early stem cell.

1.2. Other properties of monoclonal antibodies

Monoclonal antibodies have other properties apart from their specificity which may enhance or hinder their role as therapeutic manipulators.

(i) In theory they are available in indefinite amounts over an indefinite period. This is clearly an advantage over polyclonal antisera but in practice many clones have been lost due to mutation or genetic drift.

(ii) Pure antibody is easily available which facilitates the construction of antibody derivatives. This is especially important for toxin- or isotope-conjugated derivatives.

(iii) The preparation of monoclonal antibodies is much more expensive and time consuming than that of polyclonals.

(iv) Monoclonal antibodies are apt to have low dissociation constants.

(v) Perhaps because of this they are apt to be poorly cytotoxic. Cytotoxicity is often confined to a

Table 1. Cross reactions of monoclonal antibodies.

Antibody against	Potential target	Other reactors
cALLa*	Common ALL	Subpopulations of normal marrow cells Renal tubules Renal glomeruli Normal T cells
T ₁ *	All T cell tumours CLL	Normal T cells
T ₃ *	All T cell tumours	Normal T helper cell
T ₄	Mycosis fungoides Sezary's syndrome	Some macrophages
T ₆	Adult T leukaemia/lymphoma	Langerhans cells of spleen
T ₈	Some T lymphoblastic lymphomas	Normal T suppressor cells
	Some T-CLL	Sinus lining cells of spleen
	Some T-PLL	Some myeloid precursors
T ₁₀	T lymphoblastic lymphoma T-ALL	Bone marrow B cells Germinal centre blasts Plasma cells
T ₁₁ *	All T cell tumours	Normal T cells Most NK cells
Idiotype*	Most B cell lymphomas B cell CLL	The (negligible) normal cells of the same clone only

* These antibodies have already been tried in therapeutic experiments.

certain subclass of antibody, and for this reason it may be necessary to use antibody cocktails for therapy.

(vi) Monoclonal antibody will bind to only one epitope of an antigen and therefore fewer 'hits' are obtained than with polyclonal antibody.

(vii) However, multiple antibodies with different epitopic specificity may be constructed to a single antigen so that cocktails with precisely defined components may be mixed.

(viii) Idiotypic determinants are exquisitely immunogenic and form the basis of an immunological control mechanism. Unlike polyclonal antibodies, the structure of each molecule of a monoclonal antibody is identical, making it more immunogenic and thus liable to induce resistance when used *in vivo*.

2. Idiotypic immunoglobulin

The study of the idiotypic determinants of the surface immunoglobulin (Ig) of B lymphocytes has yielded much valuable information about the nature of antigens on the cell surface and how cells behave when attacked by antibody. The surface immunoglobulin of B lymphoid malignancies is monoclonal and the variable regions of the Ig molecule present antigenic determinants unique to the tumour and any residual normal lymphocytes of the same clone. The biochemical behaviour has been studied exhaustively and recently reviewed by Stevenson and Stevenson [7]. Each tumour has a specific idiotype. So far no overlap has been discovered and attempts to find 'semi-public' antigens which would be common to a number of tumours have been futile, since almost certainly such antigens would be well represented in the serum Ig. An exception to this might be IgD which is present in the serum in small amounts only and which is present on some small lymphocytes, particularly those involved in chronic lymphocytic leukaemia [8].

In general a specific anti-idiotype needs to be raised for each tumour, and although idiotype is very immunogenic, this is a troublesome chore. Most lymphoid tumours secrete small amounts of idiotypic Ig sufficient to create an extracellular

barrier to the use of anti-idiotype *in vivo* [9]. In addition excess idiotypic light chain is produced by the tumour and excreted in the urine. Since antibody to light chain idiotype cross-reacts with surface Ig, this is an easy way of producing a useful reagent [10].

2.1. Raising anti-idiotype

In the first approach [3] large numbers (10^{10}) of lymphocytes are obtained either by leucapheresis or lymph node biopsy. The Fab fragment of surface immunoglobulin is cleaved from the cells by limited papain digestion, and removed from the supernatant by complexing with sheep-anti-human Fab μ . This antibody reacts with the constant regions of the Fab, leaving the variable regions exposed as an immunogen. The immune complexes so formed are then used to immunise sheep in the conventional way. The sheep antibody requires extensive absorption to remove non-specific antibody. The final antibody contains approximately 10% anti-idiotype although pure antibody can be prepared by affinity chromatography if required.

A different approach has been adopted by the Stanford group [11], in which the patient's lymphoma cells are fused with a non-secreting myeloma cell line to produce hybrids which export useful quantities of the patient's immunoglobulin. Although this method is still capricious owing to technical problems, it has great potential. It requires only small numbers of the patient's cells; the secreting hybrids can be frozen and resuscitated as required, and the idiotypic immunoglobulin is available in quantities sufficient not only for immunisation but also for constructing affinity columns.

The main drawback of this method is the instability of the mouse-human hybrids. Unfortunately no human-human hybrids capable of secreting sufficient immunoglobulin have been constructed. The Stanford group has also preferred to use monoclonal anti-idiotype prepared in a conventional way in mice rather than polyclonal antibody.

3. Experiments with xenogeneic antibody

The blunderbus use of polyclonal antibody of limited specificity to treat malignant blood diseases continues. Anti-thymocyte globulin, a commercially available antibody raised in horses immunised with human thymocytes has been reported on three occasions as producing objective responses in Sezary's syndrome [12, 13, 14]. Its non-specificity is evidenced by the report of responses in both patients with 8 cell lymphomas treated by Fisher and his colleagues [15]. One of the two patients with T cell lymphoma also responded, but one with diffuse histiocytic lymphoma did not.

Happily a more refined approach is also taking place and Tables 2 and 3 give details of a number of important experimental systems in both man and animals. They have enabled us to dissect mechanisms of cell killing and manipulation and uncover unexpected obstacles to success.

3.1. The cytotoxicity of antibody

In general, monoclonal antibodies are only poorly cytotoxic in complement dependent assays. None of the monoclonal antibodies studied in man has been cytotoxic with human complement, although most of them were weakly cytotoxic when rabbit complement was used. Moreover, none of the

monoclonal antibodies tested mediated antibody dependent cellular cytotoxicity (ADCC). Nevertheless, all of the antibodies effectively reduced the number of circulating tumour cells when infused into patients. Since some of them are able to fix C_3 [16] even when unable to cause tumour lysis it is likely that they enhance uptake by the reticuloendothelial system. Indeed, by the use of ^{111}In -oxine labelled tumour cells Miller *et al.* [17, 18] were able to demonstrate that following antibody treatment tumour cells were sequestered in the liver and spleen and that the rebounding white count consisted of unlabelled cells probably from tissue stores.

On the other hand the polyclonal antibody of Hamblin *et al.* [19] was cytotoxic *in vitro*. The IgG₁ fraction mediated complement dependent cytotoxicity and the IgG₂ fraction antibody dependent cellular cytotoxicity (ADCC). Following infusion of this antibody there was evidence of complement utilisation with depletion of C_3 and C_4 levels and evidence of C_3 conversion. When a larger dose of antibody was given to this same patient [20] there was again evidence of complement consumption and also of the tumour lysis syndrome with increase in plasma levels of potassium, uric acid and phosphate. The density of surface antigen varies from tumour to tumour and bears a direct relationship to cytotoxicity [20].

Table 2. Important experimental systems making use of antibody in man.

Centre	Target	Antibody	Reference no.
Boston	ALL	anti-cALLa	16
Boston	ALL	anti-cALLa & ricin* **	55
Boston	Non-Hodgkin's lymphoma	Ab 89	23
Stanford	T cell leukaemia	anti-T ₁	17
Stanford	T cell lymphoma	anti-T ₁	18
Stanford	Non-Hodgkin's lymphoma	anti-idiotype	21
San Diego	CLL	anti-T ₁	22
Southampton	PLL	anti-idiotype*	19
Southampton	CLL	anti-idiotype*	20
Southampton	Non-Hodgkin's lymphoma	anti-freey*	39
Royal Free	T cells responsible for GVHD	anti-T ₃ * **	53
Royal Free	T cells responsible for GVHD	anti-T ₃ * ** & anti-T ₁₁	54

* polyclonal antibodies.

** *in vitro* systems.

3.2. Can antibodies reach their target?

Examination of peripheral blood tumour cells following antibody infusion has revealed the presence of xenogenic antibody on the surface of cells in only one patient with CLL [22]. However, Ritz *et al.* [16] were able to detect anti-cALLa antibody on the surface of bone marrow blast cells during antibody infusion, and Miller *et al.* [21] detected mouse antibody on lymphocytes from a lymph node biopsied two hours after antibody infusion.

3.3. In vivo toxicity of antibody

In most instances this mode of treatment has been remarkably free of toxicity. However, both the monoclonal antibody used by Dillman [22] and the polyclonal antibody used by Hamblin [19] caused reactions which included bronchospasm, pyrexia and hypotension. Other workers have noticed mild pyrexia and decreased in creatine clearance [16, 17, 18]. The renal toxicity seems to be related to clearance of immune complexes. In most instances toxicity can be alleviated by slowing down the rate of antibody infusion, but in Dillman's case toxicity limited the experiment.

3.4. Specificity of antibody

Apart from those using idiotype as a target most workers have found that their antibody reacted with cells other than tumour cells. cALLa is found on a small number of normal bone marrow cells as well as on normal glomeruli. The anti-T cell antibody used by Miller [17, 18] and the antibody used

against CLL by Dillman [22] both had activity against normal T cells and in both sets of experiments normal T cell numbers also fell.

3.5. Serum blocking factors

In the patient described by Nadler and his colleagues [23] the presence of circulating antigen effectively prevented monoclonal antibody from binding to lymphoma or leukaemia cells *in vivo*, and although the infusion of large amounts of antibody reduced the levels of serum antigen it could not be entirely cleared. Similarly, Miller [18] found that circulating antigen appeared in the serum for three days following antibody infusion even though it was absent prior to treatment. It was thought to have been released by the lysis of tumour cells.

The presence of serum idiotype has been extensively studied by the Southampton group. Somewhat surprisingly they have found that most B cell tumours have a secretory pathway for idiotypic immunoglobulin quite unrelated to that by which immunoglobulin is inserted into the membrane [9]. Idiotypic IgM in the pentameric form can be found in plasma at 5–200 µg/ml. Those lymphomas expressing surface IgDλ (but not those with IgDκ) also secrete idiotypic IgD [24]. However, the surface molecules are not shed into the serum but catabolised as part of a complex process of membrane recycling [25]. Those tumours which secrete enough idiotypic Ig to produce a band on serum electrophoresis (myelomas and macroglobulinaemias) are ineligible for anti-idiotype therapy, but in those without a band serum idiotype poses a problem for three reasons:

Table 3. Important experimental systems making use of antibody in animals.

Centre	Target	Antibody	Reference no.
Tenovus Southampton	Guinea pig L2C leukaemia	polyclonal anti-idiotype	7
Univ of Texas Dallas	Mouse BCL ₁ leukaemia	monoclonal anti-idiotype – ricin	5
Univ of Texas Dallas	Mouse BCL ₁ leukaemia	monoclonal anti chains – ricin	8
Weizmann Rehovot Israel	Mouse YAC lymphoma	monoclonal and polyclonal anti-YAC conjugated with Daunomycin	43
Oxford Chester Beatty ICRF	Rat T cell leukaemia	monoclonal anti-T cell ricin A chain	56
Sloane Kettering New York	Mouse TL leukaemia	polyclonal anti-TL	28

(a) It depletes antibody before it reaches the cell surface.

(b) The immune complexes formed deplete complement and may possibly cause reticuloendothelial blockade.

(c) The immune complexes pose the threat of tissue damage particularly in the form of glomerulonephritis.

Nevertheless, serum idiotype is not merely an inconvenience since it is available for monitoring the progress of the tumour [10].

Serum antigen may be depleted by plasmaapheresis [26]. However, this process also removes serum complement unless fresh frozen plasma is used as replacement fluid with its attendant risk of hepatitis and anaphylaxis [27]. Cascade filtration to specifically remove plasma IgM might be preferable.

3.6. Antibody to xenogeneic protein

One striking finding of the therapeutic experiments is the lack of evidence of the development of antibody against the xenogeneic immunoglobulin, although small quantities of anti-murine antibodies were detectable on one occasion in the patient with T cell leukaemia shortly after the first infusion of antibody [17]. The possible reasons for this lack of response, which contrasts with historical reports of cancer experiments, are many. The patients were all suffering from lymphoid malignancies and were therefore to some degree immunodeficient. Some of the antibodies cross reacted with normal T cells and were therefore immunosuppressive. Much smaller quantities of protein were given than has hitherto been the case and the antibody was in a monodisperse, aggregate-free form and given by the intravenous route which is much less immunogenic.

3.7. Antigenic modulation

Antigenic modulation was first described by Boyse and Old [28] for the TL antigen of murine thymic leukemia. Originally it referred to the phenomenon whereby tumour cells which had been susceptible to complement dependent cytotoxicity lose

that susceptibility when exposed to antibody. This may involve the temporary disappearance of the target antigen from the cell surface in the presence of the antibody and its reappearance when the antibody is reversed. The process begins with the complexing of the antibody with antigen on the cell surface. One divalent molecule of antibody links with adjacent molecules of antigen so that the antigen is rearranged into patches and caps before internalisation of the whole complex by pinocytosis.

The speed of reaction is remarkable. Incubation of the target cell antibody at 37°C for as little as two minutes can prevent complement-dependent cytotoxicity [29]. Complete clearance of the antigen is unnecessary for the antibody to be rendered ineffective, and mere persistence of the antigen on the cell surface does not mean that the cell will be susceptible to antibody induced killing or clearance [30]. The degree to which antibodies induce modulation is variable depending on both the nature of the antigen (HLA antigens, for example, do not modulate) and the nature of the antibody – whereas J5 modulates cALLa, Ba3 which has a lower affinity for the antigen does not [31]. In some systems modulation takes place so quickly that it appreciably protects the cell even from simultaneous attack by antibody and complement [32]. Antigenic modulation represents an important defence mechanism for mammalian cells confronted by antibody. It yields an avenue of escape from ADCC [33] and phagocytosis [34] as well as from complement.

When the guinea pig prolymphocytic leukaemia L₂C is inoculated into animals that have been previously immunised against L₂C idiotype, some protection against the tumour is afforded, but the leukaemic cells eventually evade the host defences in a state of chronic modulation [35]. This state results not only from antigenic clearance but from diminished delivery of new Ig to the surface, a metabolic change mediated via a surge of cellular cyclic AMP in response to the attachment of bivalent antibody [36]. In several of the clinical experiments detailed in Table 2, antigenic modulation was a major hindrance to antibody therapy and the frequency of antibody infusions was dictated by the rate of antigen resynthesis.

Experiments utilising antibody to eliminate cell populations from bone marrow grafts are also susceptible to antigenic modulation particularly when opsonisation for the host reticulo-endothelial system is relied upon for clearance.

3.8. Other escape mechanisms

The existence of a clonogenic pool of antigen free precursor cells or the capacity of the tumour to mutate to produce antigen free cells is speculative. Experiments with the murine B cell leukaemia BCL₁ have suggested that an antigen free precursor population does not exist at least for this tumour. Lethally irradiated mice were reconstituted with bone marrow from syngeneic leukaemic mice which had been 'laundered' with anti-idiotype antibody. Despite the fact that this would have been ineffective against any antigen free precursor population, no recurrence of the leukaemia occurred [5].

The patient with cutaneous T cell lymphoma treated by Miller [18] showed an initial response with clearance of Sezary cells from the blood and regression of skin lesions. However, the patient developed progressive lymphadenopathy that was unresponsive to increasing doses of antibody. The lymph node cells were described as bizarre and it is possible that the tumour may have undergone a biological modification, rendering it unsusceptible to antibody treatment. However, the target antigen persisted on these bizarre cells and it is also possible that they were showing chronic antigenic modulation.

3.9. Other methods of tumour control

Most of the *in vivo* experiments have been mainly useful in uncovering mechanisms by which tumours avoid being destroyed by antibody. However, one experiment was unexpectedly, yet decidedly, successful.

The patient with non-Hodgkin's lymphoma treated with anti-idiotype by Levy [21] showed a remarkable response to treatment. This 67-year-old man with a five year history of follicular lymphoma had

had previous partial responses to combination chemotherapy and leukocyte interference. He was treated with mouse monoclonal anti-idiotype of subclass IgG2b. There was a small serum antigenic barrier of idotypic IgM at a level of 3.5 µg/ml. Plasmapheresis had only a transient effect on this and was only tried once. Starting with 1 mg of antibody, 8 doses were given at 3–4 day intervals over a period of 4 weeks, rising to a final dose of 135 mg and a total of 500 mg. At first there was no clinical response, but after 3 weeks the tumour began to regress and continued to do so after the anti-idiotype was stopped. A complete remission ensued which has persisted for over 18 months.

There are several reasons for regarding this response as an immunoregulatory effect of anti-idiotype [37]:

- (a) The monoclonal antibody used could not invoke complement dependent cytotoxicity.
- (b) Regression continued after antibody treatment had stopped.
- (c) An early biopsy showed the presence of infiltrating T cells, consistent with an ineffectual attempt at control.
- (d) Follicular lymphoma is known to retain a degree of response to regulating influences [38].

Whether this response is unique to this type of tumour treated in this particular way remains to be clarified. We have recently treated a patient with follicular lymphoma in whom the lymphoma cells carried surface Ig that lacked light chains [39]. On the premise that normally hidden sites on the heavy chains would be exposed to antibody attack we have infused repeated doses of a polyclonal antibody raised against these sites, which therefore represent a cell surface immunoglobulin non-idiotypic antigen which was nevertheless unique to the tumour. The type of response more closely resembled the response we have seen in CLL [20] that was described by Levy [21].

4. Strategies available to by-pass resistance

The foregoing descriptions of mechanisms by which cells may escape antibody mediated destruc-

explain why previous attempts at antibody therapy have been so unproductive. However, now that these mechanisms have been uncovered we are better able to design successful strategies.

4.1. Univalent antibody

If two Fab arms of an Ig molecule engage surface antigen there is a multiple binding bonus of a lower rate of disassociation than if the linkage is confined to a single site. However, this bonus is maximal with the rigid cell wall of bacteria and less important with the mobile constituents of mammalian cell membranes. Univalent antibodies made by cleaving one Fab arm from an IgG molecule have been examined in an idiotype anti-idiotype model [40]. This derivative known as Fab/C retains the Fc portion of the molecule and is thus able to bind complement and fulfil other adjunctive functions. In *in vitro* experiments it did not cause patching, capping or pinocytosis and was very effective at inducing complement dependent cytotoxicity with no evidence of antigenic modulation. It was much more efficient than whole IgG in the immunotherapy of L₂C leukaemia of guinea pigs.

This particular derivative can only easily be made from rabbit immunoglobulin, but other procedures are available to obtain univalent antibody from either sheep or mouse immunoglobulin.

4.2. Drug-antibody combinations

Doubts about the host effector system could be overcome by arming antibody with a warhead able to kill tumour cells once they are brought into sufficient proximity. Early attempts to link drugs to antibodies were initially successful [41]. Chlorambucil linked non-covalently to anti-tumour antibodies was better able to kill target cells than antibody or drug alone. Unfortunately it became apparent that similar effects could be obtained by administering the drug and antibody separately [42]. It is apparent that such non-covalent linkages come apart in the patient.

However, covalent binding of daunomycin, adriamycin and platinum to antibody either by direct complexing or via a dextran bridge produced

conjugates which are stable *in vivo* [43]. These conjugates are at least as active as the component parts and sometimes much more so. The toxicity is much less than the drug alone.

4.3. Antibody-toxin combinations [44]

The ribosomal inactivating proteins, of which ricin, abrin and diphtheria toxin are the best known, are widely represented in both plants and bacteria. They consist of two polypeptide chains: an A chain which is the active part of the protein which enzymatically interferes with the binding of the 60S microsomal subunit to elongation factor 2 and thus inhibits protein synthesis; and a B chain which acts as a lectin binding to terminal galactose residues on cell surface glycoproteins. The entry of a single A chain into the cytosol is sufficient to kill the cell, and these toxins are among the most lethal poisons known to man. To avoid this general toxicity and direct it towards tumour cells, toxin A chains have been cleared from their built-in lectin and covalently linked to monoclonal antibody. These conjugates are variably cytotoxic *in vitro* depending on the target antigen chosen and whether a combination of the antibody with the antigen is followed by internalisation of the complex. Unfortunately, thus far the conjugates are almost always inactive *in vivo*. The reason for this is unsure; it may be that the disulphide linkage of antibody to warhead is labile in serum or possibly that this method of coupling leads to immunoglobulin aggregation and thus clearance by the reticulo endothelial system.

Toxin A chains are themselves immunogenic, and successful immunotherapy might involve rearming antibody with new warheads. There are apparently over 50 to choose from [45].

4.4. Antibody isotope combinations

Although a number of successful attempts at radio-labelling monoclonal antibody for imaging solid tumours have been reported [46], there have been difficulties in attaching isotope to antibody sufficient for a cytotoxic role. Part of this problem is that when iodine of high specific activity is used to obtain a high labelling index it binds to tyrosines

which are heavily represented in the antibody combining site, thus hindering antigen binding [47]. Investigation of other methods of binding iodine and other isotopes is continuing. Of particular interest is the use of non radioactive Boron [48] which may then be irradiated with a slow neutron flux which causes nuclear disintegration, intensely irradiating a closely confined area.

4.5. *Speculative manoeuvres*

Two further methods of using monoclonal antibodies have recently been proposed. Berken [49] has suggested macrophages or K cells might be obtained from the patient by leucapheresis and then reinfused after binding to monoclonal antibodies of appropriate specificity to direct them to their target. Watson *et al.* [50] have used a subcutaneously implanted culture chamber which contained human-human hybridomas producing small amounts of monoclonal antibody against the patient's tumour.

5. The *in vitro* use of antibody

Many of the obstacles to the *in vivo* use of antibody diminish when the experiment is reduced to the test tube. This has three important therapeutic applications in the realm of bone marrow transplantation.

5.1. *Treatment of bone marrow to reduce graft versus host disease (GVHD)*

GVHD is one of the major complications of bone marrow transplantation and seems to be entirely due to the presence of immunocompetent T cells in the graft. In animal systems removal of these cells or the antisera abolishes GVHD [51], and therefore application to the human system seemed appropriate. In three patients, Haas *et al.* [52] encountered no GVHD following treatment of their engrafted marrow with polyclonal antithymocyte globulin. In a larger series in which the marrow was treated with the monoclonal antibody OKT3, Prentice *et al.* [53] found acute GVHD in 18% of patients

compared to an incidence in historical controls of 79%. These experiments depended on opsonisation to clear the T cells, which itself depends on the integrity of the host reticulo-endothelial system.

The use of the IBM cell washer allows the bone marrow to be concentrated so that it is susceptible to complement lysis with antibody and fresh rabbit serum. With this technique it can be shown that OKT3 fails to kill 10% of T cells. However, with a cocktail of antibodies including OKT3 and OKT11a it is possible to kill virtually all T cells [54]. The results of therapeutic studies making use of this technique are awaited with interest. An important proviso of this technique is that the antibodies used should fail to kill normal marrow precursor cells. The indications are that these antibody cocktails fulfil this criterion.

5.2. *Treatment of bone marrow autografts to remove residual leukaemia*

Even in complete remission leukaemia cells frequently persist in apparently normal bone marrows to form the basis of a later relapse. In principle, treatment of such patients with supra-lethal chemotherapy and radiotherapy followed by bone marrow autografts which have been cleared of leukaemia by leukaemia specific antibodies should be potentially curative. Ritz *et al.* [55] have attempted this in four patients with ALL in second remission. J5 (monoclonal anti-cALLa) and rabbit complement were used to treat the bone marrow. Two patients remain in long-term remission but one died of recurrent leukaemia and one of persistent thrombocytopenia after engraftment. cALLa is expressed on some normal marrow cells, and it is possible that the antibody treatment delayed recovery from the autograft in two of the patients. The use of antibody-toxin conjugates [56, 57] in experimental systems suggests that this might be a more effective yet safe method of selectively killing leukaemic cells in autografts.

5.3. *Removing other tumour cells from bone marrow autografts*

High dose chemotherapy followed by bone mar-

row autograft has been attempted in a number of patients with demonstrated cancer [58]. This technique is limited by bone metastases. Early work suggesting that the marrow might be 'laundered' by monoclonal antibody prior to transplantation has been published [59].

6. Conclusion

The years of empiricism in this field have served only to deter clinicians from taking the subject of antibody treatment seriously. At long last, the availability of monoclonal antibodies and molecularly defined antigens have enabled us to appreciate the nature of the problem and to suggest solutions. It is not too much to expect that within the next ten years the manipulation of blood cell populations whether normal or neoplastic with 'off the shelf' antibodies will have become commonplace.

Acknowledgement

I am grateful to Prof. G.T. Stevenson and Dr. F.K. Stevenson for helpful discussions in the preparation of this chapter.

References

- Rosenberg SA, Terry WD: Passive immunotherapy of cancer in animals and men. *Adv Cancer Res*, 1977; 25: 323-88.
- Kohler G, Milstein C: Derivation of specific antibody producing tissue culture and tumour lines by cell fusion. *Eur J Immunol*, 1976; 6: 511-19.
- Stevenson GT, Stevenson FK: Antibody to a molecularly defined antigen confined to a tumour cell surface. *Nature*, 1975; 254: 714-16.
- Metzgar RS, Borowitz MJ, Jones NH, Dowell BL: Distribution of common acute lymphoblastic leukaemia in non-haematopoietic tissues. *J Exp Med*, 1981; 154: 1249-54.
- Krolik KA, Uhr JW, Vitetta ES: Selective killing of leukaemia cells by antibody-toxin conjugates; implications for autologous bone marrow transplantation. *Nature*, 1982; 295: 604-5.
- Bernheim A: Philadelphia chromosome positive blood B lymphocytes in chronic myelocytic leukaemia. *Leukaemia-Res*, 1981; 5: 331-39.
- Stevenson GT, Stevenson FK: Treatment of lymphoid tumours with anti-idiotype antibodies. *Springer Seminars in Immunopathology*. Springer-Verlag, Berlin, Heidelberg, New York, 1983; 6: 99-115.
- Vitetta ES, Krolik KA, Uhr JW: Neoplastic B cells as targets for antibody-Ricin A chain immuno-cytotoxins. *Immunol Rev*, 1982; 62: 159-83.
- Stevenson FK, Hamblin TJ, Stevenson GT, Tutt AL: extracellular idiotypic immunoglobulin arising from human leukaemic B lymphocytes. *J Ext Med*, 1980; 152: 1484-96.
- Pierson J, Darley T, Stevenson GT, Virji M: Monoclonal immunoglobulin light chains in urine of patients with lymphoma. *Br J Cancer*, 1980; 41: 681-88.
- Brown S, Dilley J, Levy R: Immunoglobulin secretion by mouse x human hybridomas: an approach for the production of anti-idiotype reagents useful in monitoring patients with B cell lymphomas. *J Immunol* 1980; 125: 1037-43.
- Barrett AJ, Brigden D, Roberts JT, Staughton RCD, Byron N, Hobbs JR: Anti lymphocyte globulin in the treatment of advanced Sezary syndrome. *Lancet*, 1976; 1: 940-41.
- Edelson RL, Raafat J, Berger CL, Grossman M, Trayer M, Hardy M: Anti Thymocyte globulin in the management of cutaneous T cell lymphoma *Cancer Treat Rep*, 1976; 63: 675-80.
- Fisher RI, Kubota TT, Mandel GL, Broder S, Young RC: Regression of a T cell lymphoma after administration of anti thymocyte globulin. *Ann Intern Med*, 1978; 88: 799-800.
- Fisher RI, Silver BA, Vanhaelen CP, Jaffe ES, Crossman J: Objective regressions of T- and B-cell lymphomas in patients following treatment with anti thymocyte globulin. *Cancer Res*, 1982; 42: 2465-69.
- Ritz J, Pesando JM, Sallan SE, Clavell LA, Notis-McConarty J, Rosenthal P, Schlossman SF: Serotherapy of acute lymphoblastic leukaemia with monoclonal antibody. *Blood*, 1981; 58: 141-52.
- Miller RA, Maloney DG, McKill PJ, Levy R: In-vivo effects of murine hybridoma monoclonal antibody in a patient with T cell leukaemia. *Blood*, 1981; 58: 78-86.
- Miller RA, Levy R: Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. *Lancet*, 1981; ii: 226-32.
- Hamblin TJ, Abdul-Ahad AK, Gordon J, Stevenson FK, Stevenson GT: Preliminary experience in treating lymphocytic leukaemia to immunoglobulin idiotypes on the cell surface. *Br J Cancer*, 1980; 42: 495-502.
- Gordon J, Hamblin TJ, Stevenson FK, Stevenson GT: Mechanisms of tumour cell escape encountered in the treatment of lymphocytic leukaemia with antibodies to patients' cell surface idiotype. *Br J Cancer*, 1984; 49: 547-57.
- Miller RA, Maloney DG, Warnke R, Levy R: Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med*, 1982; 306: 517-22.
- Dillman RO, Shawler DL, Sobol RE, Collins HA, Beuaregard JC, Wormsley SB, Royston I: Murine monoclonal

- antibody therapy in two patients with chronic lymphocytic leukaemia. *Blood*, 1982; 59: 1036-45.
23. Nadler LM, Strashenko P, Hardy R, Kaplan WD, Button LN, Kafe DW, Antman KH, Schlossman SF: Serotherapy of a patient with a monoclonal antibody directed against a human lymphoma associated antigen. *Cancer Res*, 1980; 40: 3947-54.
 24. Stevenson FK, Stevenson GT, Tutt AL: The export of immunoglobulin D by human neoplastic B lymphocytes. *J Exp Med*, 1982; 156: 337-41.
 25. Palade GE: Problems in intracellular membrane traffic. In: *Membrane Recycling* (Ciba Foundation Symposium 92), D Evered, GM Collins (eds). London Pitman, 1982; 1.
 26. Hamblin TJ, Gordon J, Stevenson FK, Stevenson GT: Reduction of blocking factor to immunotherapy by plasma exchange. In: *Plasma Exchange - Plasmapheresis-Plasma-perfusion*, HG Sieberth (ed). Schattauer Verlag, Stuttgart 1980; 389-91.
 27. Editorial: Hazards of Apheresis. *Lancet*, 1982; ii: 1025-26.
 28. Boyse EA, Old LJ: Some aspects of normal and abnormal cell surface genetics. *Ann Rev Genetics*, 1969; 3: 269-90.
 29. Gordon J, Robinson DSF, Stevenson GT: Antigenic modulation of lymphocyte surface immunoglobulin yielding resistance to complement dependant lysis. I Characterisation with syngeneic and xenogeneic complements. *Immunology*, 1981; 42: 7-12.
 30. Gordon J, Anderson VA, Stevenson GT: Loss of surface bound antibody accompanying the anti complementary modulation of leukaemic B cell immunoglobulin: contrasting effects of antibodies directed against idiotypic and constant regions. *J Immunol*, 1982; 129: 2663-67.
 31. Lebien TW, Boue DR, Bradley JG, Kersey JH: Antibody affinity may influence antigenic modulation of the common acute lymphoblastic leukaemia antigen in vitro. *J Immunol*, 1982; 129: 2287-92.
 32. Gordon J, Stevenson GT: Antigenic modulation of lymphocyte surface immunoglobulin yielding resistance to complement mediated lysis II. Relationships to distribution of the antigen. *Immunology*, 1981; 42: 13-17.
 33. Stevenson GT, Glennie MJ, Gordon J: The killing of lymphoma cells by univalent derivatives of tumour specific antibody. *UCLA Symposium, Molec Cell Biol*, 1982; 24: 459-72.
 34. Griffin FM, Griffin JA, Silverstein SG: Studies on the mechanism of phagocytosis. II The interaction of macrophages with anti-immunoglobulin IgG-coated bone marrow-derived lymphocytes. *J Exp Med*, 1976; 144: 788-92.
 35. Stevenson FK, Gordon J: Immunisation with idiotypic immunoglobulin protects against development of B lymphocytic leukaemia, but emerging tumour cells can evade antibody attack by modulation. *J Immunol*, 1983; 30: 970-73.
 36. Glennie M, Stevenson FK, Stevenson GT, Virji M: Cross linking of lymphocyte surface immunoglobulin inhibits its production via a cyclic nucleotide mechanism. *Nature*, 1979; 281: 305-7.
 37. Roitt IM, Cook AS, Male DK, Guarnotta G, de Carvalho LP, Hay FC, Lydgate PM, Thanavala Y, Ivanyi J: Idiotypic networks and their possible exploitation for the manipulation of the immune response. *Lancet*, 1981; i: 1041-45.
 38. Jaffe ES: Hypothesis: follicular lymphomas - are they benign tumours of the lymphoid system? *UCLA Symposium, Cell Biol*, 1982; 24: 91.
 39. Gordon J, Hamblin TJ, Smith JL, Stevenson FK, Stevenson GT: A human B-cell lymphoma synthesizing and expressing surface μ chain in the absence of detectable light chain. *Blood*, 1981; 58: 552-56.
 40. Glennie MJ, Stevenson GT: Univalent antibody to kill tumour cells in-vitro and in-vivo. *Nature*, 1982; 295: 712-14.
 41. Ghose T, Norvell ST, Gudu A, Cameron D, Bodurtha A, McDonald AS: Immunotherapy of cancer with chlorambucil-carrying antibody. *Br Med J*, 1972; 3: 495-99.
 42. Rubens RD, Dubecco R: The augmentation of cytotoxic army action by antibodies directed at the cell surface. *Nature*, 1974; 248: 81-82.
 43. Arnon R, Sela M: Targeted chemotherapy; drugs conjugated to anti-tumour antibodies. In: *Experimental Approaches to Drug Targeting*, AJS Davies, MJ Crumpton (eds). *Cancer Surveys*, 1982; 1: 429-50.
 44. Editorial: Drug targeting in cancer. *Lancet*, 1983; i: 512.
 45. Barbieri L, Stirpe F: Ribosome-inactivating proteins from plants: Properties and possible uses. In: *Experimental Approaches to Drug Targeting*, Davies AJS, Crumpton MJ (eds). *Cancer Survey*, 1982; 1: 489-520.
 46. Farrands PA, Perkins AC, Pimm MV, Hardy JD, Embleton MJ, Baldwin RW, Hardcastle JD: Radioimmuno detection of human colorectal cancers by an anti-tumour monoclonal antibody. *Lancet*, 1982; ii: 397-400.
 47. Nord S, Weissman IL: Radiolabelled anti-tumour antibodies. III Highly rodentated and highly radio rodentated antibodies. *JNC I*, 1974; 53: 959-65.
 48. Kruger PG: Boron uptake in mouse brain neoplasm. *Rad Res*, 1955; 3: 1-17.
 49. Berken A: Care for adoptive immunotherapy in cancer. *Lancet*, 1982; ii: 1190-92.
 50. Watson JV, Alderson T, Sikora K, Phillips J: Subcutaneous culture chamber for continuous infusion of monoclonal antibodies. *Lancet*, 1983; i: 99-100.
 51. Rodt H, Kolb HJ, Netzel B, Reider I, Janka G, Belohradski BH, Haas PJ, Thierfelder S: Effect of anti-T cell globulin on GVHD in leukaemic patients treated with BMT. *Transplant Proc*, 1981; 13: 257-61.
 52. Haas RJ, Janka G, Netzel B, Rodt H, Thierfelder S, Helmig M, Eife R, Belohradsky BH, Kolb HJ, Betke K: Antibody incubation of human marrow graft for prevention of graft versus host disease. *Blut*, 1980; 40: 387-97.
 53. Prentice HG, Blacklock HA, Jannossy G, Bradstock KF, Skeggs D, Goldstein G, Hoffbrand AV: Use of anti-T-cell monoclonal antibody OKT3 to prevent acute graft versus host disease in allogeneic bone marrow transplantation for acute leukaemia. *Lancet*, 1982; i: 700-3.
 54. Granger S, Jannossy G, Francis G, Blacklock H, Poulter LW, Hoffbrand AV: Elimination of T lymphocytes from

- human bone marrow with monoclonal T-antibodies and cytolytic complement. *Br J Haem*, 1982; 50: 367-74.
55. Ritz J, Salan SE, Bast RC, Lipton JM, Clavell LA, Feeney M, Hercent T, Nathan DG, Schlossman SF: Autologous bone marrow transplantation in cALLa-positive acute lymphoblastic leukaemia after in-vitro treatment with J5 monoclonal antibody and complement. *Lancet*, 1982; ii: 60-63.
56. Raso V, Ritz J, Basala M, Schlossman SF: Monoclonal antibody-ricin A Chain conjugate selectively cytotoxic for cells bearing the common acute lymphoblastic leukaemia antigen. *Cancer Res*, 1982; 42: 457-64.
57. Thorpe PE, Mason DW, Brown AWF, Simmonds SJ, Ross WCI, Cumber AJ, Forrester JA: Selective killing of malignant cells in a leukaemic rat bone marrow using an antibody-ricin conjugate. *Nature*, 1982; 297: 594-96.
58. McElwain TJ, Hedley DW, Gordon MY, Jannan M, Millar TL, Pritchard J: High dose melphalan and non-cryo-preserved autologous bone marrow treatment of malignant melanoma and neuroblastoma. *Exp Haematol*, 1979; 7: 360-71.
59. Buckman R, McChinney RAJ, Shepherd V, Patel S, Coombes RC, Neville M: Elimination of carcinoma cells from human bone marrow. *Lancet*, 1982; ii: 1428-30.

4. Therapeutic approaches to abnormalities of blood rheology in vascular disease

J. STUART and J. MARSHALL

Introduction

Historical forms of rheological therapy, such as blood letting using either leeches or the barber-surgeon's scalpel, were used as non-specific remedies for a variety of maladies which usually had no rheological basis. Haemorheology has since become an exact science but therapeutic approaches to the management of haemorheological disorders remain in their infancy. This chapter is intended as a critique of clinical trials of haemodilution and other forms of therapy for correction of the haemorheological abnormality that contributes to the prothrombotic tendency in patients with vascular disease. Some clinical studies, using older haemorheological methods for the study of erythrocyte deformability, have given misleading results and an understanding of the artefacts inherent in rheological methods is now essential for a correct evaluation of clinical trials in vascular disease.

Limitations of rheological measurements in clinical studies

The need for a global test to measure the bulk viscosity of whole blood was met by the development of microviscometers of cone-and-plate and bob-in-cup type. These instruments have recently been reviewed [1] and their performance compared [2]. Valuable as it has been, measurement of whole-blood viscosity merely reflects the sum of a number of individual components (haematocrit,

plasma viscosity, erythrocyte aggregation, and erythrocyte deformability) that should probably now be measured independently. Of these, measurement of erythrocyte deformability has posed the greatest technical problem but a number of new methods and instruments for this purpose have recently been developed [3]. Most published clinical studies have, however, employed one of the earlier whole-blood filtration tests (using a Nuclepore polycarbonate membrane filter) that were influenced, to a variable extent, by the haematocrit, plasma protein level, leucocyte count, and finally erythrocyte deformability of the test filtrate. These older methods were therefore not sufficiently specific for, or sensitive to, abnormalities of erythrocyte deformability. Of the factors, other than deformability, that affected whole-blood filterability, the fibrinogen level was probably the most important.

1. Influence of fibrinogen level

The erythrocyte filtration rate [4], and also blood viscosity measured at a low shear rate [1], are directly proportional to the fibrinogen level of the test sample. Fibrinogen is an acute-phase reactant that shows an increase in plasma level in response to such acute events as trauma, surgery, infection, tissue ischaemia, and thrombosis. Filtration or viscosity measurements made within 1–2 weeks of these acute events will largely reflect the acute-phase increase in plasma fibrinogen and other proteins; any parallel loss of erythrocyte deformability is likely to be masked.

Patients in the steady state of a chronic disease, including atherosclerosis [5], also show a haematological stress response which may again include an increase in plasma fibrinogen level. The clinical importance of the fibrinogen level in steady-state vascular disease is shown by its association with the development [6] and also extent [7] of coronary artery disease and by its role as an adverse prognostic factor in patients with intermittent claudication whether untreated [8] or after arterial grafting [9]. Any measurement of whole-blood viscosity or filterability made during the steady-state of chronic vascular disease may again largely reflect the patient's plasma fibrinogen level rather than erythrocyte deformability. The same limitation also applies to filtration studies in which the test erythrocytes are resuspended in autologous plasma.

2. Influence of erythrocytes and leucocytes

The number of blood erythrocytes (measured as haematocrit or haemoglobin level) is a known risk factor for thrombotic events in patients with polycythaemia [10] and in large-scale population studies [11]. The number of erythrocytes in the test sample is also an important variable in the measurement of whole-blood viscosity [2] and erythrocyte filterability [12]. In a clinical trial, any decrease in erythrocyte count resulting, for example, from an expansion of plasma volume, may therefore give a spurious impression that the therapeutic agent has improved erythrocyte deformability.

Similarly, a slight increase in leucocyte count as part of the acute-phase, or chronic-phase [5], stress response may be sufficient to cause increased pore-plugging of polycarbonate membranes and give a false impression of loss of erythrocyte filterability [13]. Clinical trials in which the leucocyte count falls slightly during treatment may, for the same reason, give a false impression of an improvement in erythrocyte deformability [14].

The effect of the above variables cannot be over-emphasised in the interpretation of rheological studies and of clinical trials of rheologically-active agents. Failure of authors to apply correction factors for these variables or, preferably, eliminate them entirely from their test system, will largely negate the results of their studies.

Therapeutic approaches to haemorheological abnormalities

A number of imaginative approaches to treatment of the haemorheological abnormality of vascular disease have been proposed (Table 1). The purpose of any critique of their therapeutic value should not lie in too ready dismissal of possible clinical benefit on the basis of, what is now considered to be, sub-optimal clinical trial design or the use of a rheological test that has been superceded. Rather, the purpose is to summarise what has been achieved, to indicate the limitations, and to assist in the design of improved clinical trials that are more specific for, and therefore sensitive to, the abnormality under study. Our selection for review from the agents listed in Table 1 has been influenced by either the lack of a recent review, or the availability of new data, or the potential value of a new approach.

Table 1. Therapeutic approaches to haemorheological abnormalities in vascular disease.

Rheological abnormality	Therapeutic approach
Haematocrit increased	Haemodilution
Platelet hyperactivity	Anti-platelet agents <ul style="list-style-type: none">– ticlopidine
Fibrinogen level increased	Fibrinogen-lowering agents <ul style="list-style-type: none">– ancrod– batroxobin– clofibrate
	Fibrinolytic enhancement <ul style="list-style-type: none">– urokinase– streptokinase– stanozolol– ethyloestrenol– phenformin– tetrancicotinoylfructose
Erythrocyte deformability impaired	Calcium antagonists <ul style="list-style-type: none">– cinnarizine– flunarizine– nifedipine
	Prostaglandins
	Adrenergic agonists/antagonists <ul style="list-style-type: none">– prazosin– isoxsuprine– buflomedil
	1-eburnamonine
	Oxpentifylline

1. Haemodilution

Haemodilution by blood letting has been practised for centuries, but only recently has its scientific basis been explored. The aim of haemodilution is to improve blood flow. Factors determining blood flow in a vessel include the perfusion pressure and the peripheral resistance, the latter in turn having two components, the vessel itself and the blood within it. It is the viscosity of blood that contributes to peripheral resistance, viscosity being determined mainly by the haematocrit level, with plasma proteins playing a lesser part.

Reducing the haematocrit by haemodilution increases blood flow; this might be thought to be a direct result of the lower viscosity but, in the normal circulation, this does not appear to be the case. An important function of blood is to carry oxygen to the tissues and, since lowering the haematocrit reduces the oxygen-carrying capacity of blood, it is this factor which is the prime determinant of blood flow in the normal circulation.

The haematocrit, as measured clinically, is normally that of venous blood; the haematocrit of the microcirculation, where metabolic exchanges take place, is lower. Although the haematocrit level in the microcirculation varies in relation to the autoregulatory activity of the arterioles, it does not appear to determine blood flow, this normally being a function of autoregulatory activity involving perfusion pressure and vascular tone. However, if perfusion pressure falls below the autoregulatory limits, or if the autoregulatory activity of vessels is damaged by ischaemia, the rheological characteristics of the blood then become crucial. At a haematocrit of 0.35, for example, fluidity of blood can still be maintained despite profound lowering of perfusion pressure but, in the haematocrit range of 0.45–0.55, fluidity is progressively impaired. The pressure required to prevent stasis at a haematocrit of 0.55 is 100 times greater than it is at 0.35 [15].

Therapeutic lowering of the haematocrit is therefore designed not so much to increase blood flow by lowering its viscosity (blood flow will increase but by reason of the reduced oxygen-carrying capacity) but to prevent 'rheological occlusions'

in the microcirculation. The importance of this is well demonstrated by the 'no reflow' phenomenon [16] in which reperfusion after a period of vascular occlusion leaves some areas unperfused. The extent of these areas is influenced by a number of factors: the duration of occlusion and whether it is partial or complete, the reperfusion pressure and, most important, the viscosity of the reperfusion fluid. Control of viscosity is therefore important in clinical practice and is most readily achieved by haemodilution.

1.1. Haemodilution and the cerebral circulation

Cerebral blood flow (CBF) is extremely responsive to change in haematocrit; lowering the haematocrit by venesection from 0.49 to 0.43, for example, may raise the CBF by 50% [17]. This increased flow compensates for the reduced oxygen-carrying capacity of the blood, thus maintaining oxygen delivery. But adequate oxygen delivery is not the only factor determining cerebral function. In polycythaemia rubra vera, oxygen delivery is maintained but CBF is only half that of normal [18], cerebral function is impaired [19], and there is a well recognised liability to thrombotic episodes. The incidence of the latter is clearly related to the haematocrit level [10].

The harmful effect of a high haematocrit is not confined to the extreme levels of polycythaemia. The Framingham study showed that the risk of stroke was increased by a haemoglobin level at the upper end of the normal range [11] and CBF is significantly lower in subjects with a high-normal level of haemoglobin [17]. In both the pathological and the normal situation, reducing the haematocrit by venesection significantly increases CBF [11, 17].

1.1.1. Transient ischaemic attacks.

The association between polycythaemia and transient cerebral ischaemic attacks (TIAs) has long been recognised [20], but there are, as yet, no controlled clinical trials to show whether maintenance of the haematocrit at a normal level reduces the frequency of TIAs and the risk of stroke, though there is a good natural-history study suggesting that this may be so [10]. Present evidence strongly indicates that the haematocrit should be maintained below 0.48 by

repeated venesection in all patients with TIAs, or other evidence of cerebrovascular disease, whether polycythaemic or not. Because of the risk posed by acute haemodynamic disturbance in patients with cerebrovascular disease, the amount of blood removed should be limited to 250–300 ml on any one occasion.

1.1.2. Acute cerebral infarction. The role of haemodilution by infusion of dextran 40 has been extensively studied. Infusion of 500 ml of dextran 40 within one hour was found to lower the haematocrit from 0.41 to 0.36 and raise CBF by 30%; cerebral uptake of oxygen and glucose remained unchanged [21]. An increase in CBF was achieved irrespective of its initial level; the effect, however, lasted less than 24 hours [22].

A number of controlled clinical trials of haemodilution by infusion of dextran 40 in acute cerebral infarction have now been carried out [23, 24, 25] and all have shown a reduction in acute mortality. In one, follow-up over six months showed that mortality had become equal between the treated and control groups [25]. However, the distribution of the type of lesion between the treated and control groups was not uniform. A large study, although using historical controls treated by vasodilator drugs, also showed a reduction in mortality following haemodilution [26]. There is, therefore, considerable evidence that haemodilution with dextran 40 reduces mortality in acute cerebral infarction.

A recommended regimen [27] distinguishes between those who have associated cardiac or renal insufficiency and those who have not. In the latter group, 500 ml of dextran 40 and 500 ml of crystalloid fluid are infused intravenously 12 hourly for up to 7 days. If the haematocrit is above 0.40, however, this is supplemented by one or more venesctions of 300 ml blood to keep the haematocrit between 0.35 and 0.40. Venesection is not carried out until the intravenous infusion is under way.

In patients with cardiac or renal failure, only those with a haematocrit above 0.40 are treated. Repeated venesection of 300 ml blood is carried out, with simultaneous administration of the same

volume of dextran 40 in cases of cardiac failure, or 5% human albumin in cases of renal failure. Complications of treatment have included anaphylactic reactions to dextran 40 which, though described, seem to be very rare and no example was seen in 30,000 infusions [27]. Left ventricular failure and pulmonary oedema may occasionally be encountered. Haemostasis may be adversely affected by dextran, but the risk of rendering an infarct haemorrhagic seems to be very small.

It should be remembered that an infusion of low molecular weight dextran is not generally thought to have any more specific effect on lowering blood viscosity than that of haemodilution itself [28].

1.2. Haemodilution in peripheral vascular disease
Peripheral vascular disease is commonly associated with a raised haematocrit [29], the level of which affects prognosis [8] and the results of surgery [30]. Lowering the haematocrit to 0.35 by venesection, with simultaneous replacement by an equal volume of dextran 70, was found to increase resting blood flow through the calf muscles, the peak flow during reactive hyperaemia after a period of arterial occlusion, and also the walking distance [31]. In another study employing 4% human albumin, however, resting flow was not increased, but reactive hyperaemia and clinical condition were again improved [32]. Not all patients show this benefit: patients with up to three sites of occlusion in the lower limbs are improved by haemodilution whereas those with five are not [33]. Patients with vascular lesions in the upper limbs do better.

Selection of patients with peripheral vascular disease for haemodilution is more difficult than in cerebrovascular disease because there is a much higher incidence of coronary artery disease, contraindicating haemodilution, among them. Haemodilution reduces oxygen-carrying capacity, to which the heart responds by an increase in output achieved by increasing stroke volume without an increase in heart rate. There is therefore an increase in heart work requiring an increase in oxygen supply. This can be achieved only by coronary vasodilatation and an increase in the already high rate of oxygen extraction. Reduction of haematocrit to 0.30 requires utilisation of the coro-

nary artery reserve and, if this is impaired by disease, myocardial ischaemia will develop. The aim therefore should be to reduce the haematocrit to 0.35 but not to treat patients with evidence of myocardial ischaemia. Patients with angina should certainly be excluded, but whether patients with ST changes in the electrocardiogram should also be excluded, and whether exercise tests should be carried out, is in dispute.

1.3. Haemodilution and thrombosis in surgery

Haemodilution has been extensively practised during surgery, particularly during orthopaedic and cardiac procedures. The aim is to reduce thromboembolic complications.

A significant reduction in venous thrombosis after general surgery can be achieved by the administration of low-dose subcutaneous heparin. In orthopaedic procedures, without any prophylaxis, the incidence of deep vein thrombosis, determined by ^{125}I -fibrinogen scanning, lies between 50 and 70%; low-dose heparin reduces the incidence to between 15 and 25% but there still remains an appreciable risk. Isovolaemic haemodilution further reduces the incidence [34, 35]. Combining heparin with dextran, however, increases the risk of bleeding. The evidence as to whether this outweighs the benefit is conflicting; certainly the surgeon has to be more attentive to control of haemostasis.

A secondary aim of haemodilution before surgery is to conserve blood supplies. This is achieved by combining haemodilution with autotransfusion. Blood is removed from the patient preoperatively, the volume being replaced by an equal volume of a colloid solution. The blood is stored and returned to the patient during, or after, surgery as required. Further studies of the cost-effectiveness and practicability of this approach are required.

1.4. Haemodilution in retinal ischaemia

There are two clinical situations involving the eye in which haemodilution should be considered. The hyperviscosity syndrome, whatever the cause, shows characteristic ophthalmoscopic evidence of impaired blood flow and reducing viscosity by haemodilution is logical in this situation.

The role of viscosity in central retinal venous or arterial occlusion is less clear. Whole-blood viscosity is increased in central retinal vein thrombosis [36] and haemodilution has been shown to increase retinal blood flow in the pig [37]. Small, poorly-controlled series of patients with central retinal venous occlusion treated with haemodilution, with resulting improvement in visual acuity, have been reported [38]. Experience of haemodilution in central retinal artery occlusion has been disappointing.

2. Anti-platelet agents

Ticlopidine is a potent inhibitor of platelet aggregation which, in long-term (12 month) studies of vascular disease, has been associated with a significant fall in plasma fibrinogen level [39, 40] and, in short-term studies (up to 12 weeks), with a significant reduction in leucocyte count [14]. Either mechanism could explain a report [41] that ticlopidine caused an increase in whole-blood filtration rate, and may therefore improve erythrocyte deformability, in patients with a thrombotic tendency. No effect of ticlopidine on the filterability of washed erythrocytes, after correction for leucocyte count, was found in a subsequent study [14].

3. Fibrinogen-lowering agents

3.1. Defibrination

Defibrinating agents such as ancrod and batroxobin cause a substantial, short-term decrease in plasma fibrinogen level, but double-blind trials have not shown significant clinical benefit in patients with vascular disease and either intermittent claudication [42] or ischaemic rest pain [43, 44]. The value of ancrod in the prevention and treatment of deep vein thrombosis has recently been reviewed [45].

3.2. Clofibrate

Clofibrate, in addition to its lipid-lowering effect, has been shown to cause a reduction in plasma fibrinogen in proportion to the pre-treatment level [46, 47]. In a study of 62 patients with intermittent claudication treated for a mean of 7.4 months, the

plasma fibrinogen level fell significantly ($p<0.01$) from a mean of 4.2 g/l to 3.3 g/l [47, 48]. This fall was associated with a significant reduction in whole-blood viscosity and with some evidence of clinical improvement: after an average of 11 months' treatment, 29 patients with a pre-treatment plasma fibrinogen level in excess of 4 g/l showed a significant improvement in post-exercise ankle systolic pressure index and in recovery time [48]. Unfortunately, the excess of deaths from non-cardiovascular causes in the clofibrate, multi-centre trial of primary prevention of ischaemic heart disease [49] has inhibited further studies.

3.3. Fibrinolytic enhancement

The clinical indications for short-term thrombolytic therapy using streptokinase or urokinase have recently been reviewed [50, 51, 52]. The longer-term stimulation of fibrinolytic activity is largely dependent on the use of anabolic steroids, such as stanozolol or ethyoestrenol, with or without the biguanide phenformin. Stanozolol alone can cause long-term enhancement of fibrinolytic activity [53] and a significant fall in plasma fibrinogen level has been reported after 7 days' therapy in normal subjects [54] and after 3 months' therapy in patients with venous lipodermatosclerosis [55] or Raynaud's phenomenon [56]. In the latter study of 16 patients, the mean fibrinogen level fell from 3.9 to 2.8 g/l ($p<0.001$). The rheological, as opposed to thrombolytic, effects of stanozolol have not yet been studied in detail although the expected reduction in plasma viscosity [57], but not in whole-blood viscosity [58], has been demonstrated.

Tetranicotinoylfructose is an oral agent that releases nicotinic acid in the small intestine. In a study of 20 patients with ischaemic heart disease and/or atherosclerotic peripheral vascular disease, a significant ($p<0.01$) reduction in plasma fibrinogen (from 5.1 to 4.4 g/l over six months), associated with an increase in fibrinolytic activity, was found [59]. Also, in a study of nine diabetics, a slight fall in plasma fibrinogen, plasma viscosity, and blood viscosity, with an increase in erythrocyte deformability (centrifugation technique), was found after 4–5 months' treatment [60]. In a double-blind, cross-over trial, 16 patients with Ray-

naud's phenomenon showed a significant improvement in fingertip temperature at the end of a two-week treatment period [61]. Venous occlusion plethysmography in patients with steady-state intermittent claudication has not, however, shown any increase in calf blood flow after six weeks' treatment with tetranicotinoylfructose despite a significant reduction in blood viscosity [62].

4. Calcium antagonists

Cinnarizine, and its difluoro-derivative flunarizine, are calcium antagonists that cause vasodilatation by inhibiting calcium-induced contraction of vascular smooth muscle. They may also protect the erythrocyte against calcium-dependent membrane changes [63, 64]. Cinnarizine has been shown to protect erythrocytes taken from normal individuals against the loss of deformability induced *in vitro* by hyperosmolar stress [65] or by storage at 0°C for 24 hours [66]. Patients with vascular disease who took flunarizine for 24 hours were found to have a significant decrease in whole-blood viscosity (capillary microviscometer) and increase in erythrocyte filterability (erythrocytes suspended in autologous plasma) [65]. In a further study of 25 patients with peripheral obliterative arterial disease, cinnarizine, given either as a single oral dose (14 patients) or for 7 days (11 patients), was associated with a significant decrease in whole-blood viscosity over a range of shear rates (3.75–750 s⁻¹). There were no significant changes in haematocrit, plasma fibrinogen level, or plasma viscosity and an effect on erythrocyte deformability was assumed [67].

Controlled trials of these calcium antagonists in patients with peripheral vascular disease have been small in size. In a three-month, double-blind trial of 21 patients with mild disease, there was a significant increase in resting and peak blood flow to the leg (venous occlusion plethysmography) in 11 patients who took flunarizine compared with 10 patients receiving placebo [68]. In contrast, no improvement in resting or peak calf blood flow (venous occlusion plethysmography and ¹³³Xenon clearance) was found in 13 claudicants who took cinnarizine for six weeks [62]. Self-assessed walking distance improved in two double-blind studies

of cinnarizine taken for 3–4 months [69, 70]. An objectively-measured increase in claudication distance has also been reported in a multi-centre, 12-month study of 26 patients who took flunarizine, compared with 30 who received placebo; the increase was statistically significant at three months and the difference between the groups continued to increase thereafter [71].

The design limitations of the few controlled trials of cinnarizine in cerebrovascular disorders have recently been reviewed [72].

The calcium antagonist nifedipine, taken sublingually in a pilot study of 12 patients with ischaemic heart disease, was reported to increase the whole-blood filtration rate in eight patients who initially had impaired filterability [73]. There was, however, no report of a control group.

5. Prostaglandins

There are considerable technical difficulties in evaluating the effects of prostaglandins on erythrocyte deformability [74]. In Raynaud's phenomenon associated with systemic sclerosis, a loss of erythrocyte filterability, with significant improvement following intravenous infusion of prostaglandins E₁ and I₂, has been described [75]. Heparinised blood, with the plasma removed, was filtered using a negative-pressure technique. This result should be confirmed using a platelet- and leucocyte-free filtrate in view of the cell-aggregant action of heparin and the anti-aggregant action of prostaglandins. It is also important to differentiate between any prostaglandin-mediated effect on erythrocyte deformability, and an effect on the vessel wall which may improve blood flow by precapillary vasodilatation [76].

6. Adrenergic agonists/antagonists

Prazosin, a selective post-synaptic α -adrenoceptor blocker used in the treatment of essential hypertension, has been shown to reduce whole-blood viscosity but probably as a consequence of drug-induced vasodilatation with accompanying plasma volume expansion and haemodilution [77].

Intravenous infusion of the vasodilator isox-

suprine (a non-specific, β -adrenoceptor agonist) has been found to lower plasma fibrinogen, plasma viscosity, and also whole-blood viscosity (but not corrected for a fall in haematocrit) [78]. An *in vitro* reduction in plasma-, serum-, and blood-viscosity (but not whole-blood filterability) has also been reported by this group [79, 80].

Buflomedil, a vasoactive agent which may act as a non-specific blocker of α -adrenergic stimulation of vascular smooth muscle, has been claimed to improve erythrocyte deformability. Following a 5 minute intravenous infusion, 10 patients with peripheral vascular disease showed a significant improvement in filterability of their erythrocytes when resuspended in autologous plasma; no such improvement was obtained using placebo [81]. Longer-term (20 days) intravenous administration of buflomedil to 20 patients with peripheral occlusive arterial disease caused a significant increase in whole-blood filtration rate and significant lowering of whole-blood viscosity [82]. Plasma fibrinogen level was unchanged. An increase in erythrocyte filterability and reduction in whole-blood viscosity has also been reported in 24 diabetics treated with oral buflomedil for three weeks [83]; the test erythrocytes were again resuspended in autologous plasma. Confirmation that buflomedil also affects the deformability of washed erythrocytes is therefore required. Clinical trials of buflomedil in vascular disease have mainly been small, short-term, open, or not placebo-controlled.

There has been a suggestion [84] that β -blockade may reduce blood viscosity but confirmation of this preliminary report, and further study of the effect of β -adrenergic agonists/antagonists on intracellular cyclic AMP and the prostaglandin pathway is required.

7. *l*-Eburnamoneine

This semi-synthetic vinca alkaloid is believed to stimulate glycolysis and increase intra-erythrocytic ATP and 2,3-diphosphoglycerate [85, 86]. Daily intravenous infusion during the 10 days after acute cerebral thrombosis caused a statistically significant increase in whole-blood filtration rate by day 10 in 12 patients compared with 7 patients who

received standard therapy (corticosteroids and mannitol) [87]. Further studies of its action on the metabolism and deformability of washed erythrocytes are required.

8. *Oxpentifylline*

In addition to its actions as a vasodilator and anti-platelet agent, possibly mediated at pharmacological levels by enhancement of endothelial prostacyclin release [88], this methyl xanthine derivative has been claimed to improve erythrocyte deformability. The *in vitro* evidence for a beneficial effect includes partial protection against loss of deformability when erythrocytes are stressed by either hyperosmolar conditions [89, 90], calcium loading [91, 92], artificial ageing in buffer [93] or in Blood Bank preservative anticoagulant solution [94], and incubation as whole blood at room temperature [95]. Blood taken from patients and incubated *in vitro* with oxpentifylline (10–40 µg/ml) for two hours has also shown an increase in filtration rate through 5 µm diameter pores [96, 97].

The evidence that *ex vivo* blood samples from patients taking oxpentifylline reflect its rheological action, includes an increase in whole-blood filtration through 5 µm diameter pores [98] and a decrease in plasma viscosity and whole-blood viscosity [99]. In the former study, oxpentifylline therapy for six weeks in 18 patients with chronic arterial occlusive disease was associated with a significant fall in plasma fibrinogen level which may have been responsible for the increase in whole-blood filtration rate. In the latter study, oxpentylylline was given intravenously for 10 days with mathematical correction for haemodilution; fibrinogen levels were not stated. Other clinical studies of oxpentylylline in which a significant fall in fibrinogen level has been recorded include 20 normal individuals treated for three months [100] and 22 elderly atherosclerotic patients treated for eight weeks [101].

The clinical trial evidence for a beneficial effect of oxpentylylline has come mainly from studies of patients with peripheral or cerebral vascular disease. Earlier studies have been listed by Müller [102].

8.1 *Peripheral vascular disease*

In a recent double-blind study of 128 out-patients with intermittent claudication, 42 patients given a graded daily dose (increasing from 600 mg to 1200 mg/day) of oxpentylylline for 24 weeks showed a significant increase in treadmill walking distance compared with 40 patients given placebo [103]. A significant improvement from the baseline value was found after two weeks of treatment (25% improvement in initial claudication distance for oxpentylylline compared with 6% for placebo; $p<0.01$) with no further divergence thereafter between the groups (at 24 weeks, a 59% improvement for oxpentylylline was not significantly different from the 36% improvement for placebo). No haemorheological tests were performed and no firm conclusion as to the therapeutic mode of action of oxpentylylline, at sub-maximal dosage, at week 2 can be drawn.

A second recent placebo-controlled trial, of cross-over design [104], showed a significant ($p<0.01$) 60% increase in walking distance when 24 patients with intermittent claudication were given 1200 mg/day of oxpentylylline for 8 weeks. In contrast, walking distance during placebo therapy increased by less than 3%. Again, no *ex vivo* haemorheological tests were performed to explain the mode of action of oxpentylylline.

8.2 *Cerebrovascular disease*

A number of studies of oxpentylylline in cerebrovascular disease have been reported. A significant improvement in symptoms of cerebral insufficiency in 60 elderly patients treated for eight weeks was found in a double-blind trial [105]. Also, in patients with transient cerebral ischaemic attacks, oxpentylylline given to 30 patients was significantly more effective than aspirin plus dipyridamole, given to 36 patients, in preventing further attacks over a one year treatment period [106].

Investigation of the mode of action of oxpentylylline in cerebrovascular disease has shown a significant increase in cerebral blood flow as measured by $^{99}\text{Technetium}$ scintigraphy [107, 108] and $^{131}\text{Xenon}$ clearance [109]. A significant increase in whole-blood filtration time in 28 patients given parenteral oxpentylylline in the immediate weeks

following a cerebrovascular accident, compared with 21 patients not given oxpentifylline, has also been reported [110].

The mechanism of action of oxpentifylline in patients with vascular disease is uncertain. Possible mechanisms that could alter erythrocyte deformability include an increase in erythrocyte membrane permeability to glucose with consequential stimulation of glycolysis [111] and increases in intracellular c-AMP and ATP [112, 113] and in 2,3-diphosphoglycerate [114]. Binding of oxpentifylline to the erythrocyte membrane with chelation of calcium [115] has also been proposed.

9. Suloctidil

This anti-spasmodic drug, which also inhibits platelet aggregation, has been reported to cause a significant increase in walking distance after six months' treatment in 14 patients with intermittent claudication [116]. No haemorheological studies were made but, in an earlier study of 20 diabetics treated for five months, there was a significant reduction in high-shear, whole-blood viscosity [117]. The viscosity decrease was not associated with any fall in plasma fibrinogen level, or increase in whole-blood filtration rate, but was not adjusted for significant changes in haematocrit. In a double-blind study of 45 claudicants, no improvement in treadmill walking distance was found, compared with placebo, when 300 mg/day of suloctidil was given for 6 months [118].

Conclusions

There is suggestive evidence, from the preceding clinical and laboratory studies, that some clinical benefit can be obtained in patients with vascular disease by either haemodilution or the use of a rheologically-active drug. Further clinical studies are now required to define the indications for, and optimal method of, haemodilution. The more difficult problem is to investigate the mode of action of drugs that may have a rheological effect and to devise better clinical trials in order to establish the

extent of benefit that can be achieved. Some drug effects demonstrated *in vitro* have been achieved at unpharmacologically high concentrations while other studies, at therapeutic levels, have demonstrated such a small effect that clinical benefit is unlikely. When a statistically significant improvement in an objectively measured clinical parameter has been obtained, the degree of improvement (say a 20% improvement in walking distance) may even then be of little real value to the patient.

It is notoriously difficult to design efficient clinical trials in patients with vascular disease. Localised thrombotic lesions may spontaneously lyse, regular training has a beneficial effect on exercise tolerance, and there is often a considerable placebo effect. Also, the extent of vascular damage tends to vary considerably from patient to patient and other disorders, such as diabetes mellitus, sometimes coexist. These difficulties make it mandatory to use the placebo-controlled, double-blind clinical trial format and to include a sufficiently large number of patients to ensure that a genuine drug-induced effect is not missed. Few clinical trials of rheologically-active drugs have matched these criteria, and the challenge is to devise an efficient trial format that will give a clinically-useful answer without recourse to an unwieldy and prohibitively expensive study.

Drug-intervention trials can be made more efficient, albeit more artificial, by careful selection of patients and by defining objective test parameters for assessing each patient's clinical status at the time of entry to the trial and serially during treatment. A run-in period, during which the tests are repeated, will ensure that each patient is in the steady state before randomisation. These tests should include not only an objective measurement of clinical performance but also rheological tests on *ex vivo* blood samples to show that any subsequent clinical benefit is related to a specific rheological change during treatment. Since many of the drugs in this review have multiple actions, and since patients with widespread atherosclerosis have multiple abnormalities of a prothrombotic nature [5], it is correspondingly difficult to establish which drug effect has caused the recorded clinical benefit. A further difficulty, in the study of erythrocyte defor-

mability, is that the venous blood test sample from the cubital fossa may not be at all representative of erythrocyte metabolism in an ischaemic area where stasis, local hypoxia, pH reduction, and hyperosmolarity can coexist. If a drug acts as a vasodilator and improves blood flow, then the regional metabolic improvement, as stasis and ischaemia are relieved, should eliminate any local adverse effect on erythrocyte deformability. In theory, a vasoactive drug may thereby improve erythrocyte deformability without any direct effect on the erythrocyte itself.

The basic premise that erythrocyte deformability is abnormal in venous blood from patients with atherosclerotic peripheral vascular disease [119, 120] was based on studies of erythrocyte filtration in the presence of contaminating plasma proteins and leucocytes; a more recent study of washed erythrocytes, with mathematical correction for contaminating leucocytes [13], failed to confirm an abnormality of erythrocyte filterability in patients with intermittent claudication. Similarly, the early reports that erythrocyte ATP level is reduced in venous blood from patients with peripheral vascular disease [113, 119] require confirmation before further attempts are made to influence erythrocyte deformability, via the ATP level, in vascular disease.

A combination of the double-blind clinical trial together with serial rheological studies on *ex vivo* blood samples from both treatment and placebo groups would therefore seem to provide the maximum amount of information for the initial evaluation of new rheological forms of therapy. Clinical trials which do not meet these criteria are probably not cost effective in this difficult area. Most trials in the future will require the participation of several centres with specialist expertise in vascular disease. Thus the development of rheological tests that are sensitive and specific, yet simple enough for multi-centre trial use, is now a high priority.

Many of the rheological abnormalities (plasma hyperviscosity, whole-blood hyperviscosity, impaired whole-blood filterability) described in patients with vascular disease are related to the increase in plasma fibrinogen level. If the pathogenesis of this increase, which is part of the

haematological stress response [121], were better understood, and if a non-toxic means of long-term pharmacological reduction of the fibrinogen level could be achieved, then considerable rheological benefit might result.

References

1. Stuart J, Kenny MW: Blood rheology. *J Clin Pathol*, 1980; 33: 417-29.
2. Inglis TCMcN, Carson PJ, Stuart J: Clinical measurement of whole-blood viscosity at low-shear rates. *Clin Hemorheol*, 1981; 1: 167-77.
3. Stuart J, Bull B, Juhan-Vague I: Microrheological techniques for the measurement of erythrocyte deformability. In: *Investigative microtechniques in medicine and biology*, Chayen J, Bitensky L (eds) New York, Marcel Dekker, 1984: 297-326.
4. Kenny MW, Meakin M, Stuart J: Measurement of erythrocyte filterability using washed-erythrocyte and whole-blood methods. *Clin Hemorheol*, 1981; 1: 135-46.
5. Stuart J, George AJ, Davies AJ, Aukland A, Hurlow RA: Haematological stress syndrome in atherosclerosis. *J Clin Pathol*, 1981; 34: 464-67.
6. Meade TW, North WRS, Chakrabarti R, Stirling Y, Haines AP, Thompson SG, Brozović M: Haemostatic function and cardiovascular death: early results of a prospective study. *Lancet*, 1980; 1: 1050-54.
7. Lowe GDO, Drummond MM, Lorimer AR, Hutton I, Forbes CD, Prentice CRM, Barbenel JC: Relation between extent of coronary artery disease and blood viscosity. *Br Med J*, 1980; 280: 673-74.
8. Dormandy JA, Hoare E, Khattab AH, Arrowsmith DE, Dormandy TL: Prognostic significance of rheological and biochemical findings in patients with intermittent claudication. *Br Med J*, 1973; 4: 581-83.
9. Hamer JD, Ashton F, Meynell MJ: Factors influencing prognosis in the surgery of peripheral vascular disease: platelet adhesiveness, plasma fibrinogen, and fibrinolysis. *Br J Surg*, 1973; 60: 386-89.
10. Pearson TC, Wetherley-Mein G: Vascular occlusive episodes and venous haematocrit in primary proliferative polycythaemia. *Lancet*, 1978; ii: 1219-22.
11. Kannel WB, Gordon T, Wolf PA, McNamara P: Hemoglobin and the risk of cerebral infarction: the Framingham study. *Stroke*, 1972; 3: 409-20.
12. Drummond MM, Lowe GDO, Belch JJF, Barbenel JC, Forbes CD: An assessment of red cell deformability using a simple filtration method. *J Clin Pathol*, 1980; 33: 373-76.
13. Stuart J, Kenny MW, Aukland A, George AJ, Neumann V, Shapiro LM, Cove DH: Filtration of washed erythrocytes in atherosclerosis and diabetes mellitus. *Clin Hemorheol*, 1983; 3: 23-30.
14. Neumann V, Cove DH, Shapiro LM, George AJ, Kenny

- MW, Meakin M, Stuart J: Effect of Ticlopidine on platelet function and blood rheology in diabetes mellitus. *Clin Hemorheol*, 1983; 3: 13–21.
15. Schmid-Schönbein H, Rieger H: Why hemodilution in low flow states? In: *Hemodilution and flow improvement*, Schmid-Schönbein H, Messmer K, Rieger H (eds). *Bibliotheca Haematologica* No 47, Basel, Munchen, Paris, London, New York, Sydney, S. Karger, 1981: 99–121.
 16. Ames III A, Wright RL, Kowada M, Thurston JM, Majno G: Cerebral ischemia. II. The no-reflow phenomenon. *Am J Pathol*, 1968; 52: 437–53.
 17. Thomas DJ, du Boulay GH, Marshall J, Pearson TC, Ross Russell RW, Symon L, Wetherley-Mein G, Zilkha E: Effect of haematocrit on cerebral blood-flow in man. *Lancet*, 1977; ii: 941–43.
 18. Thomas DJ, du Boulay GH, Marshall J, Pearson TC, Ross Russell RW, Symon L, Wetherley-Mein G, Zilkha E: Cerebral blood-flow in polycythaemia. *Lancet* 1977; ii: 161–63.
 19. Willison JR, Thomas DJ, du Boulay GH, Marshall J, Paul EA, Pearson TC, Ross Russell RW, Symon L, Wetherley-Mein G: Effect of high haematocrit on alertness. *Lancet*, 1980; i: 846–48.
 20. Millikan CH, Siekert RG, Whisnant JP: Intermittent carotid and vertebral-basilar insufficiency associated with polycythaemia. *Neurology*, 1960; 10: 188–96.
 21. Gottstein U, Held K: Effekt der Hämodilution nach intravenöser Infusion von niedermolekularen Dextranen auf die Hirnzirkulation des Menschen. *Dtsch Med Wochenschr*, 1969; 94: 522–26.
 22. Humphrey PRD, Michael J, Pearson TC: Management of relative polycythaemia: studies of cerebral blood flow and viscosity. *Br J Haematol*, 1980; 46: 427–33.
 23. Gilroy J, Barnhart MI, Meyer JS: Treatment of acute stroke with Dextran 40. *JAMA*, 1969; 210: 293–98.
 24. Spudis EV, de la Torre E, Pikula L: Management of completed strokes with dextran 40. A community hospital failure. *Stroke*, 1973; 4: 895–97.
 25. Matthews WB, Oxbury JM, Grainger KMR, Greenhall RCD: A blind controlled trial of dextran 40 in the treatment of ischaemic stroke. *Brain*, 1976; 99: 193–206.
 26. Gottstein U, Sedlmeyer I, Heuss A: Behandlung der akuten zerebralen Mangeldurchblutung mit niedermolekularem Dextran. Therapie-Ergebnisse einer retrospektiven Studie. *Dtsch Med Wochenschr*, 1976; 101: 223–27.
 27. Gottstein U: Normovolemic and hypervolemic hemodilution in cerebrovascular ischemia. In: *Hemodilution and flow improvement*, Schmid-Schönbein H, Messmer K, Rieger H (eds). *Bibliotheca Haematologica* No 47, Basel, Munchen, Paris, London, New York, Sydney, S. Karger, 1981: 127–38.
 28. Dormandy JA: Influence of blood viscosity on blood flow and the effect of low molecular weight dextran. *Br Med J*, 1971; 4: 716–19.
 29. Martin P: Polycythaemia and thrombocythaemia and their influence on reconstructive arterial surgery. *J Cardiovasc Surg*, 1975; 16: 371–72.
 30. Bouhoutsos J, Morris T, Chavatzas D, Martin P: The influence of haemoglobin and platelet levels on the results of arterial surgery. *Br J Surg*, 1974; 61: 984–86.
 31. Yates CJP, Berent A, Andrews V, Dormandy JA: Increase in leg blood-flow by normovolaemic haemodilution in intermittent claudication. *Lancet*, 1979; ii: 166–68.
 32. Rudofsky G, Meyer P, Strohmenger HU: Effect of hemodilution on resting flow and reactive hyperemia in lower limbs. In: *Hemodilution and flow improvement*, Schmid-Schönbein H, Messmer K, Rieger H (eds). *Bibliotheca Haematologica* No 47, Basel, Munchen, Paris, London, New York, Sydney, S. Karger, 1981: 157–64.
 33. Rieger H: Indications and contraindications of isovolemic hemodilution in clinical angiography. In: *Hemodilution and flow improvement*, Schmid-Schönbein H, Messmer K, Rieger H (eds). *Bibliotheca Haematologica* No 47, Basel, Munchen, Paris, London, New York, Sydney, S. Karger, 1981: 149–56.
 34. Gelin LE, Jansen H: Moderate preoperative hemodilution, mortality and thrombus formation in general surgery. *Bibl Haematol*, 1975; 41: 239–47.
 35. Klövekorn WP, Pichlmaier H, Ott E, Sunder-Plassmann L, Messmer K: Acute preoperative hemodilution in surgical patients. *Bibl Haematol*, 1975; 41: 248–59.
 36. Ring CP, Pearson TC, Sanders MD, Wetherley-Mein G: Viscosity and retinal vein thrombosis. *Br J Ophthalmol*, 1976; 60: 397–410.
 37. Kohner EM, Barnes AJ, Hill DW, Young S, Reid AC, Dormandy JH: Effect of viscosity on retinal blood flow. XXIIIrd International Congress on Ophthalmology, Tokyo. Amsterdam, Excerpta Medica, 1978: 172.
 38. Wiederholt M: Hemodilution in retinal hypoperfusion. In: *Hemodilution and flow improvement*, Schmid-Schönbein H, Messmer K, Rieger H (eds). *Bibliotheca Haematologica* No 47, Basel, Munchen, Paris, London, New York, Sydney, S. Karger, 1981: 185–91.
 39. Conard J, Lecrubier C, Scarabin PY, Horellou MH, Samama M, Bousser MG: Effects of long term administration of Ticlopidine on platelet function and hemostatic variables. *Thromb Res*, 1980; 20: 143–48.
 40. Aukland A, Hurlow RA, George AJ, Stuart J: Platelet inhibition with Ticlopidine in atherosclerotic intermittent claudication. *J Clin Pathol*, 1982; 35: 740–43.
 41. Abe T, Kazama M, Naito I, Kuramoto A, Taketomi Y, Yasunaga K, Kansaki M, Sakuragawa N, Takahashi K, Maekawa T, Kobayashi N, Yamazaki H, Motomiya T, Toyama K, Andoh Y, Ogawa N: Clinical evaluation of ticlopidine in the inhibition of platelet function – a multi-clinic double blind study in comparison with aspirin. *Blood and Vessel*, 1980; 11: 428–37.
 42. Martin M, Hirdes E, Avel H: Defibrinogenation treatment in patients suffering from severe intermittent claudication – a controlled study. *Thromb Res*, 1976; 9: 47–57.
 43. Tønnesen KH, Sager PH, Gormsen J: Treatment of severe foot ischaemia by defibrillation with anerod: a ran-

- domized blind study. *Scand J Clin Lab Invest*, 1978; 38: 431-35.
44. Lowe GDO, Dunlop DJ, Lawson DH, Pollock JG, Watt JK, Forbes CD, Prentice CRM, Drummond MM: Double-blind controlled clinical trial of anrod for ischemic rest pain of the leg. *Angiology*, 1982; 33: 46-50.
 45. Lowe GDO, Forbes CD, Prentice CRM: Defibrinating agents. In: *Clinical aspects of blood viscosity and cell deformability*, Lowe GDO, Barbenel JC, Forbes CD (eds). Berlin, Springer-Verlag, 1981: 241-49.
 46. Cotton RC, Wade EG: Further observations on the effect of ethyl- α -p-chlorophenoxyisobutyrate + androsterone (atromid) on plasma fibrinogen and serum cholesterol in patients with ischaemic heart disease. *J Atheroscl Res*, 1966; 6: 98-102.
 47. Dormandy JA, Gutteridge JMC, Hoare E, Dormandy TL: Effect of clofibrate on blood viscosity in intermittent claudication. *Br Med J*, 1974; 4: 259-62.
 48. Postlethwaite JC, Dormandy JA: Results of ankle systolic pressure measurements in patients with intermittent claudication being treated with clofibrate. *Ann Surg*, 1975; 181: 799-802.
 49. Committee of Principal Investigators: A co-operative trial in the primary prevention of ischaemic heart disease using clofibrate. *Br Heart J*, 1978; 40: 1069-1118.
 50. NIH Consensus Conference: Thrombolytic therapy in treatment. *Br Med J*, 1980; 280: 1585-87.
 51. Sharma GVRK, Cella G, Parisi AF, Sasahara AA: Thrombolytic therapy. *N Engl J Med*, 1982; 306: 1268-76.
 52. Stampfer MJ, Goldhaber SZ, Yusuf S, Peto R, Hennekens CH: Effect of intravenous streptokinase on acute myocardial infarction. Pooled results from randomized trials. *N Engl J Med*, 1982; 307: 1180-82.
 53. Walker ID, Davidson JF: Long-term fibrinolytic enhancement with anabolic steroid therapy: a five-year study. In: *Progress in chemical fibrinolysis and thrombolysis* vol. 3, Davidson JF, Rowan RM, Samama MM, Desnoyers PC (eds). New York, Raven Press, 1978: 491-99.
 54. Preston FE, Burakowski BK, Porter NR, Malia RG: The fibrinolytic response to stanozolol in normal subjects. *Thromb Res*, 1981; 22: 543-51.
 55. Burnand K, Clemenson G, Morland M, Jarrett PEM, Browse NL: Venous lipodermatosclerosis: treatment by fibrinolytic enhancement and elastic compression. *Br Med J*, 1980; 280: 7-11.
 56. Jarrett PEM, Morland M, Browse NL: Treatment of Raynaud's phenomenon by fibrinolytic enhancement. *Br Med J*, 1978; 2: 523-25.
 57. Preston FE, Malia RG, Greaves M, Wijngaards G, Kluft C: The effect of stanozolol on thrombotic risk factors in healthy individuals. *Haemostasis*, 1982; 11 (suppl. 1): 56.
 58. Ayres ML, Jarrett PEM, Browse NL: Blood viscosity, Raynaud's phenomenon and the effect of fibrinolytic enhancement. *Br J Surg*, 1981; 68: 51-54.
 59. Benaim ME, Dewar HA: The effect of tetrancitonoylfructose (Bradilan) on fibrinolytic activity, platelet stickiness and some other parameters. *J Int Med Res*, 1975; 3: 423-27.
 60. Sirs JA, Boroda C, Rampling MW: The effect of nicofuranose on blood rheology in diabetes. *Bibl Anat*, 1981; 20: 157-60.
 61. Holti G, Newell DJ, Poole HG: Tetrancitonoylfructose in disorders of digital blood flow. *Practitioner*, 1971; 207: 654-58.
 62. Mashiah A, Patel P, Schraibman IG, Charlesworth D: Drug therapy in intermittent claudication: an objective assessment of the effects of three drugs on patients with intermittent claudication. *Br J Surg*, 1978; 65: 342-45.
 63. Scott CK, Persico FJ, Carpenter K, Chasin M: The effects of flunarizine, a new calcium antagonist, on human red blood cells in vitro. *Angiology*, 1980; 31: 320-30.
 64. De Clerck F, Beerens M, Thoné F, Borgers M: Effect of flunarizine on the human red cell shape changes and calcium deposition induced by A23187. *Thromb Res*, 1981; 24: 1-12.
 65. De Cree J, De Cock W, Geukens H, De Clerck F, Beerens M, Verhaegen H: The rheological effects of cinnarizine and flunarizine in normal and pathologic conditions. *Angiology*, 1979; 30: 505-15.
 66. Flameng W, Verheyen F, Borgers M, De Clerck F, Brugmans J: The effect of flunarizine treatment on human red blood cells. *Angiology*, 1979; 30: 516-25.
 67. Di Perri T, Forconi S, Guerrini M, Pasini FL, Del Cippola R, Rossi C, Angnusdei D: Action of cinnarizine on the hyperviscosity of blood in patients with peripheral obliterative arterial disease. *Angiology*, 1979; 30: 13-20.
 68. Jageneau A, Haag F: Flunarizin bei Patienten mit Durchblutungsstörungen der unteren Extremitäten. Doppelblindstudie. *Med Welt*, 1977; 28: 1050-55.
 69. Staessen AJ: Treatment of peripheral circulatory disturbances with cinnarizine. A multi-centre double-blind, placebo-controlled evaluation. *Proc Roy Soc Med*, 1977; 70 (suppl. 8): 17-20.
 70. Barber JH, Reuter CA, Jageneau AHM, Loots W: Intermittent claudication: a controlled study in parallel time of the short-term and long-term effects of cinnarizine. *Pharmatherapeutica*, 1980; 2: 401-7.
 71. Schetz J, Bostoen H, Clement D, Fornhoff M, Haerens A, Roekarts P, Staessen AJ: Flunarizine in chronic obstructive peripheral arterial disease: a placebo-controlled, double-blind, randomized multicentre trial. *Curr Ther Res*, 1978; 23: 121-30.
 72. Laporte J-R: Report on cinnarizine. In: *Drug treatment and prevention in cerebrovascular disorders*, Tognoni G, Garattini S (eds). Amsterdam, Elsevier/North-Holland Biomedical Press, 1979: 181-91.
 73. Slonim A, Cristal N, Erez R, Shainkin-Kestenbaum R: The effect of nifedipine, a calcium antagonist on red blood cell filterability (R.C.F.). Abstracts, 2nd European Conference on Clinical Haemorheology, London: 1981: 88.
 74. Bull B, Stuart J, Juhan-Vague I: Normal and pathological determinants of erythrocyte deformability. In: *Investiga-*

- tive microtechniques in medicine and biology, Chayen J, Bitensky L (eds). New York, Marcel Dekker, 1984: 257-95.
75. Dowd PM, Kovacs IB, Bland CJH, Kirby JDT: Effect of prostaglandins I₂ and E₁ on red cells deformability in patients with Raynaud's phenomenon and systemic sclerosis. *Br Med J*, 1981; 283: 350.
 76. Martin MFR, Tooke JE: Effects of prostaglandin E₁ on microvascular haemodynamics in progressive systemic sclerosis. *Br Med J*, 1982; 285: 1688-90.
 77. Letcher RL, Chien S, Laragh JH: Changes in blood viscosity accompanying the response to prazosin in patients with essential hypertension. *J Cardiovasc Pharmacol*, 1979; 1 (suppl. 1): 58-520.
 78. Di Perri T, Forconi S, Agnusdei D, Guerrini M, Laghi Pasini F: The effects of intravenous isoxsuprine on blood viscosity in patients with occlusive peripheral arterial disease. *Br J Clin Pharmacol*, 1978; 5: 255-60.
 79. Di Perri T, Forconi S, Guerrini M, Agnusdei D: *In vitro* activity of isoxsuprine on blood, plasma and serum viscosity. *Pharmatherapeutica* 1977; 1: 447-52.
 80. Di Perry T, Forconi S, Guerrini M, Pecchi S, Pieragalli D, Cappelli R, Acciavatti A: Influence of non-selective and selective beta adrenoceptor blockade on isoxsuprine-dependent hemodynamic and rheologic changes. *Angiology*, 1981; 32: 257-65.
 81. Dormandy JA, Ernst E: Effects of buflomedil on erythrocyte deformability. *Angiology*, 1981; 32: 714-16.
 82. Pergo MA, Sergio G, Espureo M, Francisci A, Artale F: Haemodynamic and haemorheological effects of buflomedil in patients with peripheral occlusive arterial disease. *Curr Med Res Opin*, 1982; 8: 178-87.
 83. Coccheri S, Palareti G, Poggi M, Tricarico MG: Improvements in the rheologic properties of blood induced by medium-term treatment with buflomedil in diabetic patients. *J Int Med Res*, 1982; 10: 394-98.
 84. Dintenfass L, Lake B: Beta blockers and blood viscosity. *Lancet*, 1976; 1: 1026.
 85. Linee Ph, Hollands MA, Quiniou P, Gueguen M, Le Polles JB: Experimental approach of activity and mechanism(s) of action of drugs used in cerebral metabolic insufficiency. Application to l-eburnamonine. *Eur Neurol*, 1981; 20: 253-57.
 86. Gueguen M, Genetet B, Durand F, Cherpi J, van den Driessche J, Linée Ph, Le Polles JB: Activité de la l-éburnamonine (I) sur les propriétés hémorhéologiques et oxyphoriques du sang humain. Étude in vitro et après administration orale chez l'homme sain. *Le Praticien*, 1981; 376: 57-64.
 87. Palareti G, Poggi M, Limoni P, Andreoli A, Tricarico MG, Coccheri S: Changes in the rheologic properties of blood in acute cerebrovascular disease: effects of treatment with l-eburnamonine. *Int Angiol*, 1983; 2: 179-83.
 88. Matzky R, Darius H, Schröer K: The release of prostacyclin (PGI₂) by pentoxifylline from human vascular tissue. *Arzneim Forsch*, 1982; 32: 1315-18.
 89. Leonhardt H, Grigoleit H-G: Effects of pentoxifylline on red blood cell deformability and blood viscosity under hyperosmolar conditions. *Naunyn Schmiedebergs Arch Pharmacol*, 1977, 299: 197-200.
 90. Nishio T, Toshima Y, Matsuno Y: Effects of pentoxifylline on cell shape, ATP content and deformability in rabbit erythrocytes under hyperosmolar conditions. *Int J Biochem*, 1982; 14: 915-20.
 91. Kiesewetter H, Dauer U, Gesch M, Seiffge D, Angelkort B, Schmid-Schönbein H: A method for the measurement of the red blood cell deformability. *Scand J Clin Lab Invest*, 1981; 41 (suppl. 156): 229-32.
 92. Seiffge D, Kiesewetter H: Effect of pentoxifylline on single red cell deformability. *Klin Wochenschr*, 1981; 59: 1271-72.
 93. Seiffge D, Kiesewetter H: Filtrability investigations with red blood cell (RBC) suspensions: effects of different blood components and pentoxifylline on RBC flow rate. *Ric Clin Lab*, 1981; 11 (suppl. 1): 117-23.
 94. Werner U: Measurement of the flexibility of erythrocytes incubated with oxpentifylline and low molecular dextran. *Med Welt*, 1975; 26: 2098-99.
 95. Grigoleit H-G, Leonhardt H, Schröer R, Lehrach F: Red blood cell aging as a model to influence pharmacologically the red cell filterability. *Res Exp Med (Berl)*, 1981; 179: 249-54.
 96. Dormandy J, Ernst E, Flute P: Increase in red cell filterability after incubation with oxpentifylline. *Curr Med Res Opin*, 1981; 7: 520-22.
 97. Isogai Y, Mochizuki K, Ashikaga M: A new method of measuring red cell deformability and the effects of pentoxifylline. *Curr Med Res Opin*, 1981; 7: 352-58.
 98. Angelkort B, Kiesewetter H: Influence of risk factors and coagulation phenomena and the fluidity of blood in chronic arterial occlusive disease. *Scand J Clin Lab Invest*, 1981; 41 (suppl. 156): 185-88.
 99. Störmer B, Kleinschmidt K, Loose D, Kremer K: Rheological changes in the blood of patients with chronic arterial occlusive disease after the administration of vasoactive drugs. *Curr Med Res Opin*, 1977; 4: 588-95.
 100. Jarrett PEM, Moreland M, Browne NL: The effect of oxpentifylline ('Trental') on fibrinolytic activity and plasma fibrinogen levels. *Curr Med Res Opin*, 1977; 4: 492-95.
 101. Takamatsu S, Sato K, Takamatsu M, Sakuta S, Mizuno S: Changes in haematological and blood chemical parameters after treatment of aged arteriosclerotic patients with pentoxifylline. *Pharmatherapeutica*, 1979; 2: 165-72.
 102. Müller R: Pentoxifylline - a biomedical profile. *J Med*, 1979; 10: 307-29.
 103. Porter JM, Cutler BS, Lee BY, Reich T, Reichle FA, Scogin JT, Strandness DE: Pentoxifylline efficacy in the treatment of intermittent claudication: multicenter controlled double-blind trial with objective assessment of chronic occlusive arterial disease patients. *Am Heart J*, 1982; 104: 66-72.

104. Di Perri T, Guerrini M: Placebo controlled double blind study with pentoxifylline of walking performance in patients with intermittent claudication. *Angiology*, 1983; 34: 40-45.
105. Harwart D: The treatment of chronic cerebrovascular insufficiency. A double-blind study with pentoxifylline ('Trental' 400). *Curr Med Res Opin* 1979; 6: 73-84.
106. Herskovits E, Vazquez A, Famulari A, Smud R, Tamraoff L, Fraiman H, Gonzalez AM, Vila J, Matera V: Randomised trial of pentoxifylline versus acetylsalicylic acid plus dipyridamole in preventing transient ischaemic attacks. *Lancet*, 1981; i: 966-68.
107. Koppenhagen K, Wenig HG, Muller K: The effect of pentoxifylline (Trental) on cerebral blood flow: a double-blind study. *Curr Med Res Opin*, 1977; 4: 681-87.
108. Koppenhagen K, Wenig HG: The effect of pentoxifylline on cerebral blood flow. *Acta Neurol Scand*, 1979; 60 (suppl. 72): 626-27.
109. Hartmann A: Effect of pentoxifylline on regional cerebral blood flow with cerebrovascular disease. *Pharmatherapeutica*, 1981; 2: 528-31.
110. Martin P, Vives P: The effect of pentoxifylline on red cell deformability in cerebrovascular accidents. *Curr Med Res Opin*, 1980; 6: 518-22.
111. Stefanovich V, Porsche E, Müller E: On the influence of pentoxifylline on the permeability of rat erythrocytes for methyl-0-glucose. *Arzneim Forsch*, 1979; 29: 757-60.
112. Stefanovich V: The biochemical mechanism of action of pentoxifylline. *Pharmatherapeutica*, 1978; 2 (suppl. 1): 5-16.
113. Buchanan N, Moodley FP: The effect of pentoxifylline on human erythrocyte adenosine triphosphate. *IRCS (Med Sci)*, 1976; 5: 43.
114. Le Devehat C, Lemoine A, Crette B, Ramet M: Pharmacological influences of pentoxifylline on red cell filterability and 2-3 diphosphoglycerate. *Scand J Clin Lab Invest*, 1981; 41 (suppl. 156): 301-3.
115. Schröer R, Kiesewetter H, Müller R: Rheologically impaired microcirculation: its significance in vascular disease and drug-induced improvements. *Abstracts, 2nd European Conference on Clinical Haemorheology*, London: 1981; 87.
116. Jones NAG, De Haas H, Zahavi J, Kakkar VV: A double-blind trial of suloctidil v. placebo in intermittent claudication. *Br J Surg*, 1982; 69: 38-40.
117. Roncucci R, De Hertogh R, Dormandy JA, Doumont J, Gurewich V, Lambelin G, Lansen J, Rottiers R, Souridis E, van Stalle F, Versee L: Effects of long-term treatment with suloctidil on blood viscosity, erythrocyte deformability and total fibrinogen plasma levels in diabetic patients. *Arzneim Forsch*, 1979; 29: 682-84.
118. Verhaeghe R, Van Hoof A, Beyens Gh: Controlled trial of suloctidil in intermittent claudication. *J Cardiovasc Pharmacol*, 1981; 3: 279-86.
119. Ehrly AM, Köhler H-J: Altered deformability of erythrocytes from patients with chronic occlusive arterial disease. *Vasa*, 1976; 5: 319-22.
120. Reid HL, Dormandy JA, Barnes AJ, Lock PJ, Dormandy TL: Impaired red cell deformability in peripheral vascular disease. *Lancet*, 1976; ii: 666-67.
121. Stuart J: The acute-phase reaction and haematological stress syndrome in vascular disease. *Int J Microcirc: Clin Exp*, 1984; 3: 115-29.

5. Can we support comprehensive home care for haemophiliacs?

P. JONES and A.F.H. BRITTEN

Introduction

Twenty years ago, the child with severe haemophilia was not expected to survive beyond the fourth decade [1]. If he did he was likely to have marked haemophilic arthropathy, affecting hips and shoulders, knees, ankles and elbows. His arthritis would have crippled him, and, together with contractures due to fibrous repair following muscle haemorrhage, would have marked him out from his peers early in his adult life. He would have been less likely to marry [2] and less likely to succeed in his chosen career [3] than his non-haemophilic contemporaries. In short, unless he was lucky, he was denied both quantity and quality of life.

It was the widespread introduction of quality blood products and infusion kits, together with profound changes in medical education, which resulted in a patient- rather than a physician-orientated management of disease, that changed this picture. Today, having haemophilia still carries the threat of inopportune bleeding and of rejection by intolerant employers. However, it does not imply the probability of early death [4], is amenable to home therapy and is compatible with a full and active family life.

The legacy of inadequate treatment until comparatively recently has resulted in two broad categories of haemophilic patient [5] (Fig. 1). The first group includes the older survivors; most have severe arthropathy. The second group, over-represented when compared with normal population by age statistics, includes young people who, although severely affected in laboratory terms, are illustra-

tive of the effects of modern therapy. With time the curves should approximate; that will depend on whether the side effects of blood product therapy remain relatively benign.

From the medical viewpoint, management of haemophilia must take these two categories, with their differing needs, into account. Older patients require more of the type of support provided in good physical medicine and orthopaedic clinics, and regular check-ups directed at early recognition of the degenerative and malignant diseases of ageing. Younger patients require more counselling and more input into preventive medicine, whether it be physical, social or psychological. Both groups require careful surveillance to ensure that any harmful effects of therapy are recognised early and, when possible, treated.

Everyday life with severe haemophilia

Given the above facts, it would be simple to answer the question posed in the title of this chapter on a humane or emotional level. But, no matter what system is in operation, funding for health care cannot be infinite, and the needs of people with any chronic disorder must be the subject of regular, critical review in order to consider their priority with respect to others. Haemophilia is no exception. Once treatment is proved to be successful, pressure to make it available to everyone in need is inevitable. Among the results are increasing individual demands, and increasing numbers of haemophiliacs of reproductive age. So would it be

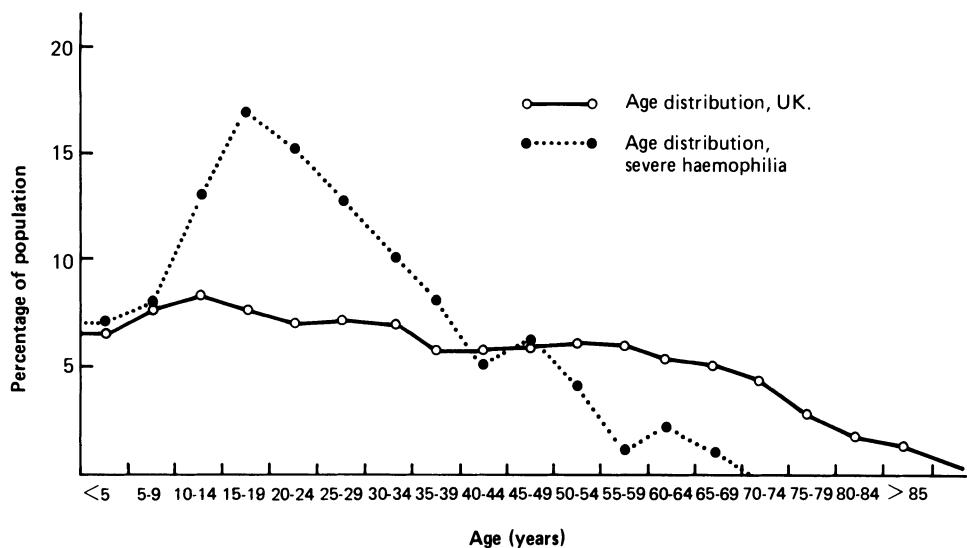


Fig. 1. Age distribution for normal and haemophilic populations. From Jones P. Ed Haemophilia home therapy. London, Pitman Medical 1980.

cheaper to provide minimal care, accepting for instance that only life-threatening haemorrhage should be treated? Such a policy might retain some increase in longevity, slow the rise in affected population, reduce the incidence of side effects and lessen the often very expensive management required to control them.

The natural history of untreated haemophilia has been well documented [5, 6, 7, 8, 9]. On average, the haemophiliac bleeds 35 times a year. The incidence of bleeding varies considerably with age, more bleeds being experienced in childhood. This higher incidence is not simply a reflection of relative physical activity. The joints of a growing boy are more unstable and less well protected by muscle than those of a young adult, and the great majority of haemophilic bleeds are intra-articular. The high incidence of ankle bleeds in pre-school children reflects this instability, some boys bleeding two or three times a week *without any evidence of a predisposing cause*. This qualification is an important one because part of any argument to cut costs might be to restrict activity in order to lessen the incidence of trauma. However, most haemophilic bleeds are spontaneous. Conversely, it is probable that only one accident in 15 results in haemorrhage.

Internal haemorrhage is painful and places an

immediate demand on the parents of an affected boy. This demand is unpredictable, help being sought at any time of the day or night. At a bleeding rate of three times a week, the family's life will be disrupted 156 times in the year. That this disruption affects the economic viability of the family has been demonstrated by the gradual decline in social status that occurs when haemophilia is present in several generations [2]. And when this happens more resource is required from state or neighbourhood aid in terms of subsidised housing, sickness and unemployment benefits.

It is inevitable that the sick role that has to be adopted by the untreated boy is extended to the rest of the family. His mother, who feels remorse as the carrier of his defective gene, may overprotect her son to the detriment of her husband and the other children. Daughters, who may themselves be carriers, will be alarmed for their future families. From these few well-recognised observations it is clear that an everyday life of restricted treatment, still evident in developing nations and in the case of a minority of patients with high titre antibodies, is unacceptable. What are the costs of the alternative?

Provision of blood products for treatment

In the United States of America and in most, but not all, developed countries much of the provision of blood products for haemophilia care has become the responsibility of commercial pharmaceutical companies. In a minority of developed countries, for instance Australia, the products needed are prepared solely from native, volunteer blood donations. The reasons for the scale of the commercial exercise are not difficult to understand, and have been well reviewed by Hagen [10]. In essence they are that large-scale industrial fractionation requires a high level of capital investment, that most haemophiliacs and their doctors demand the convenience of freeze-dried concentrates, and that the more refined these concentrates are the less their yield of active clotting factors and therefore the more the donations needed for source plasma. The only way sufficient source material can be made available in most communities is through plasmapheresis, and the most cost-effective way of ensuring large-scale plasmapheresis is by paying donors. So, despite the understandable international desire to provide blood products solely from volunteer donor sources, in the real world it has been the commercial fractionators who have helped to achieve the standards of care now possible for many haemophiliacs. The only change likely to affect this situation radically is the introduction of recombinant DNA material which should, of course, remove the need for much human plasma donation whether volunteer or paid.

At present the international cost of a unit of factor VIII derived from human plasma is around 14 US cents (10 UK pence). As with any commodity there is considerable variation from country to country, according to the local market forces operating. Thus today's price in a major haemophilia centre in the United States is quoted as 10 US cents/VIII unit, in the United Kingdom 12 US cents/VIII unit, and in West Germany 18 US cents/VIII unit. In Third World countries, any of these prices is totally out of reach of all but a minority of economically well-endowed families. Moreover, changes in manufacture claimed to reduce hepatitis transmission are already increasing treatment costs, de-

spite any clear evidence of efficacy; for instance commercial heat treated concentrate is selling in West Germany today at 28 US cents/VIII unit.

Another difficulty in calculating cost in the past have been variabilities in patient/doctor expectations, and thus in annual usage of blood product in different countries [11]. In the United Kingdom the 1982 returns from the Haemophilia Centre Directors showed that, on average, a patient required 32,500 VIII units per year. This figure showed little variation from centre to centre and included usage for home therapy and prophylaxis, as well as for surgery. It was calculated for haemophilia A patients of all severities who required treatment in that year. In the United States the equivalent figure is remarkably close, an average of 40,000 VIII units being used for all patients treated in a year [12]. As expected in both countries, more severely affected patients require more blood product; in the USA, on average 60,000 VIII units/patient/year [12].

Using these figures it is simple to calculate that the present cost of factor VIII per patient per year in a developed country is around 5,000 US\$ (3,600 UK£).

In comparison with other chronic disorders requiring intermittent treatment this cost seems reasonable. Continuous ambulatory peritoneal dialysis (CAPD) in the United Kingdom costs 7714 US\$ (5510 UK£)/patient/year, whilst hospital haemodialysis costs 17,320 US\$ (12,300 UK£) annually [13]. The cynic might like to compare these figures with the costs of keeping adult males in UK prisons – between 11,200 US\$ (8000 UK£) and, in the case of terrorists, 33,500 US\$ (24,000 UK£) a year [14].

Other resources needed

The question of providing sufficient quality blood products at reasonable cost is not the only one that has to be answered when considering haemophilia management. In the past decade the term 'comprehensive care' has become fashionable. It suggests an ideal in which the overall responsibility for a patient's well-being is taken over by a team of professionals. Not only is such a premise untrue, it

is also denigratory to the patient. The aim of comprehensive care should be to provide all the services required to treat haemophilic bleeding effectively, together with advice and help with other problems based on experience with many patients. It can work only with the co-operation of the patient and his family.

The treatment of bleeding itself is, in the case of patients without clotting factor antibodies, relatively straightforward nowadays. However, it does depend on adequate laboratory facilities with good quality control, and on rapid therapy in the event of a bleed, as well as the guaranteed supply of blood products. Management of patients with high titre antibodies is more complex, and when complications occur, should only be undertaken in major centres. It is with the other facets of management that the majority of centre staff are most likely to be involved. The staffing structure of a major centre is shown in Table 1.

Members of the core team are able to devote much of their time to haemophilia care. The overall director of the centre is usually a haematologist, but could equally be a physician or paediatrician. The senior nurse as the 'first contact' for the patients is usually the clinical co-ordinator with special responsibilities for the day-to-day running of the centre and the home therapy programme.

The ancillary team is conversant with the special problems and demands of haemophilia and may be

consulted at any time specialist advice is required. The surgical members of this team should be able to work in the knowledge that the core team will ensure optimum haemostatic control during and after invasive techniques. In many centres genetic counselling is performed by a member of the core team who is well qualified to put advice about future family into the context of modern therapy and prognosis.

Some centres have specialist careers or vocational counsellors; others rely on outside contacts via social workers or psychologists. In the United States financial counselling is provided. Every centre should have links with the local branch of the lay haemophilia society (foundation). Participation in group meetings and activities, and opportunities for up-dating families on recent advances as well as reinforcing pertinent advice are invaluable.

Clearly, staffing costs on this scale can only be justified if sufficient numbers of patients attend. The definition of 'sufficient' is debatable. In the United Kingdom, which is well served by a network of haemophilia centres, 10 reference (or comprehensive care) centres cater for a population of 56 million. In addition there are around 100 other centres, all associated with the reference organisation serving their geographical regions. Thus no patient need be isolated from day-to-day care, and every patient should have immediate access to specialist consultation when the need arises. By contrast, in the United States over 50% of haemophilic patients are treated out with comprehensive care centres, usually on a one-to-one basis with a private physician.

What are the costs of staffing a comprehensive care programme? In 1978 the cost of providing a haemophilia service from the Newcastle Reference Centre to 211 families (303 patients) was approximately 52,000 US\$ (37,000 UK£) a year [15]. Inflation has doubled this figure so that staff costs/patient/year are now approximately 350 US\$ (250 UK£). If overheads such as maintenance, heating and lighting and provision of transfusion kits and other equipment are taken into account the annual figure is probably about 420 US\$ (300 UK£) per patient. Thus the provision of blood

Table 1. Comprehensive Haemophilia care.*

Core team	Ancillary team
Haematologist	Orthopaedic surgeon
Physician	Dental surgeon
Paediatrician	General surgeon
Nursing staff	Neurosurgeon
Technical staff	Gynaecologist
Physiotherapist	Genetic counsellor
Social worker	Psychologist
Secretary	Psychiatrist

* In addition to managing haemophilia it should be remembered that most centre staff treat acquired disorders of haemostasis as well. Thus calculations based on relatively small numbers of families with hereditary coagulopathies are likely to give a false impression of the workload involved.

products together with medical and paramedical supervision, based on the British experience quoted, costs some 5,500 US\$ (4,000 UK£)/patient/year. This figure is in very close agreement with that presented by Cederbaum and his colleagues in the United States (see Table 2). The figure demonstrates that this expenditure actually *saves* money, because patients not receiving comprehensive care require more hospitalisation, and require more economic support because of their reduced capacity to work. These findings are reciprocated in the UK where an ambulance journey costs 2.9 US\$ (1.85 UK£) per mile and a bed in a major hospital 147 US\$ (105 UK£) a day. At these rates, the 5,500 US\$ (4,000 UK£) needed for comprehensive care would pay for only 13 two-day admissions for treatment a year, assuming that the patient lived 25 miles from the hospital, and this calculation does not include the cost of blood products.

Put another way, a bleed treated quickly at home with 500 units of factor VIII costs 70 US\$ (50 UK£) and the patient can usually go straight back to work or school. The same bleed treated late in hospital would require *at least* this amount plus the ambulance and hospital costs, around 493 US\$ (350 UK£), and the patient would be off work or school.

Home therapy

To be successful comprehensive care must include the provision of home therapy. Time is valuable for haemophiliacs and their families, not only because the treatment of bleeding is best when given

quickly, but also because the time spent in seeking medical care and in convalescence after treatment can interrupt and damage normal life. Education can be undermined and employment compromised. Domestic life or planned leisure may be disrupted.

Time is the essence. One reality is that hospital treatment requires travel (time) and waiting (time) for the availability of the clinical team. Another reality is that most haemophiliacs bleed often and learn to be expert in the treatment of their condition. Thus a patient's knowledge and experience can contribute a talent which can be used to solve a problem, and he can become a full member of the health care team. The essence of 'home care' is that essential treatment is available without hospital facilities or a physician's presence. It does not have to be at home; it may be in the workplace, in an airplane, at a campsite, in a car – at the convenience of private physicians or hospital staff. 'Self therapy' need not be interpreted literally; the infusion may be given by the patient but also by a spouse, a sibling, a parent or a friend. The concept is one of independence, or freedom from potentially restrictive institutional ties.

It has been said above that patients and doctors prefer freeze-dried concentrates for home therapy. The reasons for this preference are shown in Table 3. Whatever the products chosen each centre must have immediate access to them and this access should not be limited by administrative or political expediency. Prescription of blood products is no different to the prescription of any drug. Obviously questions of supply and demand of this human resource are bound to engender debate, but no patient attending a comprehensive care centre

Table 2. United States of America federally funded centres.

	Before	Year after	% Change
Number of patients receiving comprehensive care	1477	4768	+222
Number of patients on home therapy	682	2009	+203
Average number of days in the year lost from school or work	20-60	8.3	-58 to -86
Average number of days in the year in hospital	14-60	2.4	-82 to -96
Average costs of health care (USA\$)	8000-22000	5252	-34 to -76

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Table 3. Advantages of freeze dried concentrate over cryoprecipitate.

Known dosage
Smaller volume
Ease of preparation
Ease of injection by syringe
Fewer allergic type reactions
Ease of storage
Ease of carriage and handling
Longer shelf-life

should be denied the blood product best suited to his needs. It is one of the functions of careful follow up to ensure that best use is made of blood products.

The major cost involved in comprehensive care remains the provision of blood products, and this *might* be reduced in the future if the hopes for recombinant DNA are realised. For the moment some 40,000 units of factor VIII from human plasma are required/patient/year. This figure represents an annual commitment for blood transfusion services to provide between 1 and 2 units VIII per head of population.

Conclusions

Comprehensive care requires four resources:

- the provision of quality blood products,
- the availability of professional staff,
- the grouping of patients so that sufficient numbers can be followed to justify centre staffing, and
- finance.

Used properly we have shown that, at least in developed countries, we cannot afford *not* to provide comprehensive care. Not only do the economics involved support this argument; the more intangible costs to the patient should be taken into account. Untreated bleeds result in otherwise unnecessary hospitalisation and rehabilitation, loss of

potential productivity, pain and suffering, interference with normal family life and reduced life expectancy.

References

1. Biggs R (ed): The Treatment of Haemophilia A and B and von Willebrand's Disease. Oxford, Blackwell Scientific Publications, 1978: 25.
2. Morgan W: The young haemophilic adult. In: Haemophilia Home Therapy, Jones P (ed). London, Pitman Medical, 1980: 39–50.
3. Stuart J, Forbes C, Jones P, Lane G, Rizza CR, Wilkes S: Improving prospects for the employment of the haemophiliac. *Br Med J*, 1980; 280: 1169–72.
4. Rizza CR, Spooner RJD: Treatment of haemophilia and related disorders in Britain and Northern Ireland during 1976–80: a report on behalf of the directors of haemophilia centres in the United Kingdom. *Br Med J*, 1983; 286: 929–33.
5. Jones P: The haemophilia service and home therapy. In: Haemophilia Home Therapy, Jones P (ed). London, Pitman Medical, 1980: 10–23.
6. Massie R, Massie S: Journey. London, Victor Gollancz, 1975.
7. Biggs R, Macfarlane RG (eds): Treatment of Haemophilia and Other Coagulation Disorders (1st edn.). Oxford, Blackwell Scientific Publications, 1966.
8. Hardisty RM, Ingram GIC: Bleeding Disorders. Oxford, Blackwell Scientific Publications, 1965.
9. Duthie RB, Matthews JM, Rizza CR, Steel WM: The Management of Musculo-skeletal Problems in the Haemophiliac. Oxford, Blackwell Scientific Publications, 1972.
10. Hagen PJ: Blood: Gift or Merchandise. New York, Alan R Liss, 1982.
11. Jones P: Factor VIII supply and demand. *Br Med J*, 1980; 280: 1531–2.
12. Aledort LM, Diaz M: The cost of care for haemophiliacs. In: Haemophilia in the Child and Adult, Hilgartner MW (ed). New York, Masson Publishing, 1982.
13. Williams AJ, Smith BA, Walls J: End stage renal failure: coping with the demand. *Health Trends* 1984; 16: 1–3.
14. Smith R: The state of the prisons; crisis upon crisis. *Br Med J*, 1983; 287: 1705–8.
15. Jones P: The haemophilia service and home therapy. In: Haemophilia Home Therapy, Jones P (ed). London, Pitman Medical 1980, 19.

6. Transfusion support in a community disaster

P.J. SCHMIDT and W.L. BAYER

Introduction

The planning of the supply of blood which may be needed in a disaster situation is complex since it is based on so many unknowns. How many victims will need care? How many of those will require transfusion? Will transfusion be necessary immediately after the disaster, or some hours later? Where will the transfusion be given? How will the blood be transported to the points of need? And finally, how can records be kept?

These considerations will be discussed with knowledge of disasters that have occurred in the United States in the past six years. We have not treated the matter of organizing transfusion services in support of military undertakings or in the event of a large-scale nuclear disaster. We believe that for the latter, planning transfusion support is either hopeless, or low on the list of immediate priorities.

It is our opinion that there is almost no conceivable disaster in the U.S.A. in which the *victims* would benefit from a call for emergency donors. That is because any community will have on the shelf at least a three-day supply of blood for its normal medical care facilities. If there is a disaster that involves all the hospitals of the community and all the surgeons and triage physicians, there will be no elective surgery the next day. There is no community in the country that could marshall enough care to deliver in three hours what it usually does in three days, and furthermore another source is only three hours away in neighboring communities.

Blood processed and ready to use can be shipped

in at a rate that will far outpace the usage and the ability to collect. The people in those neighboring communities can, and will, replenish and over-replenish the blood supplies. Let the people in the disaster community take care of the victims and not the healthy donors. If there is a disaster so large that it affects several neighboring communities, then blood is probably one of the last things needed.

Fires, floods, tornadoes, hurricanes, marine and mine disasters do not create an immediate need for blood. When a city is flooded or impassable with snow so that donors cannot attend to give blood, then patients are unable to get to the hospital for elective surgery, and there is no automobile trauma. Neither frostbite nor drowning victims need blood; nor do dead people. A reporter called the Chicago blood center on May 25, 1979 asking what was going to be done about blood for the DC-10 take-off disaster. The answer was simple since, of the 272 persons on board, there were no survivors.

In most situations it is to the detriment of disaster care to announce and mobilize local emergency blood collections at the time of a disaster. Even if blood collection is ordinarily done at a location away from the hospitals where the victims are treated, the hospital switchboard and access roads will be swamped. There will be calls and appearances by those who did not get the message right, or who want to be sure that their blood is delivered 'where it is needed'.

Fire, storm and wreck

In the Chicago elevated railroad disaster of January 1977, notice was given by the media that adequate blood was on hand. Nevertheless, one of the large hospitals opened its donor center as a place to send relatives and friends who were waiting on casualty information. Approximately 50 people donated blood between 6:30 p.m. and 1:30 a.m. Total usage at all the hospitals for the victims was 20 to 30 units.

The situation that everyone fears happened in Portland, Oregon on December 28, 1978. A DC-8 jet attempted an emergency landing and crashed into a residential area at 6 p.m., five miles south of the airport. There were 185 persons on board and 10 died. Four hundred donors appeared at the blood center and 250 units were collected. Six pints of blood were used for crash victims. Except for the victim list, i.e. 78 died and five survived, the jetliner crash in Washington D.C. in January 1982 had the same scenario, the same blood donor and blood center response.

At 6 p.m. on an April day in 1979, a tornado destroyed most of Wichita Falls, Texas. There were 47 deaths and almost 2,000 injuries. During the confusion of the storm, people went directly to hospitals to donate blood as they had done thirty-five years earlier. Within an hour and a half after the storm hit, over 500 people had presented themselves to be blood donors. They were waiting in the driving rain outside of the community blood center. Nurses, operating by lantern, withdrew blood from 364 donors during the next 36 h. But, before the storm hit, there were already 400 units of blood on hand in the blood center; more than twice as much as was used.

In the November 1980 Las Vegas, Nevada hotel fire, although the community blood services did not request donors, the radio station did. Accordingly, there was a flow of donors; 342 on that Friday and Saturday, which was five times the normal collection. Meanwhile, exactly the expected happened in San Bernardino, California two hundred miles away. They were also inundated with donors. If there had indeed been a need for extra blood in Las Vegas, then San Bernardino could have supplied

the stock from the shelf and still be more than replenished.

There was *no* usage of blood or even plasma in Las Vegas for the victims of that disaster. In fact, blood usage decreased in the community since medical resources were occupied with helping the victims instead of the regular work. But we have told people that 'blood is life' so we do not fault those who want to give 'life' at a time of public frustration with death. The donation of blood in time of crisis is a way for that donor to be 'doing something'. If there is no danger that the secondary purpose interferes with the primary purpose of taking care of disaster victims, perhaps it should be allowed. Certainly an organisation not at the site of the disaster should not collect blood for a disaster in the absence of a legitimate request for assistance from proper authorities and a means of shipping to the disaster area. No blood center should make massive appeals for donors when there are two or three survivors, no matter how large the disaster. That happened at least twice in 1982 when the survivors were children.

Disaster planning *should not* mean planning for large-scale blood collections. There will be enough confusion at the time of disaster without planning for emergency blood collections. There will be more than enough blood in a community at any time to take care of any disaster that the available medical forces can service. That was proved in the handling of the hotel collapse in 1981, in Kansas City, Missouri.

The Kansas City experience

On Friday night at 7:05 p.m., July 17, 1981, two skywalks at the Hyatt Regency Hotel collapsed during a dance attended by about 1,000 people. The medical needs of those patients caused the largest civilian blood-using disaster in the U.S.A. No one could predict immediately, how many people would be injured or how much blood would be necessary to support the emergency surgery. The final totals for this disaster were 114 dead and 188 injured. During the first 30 h after the disaster, 126 units of whole blood and red blood cells, 32 units of

platelets, 23 units of fresh frozen plasma and 10 units of cryoprecipitate were transfused to disaster victims. Blood use for disaster victims continued for weeks and eventually totaled 249 units of whole blood and red cells, 67 units of platelets, 32 units of fresh frozen plasma and 10 units of cryoprecipitate. Twenty-eight people were transfused, six of whom required more than 10 units within the first 30 hours.

How was this disaster handled and what can be learned from this experience?

The Community Blood Center of Greater Kansas City is a free standing regional blood program with a large central facility in Kansas City and a smaller collecting substation 60 miles north of Kansas City in St. Joseph, Missouri. The Center totally supplies the blood needs of all patients transfused in 69 hospitals in a geographical area that extends up to 150 miles from the main facility. Approximately 2.2 million people reside in this region and 1.5 million in the immediate metropolitan area served by 19 hospitals. Daily transfusion use in 1981 was about 300 whole blood and red cell units during each weekday and about 200 on the weekends. Twenty-one thousand different patients were transfused in 1981 throughout the region. Thirty percent of the blood is collected at the Center's main facility. Blood is kept on consignment in the hospitals and can be transferred for use to another institution as needed. Twice daily, in the morning and at night, an inventory check is made of the 19 metropolitan hospitals, where 70% of the units are transfused, to determine the units reserved for patients or otherwise available, should transfer be necessary. On July 17, 1981, the morning inventory showed the metropolitan hospitals had 915 units and the Center's distribution department another 419 units. The entire system had 2,500 units of blood available.

The Community Blood Center of Greater Kansas City had two objectives at the time of the disaster: first, to meet the immediate transfusion needs of the victims; and second, to meet the ongoing needs of those injured as well as all the needs of every patient in the region.

The metropolitan Kansas City area hospitals each have disaster plans, coordinated by the Kan-

sas City Area Hospital Association's emergency radio network. The Blood Center is a participant in the radio network and, depending upon a disaster's magnitude, different alert stages are broadcast. The Blood Center can also monitor the hospitals to which injured patients are transported. The disaster struck at 7:05 p.m. and the first order for blood specifically for patients from the hotel came at 8:25 p.m. Based on the level of the radio alert, a major disaster was expected and there was just over an hour to put the Center's emergency plan into motion.

Senior administrative and medical staff were alerted and reported immediately to the Center's main facility. The medical staff began communication with the hospital transfusion services and the following actions were taken. The blood availability status of the metropolitan hospitals was updated and information as to availability was obtained from other hospitals within a reasonable distance of the Center. Additional distribution department staff was called in to help handle the anticipated increase in blood orders. The delivery service was alerted to have additional drivers available to transport blood. Part of the processing laboratory staff was called in to process 300 units of blood that had been collected during the day. The State Highway Patrol was alerted and 100 units of blood were recalled from hospitals outside the immediate metropolitan area not likely to receive patients. The director of public relations was brought in to coordinate and to assure that all media communication would be accurate. Eight nurses were called in to handle blood donors and receptionists to handle calls. In the first five hours after the skywalk collapse, 194 units of blood and blood components were ordered by the eight hospitals handling the injured patients.

The second objective, to meet the ongoing needs of those injured as well as all the needs of every patient in the region, proved to be the most challenging – not because there was not enough blood, but because there was, and will always be, a community response. The expected needs of the victims were still not known two hours after the collapse. Eight nurses had been called in to handle donors and the remaining nurses attached to the

main facility not scheduled to work Saturday were put on alert. By 9:20 p.m., because of the large number of phone calls and blood donors who arrived at the center, the additional alerted nursing staff was brought in. The charge nurse at the St. Joseph substation was told to prepare her staff to serve as relief in Kansas City on Saturday. Department supervisors, not usually involved in collection or processing, were asked to call in part of their staff that night, and others the next day to assist as needed. During the night, not only did employees report to the Center, but their spouses also and even former employees wishing to help. These people were quickly put to work answering the phones, directing traffic in the parking lot and assisting in the movement of donors. By 11:00 p.m. the line of donors extended three blocks around the Center and there was a 2-3 h wait to donate. We decided at that time to remain open all night and to bring the St. Joseph staff to Kansas City on Saturday morning. A large classroom was set up with our blood mobile equipment to help process donors. A smaller seminar room was utilized to help donors complete part of the medical history questionnaire. Although donors were informed that there would be a 2-3 hours wait, most chose to stay to donate.

The staff answering Center telephones were instructed to ask donors not to come directly to the Center, but rather to make appointments for Saturday or the following week. At the same time, the media was told to inform the community that there was an adequate blood supply and those wishing to donate should call the Center and make an appointment. As the night proceeded, live TV broadcasts were produced at the Center and many reporters either came to the Center or called to obtain updated information. We conferred several times with the press during the night to keep the public informed and prevent the issue of conflicting reports. From 9:20 p.m. Friday to 4:30 a.m. Saturday 855 donors had registered and many more made appointments for the following week. By not using the entire staff on Friday night and utilizing the St. Joseph staff on Saturday, it was possible to process the large numbers of donors without compromising professional standards. The outpouring of blood donors showed the need and desire of the

community to be involved and help. Between Friday night July 17th and the following Friday July 24th, 2,116 donors registered at the Center's main facility. Normally for a similar period, 650 donors would have been registered. Of the 2,116 donors, 43.3% were active donors. 31.2% were first-time donors and the remaining 25.4% were donors who had not donated since 1979. The week after the disaster our telephone recruitment department enjoyed the unique experience of having donors call for an appointment rather than calling donors.

Analyzing the events of that night and the weeks after, the Center did accomplish its objectives and met the immediate and continuing blood and blood component needs of all patients. No blood was brought in from outside the region and there was no interruption in any hospital activity. The Hyatt disaster illustrated the value of a regional blood program in the following ways. Blood was immediately available for the disaster victims. Because of the trust and coöperation that existed between the hospitals, and the hospitals and the Blood Center, it was possible to call upon the entire region to meet the blood needs of the injured. The 100 units of blood brought in from the intermediate distance out-of-town hospitals to the Center's main facility illustrates that point. Those units served as a necessary back-up to the existing inventory.

There was coördination in collection. The Community Blood Center had the facilities and the expertise to process large numbers of donors, and the experience in communicating with both the media and the community on current and future blood needs. The hospitals had the facilities and expertise to treat the injured.

After the collapse, the hospital blood banks were faced with crossmatching large amounts of blood and blood components. They would not have had the time or the facilities to deal with blood donors. Hospitals at that time were deluged with phone calls from concerned relatives and the media wanting and needing information. Thousand of donors calling and arriving at the hospitals would have only added to the confusion. Needless to say, the disaster had a major impact on the people of Kansas City. With the on-site media coverage, the horror of the disaster was immediately brought into

homes by radio and television. Many felt the need to help. By being available, the blood center gave several thousand people an opportunity to do something. Even though the donors were informed that their donations were not going to those injured at the disaster, they knew it would go to some patient equally in need. These donors would not be denied. At the same time, because of the regional blood program, it was possible to adjust blood collections the following week to utilize the over collection.

Local applications

The total Kansas City story emphasizes that there will always be as much blood available as the community's medical triage teams can manage to use. It is the proper management and distribution of that supply that must be planned. The role of a good blood bank is always to have enough blood. That fact, which applies daily, will apply also in a disaster situation. Since there is no excuse for being unprepared for the unexpected, a hospital will want just a little more blood than reasonable expectations require. 'Just a little more' is relatively easy to measure when the blood bank has an operating room schedule, knowledge of the bleeders in the community and their types, and visual evidence of what is on the shelves, crossmatched and 'available'.

How does the daily exercise in inventory relate to the real day of disaster? If it is a multi-hospital disaster or if that fact is not yet known, then no hospital will know where the victims will go. The last person to hear in some hospitals may be the blood bank technologist. The good technologist has no way of knowing what is 'just a little more' than can be used, and will proceed immediately to get all the blood possible, from wherever possible. Once there, that blood is not going to leave except to the victims brought to that hospital.

In the United States, many of the 5,000 hospitals routinely obtain blood from several suppliers. However, since the more usual pattern is for a major source supplier for a number of hospitals, then the matter can be considered on the regional level.

The outline for a regional blood center such as was followed in Kansas City is shown on Table 1. The proper first step is to determine the magnitude of need. If the disaster is big enough to require an invasion of the crossmatched supply at any hospital, that should not be a cause of worry. Elective surgery is going to be cancelled because the same surgical teams working on the victims are the ones who would be doing the elective surgery. For that reason, the total stock of blood is 'available'.

The next information needed is the kind of injuries, possible number of victims, and which hospital(s) will receive them. First knowledge of the type of disaster will carry information on the type of injuries and that will decide what the transfusion needs may be. Smoke inhalation, drowning and crush victims will not (or should not) require transfusion. The efficacy of the on-site emergency team will determine how many victims of mangled accidents will survive to get to a hospital.

In an enormous disaster situation transfusion may not be given to the 'expectant' patients, i.e. the ones with minimal chances for recovery. However, in most situations, victims with evidence of major hemorrhage and shock will be given immediate resuscitation with fluids and blood before going to surgical repair.

It is quite probable that the hospitals which usually have the busiest emergency rooms will also get the most victims, even if the hospitals are not those closest to the disaster. The teams picking up victims should and will bring them to locations where they know the most care exists. It is when the usual

Table 1. Guidelines for the community blood center.

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1. At first word of disaster, try to determine the number of victims, types of injuries, and hospitals that will receive the victims.
 2. Establish a 'single voice' to maintain brief, consistent and authoritative communication with area hospitals and disaster response teams.
 3. Move blood to where it is needed.
 4. Discourage attempts to call in donors, especially at the active hospitals.
 5. Consider contacting hospitals and communities unaffected by the disaster to arrange back-up supplies.
-

emergency care capacity is taxed that unusual facilities will be used.

Some of the problems of distribution and use of blood in a disaster relate to problems of the delivery and temperature control of the blood. At the present time, blood banks discard blood which has been outside controlled refrigeration for 30 minutes. That approach must be tempered by reason in a disaster. Of course, nothing happens at 31 minutes or even 131 min which makes that blood lethal, or perhaps even any less potent. The usual worry is the effect of storage temperature on red cell survival. But when blood is used in support of acute blood loss due to trauma, the life span of the red cells is not crucial. For most such situations, immediate volume and oxygen-carrying capacity are what is desired and there can be a great leeway in red cell survival. In fact, the usual criteria of storage survival are predicted on a 70% loss in 24 hours.

It becomes necessary to know what would happen to blood which might be left out of controlled temperature for longer periods in a disaster. The experiment was performed ten years ago by the U.S. Army. ACD blood was stored at 10°C (50°F) and rotated on shakers for 24 hours and also stored at 22°C (72°F).

Mechanical stress was found to produce minimal adverse effects, although damage increased with the hematocrit and with the age of blood. Red cells, already three weeks old, moved to room temperature (22°C) for 24 hours before transfusion still gave 78% survival. Survival was 62% for similarly treated four-week-old blood[1]. It is possible then, to stock, ship and use blood in a disaster without the rigid time and temperature control used daily. In fact, it would be better to use no refrigeration since freezing can result from the use of unknown refrigerators. Wet ice packing can destroy labels or cause bags to drip contaminated water into the patient's wounds.

The plan for the transfusion service inside the hospital is given in Table 2. One major problem will be the identification of the victim-recipient. Are all victims transported to a hospital going to be tagged at the disaster site? If so, is that tag going to be used at the hospital door or is the patient going

to be retagged? When the patient gets to the operating room, is there going to be another identification set? Unless the hospital has a procedure plan tied into the plan of field personnel, the only safe approach may be to give nothing but group 0 for the first transfusions[2]. Group specific blood should be used when there is a guarantee that sample identity has been established. When there is breathing time the 'immediate spin' crossmatch should ensure vital ABO compatibility. Later the type and screen procedures should serve to find those few victims who might present with antibody problems.

After the situation has stabilized, it would be ideal if the Medical Director consulted with the patient care physicians of any patients given less than optimal transfusions. If ABO and Rh lines were crossed, or a weak antibody found in retrospect, the potential delayed hemolytic reactions should be recognized by the clinical staff.

Table 2. Guidelines for the hospital transfusion service.

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1. At first word of the disaster, count the blood on hand.
 2. Establish a command post with the person in charge readily identifiable; insure that the communication person has an open line to the outside.
 3. Call in necessary personnel under a predetermined response plan. The entire staff will not be needed at first. If the disaster occurs during the day shift, adequate personnel should already be on duty. Evening personnel might report a few hours early. If the disaster strikes in the evening, call back designated day personnel. If it occurs during the night shift, all day personnel should report. No one should remain on duty more than 12 h.
 4. Prepare to issue group 0 red cells at first.
 5. Use group specific blood when satisfied that the patient-sample identification system is operating properly. Non-0 patients who have received more than four units of group 0 red cells should not return to their own group without a compatible 'immediate spin' crossmatch.
 6. When the situation stabilizes, use 'immediate-spin' crossmatches to verify patients' groups.
 7. Screen all samples for antibodies. Identify positive patients so their transfusions can be handled with care.
 8. Personnel in charge of follow-up will review status of all transfusion recipients. The medical director will alert clinical staff to possible delayed hemolytic reactions. The staff will perform routine crossmatches for blood still on hand and record instances of less than optimal transfusions – and the reasons for them.
-

The records on individual patients or the overall laboratory records should clearly state the shortcuts taken and the reasons. Presumably any legal problems as to why an action was taken would be better explained, with the facts, at the time. Finally, there should be later a meeting of those involved, in a relaxed atmosphere to plan for the next time. In Las Vegas it was less than three months before a second major hotel fire occurred.

Drills

There is a real advantage in not waiting for a disaster in order to design a system. A regional disaster drill is the ideal training point. Contact should be made with the administration of a local sport stadium or performing arts auditorium if a drill cannot be initiated among hospital administrators. People who have the responsibility for large crowds are keenly aware of the planning necessary to treat possible victims of terrorist or natural and accidental disasters.

In Tampa, such drills have evolved a radio system that drills with the hospitals around the scenario of a jetliner crash at our sport stadium which holds 72,000 people. It has been decided to go immediately to an all group 0 service, quarantining all other blood groups for the first few hours. At the same time inundation by potential blood donors would be discouraged by assuring them of the adequacy of supply. All the group 0 that neighboring blood centers could spare would be brought into the center.

In a system that uses, routinely, from 1,000 to 1,200 pints of red blood cells or whole blood per week, the 'in stock' supply of group 0 has been between 400 and 650 units on the days of actual disaster drills. At least 600 more were identified as available from neighboring blood centers to arrive within three hours. Meanwhile, the plans call for a diversion of all prospective blood donors away from the hospitals at the time of crisis, offering them appointments during the following week. These people who cannot be refused in the post-disaster hours, without creating more problems, can be accommodated at a blood donor station well removed from the major medical complexes.

Conclusion

The major disasters in the United States between 1977 and 1982 could all be readily handled by the blood system in place, without compromising any of the medical activities of the region. Even larger disasters could have been handled. Large sports arenas with attendance capacity of 15,000–20,000 have collapsed in the United States; luckily, they were not in use at the time. Extrapolating from the Kansas City experience, which was the largest one, it can be seen that even a disaster 10 to 15 times that magnitude could have been handled by the blood supply and the hospitals without having to discontinue the usual medical activities of the institutions in the metropolitan area. It also showed that the supplies of blood could have been replenished for a disaster of such magnitude, not only from the neighboring blood centers within a few hours but even without the need to go beyond the region.

One hopes that a disaster is not needed to unite a community in an effort, but it will do so. Disaster drills can be emphasized in discussions of such events with the public and can serve to make people aware of the need to have a continuing constant supply. In Kansas City, the event also made the public proud of their medical and blood delivery system and has given them the comfort of knowing that a disaster can be handled.

Acknowledgement

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References

1. Shields CE: Studies on stored whole blood: IV effects of temperature and mechanical agitation on blood with and without plasma. *Transfusion*, 1979; 10: 155–62.
2. Camp F: Lessons learned applicable to civil disaster: recipient identification and blood transfusion. *J Trauma*, 1975; 15: 743–44.

7. Comprehensive haemotherapy program for developing countries

F.A. ALA

In the developing countries, the introduction of modern medicine and surgery, patterned after European models, and frequently irrelevant to the main indigenous problems, gradually occurred in the course of this century, but these clinical services developed in a haphazard fashion and were usually driven by monetary gain. Curative or symptomatic medicine predominated and was delivered by a few prestigious doctors whilst public health or primary health care were neither taught nor catered for.

It is only latterly that government planning and the investment of substantial financial resources in health care have become prevalent. Initially, the implementation of these plans took the form of hospital building programmes and the creation of increasingly large, complex medical centres. This is where Blood Transfusion Services ought to have come in, but it is commonly seen that the creation of a sophisticated medical super-structure has not been supported by the development of laboratory and transfusional disciplines to serve and sustain it.

It is often asked what degree of priority Blood Transfusion should enjoy in the overall health care planning of developing countries. The question should really be re-phrased to read: what is the priority of hospital health services? Blood Transfusion does not, after all, exist to serve itself and cannot be considered in isolation from its medical, scientific and social context.

There has been considerable evolution in the way in which transfer of biomedical technology and health planning for developing countries has been perceived over the past two decades. An increasing awareness has recently developed of the failure of

strategies which only foster expensive hospital based health care, curative medicine and the formation of an elite of specialized medical practitioners. More thought has been devoted to a community-based, holistic approach and to the greater allocation of resources to Primary Health Care at the grass roots, to improved nutrition, mother/child services, immunoprophylaxis, environmental sanitation, occupational health rather than to the creation of prestigious hospital-centered health services where the patient comes as a supplicant. Perhaps the most notable example of this philosophy is the 'Blue Nile' project for water-borne disease in Sudan [1] which really illustrates inter-sectorial multi-disciplinary planning at its best, and involves the cooperation of sanitation engineers, irrigation experts, parasitologists and even ichthyologists. This environment also serves as a 'laboratory' without walls and a university campus for a new breed of medical students.

Even though one must fully endorse this approach to planning for the Health Sector, it cannot be denied that hospital services are required to provide diagnostic and curative skills and to provide a back-up to the Primary Health Care system. The whole thrust of change in developing countries is towards greater urbanisation and industrialisation; more accident services, more sophisticated surgery and cancer therapy. Medical insurance schemes are bringing modern medical care within reach of everyone whilst the expectations of the people are ever increasing. It is illusory to expect that the paternalistic state can confine its support to planned priorities; the clam-

our of patients for the latest diagnostic surgical and medical techniques cannot easily be denied.

All this inexorably leads to the absolute need for underlying support services such as Clinical Pathology and Blood Transfusion, which are all too often ignored. Clearly there is a genuine place for both developmental approaches – primary health care and hospital-based services.

Having established that Blood Transfusion Services deserve priority rating in developing countries, what are the desirable features which should be fostered from the outset?

Blood Transfusion Services should be based upon voluntary, altruistic blood donation, even though there are those in Western countries who hold that moralizing in this regard is inappropriate and exaggerated, pointing to the failings and inefficiencies of some non-profit organisations in order to justify the purchase of blood from professional donors. Probably they are not aware of the prevalence of degrading poverty, sickness and anaemia amongst the donors who may sell their blood four or five times a week, or of the rapacity of the 'agents' who manage them in some developing countries. Voluntary donation, indeed, in the parlance of the World Health Organisation and Primary Health Care planners, is an admirable model of community motivation; of community participation in health care provision which cannot be achieved by money, legislation or by browbeating.

It is best to conceive of Blood Transfusion as a National Programme (and in this to include the Armed Forces which so often form an insular, totally separate part of the community). There are many compelling reasons to support this concept:

1. Small, scattered centres lead to mediocrity and poor standards, a high level of blood and plasma wastage and poor utilization of blood and financial resources, together with duplication or even competition in donor recruitment programmes, purchase of equipment and enlisting skilled personnel.
2. If one subscribes to the 1975 resolution [2] of the International Society of Blood Transfusion that national self-sufficiency in the provision of blood and blood products must be the ultimate goal, then it follows that only a National Programme

can promote and achieve this aim. It is only in this way that overall and changing patient needs can be perceived and met; and that long-term policies can be formulated and capital investment can be planned to sustain growth.

3. A National Service can establish one set of standards for blood donor selection, collection, processing and distribution. The responsibility and accountability to the donor and the public is by a single agency. One knows so well of countries where each hospital is running its own blood collection programme!
4. A National Service can promulgate and, through appropriate legislation, implement a standard 'Transfusion Code', fostering good transfusion practice; control of hospital blood banks and the charges they may levy; standardisation of equipment, laboratory methodology, reagents and reference materials and ensure the implementation of Good Manufacturing Practice, high standards and quality assurance programmes.
5. Manpower development and the establishment of an official training and accreditation programme are amongst the most vital roles of a National Service and to this must be linked the all-important development of a career structure and attractive career prospects for transfusion personnel whether they are doctors or technicians. This may well be a difficult task where blood transfusion has remained at an artisanal level or where it is entirely in commercial hands.
6. The collection of plasma for fractionation under optimal and standard conditions can only be satisfactorily carried out by a National agency, and if fractionation capacity is insufficient or poorly developed, the Service can negotiate equitable terms for contract fractionation which will protect the best interests of donors and make the best use of a precious human resource.
7. Planning for National disasters or emergencies can be carried out in close cooperation with the Armed Forces and other appropriate services, ensuring, above all, that compatible equipment and procedures are selected for use.
8. The Service may act as a central purchasing agency, reducing costs by bulk purchasing and ensuring uniformity of quality by batch-testing

of all materials and reagents.

There would also be every justification for carrying out the design, specification and creation of prototypes for simple developmental engineering projects such as donor beds, stands and furniture which, if they prove successful, can be contracted out for larger scale production.

One of the problems afflicting the laboratory services of 'Third World' countries is the poor maintenance and repair of electrical and electronic equipment and the dearth of spare parts. It is not always possible to obtain adequate and speedy service from the local representatives of manufacturers who are often merely salesmen and entrepreneurs with little knowledge of the equipment they sell and are reluctant to carry an expensive inventory of spares. Here again, the National Service would be well placed to store a supply of spare parts on behalf of peripheral centres, to train technicians in 'diagnostic' and repair work and to run a reference 'flying doctor' service for major breakdowns of equipment. Indeed, the costs of establishing such a unit can either be divided in partnership with other organisations (university laboratories or research institutes) sharing a similar need or they can be partially defrayed by charging other users for services.

9. Finally, a National Service is in a unique position to capture a great deal of important demographic and epidemiological information of great value to the Ministry of Health or Public Health Authorities and to foster biomedical research.

It is perhaps worth emphasizing the role which a well-founded and dynamic Blood Transfusion Service can have in promoting developmental and pure research. It not only adds an intellectual dimension and career attractions of its own work but it can galvanize biomedical research and teaching in the country as a whole.

Many of the problems of Blood Transfusion and indeed of Medicine and the quality of teaching and research in these disciplines cannot be addressed without reference to the broader problems, some of them cultural and philosophical, which beset the development of science in 'Third World' countries. Thus, it is not merely a matter of purchasing equip-

ment and importing know-how (although some would argue that this is the easiest and cheapest option) but rather of examining the entire social fabric and context within which this microcosmic service must be embedded and incorporated so as to remain viable and creative – what Charles Weiss calls the 'software' of technology [3].

It is often the case that behind a cosmetic, political facade of scientific progress, there are only fragments of a scientific community which is disorganized, disunited and intellectually isolated. Science is not an industrial organization or a bureaucratic institution; it grows and lives by close interpersonal relations, collaboration, competition and criticism. This isolation and fragmentation is compounded by the conventional wisdom prevalent in many Third World countries which regards science with suspicion as one of the roots of their social malady threatening to undermine traditional values, or, at best, as irrelevant to their immediate problems. Teaching is carried out by those who no longer have (or never had) a continuing involvement in scientific research so that they are divorced from the spirit of science and, above all, from the art of problem-solving. Learning is by rote, with regurgitation of knowledge at examinations, and little research guidance is available to the more gifted pupils.

The slow-maturing character of science and education means that the all important development of a viable national infrastructure in the sciences is a lengthy process, even when it is favoured by cultural, material and social factors. It is impossible simply to 'import' science and technology in the absence of an indigenous scientific, technical community, and even if it were possible, it would be undesirable because of economic, political and psychological considerations. For all these reasons and far beyond the solution of specific material problems, the development of this indigenous scientific community is even more essential for science to become firmly planted in new soil and to achieve a broad social impact.

There is much that a National Transfusion Service can do to help in cultivating the biomedical arm of this all important scientific community and in providing a 'culture medium' for its gradual de-

velopment, for it is an uncommon amalgam of many things. It has an academic and university teaching commitment; it takes on exemplary roles, establishing and monitoring standards of practice through a national network; it has an industrial role as a manufacturer of clinical products and has its feet firmly planted in clinical medicine, blood coagulation, organ transplantation, virology, epidemiology, population genetics. With this polymath role, the Service can act as a nexus for bringing together a number of scientific disciplines, fostering interactive, cross-disciplinary research by utilizing local or regional subjects as models for problem solving, not only in order to address relevant clinical and scientific problems, but also to offer training and career opportunities to young scientists.

On a more practical plane, the Transfusion Service can help to overcome some of the chronic deficiencies which so often materially inhibit biomedical research in developing countries by cooperating with sister scientific organisations in the establishment of modern library facilities, providing quick access to current scientific literature; in offering expertise in statistical analysis and data processing and in the creation of a good laboratory animal breeding station for common use and comprising facilities for the provision of training in the proper breeding, nutrition and care of pure bred strains.

How can these broadly stated objectives be attained and how can these policies be implemented?

Generally speaking, the transfer of technology or development and training programmes always comprise three essential components: (i) The transfer of expertise or skills, (ii) The imparting of knowledge, and (iii) Influencing attitudes.

This last element is by far the most important one, the most frequently overlooked, and the most difficult one to alter, for attitudes partake of the cultural make up of individuals and communities. Nevertheless, all three aspects must, clearly, be taken into consideration.

Establishment of a Blood Transfusion Service

The absolute precondition for the establishment of Blood Transfusion Services is a genuine sense of need, whether explicit or unspoken, shared by the medical profession, the authorities and the public. In most developing countries, the spotlight is on hospital beds, whereas Blood Transfusion has little visibility or glamour and evokes only the shady associations of back street 'dealers' and sick, anaemic donors. It is usually represented by exponents whose stature and standing perfectly reflect the lack of importance attributed to this service and the administrative isolation which, more than anything else, relegates it to obscurity and mediocrity, whilst other rapidly developing medical specialities such as open-heart surgery, cancer therapy or neurosurgery are well funded and eagerly over-subscribed by the most promising medical graduates and scientists. If it is to become rooted and integrated into the medical fabric of the country, a Blood Transfusion Service cannot come before its time, no matter how much money or expertise is applied. Provided that the time is ripe, and that medical services have evolved to reach a stage of sophistication where the dearth of a safe, efficient and science-oriented service is palpably inhibiting further progress, two more conditions must be fulfilled before the preliminary steps towards establishing the Service can be taken. First, an appropriate, sound administrative vehicle or political 'mould' must be conceived to ensure financial security, independence, stability and future growth. The nature of this administrative base will obviously vary from nation to nation and may take the form of an affiliation with the Ministry of Health or the National Red Cross (or Red Crescent) Society. However, the greatest freedom to grow and develop, to determine its shape and destiny and to establish rich, unprejudiced associations with all sectors of the community can be achieved by the creation of an independent National Organisation with its own Board of Trustees.

As to the second requisite, it is only rarely that a widely respected, influential member of the medical profession can be enlisted to devote the dedication, time and effort needed to lead blood transfu-

sion technology and practice out of the hands of commerce into the realm of science, to bring unity to a disparate artisanal activity and to spearhead the minor social revolution which is required to introduce voluntary blood donation where it did not exist before. Yet, in the early stages of development, it is absolutely crucial that such an individual be found.

The second phase of development may be termed Political and must involve influencing the attitudes of national leaders, politicians and governmental financing and planning authorities at the highest level, focussing their attention and mobilizing their support for the establishment of Blood Transfusion Services as a national priority. In these discussions between planners and clinicians, it is important for both sides to realize that they often utilize different premises and criteria in the evaluation of priorities. The judgement of clinicians is usually made in terms of benefit to the individual patient. Policy makers face questions of a different sort: are there nett benefits to the health of a large number of patients from the use of a particular technology? Are these benefits sufficiently worth having at some particular scale of provision if this means not providing other health services or providing them on a smaller scale? At this macro-level, a judgement is made in terms of a wider set of advantages and disadvantages, and their very diversity raises questions of how to make them commensurate (by using money measures?) and how to judge their value to different types of persons (e.g. patients, patients' families, the public at large, city dwellers or rural populations).

These issues can be resolved by approaching them from the disciplinary viewpoints of medicine, epidemiology and economics. The greatest commonality of interest between the three disciplines lies in considerations of cost-effectiveness, and in this regard, powerful arguments favouring the early establishment of Transfusion Services can be advanced. In comparison, let us say, with a 500 bed hospital building programme (at a cost of some \$ 60–100,000 phase 1 per bed), the financial resources required for the establishment of Transfusion Services are quite small, particularly in consideration of the profound and wide ranging impact they can have upon all the various branches of medicine.

Donor recruitment

The third is a Social or Cultural phase, that is, obtaining the participation of the people, all the people, including the armed forces and religious communities in helping to establish decent blood transfusion services for themselves by voluntarily donating blood.

The public in developing countries will have had many apparently worthy, progressive projects foisted upon it which are not necessarily dependent upon their direct support and participation, such as the establishment of atomic energy centres, petrochemical industries, and national airlines. Even though one knows that a man who has planted trees with his own hands will nurture them tenderly and defend them with his life-blood, when the state wishes to create a 'green belt' or a aforestation programme, it is met with public indifference; branches are broken and the trees are neglected because the people have not taken part in the process of establishing them. This process of participation is, of course, central to blood transfusion, which is absolutely dependent upon public support. Legislation, intimidation or importing blood in tins will not do!

Certainly, donor recruitment is the most important, onerous and neglected aspect of the service, and all too often inadequate funds are devoted to this facet in favour of purchasing costly and sometimes unnecessary laboratory equipment.

There is no magic formula for success in donor recruitment, unless it is sheer enthusiasm and perseverance. Certain principles and modes of approaching the problem are valid the world over, and systematic planning together with continuous, regular campaigning are essential, if only to disabuse people of the commonly held belief that blood is needed only in times of emergency and disaster.

First of all, one must realize that this is an urban activity. Hospitals are urban; laboratories are urban and the needs for and applications of blood are largely confined to this environment. Rural people consider city dwellers as an alien race: predatory, egocentric and unreliable – outside the inbred fabric of interdependence and atavism of the village

community. To take their blood away, even were one to overcome fear and prejudice, would be like the plunder and rapine of the 'Mongol Hordes'. It is no use talking about 'compatriots' or 'co-religionists'. To them, city dwellers are like extraterrestrial beings! It is with the experience and the confidence that blood, which is given in trust, is returned to the community, to a neighbour or relative in time of need which acts as the most effective motive force. Since in most developing countries there are few hospitals in rural areas, it is difficult to motivate people in this way.

Broadly speaking, two basic approaches may be employed: i) The diffusion of a constant, low-key stream of background information and educational material regarding blood donation, its innocuous nature, the increasing needs and uses for blood, and exploiting every possible means for enhancing public awareness. Media such as public meetings, television and cinemas can be used to project 15 to 20 min films as well as 60 to 70 s 'spot' films which can be extremely effective. Although costs for good quality films tend to be high, there are distinct advantages in staging them locally, utilizing the services of public heroes, athletes and well-loved singers. Politicians should be avoided for they are transient and frequently mistrusted. These films must be carefully crafted and pitched to reach every sector of society.

Where religion is a powerful social force, as it is in Islamic countries, religious leaders should be persuaded to assist in lending dignity and legitimacy to the act of blood donation by giving blood themselves, providing appropriate quotations from holy writs for posters and allowing filming in sacred places. It is often possible to exploit local, age-old customs with benefit. In Iran, for instance, scariification and cupping or blood-letting is an ancient tradition said to purify the blood and purge evil humours. It has deliberately been linked to the modern practice of giving blood, with the suggestion (emphasized by sayings from Emam Ja'afar-e-Sadeq) that there is a dual virtue in donation – cleansing the body and spirit, and helping to save a fellow Moslem (Eethar).

Prizes for the best posters may be offered at schools and universities, and every opportunity

must be seized for putting up stands and exhibitions wherever large numbers of people are gathered (e.g. industrial or agricultural shows; stations, athletic meetings), where pamphlets and other publicity material may be distributed. The young are impressionable and they are the blood donors of tomorrow. They also serve as a convenient conduit for introducing the message of the Transfusion Service into their homes. School field trips to the blood centre or promotional visits to the schools should therefore be encouraged, and educational material concerning the functions of blood, civic responsibilities, and blood donation should be included in standard primary school textbooks.

The establishment of 'Blood Donor Organisations' which must include priests, teachers, students, representatives of different ethnic groups, can serve to great advantage in converting the non-donor organizing ceremonies to honour the most faithful donors, and as a source of voluntary recruiters and helpers.

Finally, it is worth considering the institution of a simple biochemical screening programme for attracting donors (blood urea, cholesterol, uric acid, blood sugar) which will also yield valuable population-based data.

Superposed upon this general educational publicity is a more specific campaign which is focussed, for a few days or even a week, upon a certain quarter of the city, or a particular organisation such as a ministry, a bank, an industry, the Scouts etc. Thus it is important to be able to address oneself, in turn, to various groupings in society whose members are linked by some common bond of allegiance: a common employment, a shared sporting association.

It is most effective to go right to the top when setting up blood donor sessions; persuading the director or the most senior officials that they must set an example by exhorting their employees and giving blood themselves. The same principle applies to the Armed Forces where officers must be urged to fulfill their leadership role and improve confidence and morale of their men by being first in line. The peace-time military will usually welcome this opportunity for overtly demonstrating solidarity with the civilian population. The Army, of

course, is a particularly valuable source of hyper-immune plasma, for large groups of men are simultaneously immunized at induction into the forces, and for this reason they must be carefully cultivated.

The various modes of blood collection will, of course, include the creation of Mobile Teams. Needless to say, the greatest care must be taken to ensure that donors are courteously and pleasantly received; that the members of the team are disciplined, enthusiastic and impeccably uniformed, and that venesects are faultless. It is crucial to convey the image of a caring, meticulously well-ordered organisation, and potential donors are always impressed to find that numbers of volunteers are rejected for medical reasons, demonstrating that integrity overrides the desire to inflate the numbers of donors bled.

Where accommodation is difficult to obtain, Mobile Trailers (with 6 couches, doctors' examination cubicle, refrigerators), accompanied by a good team of recruiters and placed in a busy part of town, can be extremely cost-effective, serving also as an eye-catching, ambulant advertisement.

Many hospitals or transfusion centres in developing countries institute a 'blood replacement programme' in an effort to overcome blood shortages and to avoid the difficulties of recruiting by putting the onus of finding donors upon the patients. In the long run, this kind of inflexible coercive approach tends to be self-defeating, causes resentment and favours perpetuation of the professional donor system.

Staff training

Developing skills and the necessary knowledge form the next phase of development and clearly, as mentioned before, manpower development programmes, both short and long term, together with career structure planning cannot be dissociated from this phase.

In countries where there is a dearth of skilled technicians and no national structure or statutes governing the conditions of pay, advancement and benefits which are linked with recognized qualifi-

cations and experience, technician 'rustling raids' are often carried out by more recently established institutes and research centres which can seriously disrupt plans for development. The establishment of a technical college with a nationally recognized qualification is essential, and in doing so it would be preferable to design an internationally compatible curriculum to allow for the future possibility of further training and qualification of the ablest candidates abroad.

It is worth interjecting, at this stage, that there are dangers inherent in sending doctors and scientists abroad, because the most talented workers may remain there. On the other hand, those who have been impelled to go abroad merely to win a degree and have not learned problem solving, may return to find that their skills, which appeared to be of riveting importance in the U.S.A. or Europe, are not relevant to their own country, or else, cannot be utilized for lack of funds, equipment or reagents. This inevitably leads to frustration. The happiest case is where an already mature scientist, with the requisite drive and intellectual curiosity travels abroad to a specific place and for a specific and relevant purpose which, once it is achieved, can immediately be put to use upon returning home.

Perhaps the most significant contribution to manpower development and the maturation of the newly established technical college already alluded to, can be made by developing a political, administrative and fiscal device that can open up a wide range of temporary teaching and scientific appointments and make them attractive to relatively young Western doctors, scientists and technicians. The prestige visits by Nobel laureates who deliver two lectures and dine with the Minister of Health and the University Rector before flying on, are worse than useless.

Quality assurance

Fostering the creation of that state of mind and that consciousness of Quality Assurance which is, more than anything else, an attitudinal and cultural concept, is possibly one of the most difficult and important phases of development.

Although a Blood Transfusion Service should partake of the nature of a university department, where developmental research and teaching co-exist with service, it must also be perceived as an industrial activity rather than merely as a laboratory function and, like the pharmaceutical industry, it must be concerned with the principles of Good Manufacturing Practice and Quality Assurance, both of which are forms of accountability.

Accountability has many facets in the context of Blood Transfusion in connection with:

1. Costing and budgeting; monitoring the cost-effectiveness of expenditure and determining whether financial targets and priorities are being met.
2. The demonstrable safety and efficacy of laboratory procedures and manufacturing processes by objective scientific evidence.
3. Accountability to the patient through the capability for tracing errors and identifying trends or deviations from established norms; objectively and continuously demonstrating the standards and safety of processing and laboratory procedures; the efficacy and purity of reagents and materials utilized.
4. Accountability to the staff, manifested by good design of the work environment and equipment, utilising working habits which will ensure the safety of procedures by planned training programmes; defining staff responsibilities clearly.
5. Being answerable to the public who donate the blood and ultimately provide the funds for the best use of both blood and money.

In brief, this accountability is a concept which ideally engenders confidence in the efficacy, safety and economy of the manufacturing process vis-à-vis the government or funding agency; the staff and the public, whether taken as donor, blood recipient or merely taxpayer. One of the important steps towards establishing this confidence is the detailed documentation of Standard Operating Procedures which become a management tool for legitimizing or validating the process of manufacture and which are every bit as telling as the results of conventional end-product testing. They become a means for minimizing the arbitrary, subjective aspects of management, providing a framework for

systematic, controlled change, whilst still allowing for the cultivation of initiative in order to avoid stultified regimentation. The familiar 'Chinese whispering game' distortion of laboratory procedures which can gradually occur in repetitive work or as one technician passes techniques on to another, illustrates the importance of rigorous documentation and adherence to minimal blood banking standards.

Cross reference should be made in all Operating Procedures to calibration, maintenance and cleaning of equipment; methods for monitoring and controlling the laboratory environment; methods for quality control of all laboratory materials and reagents employed; storage and transport conditions; safety measures; quality control of final products; and methods of documentation.

Certainly the exhaustive definition of Standard Operating Procedures covering the entire spectrum of blood procurement, testing, processing and issue is a tedious, time-consuming business and it is difficult to ensure that, having defined procedures, they are really followed and not merely retained as a cosmetic exercise.

The clinics and blood uses

Finally, one of the most crucial aspects of establishing a Blood Transfusion Service in the developing countries is the 'downstream' work which is so necessary amongst the medical community and its sub-populations of nurses, hospital blood bank technicians and doctors, who order and utilize blood and blood products.

A blood centre cannot confine itself to behaving like a supermarket, merely putting products, however wonderful, on the shop counter. The aim must be to exert integral control over the entire continuum of transfusion practice, ranging from donor recruitment, all the way to hospital practice and preparation of blood for the individual patient, without fragmentation of the service. The transfusionist must influence medical users and tactfully educate them in the proper utilization of blood and, having won their confidence, ensure their active participation in the delivery of a high quality

service to the patient – gaining influence by fostering a sense of need and cultivating this dependence, in turn, by providing a good service. This takes one back to the need for impeccable quality assurance and the confidence this inspires in the medical user and, ultimately, in the public, which must, after all, supply the blood donors.

Where a commercial contract supplier is employed, blood is frequently delivered at the back door of hospitals, rather as the laundry or kitchen supplies are brought in, and reliable compatibility and matching tests for the individual patient are often not carried out. It is this isolation and dissociation which must be overcome by deliberately involving the physicians, the surgeons and the hospital pathology laboratory in the whole process of judiciously selecting blood products, and preparing and transfusing them with safety.

A consultation or referral service for cross matching problems and antibody identification must be offered, but the pressure to carry out routine matching for hospitals, in my opinion, must be resisted, so far as possible – they cannot be absolved from this token of a shared responsibility without lapsing into indifference.

Vested with the authority of the national health regulating agencies, the Transfusion Service must initiate and administer a formal system of accreditation for hospital blood bank personnel, premises, equipment such as storage refrigerators, their alarms and recording devices, minimal testing procedures and standards for the reagents which are utilized. Great efforts must be devoted to providing hospital blood banks with some of the reagent and reference materials which they require such as screening and panel cells and antisera from local sources.

Medical College curricula go into considerable details regarding chemotherapy or fluid and electrolyte replacement in various medical and surgical conditions, but very little is usually taught about the indications and appropriate use of blood and blood products. The same deficiencies apply to the nurses, training courses. In consequence, whole blood is overused; inappropriate demands for fresh blood are insistent; albuminoids are misused for cases of malnutrition; reserves of blood group 0 Rh

negative blood are widely employed in non-homologous cases as a transfusional panacea; miracles of hemostasis are expected, in the adult thrombocytopenic, from only one unit of platelet concentrate; blood is stored together with watermelons . . . the catalogue of sins and misapprehensions is prolonged. It is necessary, therefore, to incorporate lectures and demonstrations of blood component therapy in courses for doctors and medical students, to hold regular seminars for nurses regarding the safe and correct use of plastic blood bags, and workshops for hospital blood bank technicians.

Finally, many of the areas of expertise available at the Blood Transfusion Centre can subserve the creation of valuable services such as a diagnostic clinical immunology service, a clinical haemostasis laboratory which may underpin the establishment of haemophilia care, or screening tests for thalassaemias and haemoglobinopathies, further enhancing the links with clinical medicine and clinical investigation which are so important.

In sum, blood transfusion is a service of fundamental significance which underpins all modern hospital practice, finding direct and immediate use at all levels of health management susceptible to intervention: screening, prophylaxis and therapy – hence its ubiquitous role and the multi-levelled structure of a blood transfusion service.

Much is usually made of insufficient funds to support the establishment of a modern service in developing countries, and a good deal of ingenuity has been used to devise a simplified, compromise technology which will be less of a financial burden to them. Yet, although the term 'developing country' embraces many different degrees of sophistication and levels of financial capacity and service requirement, it is difficult to subscribe to the view that they cannot afford a good blood transfusion service. The spectrum of products which can be provided (e.g. red cell and platelet concentrates; pooled buffy coats, filtered leucocyte-poor blood; fresh plasma, cryoprecipitate; cryosupernatant for blood volume expansion) with no more than multiple plastic bag assemblies, hand sealers and presses, a large capacity refrigerated centrifuge, freezer and 4°C refrigerator with appropriate volt-

age stabilizers, together with a handful of disciplined, dedicated technologists is very broad and will cover most clinical requirements.

Visiting consultants from the West frequently (and with some condescension), discourage the use of advanced and sophisticated techniques by developing countries. Many of the reasons adduced to justify this stance have already been discussed and are undeniable. Yet, sophistication should not be rejected out of hand and it is more a matter of making a wise selection of appropriate technology – a twentieth century heritage which is available to us all, the world over, for exploitation. For instance, why should not the National Transfusion Service, in a country where skilled, experienced clerical staff is not available, utilize microcomputers and bar-coded labels to ensure accuracy and to eliminate transcription error, at an early stage? Why should not the few highly trained scientists available in a large number of developing countries, be pooled in a 'high-technology' core to the National Service in order to produce monoclonal grouping antisera which provide a guaranteed, unlimited supply of sera with great potency, constant

specificity and reliability at low cost, when compared with polyclonal, immune reagents which are not easy to standardize? This 'high-technology' can, after all, be utilized in a perfectly conventional, 'low-technology' manner at the periphery.

It is, perhaps, more reasonable to say that developing countries cannot afford to be without even a small but high quality service. To over-compromise is only to exaggerate what is already a far too humble posture, and it does nothing to limit the rapid, disproportionate expansion of expensive and advanced clinical services.

References

1. Gaddad AA: Blue Nile Health Project, Ministry of Health, Wad Medani, Sudan. Annual Report, 1981.
2. The International Society of Blood Transfusion. Resolution passed by the General Assembly during XIVth International Congress in Helsinki, 1975.
3. Weiss C Jr: Mobilising technology for other countries. *Science*, 1979; 203: 1083-89.

8. Artificial blood substitutes

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Biological carriers of oxygen are generally either metal chelates that combine reversibly with this gas or solvents that simply dissolve it. Those that chemically bind oxygen are usually related to heme, and of course, the primary example is hemoglobin itself. Although reversible oxygen binding synthetic iron chelates have been made [1] they are not yet available in practical form. Stroma-free hemoglobin [2] and hemoglobin derivatives [3] have been produced, but there are still no uniform products for general use. Hemoglobin must be presently derived from out-dated human red cells. This supply is not unlimited, and in fact, has been dwindling because of more efficient use of erythrocytes by the blood banks. The use of hemoglobin from other species has been proposed by a number of investigators, but the immunological aspects of doing so have not been fully addressed. A desirable characteristic of oxygen binding chelates is their functioning in ambient concentrations of oxygen. It is unclear at present when usable hemoglobin products or synthetic oxygen binding chelates will become available in adequate practical quantities.

Foremost among oxygen carriers that simply dissolve this gas are the liquid perfluorochemicals. These compounds are organic molecules in which all hydrogens have been replaced by fluorine atoms. A few of the compounds that have been used in artificial blood replacement preparations have residual hydrogen atoms present. These are the exception, however. Gollan and Clark [4] first demonstrated that mice *totally* immersed in an oxygenated compound of this kind would remain alive by breathing the liquid itself. For perfusion pur-

poses it is necessary to finely emulsify these liquids, a technique first reported by Sloviter and Kamimoto [5] in their studies on rat brain perfusion. These authors were unable to use their product intravenously in rats. Meanwhile, Geyer *et al.* [6, 7] reported that intact living rats could be totally perfused with a liquid perfluorochemical which was highly dispersed in an aqueous solution of Pluronic polyol, electrolytes, glucose, and antibiotics. An elevated oxygen atmosphere was also provided. Animals survived for as long as eight hours without any plasma proteins or blood cells in circulation. The perfluorochemical employed was perfluorotributylamine (FC-43 or FC-47, 3M Co., St. Paul, MN). The Pluronic polyol served not only to help emulsify the perfluorochemicals, but also to furnish temporarily part of the needed oncotic pressure.

It was recognized that a satisfactory artificial preparation should not only allow the total replacement of an animal's blood but should also allow such bloodless animals to remain alive without any supplementation and to renew their own missing blood cells and proteins. After much additional effort [8, 9], elimination of all red cells and blood proteins was possible with the animal surviving. Because of the short-lived oncotic effect of the Pluronic F-68, hydroxyethylstarch was introduced as an oncotic agent. It also appeared to have a beneficial influence on the flow properties of the final preparation. Thus, with a preparation consisting of emulsified F-tributylamine, Pluronic F-68, hydroxyethylstarch, electrolytes and bicarbonate buffer, it was possible to obtain 'bloodless' rats.

These animals not only lived, but regenerated erythrocytes, leukocytes, and plasma proteins at a rapid rate. They were very active and alert, and performed all normal functions such as eating, washing, urinating, and nest building. They continued to grow and develop. These studies showed for the first time that a completely artificial preparation could be used for obtaining bloodless animals that survived without any further treatment. Although partial rather than total substitution of blood is more likely in the clinic, experimentally the true capabilities of the artificial material may be obscured by the presence of the normal blood. Total blood replacement has proven to be an excellent way to test the efficacy of preparations designed to take the place of blood either partially or completely.

The preparation containing F-tributylamine proved very useful for research purposes in animals and for organ perfusion *in vitro*. A serious drawback for possible clinical use was the long retention time of this fluorocompound in the tissues of animals [10, 11]. An important development was the finding that F-decalin in such preparations left the body rapidly [10, 11]. For a period of time it appeared that heteroatoms such as nitrogen in perfluorochemicals caused long retention of these compounds in tissues, but later work showed this was not necessarily true, for F-N,N-dimethylcyclohexylmethylamine was readily transpired [12].

Mixtures of perfluorochemicals were found to have some advantages over the individual compounds [12]. For example, F-dihexylether which forms stable emulsions could be used together with F-decalin which forms unstable emulsions, and result in an emulsified product that had good stability. Thus, the role of the dihexylether was to act as a 'helper' compound in terms of emulsification. The Green Cross Corporation (Osaka, Japan) adopted this technique in formulating the product, Fluosol-DA (20%), that is currently being tested clinically [13]. This preparation contains F-decalin mixed with F-tripropylamine. The latter serves as the 'helper' compound and has a tissue half-life of about 60 days while that of F-decalin is 7 days. Although its stability has thus been enhanced, the emulsion of Fluosol-DA must be stored in the

frozen state and only thawed before use. This preparation has been used in more than 800 patients in Japan, the United States, and Europe [14]. New and better preparations will undoubtedly be developed. For example, the need for freezing perfluorochemical emulsions needs to be overcome, and the eventual use of high perfluorochemical concentrations should decrease the level of oxygen needed in the inspired air.

Emulsions of perfluorochemicals are made by means of high pressure homogenization or sonication [15]. The latter suffices for small volumes while the former is most used for medium and large scale quantities. It is general practice to carry out the emulsification by dispersing the perfluorochemical in an aqueous solution of the emulsifying agent. Other ingredients such as electrolytes and hydroxyethylstarch are not added until the desired size of the fluorocompound particles has been achieved. This is usually approximately 0.2 micron in diameter or lower. To date no method has been reported that yields all particles of a given size, nor is there evidence that such an end point would have advantages of a practical nature. Most perfluorochemical emulsions are off-white in appearance, but some can be made that are transparent and resemble microemulsions in this regard. They are not, however, true microemulsions. As a general rule, the finer the particle size, the greater the stability of the emulsion, not only during storage, but in the presence of other materials. It is clear that with a given emulsifying system different perfluorochemicals can be emulsified to form either turbid or transparent products, others always yield turbid ones no matter how vigorously the emulsification is pursued. It is likely that with a wide selection of emulsifying agents any perfluorochemical could be obtained in any desired particle size range. However, there are few emulsifying agents that can be employed intravenously. Additional ones may become available as a result of new synthesis programs aimed at enlarging the options in the area of perfluorochemical blood replacement preparations. Self-emulsifying perfluorochemicals would be of great interest, but none have so far been developed.

As mentioned earlier, different perfluorochemi-

cals remain in the body for different lengths of time. There is no agreement as to the most desirable rate at which these compounds should leave the body. It would appear that the primary concern is that they remain in circulation sufficiently long to serve the purpose for which they are given. Currently available preparations have circulatory half lives of about 30 to 48 hours depending upon the dose administered. This is adequate for many short-term needs. Other preparations having a long circulatory dwell time would be needed for many applications. It has been shown [16] that the disappearance rate of a lecithin stabilized emulsion from the bloodstream can be greatly prolonged by daily administration of generous quantities of lecithin itself. Presumably the supplemental phospholipid replenishes that which was lost to metabolism. The practicality of this approach remains to be determined. Specially designed surfactants should be another approach to controlling the circulatory dwell time of any given perfluorochemical.

The particles of perfluorochemicals have a high density, and when the artificial preparation is centrifuged they are sedimented to yield what is termed a fluorocrit. This is analogous to the hematocrit, and does not indicate the true perfluorochemical concentration since the packed particle layer includes surfactant and water. Usually the final preparations will have a fluorocrit of approximately 14 volume percent. An animal completely exchange perfused will also have a fluorocrit of 14. On the other hand, the quantities of perfluorochemical preparations given to patients is such that the fluorocrit generally is 2 to 5 volume percent [17]. The actual perfluorochemical concentration can be obtained by means of gas-liquid chromatography. In spite of its limitations the fluorocrit is a useful means of monitoring the disappearance of the perfluorochemical from the bloodstream.

The perfluorochemicals are taken up by the reticuloendothelial cells and macrophages. This can be seen in histological sections of the liver and spleen where the compounds appear as clear droplets of various sizes that do not stain. The perfluorochemical content of the tissues can be deter-

mined by extraction and gas-liquid chromatography. The rate at which the material leaves the tissues depends upon the compound administered. When mixtures of perfluorochemicals are given, each compound behaves as though it is present alone, and its rate of disappearance from the tissue appears to be unaffected by the presence of other perfluorochemicals. The mechanism by which the fluorinated molecules leave the cells and the body (via the lungs) is unknown. Molecular weight and size, vapor pressure, actual structure, and lipid solubility have all been thought to play important roles. More investigation is needed to fully understand this phenomenon. There is no compelling evidence that these perfluorochemicals are metabolized in any way. Therefore no metabolites need be dealt with, greatly simplifying the study of the fate of the compounds. Since the Pluronic F-68 is also not metabolized, the problem of its metabolites is also moot.

Hydroxyethylstarch is employed for its oncotic pressure effect, and maintenance of blood volume is adequate even in animals totally exchange perfused. Of interest is the observation that this plasma volume expander does not promote 'weeping' or 'bleeding' when used as a component of the perfluorochemical blood replacement preparations. This is in contrast to findings in which the hydroxyethylstarch is used alone. Other products such as Dextran have not been used very much with emulsified perfluorochemicals because they often cause marked aggregation of the particles when mixed with the emulsions. Serum albumin, plasma, and serum are all compatible with properly made perfluorochemical emulsions.

Many different species have now been used in studies with perfluorochemicals. These include humans, baboons, monkeys, dogs, cats, rabbits, rats, mice, gerbils, and frogs. A great many different kinds of experiments have been done [18]. Most have not addressed the issue of bloodless animals and relatively few have involved investigator-produced preparations. Instead, reliance has been upon the available commercial products. On the one hand this has the advantage that common formulations are being used by a number of investigators, provided of course, that the products are simi-

lar batch to batch. Since the commercial preparations are fixed in composition, however, freedom on the part of individual investigators to manipulate the formulation is limited. Such manipulation might be necessary to accommodate specific experimental protocols. It seems logical that results of experiments can be no better than the product used will allow. It is also apparent that the techniques and skills of the research personnel involved are of extreme importance.

Many of the applied uses of perfluorochemical preparations involve only partial blood substitution [18]. Under these conditions the animals or humans presumably receive their oxygen from the hemoglobin in the erythrocytes, from that dissolved in the perfluorochemicals, and a very small amount that is dissolved in the water. Assuming that the recipient of the artificial product is breathing an elevated oxygen concentration, the circulatory pO_2 should be elevated and the hemoglobin essentially saturated with oxygen. Obviously any hemoglobin that is deoxygenated can also pick up oxygen derived from the perfluorochemical phase. As the mixture circulates through the tissues, the dissolved oxygen, not requiring any particular pO_2 for off-loading, should be preferentially utilized. It is therefore not surprising that in the studies of Tremper *et al.* [17] the conclusion was reached that their patients derived about 15% of their oxygen needs via transport by the circulating perfluorochemical. This was accomplished even though the fluorocrit of these patients was only slightly above 2 volume percent.

Many kinds of experiments can be carried out with perfluorochemical-type blood replacement preparations. The lack of chelation of oxygen and the high oxygen partial pressures enable animals to live in carbon monoxide concentrations of 10 percent or higher [19, 20, 21]. Because these animals depend upon a perfluorochemical for oxygen transport instead of hemoglobin, sufficient amounts of this gas obviously reach the tissues to prevent anoxia and to prevent CO from competing successfully for cytochromes and related compounds. Thus, an artificial blood has been used to establish the answer to the long-standing question of whether CO kills at the cellular level or by means of

fatal anoxia. The latter seems to be the case. The linear relationship between the amount of oxygen that dissolves in the perfluorochemical and external pO_2 , makes the use of these compounds of particular interest in hyperbaric oxygen studies. It was shown that instead of the 4 to 5 hours required by normal rats to develop convulsions in 2 atmospheres O_2 , bloodless animals convulsed within 20 minutes [22]. The treatment of anaerobic infections by using hyperbaric oxygen and perfluorochemicals would appear to have clinical potential.

From the onset of work with perfluorochemicals organ perfusion has received much attention. Sloviter and Kamimoto [5] first used them in emulsified form with rat brain. Since then, liver [23], heart [24], kidney [25], and pancreas [26] have been among the isolated organs studied. As with the perfusion of whole animals there is a great need to have the means to carry out perfusions for very long periods.

Clinical studies have been carried out in Japan, Europe, and the United States with the commercial product, Fluosol-DA 20%. From Table 1 it can be seen that this preparation provides oxygen and carbon dioxide transport via the perfluorochemicals, osmotic pressure from a spectrum of electrolytes, and oncotic pressure from hydroxyethylstarch. Based on experimental studies with other perfluorochemicals [27] there should be no problem in adding other biologically important substances such as amino acids, vitamins, carbohydrates, and antibiotics to the preparation. However, that should not be done even in experiments with animals without first consulting with the manufacturer. Patients have received as much as four liters of this product, but most have been given 500 to 1500 ml. Approximately 10 percent of the persons receiving the Fluosol-DA 20% in the United States experience a transient decrease in the number of circulating white cells and platelets. This phenomenon is associated with as little as 1 ml of the product and is thought to be related to complement activation [28]. Because it occurs within the first 5 to 15 minutes, it is easily overlooked. Predosing with steroids prevents this transient reaction. Many investigators give a test dose

of 0.5 to 1.0 ml of the Fluosol-DA 20% and then follow with larger quantities. In a few patients the phenomenon has been accompanied by a temporary change in respiratory rate, but most display no noticeable reactions and the true etiology of this reaction is still not known.

The Japanese reported using this product in volunteers [29] and a series of 186 patients [14, 30]. Both hemorrhaging and non-hemorrhaging cases were involved and the spectrum of illnesses included cerebral anoxia, malignant tumor surgery and hemolytic anemia. Some received the material because they refused blood; others, because their type of blood was unavailable; still others, due to the choice of the physician for 'bloodless surgery' to avoid the risk of hepatitis infection. Only four were listed as having had any kind of meaningful reaction, the most serious of which was a decrease in liver function which returned to normal within a

week. Results obtained with Fluosol-DA were compared to those in which hydroxyethylstarch alone was administered. Blood pressure, PaO_2 , CaO_2 , cardiac output, and oxygen consumption were all more favorable when the perfluorochemical preparation was employed.

In the U.S.A. Tremper and colleagues [17] reported on a series of seven patients given Fluosol-DA. Two experienced reactions to the preparations and were discontinued from the study. The remaining five had been hospitalized for anemia and in need of surgery. They received up to 20 ml/kg body wt. of the product and ended with a mean fluorocrit of 2.9. Hemoglobin before the infusions ranged from 1.9 to 7.5 g/dl. The mean PaO_2 increased from 291 to 361 torr and the CaO_2 from 7.1 to 8.6 volume percent ($p < 0.001$). The conclusion was reached that approximately 15 percent of the oxygen consumed was transported by the perfluorochemical. As pointed out earlier, it is of interest that in spite of the low fluorocrit a positive result was obtained.

Gould and coworkers [31] have carried out an instructive study designed to determine whether or not Fluosol-DA would allow a reduction in the inspired oxygen concentration of patients with hemoglobin so low that such a reduction would not be possible ordinarily. Having such patients breathing 100 percent oxygen was considered a risk, whereas, an FiO of 0.60 was considered safe. It was found that indeed the administration of the Fluosol-DA made it possible to reduce the FiO to 0.60. The authors concluded that in these instances the perfluorochemical preparation was both safe and effective. They pointed out, however, that the relatively short circulatory half-life of the material restricted its usefulness in long-term applications.

In addition to the interest in perfluorochemicals for blood replacement purposes, a number of other areas have come under study. One of these involves the visualization and/or treatment of tumors. Long and colleagues [32] have reported that emulsified F-bromo octane particles are taken up by macrophages. These cells are often found in the vicinity of tumors, and if they have taken up the F-bromo octane, the tumor can be imaged by means of x-rays. An alternative means of visualiza-

Table 1. Composition of commercially available perfluorochemical preparations.

Component	W/V%
Oxypherox*	
F-tributylamine	20.0
Pluronic F-68	2.56
NaCl	0.60
KCl	0.034
MgCl ₂	0.020
CaCl ₂	0.028
NaHCO ₃	0.21
Glucose	0.180
Hydroxyethylstarch	3.0
Fluosol-DA (20%)*	
F-decalin	14.0
F-tripropylamine	6.0
Pluronic F-68	2.7
Yolk phospholipids	0.4
Potassium oleate	0.032
Glycerol	0.8
NaCl	0.60
KCl	0.034
MgCl ₂	0.020
CaCl ₂	0.028
NaHCO ₃	0.210
Glucose	0.180
Hydroxyethylstarch	3.0

* Water added to a total of 100 ml.

tion is to use emulsified perfluorochemicals which though not opaque to x-rays, can be imaged by means of nuclear magnetic resonance [33]. Again it is the perfluorochemical-laden macrophages that are imaged not the tumor itself. Thus, a perfluorochemical product made available for blood replacement might also be used for tumor diagnosis. The product could also be used for tumor therapy by increasing the oxygen tension in the more anoxic areas of tumors. Thus, in studies with tumor bearing mice, Teicher and Rose [34] have reported that even small intravenous concentrations of perfluorochemicals (Fluosol-DA) enhanced the effectiveness of radiation therapy when the animals also breathed oxygen. Controls breathing oxygen but given no Fluosol-DA showed only modest improvement over air-breathing animals. Fluosol itself had little effect in the absence of oxygen. The mice had low fluorocrits and still effectiveness was obtained. This could indicate that this means of delivering oxygen has an advantage over that provided by normal concentrations of red cells and hemoglobin.

Such an advantage is also indicated by studies on the rescue of ischemic heart tissue [35, 36] and by the use of perfluorochemical emulsions in cardioplegic solutions [37, 38]. In all of these investigations improved results were obtained when the fluorocompounds were present. The over-all conclusion was that better oxygenation was the reason for the superior results. Similar studies are being conducted in research dealing with ischemic brain. Of many other applications of emulsified perfluorochemicals one other may be mentioned. This involves the use of transparent emulsions of fluorocompounds to aid in intravascular visualization *in vivo*. By using such a preparation to wash all blood out of the vessel of a dog, it was possible to examine the interior of the vessel by means of fiberoptics without the usual interference from red cells [39]. This may afford a way of facilitating the use of laser surgery intravenously.

Interest in artificial blood replacement preparations that carry oxygen has increased greatly in recent years. Freedom from infectious agents, lack of typing requirements, and essentially unlimited availability, have contributed in part to this inter-

est. Where effective natural blood programs exist, such artificial products could be of help when blood supplies prove inadequate temporarily, or rare type bloods are unavailable. In countries having poor or no blood programs, the artificial preparations would offer an alternative solution to the problem. It appears prudent to consider the artificial products as a way of delivering unbound oxygen at a higher partial pressure than normal, and by means of very fine particles that can penetrate areas that may be unaccessible to red cells. Thus, the perfluorochemical preparations offer, for the first time, an additional approach to the long-standing problem of furnishing adequate oxygen to biological systems both *in vivo* and *in vitro*.

Acknowledgements

This work has been supported by NIH Grants HL 15520 and HL 17844 and NIH contract N01 HB 9-2917. The author acknowledges the excellent technical assistance of Kenneth Taylor, Robert Eccles and Therese Zerbonne and assistance in preparation of the manuscript from Jeanne MacLaren.

References

1. Baldwin JE, Crossley MJ, Klose T, O'Rear (III) EA, Peters MK: Syntheses and oxygenation of iron (II) 'strapped' porphyrin complexes. *Tetrahedron*, 1982; 38: 27.
2. Bolin R, DeVenuto F: Hemoglobin solutions as a blood substitute. In: *Progress in Clinical and Biological Research*, Vol. 122: *Advances in Blood Substitute Research*, R.B. Bolin, RP Geyer, GJ Nemo (eds). Alan R. Liss, Inc., New York, 1983: 1-7.
3. Rosen AL, Gould SA, Sehgal LR, Sehgal HL, Moss GS: Evaluation of efficacy of stroma free hemoglobin solutions. In: *Progress in Clinical and Biological Research*, Vol. 122: *Advances in Blood Substitute Research*, RB Bolin, RP Geyer, GJ Nemo (eds). Alan R. Liss, Inc., New York, 1983: 1-7.
4. Gollan F, Clark LC Jr: Organ perfusion with fluorocarbon fluid. *Physiologist*, 1966; 9: 191.
5. Sloviter H, Kamimoto T: Erythrocyte substitute for perfusion of brain. *Nature*, 1967; 216: 458-60.
6. Geyer RP, Monroe RG, Taylor K: Survival of rats totally perfused with a fluorocarbon-detergent preparation. In: *Organ Perfusion and Preservation*, JC Norman, J Folkman, WG Hardison, LE Rudolf, FJ Veith (eds). Appleton-Cen-

- tury-Crofts, New York, 1968: 85–96.
7. Geyer RP: Whole animal perfusion with fluorocarbon dispersions. *Fed Proc*, 1970; 29: 1758–63.
 8. Geyer RP: Potential uses of artificial blood substitutes. *Fed Proc*, 1975; 34: 1525–28.
 9. Geyer RP: Oxygen transport *in vivo* by means of perfluorochemical preparations. *NEJM*, 1982; 307: 304–5.
 10. Clark LC Jr, Becattini F, Kaplan S, Obrock V, Cohen D, Becker C: Perfluorocarbons having a short dwell time in the liver. *Science*, 1973; 181: 680–82.
 11. Naito R, Yokoyama K: Improvement of perfluorodecalin emulsion with special regard to *in vivo* stability, offering Fluosol-DA. In: *Research on Perfluorochemicals in Medicine and Biology*, V Novakova, L-O Plantin (eds). Karolinska Institute Research Center, Huddinge Univ Hosp, Huddinge, Sweden, 1978: 42–92.
 12. Geyer RP: Studies and uses of perfluorochemical emulsion as blood substitutes. In: *Research on Perfluorochemicals in Medicine and Biology*, V. Novakova, L-O Plantin (eds). Karolinska Institute Research Center, Huddinge Univ Hosp, Huddinge, Sweden, 1978: 229–79.
 13. Naito R, Suyama T, Yokoyama K: Fluosol-DA. The Green Cross Corporation, Technical Information Ser No 4, December 1976.
 14. Mitsuno T, Ohyanagi H, Naito R: Clinical studies of a perfluorochemical whole blood substitute (Fluosol-DA): Summary of 186 cases. *Ann Surg*, 1982; 195: 60–69.
 15. Geyer RP: The design of artificial blood substitutes. In: *Drug Design*, EJ Ariens (ed). Vol VII. Academic Press, Inc, New York, 1976: 1–58.
 16. Sloviter HA, Mukherji B: Prolonged retention in the circulation of emulsified lipid-coated perfluorochemicals. In: *Progress in Clinical and Biological Research*, Vol 122: Advances in Blood Substitute Research, RB Bolin, RP Geyer, GJ Nemo (eds). Alan R Liss, Inc, New York, 1983: 181–88.
 17. Tremper KK, Friedman AE, Levine EM, Lapin R, Camarillo D: The preoperative treatment of severely anemic patients with a perfluorochemical oxygen transport fluid, Fluosol-DA. *NEJM*, 1982; 307: 277–83.
 18. *Progress in Clinical and Biological Research*, Vol 122: Advances in Blood Substitute Research, RB Bolin, RP Geyer, GJ Nemo (eds). Alan R. Liss, Inc, New York: 1983.
 19. Sloviter HA, Petkovic M, Ogashi S, Yamada H: Dispersed fluorocarbons as substitutes for erythrocytes in intact animals. *J Appl Physiol*, 1969; 27: 666–68.
 20. Geyer RP, Taylor K, Eccles R, Duffett E: Survival of bloodless rats in 10 percent carbon monoxide. *Fed Proc*, 1976; 35: 828.
 21. Yokoyama K: Effect of perfluorochemical (PFC) emulsion on acute carbon monoxide poisoning in rats. *Jpn J Surg*, 1978; 8: 342–52.
 22. Geyer, RP: Perfluorochemicals as oxygen transport agents in partial and total blood replacement. In: *Oxygen and Life: Lectures Delivered at the Second BOC Priestley Conference*, A Cubitt (ed). Royal Society of Chemistry, Burlington House, London, 1981: 132–41.
 23. Hoper J, Ji S, Kessler M: Tissue oxygen supply of the isolated rat liver perfused with fluorocarbon. In: *Oxygen Carrying Colloidal Blood Substitutes*. 5th Intl Symp on PFC Blood Substitutes, Mainz, R Frey, H Beisbarth, K Stosseck (eds) We Zuckschwerdt Verlag Munchen, 1982: 19–29.
 24. Segel LD, Rendig, SV: Isolated working rat heart perfusion with perfluorochemical emulsion Fluosol-43. *Am J Physiol: Heart & Circ Phys*, 1982; 242: H485–9.
 25. Lutz J, Baum M, Schulze HG: Comparison of Fluosol DA 35% with -DA 20% in the circulation of the liver. In: *Proceedings of the IVth Intl. Symp. on PFC Blood Substitutes*, Kyoto, 1978. Excerpta Medica, Amsterdam, 1979: 123–36.
 26. Ikeda T, Hamasaki N, Ando Y, Mashiba H: Perfusion of isolated rat pancreas with Fluosol-43. In: *Proceedings of the IVth Intl. Symp. on PFC Blood Substitutes*, Kyoto, 1978. Excerpta Medica, Amsterdam, 1979: 173–78.
 27. Geyer RP: Utilization and tolerance of intravenous fat emulsions. In: *Parenteral Nutrition in Infancy and Childhood*, HH Bode, JB Warshaw (eds). Plenum Publishing Corp, New York, 1974: 98–111.
 28. Vercellotti GM, Hamerschmidt DE, Jacob HS, Craddock PR: Activation of plasma complement (c) by perfluorocarbon artificial blood (Fluosol-DA). Mechanism and prevention of resulting adverse pulmonary reactions. *Clin Res*, 1982; 29: A572.
 29. Ohyanagi H, Toshima K, Sekita M, Okamoto M, Itoh T, Mitsuno T, Naito R, Suyama T, Yokoyama K: Clinical studies of perfluorochemical whole blood substitutes: Safety of Fluosol-DA 20% in normal human volunteers. *Clin Ther*, 1979; 2: 306–12.
 30. Yokoyama K, Suyama T, Naito R: Development of Fluosol-DA and its perspective as a blood substitute. In: *Oxygen and Life. Lectures Delivered at the Second BOC Priestley Conference*. The Royal Society of Chemistry, Burlington House, London, 1981: 142–52.
 31. Gould SA, Rosen AL, Sehgal LR, Sehgal HL, Moss GS: Clinical experience with FluosolDA. In: *Progress in Clinical and Biological Research*, Vol 122: Advances in Blood Substitute Research, RB Bolin, RP Geyer, GJ Nemo (eds). Alan R Liss, Inc, New York, 1983: 331–42.
 32. Long DM, Lasser EC, Sharts CM, Multer FK, Nielson M: Experiments with radiopaque perfluorocarbon emulsions for selective opacification of organs and total body angiography. *Investig Radiol*, 1980; 15: 242–47.
 33. Longmaid HE III, Adams DF, Neirinckx R, Harrison C, Brunner P, Seltzer S, Davis M, Neuringer L, Geyer RP: *In vivo*, non-proton, ¹⁹F-NMR imaging of liver, tumor and abscess. *Investig Radiol*. In press. (Abstract).
 34. Teicher BA, Rose CM: Perfluorochemical emulsions can increase tumor radiosensitivity. *Science*, 1984; 223: 934–36.
 35. Glogar DH, Kloner RA, Muller J, DeBoer KWF, Braunwald E, Clark LC Jr: Fluorocarbons reduce myocardial ischemic damage after coronary occlusion. *Science*, 1981; 211: 1439–41.

36. Menasche Ph, Fauchet M, Lavergne A, Commin P, Masquet C, Lorente P, Birkui P, Geyer RP, Piwnica A: Reduction of myocardial infarct size by a fluorocarbon-oxygenated reperfusate. Submitted.
37. Kanter KR, Jaffin JH, Ehrlichman RJ, Flaherty JT, Gott VL, Gardner TJ: Superiority of perfluorocarbon cardioplegia over blood or crystalloid cardioplegia. *Circ.*, 1981; 11:75.
38. Menasche Ph, Fauchet M, Lavergne A, Commin P, Masquet Ch, Birkui P, Lorente P, Geyer RP, Piwnica A: Applications of a perfluorocarbon to heart ischemia. In: *Progress in Clinical and Biological Research*, Vol 122: *Advances in Blood Substitute Research*, RB Bolin, RP Geyer GJ Nemo (eds). Alan R Liss, Inc, New York, 1983: 363-72.
39. Spears JR, Marais HJ, Serur J, Pomerantzoff O, Geyer RP, Sipzener RS, Weintraub R, Thurer R, Paulin S, Gerstlin R, Grossman W: In vivo coronary angioscopy. *J Am Coll Cardiol*, 1983; 1: 1311-14.

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