THROMBOSIS RESEARCH, Supplement V; 105-145, 1983
0049-3848/83 $3.00 + .00 Printed in the USA.

THE PHYSIOLOGICAL SIGNIFICANCE OF THE ENDOENDOTHELIAL FIBRIN LINING (EEFL) AS THE CRITICAL INTERFACE IN THE 'VESSEL-BLOOD ORGAN' AND THE IMPORTANCE OF IN VIVO 'FIBRINOGENIN FORMATION' IN HEALTH AND DISEASE

Alfred L. Copley

Laboratory of Biorheology, Polytechnic Institute of New York, Brooklyn, New York 11201, USA

ABSTRACT

The author's theory of the endoendothelial fibrin lining (EEFL), first advanced in 1953 and developed by him ever since, localizes the homeostasis between steady fibrin formation and deposition, or 'fibrination', and continuous fibrinolysis in the more or less immobile portion of the plasmatic zone next to the vessel wall. In 1971, the author advanced, in relation to the EEFL, the theory of fibrinogen gel clotting without thrombin action or 'fibrinogenin' formation in vivo. Considerable direct and indirect experimental evidence, secured by the author and by several other investigators, advanced markedly the knowledge of the normal physiology and the pathophysiology of various disease processes involving the vessel wall and blood circulation.

The information presented is an extension to that given in the author's recent overview (Clin. Hemorheology 1, 9-72, 1981). It deals both with new data by several investigators including those by the author, as well as with older data from the literature. The author maintained already in 1960 that the blood together with the blood vessels, in which it circulates, constitute 'an entity'. In 1981 he postulated this entity to be a very special organ, named conveniently 'vessel-blood organ', which is ubiquitous and penetrates all other organs and adjacent tissues. The EEFL of the vessel-blood organ is considered by the author as the crucial critical interface between the blood and the vessel wall. It is the primary barrier, followed by the

KEY WORDS: Antithrombogenic action, basement membrane, capillary fragility, capillary permeability, capillary wall structure, endoendothelial fibrin lining, fibrinogenin formation, glycosaminoglycans, leukocyte emigration, vessel-blood organ.

endothelium (comprising the endothelial cells and the interendothelial cement substance which contains or is identical with 'cement fibrin') and the basement membrane for the exchanges between the blood, the vessel wall and its surrounding tissues and spaces. The EEFL acts as anticoagulant, is antithrombogenic, maintains vascular patency and aids cardiac action by decreasing significantly the apparent viscosity of blood, referred to in the literature as the 'Copley-Scott Blair phenomenon'.

A new concept of leukocyte emigration traversing the capillary wall is presented, affecting focal fibrinolysis of the EEFL and of fibrin contained in the interendothelial cement substance and in the basement membrane.

The physical property of capillary (or vascular) permeability is related to the existence of the EEFL, since, as found by Copley et al, both fibrinopeptides, liberated in the transition of fibrinogen to fibrin, and plasminopeptides, freed in the conversion of plasminogen to plasmin, enhance capillary permeability. Capillary fragility, which is antagonistic to capillary permeability, is in great part due to fibrinolytic action on fibrin as a constituent of the basement membrane. Pseudohemorrhages occur as minute microaneurysms in augmented capillary permeability, when the basement membrane remains intact and blood escapes through the interendothelial junctions into the exoendothelial space between the endothelium and the basement membrane. The occurrence of petechiae can be either due to focal lesions in the basement membrane leading to extravasation of blood or due to these pseudohemorrhages. Thus, petechiae are not necessarily identical with capillary hemorrhages.

New surface hemorheological findings, obtained by Chien, King and Copley with a modified Weissenberg rheogoniometer, are presented. We found Ca^{2+} to increase markedly the viscous and elastic moduli of fibrinogen surface layers. Other studies concern marked decreases of these surface rheological moduli by the addition to fibrinogen solution of the following substances: heparins of low molecular weights of 4400, 5300, and 5900; chondroitin A, B and C; dextran MW 20,000; dextran sulfate MW 17,000; and sodium hyaluronate. The author considers these findings of inhibition of fibrinogenin formation to mirror antithrombogenic actions of these different agents, which are expected to prevent thrombogenesis.

The pathogenesis of different disease processes, such as thrombosis, hyperthermia therapy of cancer, (non-thrombocytopenic) vascular purpura, and atherogenesis is briefly discussed. The author relates these disease processes to the theories of the EEFL and of in vivo fibrinogenin formation. In his conclusions he suggests a number of studies to be made towards the advancement of knowledge pertaining to these theories involving health and disease.

CONTENTS

I. Introductory remarks

Earlier at this Symposium the author has given a historical account (1) of the endoendothelial fibrin lining (EEFL). It is advisable to consult his overview on the EEFL, published in 1981 in CLINICAL HEMORHEOLOGY (2), as it is impossible in this presentation to refer to the many findings and aspects dealt with in the overview. This presentation is limited to several aspects of the EEFL and includes certain findings, not previously referred to. New work by different authors, published since the overview appeared and also earlier, will be presented, as well as some new findings by the author and associates, particularly in connection with fibrinogen gel clotting or so-called 'fibrinogenin' formation extra vivum and in vivo.

As the author already referred to earlier in the historical account (1), the word 'blood capillary' is used as a non-committal, generic term for all minute or capillary blood vessels which some authors also refer to as 'microvessels'. It is reemphasized that only after the particular capillary blood vessel has been clearly identified, it should be characterized by its specific name.

II. The structure of the capillary wall

The blood capillary wall consists of several structures. It is generally believed that it is the endothelium which faces the vascular lumen. However, Copley extended the composition of the blood capillary wall to include the EEFL as the structure closest to the vascular lumen (2).

According to Copley's concept, several layers of different components, structures and spaces constitute the architecture of the capillary wall. The proposed spaces and layers of the capillary vessel wall (1-3) are: (A) The more or less immobile portion of the plasmatic zone, (B) the porous endoendothelial fibrin lining, (C) the endothelial cell proper, and the interendothelial porous cement substance filling the intercellular spaces or junctions, (D) the exo-endothelial space, (E) the basement membrane, and (F) the adventitial tunic.

The EEFL, as well as the interendothelial cement substance, which the author proposed to contain the so-called 'cement fibrin' (31,61), may also contain certain other plasma proteins due to their affinity to fibrin surface. It may likewise contain calcium, as the so-called 'calcium fibrinate', and non-protein substances (61).

III. The endoendothelial fibrin lining (EEFL) as the crucial interface of the vessel-blood organ

In a plenary lecture of the 4. International Congress of Biorheology in 1981 in Tokyo, Copley postulated that the blood together with the blood vessels, in which it circulates, constitute one single organ (4). It was named conveniently the 'vessel-blood organ', since hitherto no name existed for it. This very special organ is ubiquitous and penetrates, similar to the nervous system, all other organs and adjacent tissues. Copley proposes the EEFL as the crucial interface between the two portions of the vessel-blood organ for the exchanges between the blood and the tissues and vice versa A number of data in support of this rather extraordinary claim are presented.

The idea that 'the relationship and interaction of blood and the vessel wall in health as well as in disease' constitute an organ was already reported by the author in 1960 (12) in the first overview on the EEFL in a plenary lecture before the VIII. International Congress of Hematology in Tokyo. At that time he considered 'blood and its more or less leaky envelopes, the variety of blood vessels in a variety of tissues and organs' to be 'an entity'. However, it was twenty-one years later when he defined this entity as an organ (4). (It was inadvertently not referred to in the 1981 plenary lecture. It is likewise regrettable that in a clarification of the term 'hemorheology', Copley and Seaman (112) inadvertently did not refer to the introduction of the term 'vessel-blood organ' in Copley's plenary lecture, held in Tokyo in 1981).

IV. On the role of calcium as a constituent of the EEFL and the interendothelial cement substance

The significance of calcium in the integrity of the vascular wall was discussed in the Historical Account (1). Calcium ions were found to increase the rigidity of fibrin gels both with plasma (5) and upon the activation of fibrinogen by thrombin (113). As no studies have thus far been made on the action of calcium on gels of fibrinogen, formed without thrombin at an interface, the recent preliminary studies by Copley and King are of interest (114).

Fig. 1 shows a plot of the viscous modulus and the elastic modulus of surface layers of fibrinogen versus frequency measured from a preparation of 0.2 per cent highly purified fibrinogen (98-100 per cent coagulability) as control. The fibrinogen was prepared according to Blombäck and Blombäck (6) and manufactured by IMCO, Stockholm. The second plot is from the addition of 5 mM $CaCl_2$ to the above fibrinogen solution which increases the viscous and elastic moduli of the surface layer by approximately 30 per cent.

We also used varying concentrations of Ca^{2+}, viz., 5 mM, 20 mM, 40 mM, and 100 mM $CaCl_2$. However, the higher concentrations did not bring about increase in values of viscous and elastic moduli, which are similar to those obtained with 5 mM $CaCl_2$ (114).

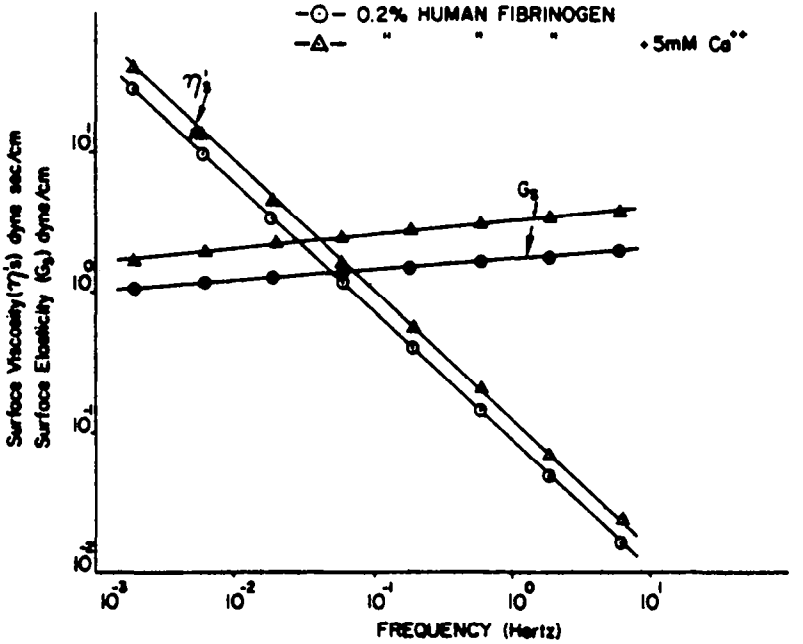

FIG. 1

Viscous and elastic moduli versus frequency measured from surface layers of 0.2% fibrinogen solution as control (circles). The second preparation (triangles) contains the additional 5 mM $CaCl_2$. The open circles and triangles depict viscous moduli, while the solid circles and triangles denote elastic moduli. Temperature was controlled at 25 ±0.5°C. A Tris-saline buffer at pH 7.4 was used for the preparation of the 0.2% fibrinogen solution.

V. The porosity of fibrin gels and the EEFL

Recently, Birger Blombäck and Okada presented several communications pertaining to fibrin gel structure and pores in these fibrin gels (7-9). Their liquid permeation studies led to the following results.

Gels of Batroxobin-induced fibrin I and gels of thrombin-induced fibrin II have different flow properties. Blombäck and Okada found a permeability index which is the ratio of clotting time versus fibrin concentration. They concluded in their original report (7) that the coagulant forces in blood leading to thrombin generation, and expressed as clotting time, will, together with the fibrinogen concentration, determine the overall permeability characteris-

tics of the gel phase, for which they introduced a permeability index. These authors reasoned that a very tight layer may impair flow through the endothelium or limit the access of plasma proteins to specific receptors on the endothelium. They considered this to lead to disturbed cell function with tissue damage as a result, and thought that vestiges for local pathological processes such as thrombosis or atherosclerosis would be created.

In subsequent communications on gel pores Blombäck and Okada (8,9) stated that the effective pores in fibrin gels are much smaller than theoretically calculated for a parallel capillary system and that the pores in fibrin gels appear to be remarkably uniform. They used also spherical latex particles of known size, suspended in the buffer, for permeation experiments.

Blombäck and Okada found that clotting time, i.e. thrombin concentration, was directly related to the permeability coefficients of the final gels. Gels with low permeability coefficient and small average pore size were produced with high pH and average pore size. Their finding that clotting time was directly related to the permeability coefficients of the final gels appears to be particularly important. They concluded that events preceding gel formation determine the gel structure. Furthermore, Blombäck and Okada (7) found that the pH and ionic strength influenced the pore size of the gel.

Of great interest is that the addition of calcium produced gels with higher permeability coefficients and wider pore sizes than those formed without the addition of calcium (9).

The findings by Blombäck and Okada support the thesis of the EEFL as a crucial interface for capillary permeability. The latter has been associated in the past solely with the endothelium, whether via transendothelial channels and plasmalemmal vesicles (10,11) or the interendothelial porous cement substance (2, 31, 61). The role of the EEFL as the first filtration barrier of the capillary wall will have to be established in future studies and may prove to be highly significant.

VI. Certain observations affecting the basement membrane

It is emphasized that the basement membrane (BM) contains fibrin and other substances, such as collagen, elastin, glycoprotein molecules (13). The existence of fibrin as a constituent of the basement membrane was shown in the production of vascular purpura with antifibrin serum (14-16,74) as well as in the production of petechial hemorrhages by fibrinolysin (plasmin), injected intradermally into the nictitating membrane of the rabbit's eye (17). However, more detailed studies will need to be made on fibrin as a constituent of the basement membrane. The porosity of gelated fibrin (7-9) contributes to the permeability of the BM.

Zweifach (18,19) employed micromanipulative technique which makes it possible to separate the various sequelae of tissue damage. Under normal conditions, the sequelae develop with unusual rapidity which he demonstrated. In his biomicroscopic observations on the

BM, which he called the pericapillary sheath, Zweifach introduced testicular extracts with hyaluronidase activity with a micropipette onto the vessel wall (18). As a result, the BM becomes eroded at this point and the vessel frequently develops petechial hemorrhage. As he also found that the particular extract has no visible effect on the interendothelial cement, he considered both the BM and the interendothelial cement as two distinct chemical entities.

In Copley's view, the EEFL extends to the interendothelial spaces and fibrin(ogen) is contained, if not fully, as part of the cement substance. In a study with Robert Chambers, we injected into the nictitating membrane of the rabbit's eye various hemorrhagic agents of animal, plant, bacterial and chemical origins (17). Their administration must have acted on constituents of the basement membrane (17) leading to petechial hemorrhage and, in certain cases, to complete breakdown of the basement membrane (12,13).

Some of these hemorrhagic agents may well have had fibrinolytic action, while others attacked other constituents of the basement membrane. We also observed white thromboemboli in the microcirculation of the nictitating membrane which might have been embolizing fibrinogen gels, formed without thrombin participation. Although the author refers to in vivo fibrinogen gel clotting later in this presentation, fibrinogen gels in addition to fibrin may also be contained in the interendothelial cement substance.

VII. The three barriers in capillary permeability

There is no question that the vascular endothelium has acquired a remarkably high permeability to water and water soluble solutes including macromolecules through a characteristic process of differentiation of its cells according to Palade, Simionescu and Simionescu (10,11). These authors also contend that this differentiation involves an unusually large population of plasmalemmal vesicles.

Of special interest are the recent findings by Simionescu, Simionescu and Palade (20,21) pertaining to differentiated microdomains on the luminal surface of the capillary endothelium. The authors expect that the structures corresponding to these differentiated domains will select, in addition to size, permeant molecules according to charge. They anticipate that fenestral diaphragms discriminate against anionic molecules. They also consider stomatal diaphragms, plasmalemmal vesicles, and transendothelial channels to favor the penetration of anionic molecules for diffusion, filtration, or vesicular transport. The majority of plasma proteins, as they point out, are anionic and that the size range remains to be established at which discrimination becomes effective (20).

It is emphasized that fibrinogen, which is negatively charged, appears to be selective regarding the adsorption of plasma proteins to its surface layers. Copley and King (22) found that highly purified β-lipoprotein nullifies the extremely high rigidity and elasticity of fibrinogen surface layers. This is shown in Figs. 2 and 3. They also found that highly purified γ-globulin behaves similarly, as demonstrated in Fig. 4 (22,90).

In general, data on the elasticity of surface layers of fibrinogen systems are given in this presentation, since the elastic modulus is a function of the cross-linking within the gel regardless of whether van der Waals forces or covalent bonds are involved The magnitude of the elastic modulus provides data concerning the organization of the gel structure. In order to show the action of β-lipoproteins and γ-globulin on surface gels on these fibrinogen systems, this information if provided in Figs. 3 and 4.

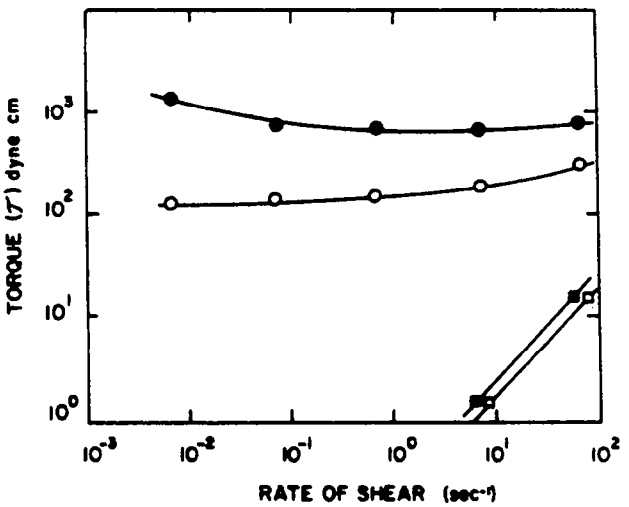

FIG. 2

Comparisons of torque values from surface layers of -●- 0.4% bovine fibrinogen and -□- 0.25% β-lipoprotein as controls. -○- 0.4% fibrinogen plus 0.025% β-lipoprotein; -■- 0.4% fibrinogen plus 0.25% β-lipoprotein.

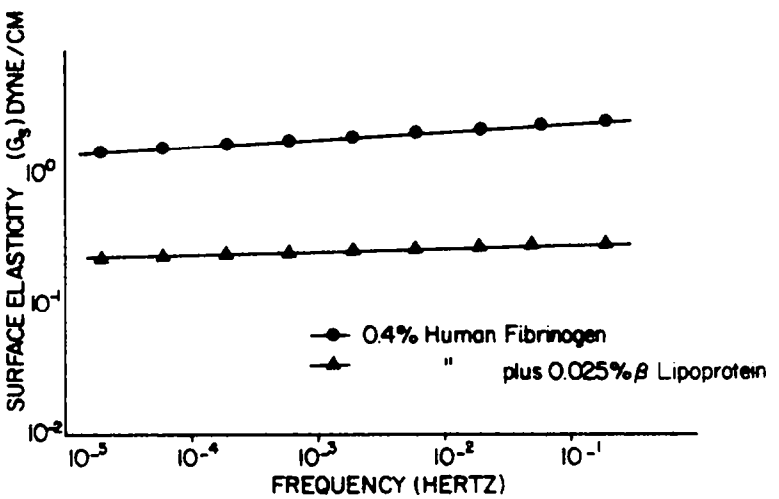

FIG. 3

The effect on elasticity (G_s) of the surface layer of a solution of 0.4% fibrinogen when β-lipoprotein is added at a concentration of 0.025% and 0.25%. The addition of 0.25% appears to eliminate the layer altogether (81).

There are gradations pertaining to the rigidity of fibrinogen surface layers by the use of other plasma proteins, added to the fibrinogen solutions (23,24). Fluorescent labeled fibrinogen and fibronectin, injected intravenously into rats, were found by Witte (25,26) to be adsorbed at the luminal site in the microcirculation of the mesentery, i.e., on the EEFL.

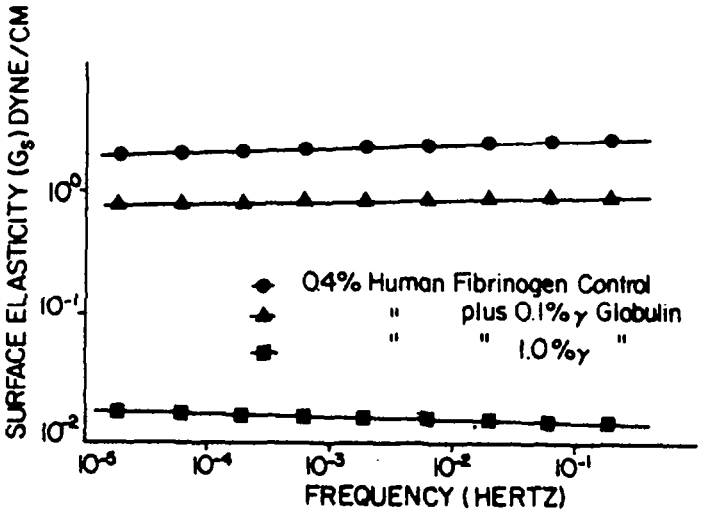

FIG. 4

The effect on elasticity (G_s) of a surface layer of a 0.4% fibrinogen solution, when γ-globulin at 0.01 and 1.0% concentration is added (81).

We are in the beginning of in vivo explorations pertaining to the EEFL. The microdomains on the luminal site of the endothelium, observed by Simionescu, Simionescu and Palade (20,21), may well be related to, intertwined with, or in close proximity to the EEFL. Of particular interest in this connection are our recent studies with Shu Chien and R.G. King on fibrinogen surface layers with glycosaminoglycans, which will be referred to later in this presentation.

Students of capillary permeability and, in general, of the microstructure of the capillary wall and its functions can no longer ignore the EEFL as the first barrier in the exchange between the blood and the tissues.

As the author conceives it, the capillary blood vessel wall has three barriers. The first barrier is, as already stated, the EEFL which may extend to the interendothelial junctions, comprising there the cement substance or part of it. The second barrier represents the endothelial cells, in which transendothelial channels and plasmalemmal vesicles play major roles as compared to the interendothelial cement substance which is part of the endothelium. Although the cement substance in the intercellular junctions involves a small part of the total endothelium, it is important in capillary permeability. The third barrier is the basement membrane, which has a porosity of its own.

In certain blood vessels, in which the endothelium is discontinuous or where it is fenestrated, the first barrier would be an endovascular fibrin lining similar to the EEFL. Certain blood vessels have no basement membrane such as the sinusoids of the liver. However, the glomerular capillaries have a basement membrane and endothelial cells with large fenestrae (27). Thus, an endovascular fibrin lining would exert its role in vascular or capillary permeability as the first barrier, followed by the basement membrane as the second barrier.

Renkin and Curry (28), who recently discussed capillary permeability, stated that histamine and bradykinin produced transient widening of interendothelial junctions in postcapillary venules of the skin. These gaps narrowed subsequently, but the transport of protein remained elevated. They concluded that a different pathway was involved, because protein flux is not coupled to volume flow. These authors found that moderate dilution of plasma proteins by plasmapheresis increased the permeability to fluid, but decreased protein leakage. They also observed that extreme dilution of plasma led to increased transport of both fluid and proteins. They considered this change to be junctional and related the involvement of interaction of plasma proteins with a junctional fiber matrix model, proposed by Curry and Michel (115). Such a matrix might be identical with the interendothelial cement substance containing calcium fibrinate and possibly also fibrinogen gels or so-called fibrinogenin.

Michel reported in 1979 measurements of filtration coefficients and osmotic reflexion coefficients of single capillaries in the frog mesentery (29). He stated that his investigations suggest that fluid flows through the walls of these capillaries in two types of channels. Michel considers one of these channel types, representing about 10 per cent of filtration coefficients, to be exclusively for water, whereas the other channel type is available for both water and hydrophilic solutes. He concludes from the effects of proteins in the perfusate upon filtration coefficients and investigations on the passage of ferritin into the surface vesicles of the endothelial cells that an endocapillary layer provides an important component of capillary permeability. Michel refers to the hypothesis of the intercellular cement by Chambers and Zweifach, proposed in 1940 (30).

Michel postulated that the "selectivity of the capillary pore system lies in a three-dimensional net of extracellular material which covers the surface of the endothelial cells and fills the cracks between them". This evaluation of his experimental findings supports strongly the concept of the EEFL as well as its extension, proposed by Copley (31) twenty years ago, as so-called 'cement fibrin' filling the interendothelial junctions and surrounding the endothelial cells on their exoendothelial site in the exoendothelial space, i.e., between the endothelial cells and the basement membrane (2, 12). What Chambers and Zweifach (30) referred to as 'cement substance', Copley identifies mainly as cement fibrin (31), composed of fibrin particles in initial stages of polymerization, a calcium containing fibrin or 'calcium fibrinate' and fibrin breakdown particles due to fibrinolysis (see also IV).

In the discussion of his paper, Michel (29) commented that it may be the protein coat or the so-called "fuzz" that determines passage and not the pore itself. However, the findings on the pore size in fibrin gels, published recently by Blombäck and Okada (7-9), when applied to the EEFL support it as a crucial barrier in capillary permeability.

VIII. New findings pertaining to homeostasis of fibrin formation and fibrinolysis

The hypothesis of intravascular homeostasis between fibrin formation and fibrinolysis in healthy subjects continues to be discussed in the literature. Nolf was among its first originators in the beginning of this century (32). Some investigators contended that there has not been sufficient evidence available to substantiate this hypothesis. However, during the past decades additional evidence in favor of the hypothesis accrued with the development of new methods and techniques, resulting in new quantitative data pertaining to the two processes of steady fibrin formation and continuous fibrinolysis in vivo.

Recently Moroz (33) concluded on the basis of a quantitative analysis of different factors and products, present in the circulation, that the polymorphonuclear leukocytes (polymorphs) are "functionally equipped, numerically adequate and appropriately situated for removal of physiological fibrin deposits necessary for intravascular homeostasis in healthy individuals".

Moroz based his conclusions merely on calculations pertaining to the polymorphs observed by Stewart et al (38) in proximity to the endothelium in normal veins. It should be noted that Moroz (33) ignored entirely the EEFL in the discussion of his findings.

The vast number of endothelial cells in the microcirculation may contribute to steady fibrinolysis on the luminal site of the vascular wall. Such an involvement of the endothelial cells may even be more significant than that of the polymorphs. It is not known whether the endothelial cells promote steady formation and deposition of fibrin which the author named 'fibrination' on the vascular endothelium. (The term 'fibrinization' was used in the past by Copley (12) for both the coating of foreign surfaces with fibrin as well as the lining in vivo of the endothelial cells with fibrin. However, the term 'fibrinization' has been redefined by him to apply solely to the coating of surfaces foreign to blood with fibrin).

Regulatory mechanisms for both processes of fibrination and fibrinolysis probably exist for the maintenance of the EEFL and in providing the delicate balance between fibrination and fibrinolysis in the more or less immobile portion of the plasmatic zone.

IX. Fibrinopeptides and plasminopeptides in the physiology of capillary permeability

Of considerable importance are the findings by Copley et al that fibrinopeptides, freed in the conversion of fibrinogen to

fibrin, as well as plasminogen and plasminopeptides, freed in the conversion of plasminogen to plasmin, enhance capillary permeability (34). These studies were made with horseradish peroxidase which Copley and Carol (35) introduced in 1964 as a new tool in the physiology of the microcirculation, as compared to the Aschheim-Zweifach method (36). In employing horseradish peroxidase, we devised a quantitative method for capillary permeability which was found to compare well with the Aschheim-Zweifach method using radioactive iodinated albumin. Several years later, horseradish peroxidase was selected by Karnovsky as a tracer in electron microscopic studies of capillary permeability (37).

Since the author contends that homeostasis between fibrin production and fibrinolysis occurs steadily in the formation and maintenance of the EEFL, taking place in the immobile portion of the plasmatic zone, the released fibrinopeptides and plasminopeptides are expected to contribute directly to the physiology of capillary permeability. This conception needs further experimental exploration, as does the relationship, if it exists, between these peptides and substances such as histamine, bradykinin or serotonin, long known for their roles in augmenting capillary permeability.

X. The EEFL and the concentration of fibrinogen in the plasmatic zone

Fry (42,43) pointed out that blood contains a mixture of complicated molecules which apparently have different degrees of affinity for water. He states as an example that a neutral salt, if added to plasma, competes for the available water. Fry emphasized that molecules with a lesser affinity for water are forced out of the liquid phase, i.e., they precipitate. Among plasma proteins, he considers fibrinogen to have the least affinity for water, since during such a salting out process it precipitates first.

As contemplated by Fry, fibrinogen is competing for water even in the normal situation. It, therefore, tends to be forced by more 'aggressive' hydrophilic substances, e.g., albumin, into regions where the fewest free water molecules occur. He considered such regions to be at an interface where water molecules are 'more tightly bounded to themselves'.

According to Fry, an increased concentration of fibrinogen might occur in an interfacial region, and that this would exist normally at the interface between the blood and the endothelium. He considered it possible that the maintenance of these chemical concentration gradients within certain limits is vital to the survival of the endothelial cell. Although Fry did not refer to the EEFL, his considerations appear to be in support of its existence.

XI. Theoretical considerations of the Copley-Scott Blair phenomenon

In several publications Oka (44-46) proposed several theoretical treatments of what he termed the 'Copley-Scott Blair phenomenon'. He referred to experimental studies by Copley published in 1958 (47)

and to subsequent ones by Copley, Scott Blair et al (2,48) of apparent viscosity of blood, plasma and serum in contact with glass and fibrin coated or 'fibrinized' glass capillaries. There was always a decrease in apparent viscosity when the blood systems were in contact with fibrin surfaces, as compared with glass and other surfaces such as silicone.

Oka referred to studies by Koyama et al on the decrease of apparent viscosity on fibrinized or fibrin coated surfaces. Since the decrease which these authors found was about 4 per cent, their findings cannot be considered to be significant. These investigators may not have fibrinized adequately their viscometers. It should be emphasized that, although fibrinization is a simple procedure, it takes considerable skill and experience in securing adequate coatings of capillary viscometers with fibrin. Copley and Thorley described in 1962 (116) the method of fibrinization of capillary viscometers. We found a decrease in apparent viscosity of blood and plasma in the fibrinized viscometers to be about 20 per cent, which is a significant reduction. (These combined studies were made independently by G.W. Scott Blair and F.A. Glover at the National Institute of Research in Dairying, Shinfield, while A.L. Copley and R.S. Thorley made them at Charing Cross Hospital Medical School, University of London. R.S. Thorley made the fibrinization of the viscometers for the combined studies.

Ossoff and Charm (117) tested the possibility whether the vessel wall and the red cell charge could influence red cell-vessel interaction and the resistance to flow. They determined the apparent viscosity of blood in electrically charged and neutral tube viscometers and compared it with viscosity measured in a cone and plate viscometer. They found a viscosity reduction in negative charged capillary viscometers in blood, but not in plasma. Although such a reduction was found by Copley and Scott Blair in fibrinized capillary viscometer tubes both with blood and plasma, the findings by Ossoff and Charm of about 18 per cent reduction in blood viscosity appear to confirm our findings with blood. However, the reduction in plasma viscosity which we also noted cannot be explained by a red cell-wall interaction. There may be still another explanation of the Copley-Scott Blair phenomenon which is not based on electric charge effects.

It is not possible to present adequately the different theories on the Copley-Scott Blair phenomenon in this communication. The phenomenon cannot be explained by the Poiseuille Law. Oka (44-46) presented several explanations, based on (a) a slip model, (b) an electrostatic model and (c), also for this Symposium (118), an electric double layer, which exists at all solid-liquid interfaces. It is formed at the surface of a colloidal particle or a cell. The electro-kinetic potential or so-called zeta potential is decreased, when an ion or opposite charge to that of the fixed charge is adsorbed to the particle surface. The mutual repulsion diminishes between the particle and coalescence becomes possible with consequent precipitation or aggregation. Nevertheless, Oka emphasized that there are difficulties in explaining the Copley-Scott Blair phenomenon merely on the basis of an electric double layer. Blood has non-Newtonian behavior, whereas Oka's theory is limited to Newtonian liquids. Furthermore, effects of hematocrit and plasma layer need to be taken into account.

Tamamushi (49) offered an explanation for this phenomenon which is based on surface chemistry. He proposed that protein molecules may be adsorbed from the plasma on the fibrin surface, resulting in the build-up of protein multilayers. A decrease of the protein content in plasma will take place and thus decrease the apparent viscosity of blood and plasma.

Still another explanation has been offered by Müller who considers a functional coupling between capillary flow and the EEFL (50).

XII. The Blombäck-Copley hypothesis of conformational changes of fibrinogen at high shear forces at the vessel wall

In Fig. 5, values of wall shear rates are compiled (51) from data by Whitmore (52), Chien (53) and Charm and Kurland (54). The high shear forces, present at the walls of all blood vessels, in particular of arterioles and capillaries, may bring about conformational changes of fibrinogen molecules at certain sites in the circulation.

Blombäck et al (55,56) demonstrated that fibrinogen contains polymerization sites in its fragment D and N-DSK portion. Treatment with thrombin or thrombin-like enzymes activate the sites residing in the N-DSK portion, apparently due to unfolding of the polymerization sites or their exposure after release of fibrinopeptides. It appears likely that enzymatic or non-enzymatic polymerization processes involve always conformational change in N-DSK, with the exposure of polymerization sites. Since the shearing forces are especially high at the vessel wall, the Blombäck-Copley working hypothesis may prove to be pertinent in bringing about conformational changes of fibrinogen.

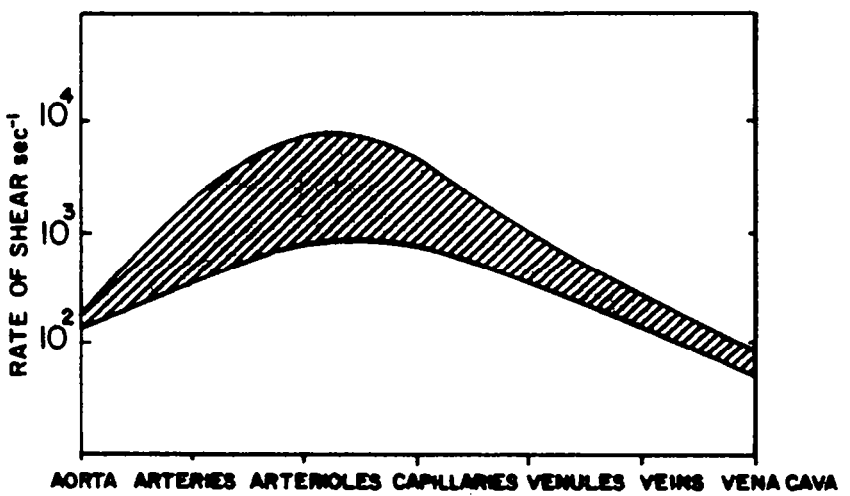

FIG. 5

Compilation of values of wall shear rates in different blood vessels from data by Whitmore (52), Chien (53), and Charm and Kurland (54).

Whether shearing forces can open up polymerization sites and thus simulate enzymatic action, as proposed in the Blombäck-Copley hypothesis, will need to be studied.

Of interest are the findings by Charm and Wong (57) that fibrinogen in plasma subjected to sufficient shear looses its ability to clot. Charm and Kurland (54) stated that these findings indicate that plasma undergoes a shear degradation. They explained the half-life of fibrinogen in the circulation to be due to shear degradation.

XIII. The problem of afibrinogenemia as related to the EEFL

In view of the concept of the EEFL, the author was asked, on many occasions (12), the question to explain the low incidence of spontaneous bleeding in congenital afibrinogenemia. It is known that in this condition, the platelets agglutinate well (58). There may be still some other compensatory mechanisms (12). Studies are needed to find out whether fibrin(ogen) is substituted by fibronectin and/or other proteins.

There is the possibility that an atypical fibrinogen may be contained in the endovascular layer. Such an atypical fibrinogen might lack the immunologic determinants and the special configuration required for clotting, but still retain the interaction with the endothelium.

XIV. Cultured endothelial cells and the EEFL

The endothelium, which is a special structure of the living vessel wall, is usually equated with cultured endothelial cells. Such an identification is not justifiable particularly when conclusions are drawn from findings secured with cultured endothelial cells and related to the living endothelium in situ for the following reasons. Although the endothelial cells and the interendothelial cement substance comprise the endothelium, the cement substance is frequently not taken into consideration by such conclusions. Furthermore, the EEFL is entirely ignored and the basement membrane is often hardly mentioned. The involvement of these structures in the architecture of the capillary wall is important for the dynamic interactions pertaining to the exchanges between the blood and the tissues.

Recently Shuman et al (59) used umbilical vein endothelial cells, grown in tissue culture wells, coated with fibrin, and preincubated with thrombin in physiological concentration. They found that plasminogen activation is markedly inhibited. Similar findings were also secured by Loskutoff (60) who employed cultured bovine endothelial cells.

The above findings support extra vivum studies of the inhibition of fibrinolytic activity by fibrin surface, reported by Copley and Stefko in 1962 (2,12,61). Such fibrin contact plasma lost its fibrinolytic activity. We also found the inactivation of plasmin in blood vessel chambers, produced experimentally in dogs (2,12). It appears that the immense surface of the EEFL on the luminal site of the blood vessels would bring about this antiplasmin action.

XV. A new hypothesis of leukocyte emigration traversing the capillary wall

In accordance with findings by Gert Schmid-Schönbein et al (62, 63) the white blood cells (WBC) are not larger than the red blood cells (RBC) in the flowing blood. These authors proposed a standard solid viscoelastic model to characterize the deformation of the cell for small strains. Because of the high rigidity of the WBC, Chien considered them to have more difficulty traversing the blood capillary wall (64).

Of great significance is the mechanism for the actual emigration of leukocytes from the blood vessels as it occurs in an inflamed area. It is not yet clear whether there is bacterial or thermal damage which would alter the rheological properties of the leukocytes and induce their emigration. It was speculated in 1908 by Nolf (32) that thus provoked leukocytes may bring about fibrinolysis.

Plow and Edgington (65) emphasized that the migration of leukocytes to sites of injury where fibrin has accumulated is a central feature in inflammation. They cited several groups of investigators who demonstrated that fibrinolytic activity of leukocytes is observed at neutral pH. At this physiological pH they found an alternative fibrinolytic system to be present in leukocytes in the peripheral circulation. They claimed that fibrinolytic proteases which are localized predominantly in the leukocyte granules can degrade both fibrinogen and fibrin. The specificity of the alternative fibrinolytic proteases for fibrinogen can be clearly distinguished from the plasmin system. Riddle and Barnhart (66) already had emphasized in 1964 that the interaction of the leukocytes with fibrin may be an active process and of physiological significance.

The author proposes that fibrinolysis produced or initiated by the leukocytes (67) either via the plasmin system or via the alternative pathway of fibrinolytic proteases would act as follows: There would be lysis of the fibrin constituent in three structures of the blood capillary wall, viz., the EEFL, the interendothelial cement fibrin and the basement membrane. These fibrinolytic processes, directly affecting the capillary wall and occurring concomitantly with alterations in the rheological properties of the leukocytes, would thus be responsible to cause leukocyte emigration, traversing the capillary wall.

In 1907 Opie (68) called attention to the association of fibrin deposition, leukocytes and inflammation. This was later emphasized by Menkin (69) and Jansco (70). Barnhart (71) tested the kind of response of leukocyte emigration into skin window collection chambers placed surgically on the back of dogs. In a quantitative study she correlated experimentally the leukocyte response to the chemotactic power of fibrin, fibrinogen and their proteolysis products. Barnhart found that fibrin-related materials in acute inflammation were the most potent chemotactic agents for granulocyte (predominantly neutrophil) emigration. It appears that her findings offer support for the author's concept of leukocyte emigration traversing the blood capillary wall. As fibrin is considered in Copley's concept to be contained in all three barriers of the blood capillary wall, it serves as an attractant for leukocytes in their emigration across the wall of capillary blood vessels.

XVI. Vascular purpura and the EEFL

Clark and Jacobs reviewed the Japanese literature on experimental (non-thrombocytopenic) vascular purpura (72). For the production of a vascular antiserum, the endothelium containing material was scraped off from the aorta and vena cava of healthy dogs. This endothelium containing material which Clark and Jacobs called "endothelium", was prepared as an antigen and injected into rabbits. The resulting antiserum was injected into dogs and a marked hemorrhagic, non-thrombocytopenic purpura was produced.

The above experimental vascular purpura in dogs appears to be similar to the vascular purpura we produced with antifibrin rabbit serum in guinea-pigs and hamsters (16,73,74). It is possible that the vascular purpura produced by the Japanese investigators, and experimentally confirmed by Clark and Jacobs (72), may well be, fully or in part, due to any remnants of the EEFL, the interendothelial cement fibrin, and the fibrin containing basement membrane, possibly present in the scraped-off endothelium containing material, which served as antigen. There is also the possibility that fibrin degradation products, which could have resultad, might have been contained in the scraped-off material used as antigen. These products still could have had the ability to form antibodies, similar to those we obtained with fibrin for the production of vascular purpura (16,73,74).

XVII. On similarities between fibrinogen and fibrin

X-ray crystallography is expected to reveal precise polypeptide chain folding and arrangement of the various domains in the hydrated molecule. Crystallographic studies were made by Cohen et al (39) with a variety of crystals and microcrystals of fibrinogen, modified by a protease in Pseudomonas bacteria. However, such modified fibrinogen may differ markedly from non-modified fibrinogen, of which crystals for x-ray crystallography could not yet been obtained by these authors. It may be necessary to secure for the production of such crystals a fibrinogen preparation which is entirely free of any contamination, such as calcium, plasminogen, among other substances.

Cohen et al (39) claim that their combined studies of x-ray crystallography and electron microscopy of modified fibrinogen preparations can account as well for the architecture of fibrin. These authors emphasized recently that the actual design in the molecular structure and the complexity of fibrinogen is not yet known.

Fibrin-like aggregates of unmodified fibrinogen, that appear identical to fibrin in the electron microscope, were produced by the precipitation of fibrinogen. These fibrin-like arrays were obtained at high ionic strength, where the charges on fibrinogen are shielded, allowing interactions between fibrinogen molecules that do not occur otherwise. Weisel et al (40) emphasized that the most common aggregates show a fibrin-like structure and concluded that they provide additional evidence that fibrinogen is not greatly different from fibrin. However, their fibrinogen preparation is altered or modified. Gollwitzer (41) found aggregation of non-altered or non-modified fibrinogen molecules, as compared to the altered fibrinogen molecules employed by Cohen and her associates in their studies (39,40).

XVIII. Fibrinogenin formation comprising both fibrinogen aggregation and fibrinogen gel clotting

Fibrinogenin formation comprises two phases in the clotting of fibrinogen without thrombin participation. This is shown diagrammatically in Fig. 6. The initial phase is the aggregation of fibrinogen molecules and the subsequent phase is fibrinogen gel clotting. The term "fibrinogenin" is a new generic term which the author introduces for these two phases of fibrinogen clotting. The suffix 'in' added to the word 'fibrinogen' indicates that the term fibrinogenin differs as a clot from the thrombin-induced fibrin. It should be noted that fibrinogenin formation in initial hemostasis was first observed in vivo by Zweifach (18). Witte observed fibrinogenin formation recently in the living capillary circulation and referred to it as "thrombosis in statu nascendi" (25). It is emphasized that, both in thrombogenesis and in the initiation of hemostasis, the white thrombi which were observed biomicroscopically have been interpreted in the past to be agglutinated platelets undergoing viscous metamorphosis. Although the platelets can undergo such rheological changes following their clumping together, many of these observations in the literature probably are not due to viscous metamorphosis of platelets but to in vivo fibrinogenin formation at certain sites of the microcirculation.

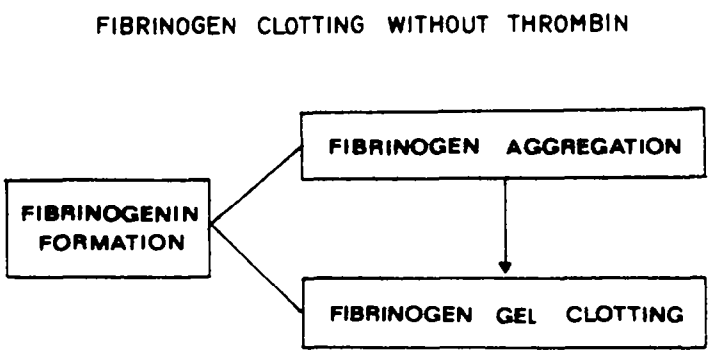

FIG. 6

The two phases of fibrinogenin formation.

XIX. Focal intravascular acidosis as a trigger in thrombogenesis

Thrombus formation may be induced at certain sites of the vessel wall, in particular in the microcirculation, at a pH below 6.4. This claim was made by Copley, King, Kudryk and Blombäck (76-80) on the basis of their studies on the role of pH on the rigidity of surface layers of highly purified fibrinogen.

From Fig. 7 it can be seen that at a pH below 6.4 the rigidity of these surface layers is markedly increased, while at pH above 7.8 a marked decrease of rigidity occurs.

Elastic modulus measurements of surface layers of fibrinogen, made by King (81), at varying pH resulted in similar increases below pH 6.6 and decreases above pH 7.6, as shown in Fig. 8. The highly purified fibrinogen (98-100 per cent clottability) was prepared according to Blombäck and Blombäck (6) and manufactured by IMCO Corporation, S-113 30 Stockholm, Sweden, as already mentioned under IV.

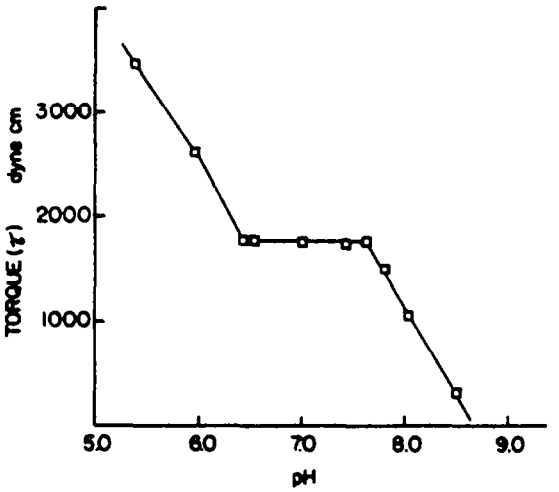

FIG. 7

The effect of pH on the rigidity of surface layers of 0.4% highly purified human fibrinogen solutions employing Tris-maleate buffers.

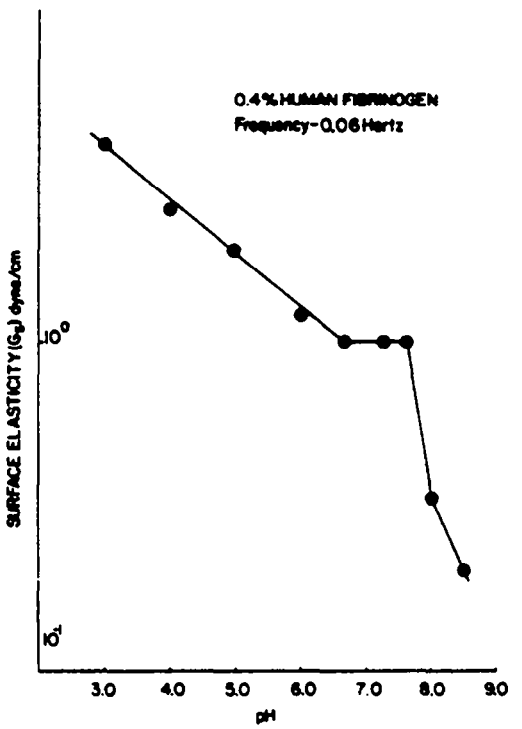

FIG. 8

The effect of pH on the elasticity of surface layers of 0.4% highly purified human fibrinogen solutions at frequencies of 0.06 Hertz. The pH was adjusted to values over the range of 3.0 to 8.5, employing buffers (81). The low frequency of 0.06 Hertz was chosen because it is unlikely to disturb the development of the surface layer.

It is emphasized that most biological cells and surfaces, such as the endothelial cells, are negatively charged and, therefore, the pH at the surface, or in the region where the charges are located, are lower than the bulk pH. Charges occur, according to Seaman (82,83), in patches, arrays or clusters and in such micro-regions the pH could be 2 or 3 or more pH units lower than in the bulk (80,83).

Copley, as well as several other authors, claimed that thrombogenesis can be initiated without an injury to the vascular wall (2, 84). The high wall shear forces may, according to the Blombäck-Copley hypothesis, mentioned earlier under XII, bring about conformational changes of fibrinogen molecules at certain sites of the circulation. Blombäck and Copley suggested that these sites may well be, where there is a lowering of pH (80).

XX. Prostacyclin, platelet clumping, the EEFL and in vivo 'fibrinogenin' formation

The generation of prostacyclin found in layers of the aortic wall, and particularly in the intima, was interpreted by Moncada et al (85,86) to offer an explanation for the antithrombotic properties of the vascular endothelium. They based this interpretation of their findings on the inhibition of platelet clumping by prostacyclin. In several publications Copley discussed these findings (2,87,88). Since Copley and Witte demonstrated (89) that the platelets do not play the primary role in thrombogenesis, it needs to be clarified whether prostacylin prevents fibrinogenin formation and in this way might act as an antithrombogenic agent.

XXI. The differentiation between antithrombogenic and antithrombotic actions

The term 'antithrombogenic' is introduced for the inhibition of thrombogenesis. Such inhibition in the initiation of thrombus formation can be brought about by a property of a surface, such as that of the EEFL, or by an agent.

An antithrombogenic agent would be primarily one which would be used prophylactically for the prevention of fibrinogen gel thrombus formation or of a fibrinogenin thrombus.

The term 'antithrombotic' was redefined by the author who recommended it as a generic term for the inhibition of any of the processes in the formation of a mixed thrombus, similar to that of mixed clotting, as well as for the inhibition in the further development of the pathologic condition of thrombosis. The term 'antithrombotic' should therefore be used for the inhibition or prevention of any of the three clotting processes in vivo, leading to thrombosis. It is applicable as well to counteract any hemorheological and hemodynamic disturbances, which would augment the propagation of the mixed thrombus and lead to the pathological condition of thrombosis.

XXII. On the inhibition of fibrinogenin formation by glycosaminoglycans, dextran and sodium hyaluronate. Aspects of antithrombogenic action

In the author's view, fibrinogenin formation in vivo can be

detected extra vivum with surface hemorheological methods. Measurements of surface viscosity and surface elasticity of fibrinogen systems are, therefore, made for which Copley and King (51) developed a modified Weissenberg rheogoniometer. The viscous and elastic moduli obtained in these measurements appear to give a measure for antithrombogenic activity of substances added to the fibrinogen solution in order to secure these data. Thus far, only this sophisticated research tool, viz., the modified Weissenberg rheogoniometer, is available for such surface hemorheological studies. There is, therefore, a need for the development of an apparatus for clinical use which measures viscous and elastic moduli of surface layers of fibrinogen systems and of plasma. In the near future, there will be an urgent need for such a device which could be similar to that proposed by Copley and designed by King (94).

In an experimental survey, conducted over more than ten years, Copley and King (91) studied the effect of many non-commercial and commercial preparations of heparin on the rigidity and viscoelasticity of fibrinogen surface layers. Among many heparin preparations there are only a few heparins which exhibited decreases in rigidity ($\mathcal{T}$ or torque values), viscous and elastic moduli. In all studies of surface viscosity and surface elasticity of fibrinogen systems we employed highly purified human and bovine fibrinogen (IMCO). Tris-saline buffer at pH 7.4 was used for the fibrinogen systems.

Recent studies by Copley, King and Chien (119) on chondroitins A, B and C and on low molecular weight depolymerization products from commercial heparin, prepared by Lasker (92) are of particular interest. The low molecular weight (LMW) heparins and the chondroitins demonstrated a strong inhibitory action on fibrinogen surface gels.

Figs. 9 and 10 show surface viscosity and surface elasticity values of surface layers of 0.4 per cent fibrinogen with 0.2 percent heparin derivatives of LMW of 4400, 5300 and 5900. While the 4400 MW preparation exhibits a decrease of about 30 per cent (curve with triangles) of the viscous and elastic moduli, as compared to the fibrinogen control (curve with circles), the 5300 and 5900 MW heparin preparations showed decreases of about 75 per cent.

In a just reported study Ockelford et al (93) did not find a relationship between the in vivo antithrombotic effect of low molecular weight (LM) heparin, employing animal thrombosis models, and extra vivum anti-Xa activity. These authors concluded from their findings that "other properties of LM heparins contribute to their antithrombotic effectiveness", since anti-Xa activity, measured extra vivum, does not always predict in vivo antithrombotic efficacy. The efficacy which Ockelford et al observed may be, therefore, due to the antithrombogenic action of LM heparins as our findings of marked decrease in viscous and elastic moduli of surface layers of fibrinogen appear to indicate.

The author appraises the findings by Ockelford et al (93) that in vivo fibrinogenin formation was inhibited in their study and that any measurements of anticoagulant factors could not have mirrored the antithrombogenic action of LM heparins.

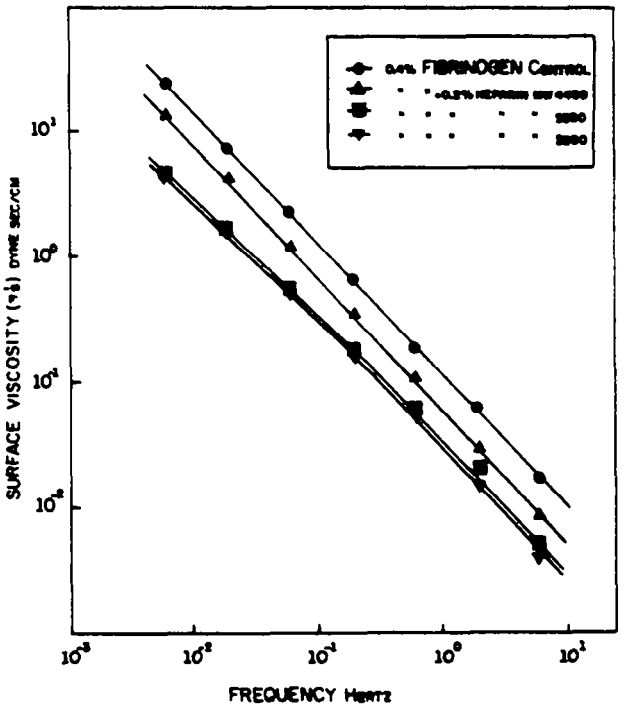

FIG. 9

Surface viscosity (η'_s) dyne sec/cm versus frequency for 0.4% fibrinogen solution as control and with 0.4% fibrinogen plus 0.2% heparin MW 4400, 5300 and 5900.

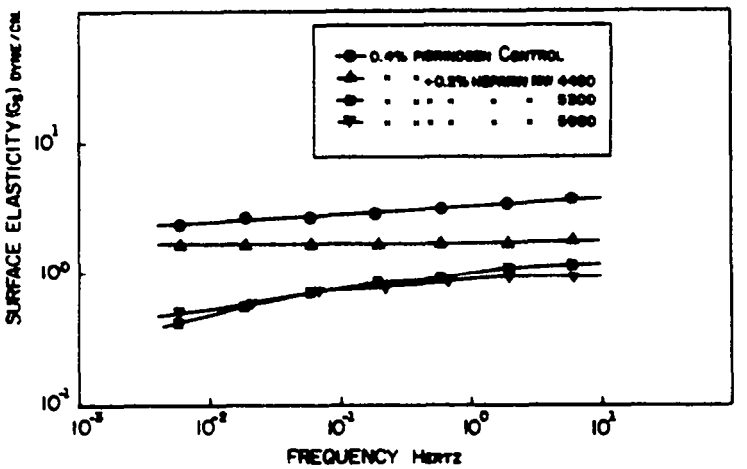

FIG. 10

Surface elasticity (G_s) dyne/cm versus frequency in Hertz for 0.4% fibrinogen solution as control and with 0.4% fibrinogen plus 0.2% heparin MW 4400, 5300 and 5900.

We studied also preparations of glycosaminoglycans other than heparin. Figs. 11 and 12 show the surface viscosity and surface elasticity of fibrinogen-chondroitin systems. These figures demonstrate that chondroitin A, B and C reduce both the viscous and

elastic moduli by about 40 per cent when compared to the fibrinogen control.

In 1968, Morrison et al (95) reported in vivo findings in rabbits of marked antithrombotic action of chondroitin-4-sulfate (chondroitin A), which is one of several natural glycosaminoglycans (96). Recently, Bjornsson et al (96) published their extra vivum

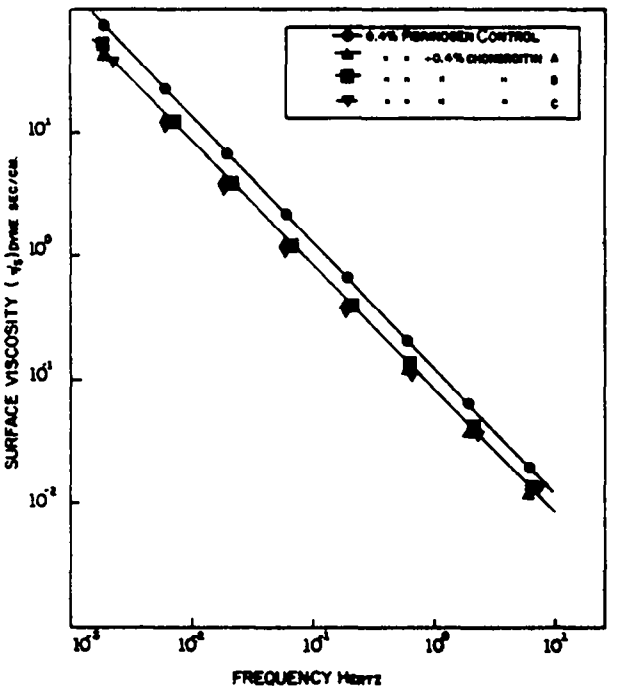

FIG. 11

Surface viscosity (η^{i}_{s}) dyne sec/cm versus frequency for 0.4% fibrinogen solution as control and with 0.4% fibrinogen plus 0.4% chondroitin A, B and C.

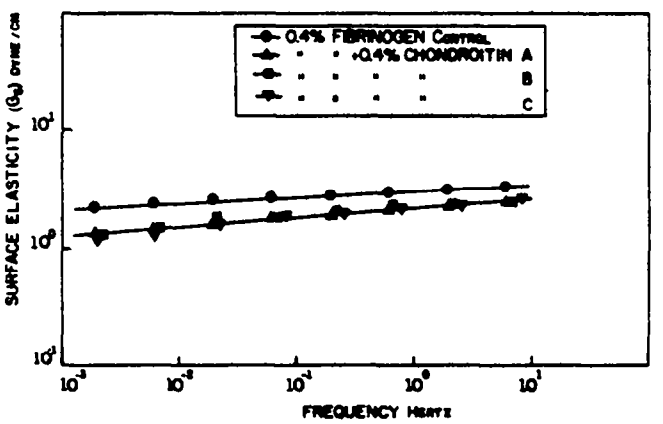

FIG. 12

Surface elasticity (G_s) dyne/cm versus frequency in Hertz and with 0.4% fibrinogen solution plus 0.4% chondroitin A, B and C.

studies with chondroitin A that showed this substance to have certain anticoagulant properties, but that its antithrombotic action could not be fully explained. These authors stated that chondroitin A is presently undergoing clinical trials as an antithrombotic agent, administered orally in daily doses of several grams.

It is quite possible that the antithrombotic action, found by Morrison et al, is in great part antithrombogenic, because of our findings in lowering the rigidity (91) and the viscous and elastic moduli of surface layers of fibrinogen by chondroitin A, as well as by chondroitin B and C. Similar antithrombogenic actions may be expected by the use of other glycosaminoglycans which showed marked decreases in the rigidity and viscoelasticity of surface layers of fibrinogen.

Whether there exists a relationship involving glycosaminoglycans between the EEFL and the microdomains, found by Simionescu, Simionescu and Palade (11,20,21), on the luminal site of the endothelium will need to be clarified.

In preliminary studies of other substances, we found dextran MW 20,000, shown in Fig. 13, to reduce both the viscosity and elasticity of surface layers of fibrinogen by about 50 percent as compared to the fibrinogen control. Dextran sulfate MW 17,000 exhib-

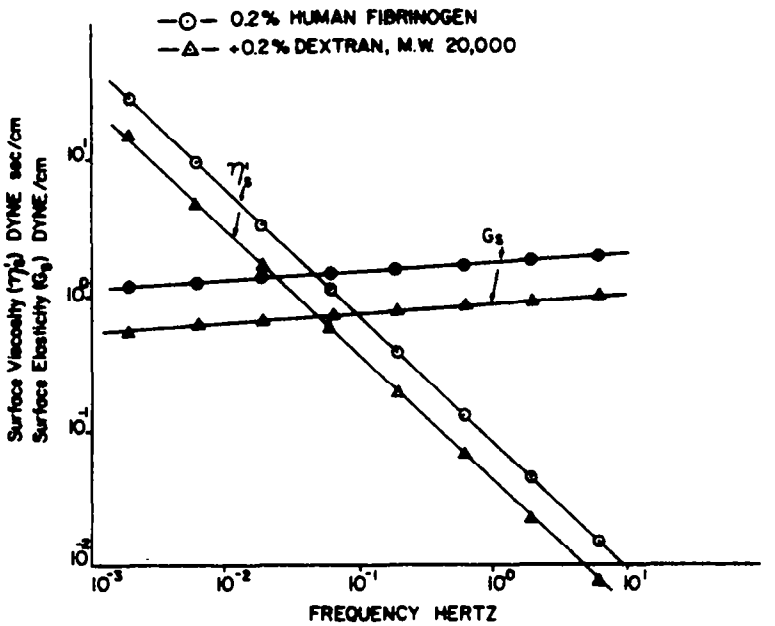

FIG. 13

Viscous and elastic moduli versus frequency, measured from surface layers of a 0.2% human fibrinogen solution (circles) as control. The second preparation (triangles) contain the additional 0.2% dextran, MW 20,000. The open circles and triangles depict viscous moduli while the solid circles and triangles denote elastic moduli.

ited a decrease in elastic moduli by about 50 percent, as shown in Fig. 14, and there was a similar reduction in surface viscocity values, not shown in the figure.

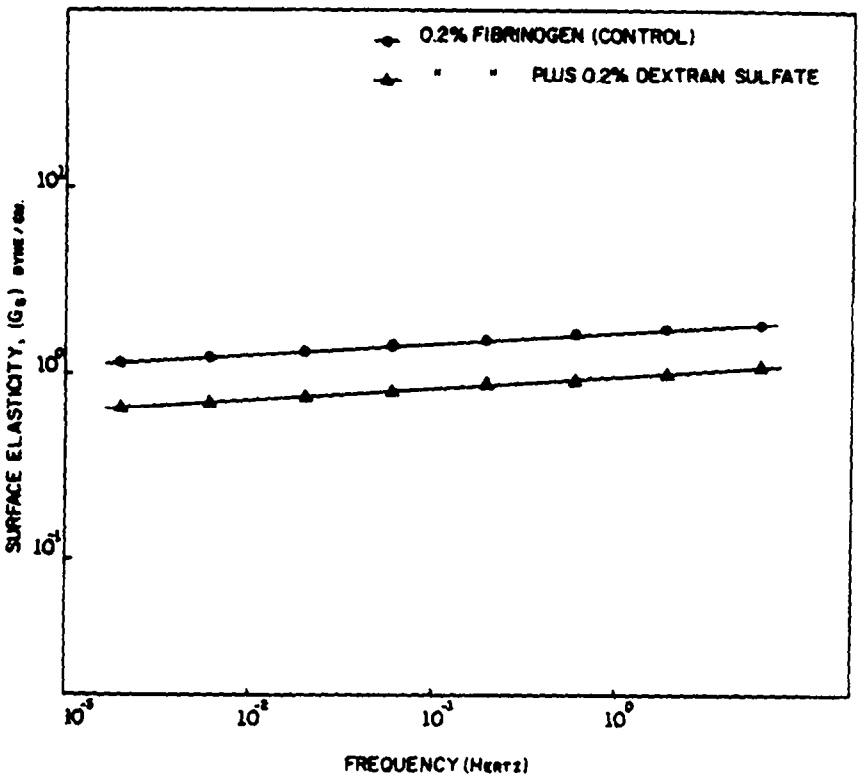

FIG. 14

Surface elasticity values of surface layers of 0.2% fibrinogen solution as control and with the addition of 0.2% dextran sulfate. The fibrinogen control is in circles and the addition of dextran sulfate is in triangles.

Figure 13 shows a reduction of about 50 per cent in viscous and elastic moduli of surface layers of fibrinogen solutions to which sodium hyaluronate was added, as compared to the fibrinogen control.

Our above findings with glycosaminoglycans, dextran and sodium hyaluronate appear to be of great significance in considerations pertaining to the prevention of thrombogenesis. Marked decreases in viscous and elastic moduli of fibrinogen surface gels may provide a tool in establishing the clinical value of an antithrombogenic agent.

The oral administration of agents, such as low molecular weight depolymerization products of heparin or other low molecular glycosaminoglycans, and the intravenous administration of certain dextran preparations or certain other substances may prove to be important in the prevention of thrombotic conditions.

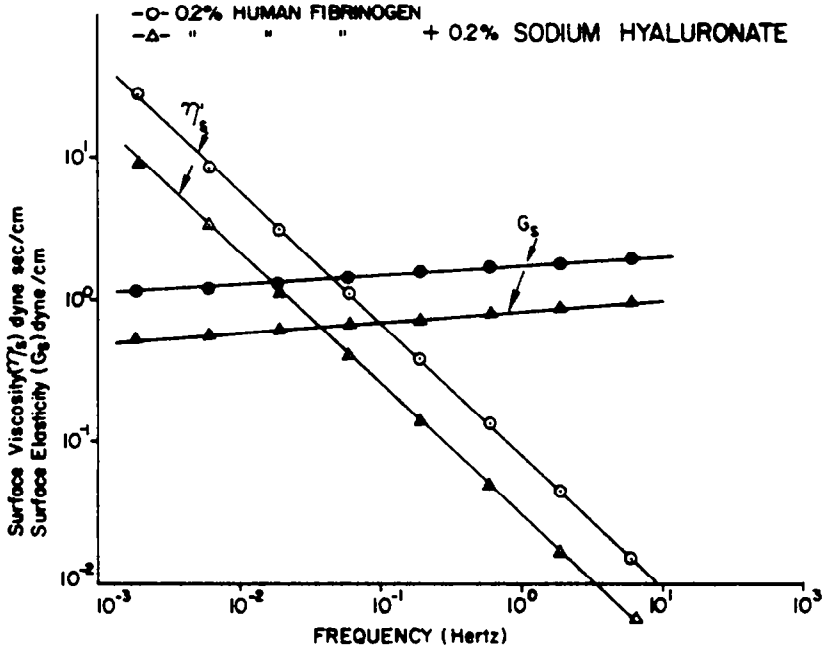

FIG. 15

Viscous and elastic moduli of surface layers of 0.2% sodium hyaluronate added to 0.2% solution of human fibrinogen with 0.2% human fibrinogen as control. The open circles and triangles depict surface viscosity, while the solid circles and triangles denote surface elasticity.

XXIII. The EEFL and extra vivum fibrin surface in relation to wall adherence and wall adhesiveness

Wall adherence and wall adhesiveness are different surface rheological phenomena which appear to be related to each other. Wall adherence of blood or plasma extra vivum may mimic the immobile portion of the plasmatic zone in vivo in which all kinds of processes can take place, undisturbed by the flow of blood. Conversely, wall adhesiveness pertains to rheological properties of the EEFL, and to extra vivum fibrin surfaces.

The phenomenon of wall adherence was first reported by Copley in 1958 (47). Our investigations (120) showed wall adherence to fibrin surface to be lower than to glass or other surfaces (perspex, lusteroid, paraffin). Copley employed also etched glass as a rough surface and reported that glass surface, irrespective of whether smooth or roughened by etching, exhibited always higher wall adherence than fibrin surface (120).

The measurement of wall adherence is as follows: A precision bore tube, 1 mm in diameter and long enough to allow runs up to 500 mm was surrounded by a water jacket and clamped in horizontal position. Air pressure was applied to one end so as to move a 100 mm index of blood, plasma or plasma protein system along 500 mm of the tube at standard velocity of about 96 mm/sec or 63 mm/sec, res-

pectively. The thickness of the adherent layer was calculated from the loss in length of the column or index at the end of its journey. All tests were done in triplicate and averages taken. Agreement between triplicate runs was extremely close. For the calculations of the layer thickness, δ, the formula used was

$$\delta = \frac{R\Delta l}{2\lambda},$$

where R is the radius of the tube, Δl is the length of index lost and λ is the entire length of the adherent layer.

A detailed description of the wall adherence apparatus was given by Copley and Thorley (121). Although many papers on wall adherence by Copley et al were published from 1958 to 1961 (122), the phenomenon has been ignored by many investigators.

In the discussion of Copley's paper Maude (120) considered the phenomenon of wall adherence to be of importance also during dilution of blood and other suspensions for such purposes as counts of blood cellular elements. Maude pointed out that it is common practice to draw the suspension into a pipette, and allow it to run out into a clean vessel. He emphasized that the rise in the concentration of the suspension leaving the pipette will cause an error in dilution values.

Wall adherence may be an important factor in thrombogenesis. This appears to be especially significant with regard to the biomaterials used in cardiovascular prosthetic devices, a point which hitherto has not been dealt with, but needs to be clarified.

Of significance are the findings on wall adhesiveness by Copley et al (123). We found that gels of blood, formed in fibrin-coated or fibrinized glass tubes, exhibited less adhesiveness than on other surfaces and a much higher degree of clot retraction. These findings are similar to experimentally produced non-infected coagulation thrombi in artery and vein segments in dogs which Copley and Stefko (124) reported in 1947. They indicated that such thrombi are potentially more dangerous in causing thrombo-embolization than those which are adhesive to the vessel wall. The markedly lowered adhesiveness of fibrin suface extra vivum explains in great part the non-adhesiveness of the luminal surface of the normal vessel wall which constitutes the EEFL.

Studies on the nature of wall adherence and wall adhesiveness and their relationship to each other are very much needed.

XXIV. Fibrin(ogen) at interfaces and its significance in cardiovascular prostheses

It is not yet generally known - or, if known, not appreciated - that it is the EEFL which provides the most thromboresistant surface Thromboresistance is generally attributed to the endothelial cells in vivo or the endothelium, both words being used interchangeably. The adsorption of fibrin(ogen) at the endothelium interface in the relatively immobile portion of the plasmatic zone is also of considerable relevance to the quest of securing suitable biocompatible materials for prosthetic devices and implants.

The EEFL possesses net negative charges under physiological conditions. These negative charges determine the distribution of other charged components of the system in the immediate vicinity of the EEFL, i.e., endothelial cells, blood cellular elements and plasma proteins. According to G.V.F. Seaman (83) the effective thickness of the electrical double layer is about 8 Å under physiological conditions and consequently such charge effects will be operative over only such short distances. Slip between the molecular layers of water would occur in the suspending medium (83).

The adhesion of platelets is not the initial event at the blood-biomaterial border or at non-physiological boundaries (97-101). It is the spontaneous adsorption of proteinaceous matter which is considered by Vroman et al to be fibrinogen, and it may very well be fibrin or a fibrin-like material. The latter could be identical to fibrinogen which may have undergone conformational changes according to the Blombäck-Copley hypothesis due to the high shear forces at the wall of the prosthesis (2,51).

Normal plasma or blood deposits predominantly fibrinogen within 2 seconds onto glass and similar surfaces according to Vroman et al (97-100). These authors found in normal human plasma deposits that fibrinogen is preferentially deposited on many surfaces, but that this fibrinogen is rapidly displaced by high molecular weight kininogen on wettable activation surfaces (100). Platelets adhere only where fibrinogen remains. Vroman et al found most recently (101) that fibrinogen, deposited by plasma in narrow spaces (between 20 μ and 1 μ) where flow was minimal, remains for several hours. This suggests, according to these authors, that diffusion of high molecular weight kininogen fails to reach the surface and that platelets adhere.

XXV. The EEFL and/or in vivo fibrinogenin formation in the pathophysiology of various diseases

Thrombogenesis, (non-thrombocytopenic) vascular purpura, and leukocyte emigration in inflammation have been dealt with above.

Although there does not appear to be a direct relationship between the EEFL and decompression sickness (DS), our data on the rigidity of surface layers of fibrinogen in contact with air provide a new basis for the understanding of DS (2). The viscosities of these surface layers were several million poises (1,2), as calculated on the basis of an estimated layer thickness of 100 Å. The coating by fibrinogen and other plasma proteins make the embolizing air bubbles behave like highly rigid bodies. This knowledge pertaining to the formation and physical nature of these emboli may open new ways in the prevention and treatment of DS.

Since more than one hundred years, hyperthermia therapy of cancer is known to be responsible for the destruction of cancerous tissue (103). Ardenne et al (2,102,103) maintained that decrease in pH below 6.5 was responsible for the destruction of cancer in patients. We found that at these low pH values fibrinogen surface layers would become highly rigid, as can be seen in Fig. 7. These findings were related by the author to the triggering of thrombogenesis (103). The so-called Cancer Multistep Therapy (CMT) was

introduced by von Ardenne et al (102). They claimed that the selective destruction of the cancer tissue interrupts the microcirculation by the administration of a combination therapy of selective hyperacidification, brought about by an increase of the blood glucose concentration up to 500 mg/100 ml for about 4 hours, combined with local hyperthermia, employing their CMT. Their treatment was discussed by Copley (3,103), who recommended strict safeguards by the additional infusions of fibrinogen and platelet suspensions to prevent hemorrhages in different parts of the body. Such infusions are also needed to replenish the supply of fibrinogen and platelets in addition to the acidification of cancerous tissues by the combined glucose infusions and CMT. The clinical management in the therapeutic production of thrombi in regions of the body affected by cancerous tissues will need to be well controlled.

In 1978, a new theory of atherogenesis was proposed by the author (84,104,105), diagrammatically shown in Fig. 16. It is based on the adsorption of β-lipoprotein or low density lipoproteins (LDL) via two pathways which are: (A) on the EEFL (106,107) and (B) on fibrinogen gels, loosely structured due to an action of β-lipoprotein which we discovered (22). Furthermore, the theory is based on recently postulated theoretical approaches by Oka (46, 108-110), involving hemorheology, polymer physics and other physical factors, as well as on recent surface chemical findings by Miller et al (111).

It is emphasized that of particular significance for the author's theory are the adsorption experiments by Israel Miller et al of the Weizmann Institute, who studied cholesterol exchange

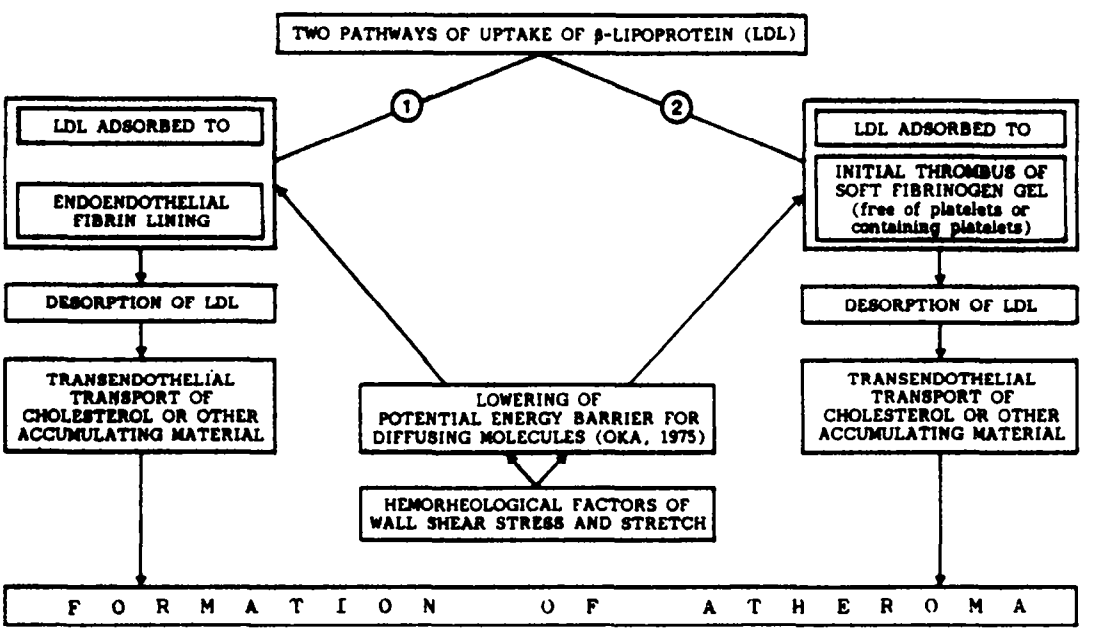

FIG. 16

Schematic diagram of the author's theory of the genesis of atheroma formation.

between surface layers and plasma proteins in bulk. These authors found that lipoprotein molecules can be desorbed readily and may maintain transient contact with the proteinaceous blood vessel surface. Copley (84,105) considers such a surface to be mainly fibrin and/or fibrinogen. Their metabolic digestion, leaving the lipids behind, may then result in the formation of fatty streaks, lesions and atheromas. Hemorheological and other physical factors of wall shear stress and stretch, proposed by Oka (109,110), come into play prior to or simultaneous with the processes of adsorption and desorption of β-lipoprotein and other accumulating material.

XXVI. Conclusions

There are a number of problems pertaining to the endoendothelial fibrin lining (EEFL) and fibrinogen gel clotting or fibrinogenin formation in vivo, which need to be attacked experimentally. Some of these problems relate to the production of the EEFL, the regulation between fibrin formation and fibrinolysis in the more or less immobile portion of the plasmatic zone, the relation of fibrinogen to the EEFL and/or the endothelium (endothelial cells plus interendothelial cement substance), as well as the EEFL's role as the first filtration barrier, followed by the endothelium and the basement membrane as second and third barriers, respectively. Other problems concern wall adherence and wall adhesiveness and their relationship to thrombogenesis and thromboembolization, respectively. Studies are needed which relate both fibrinogen and in vivo 'fibrinogenin' formation to the EEFL. We know already that a low pH may trigger fibrinogenin formation in vivo, but this may be only one of the mechanisms in the initiation of physiological hemostasis and in thrombogenesis. In future studies, it will need to be clarified to which extent fibrin(ogen) is contained in the interendothelial cement substance and in the basement membrane and in which ways it plays a role in capillary permeability.

During the past forty years blood coagulation or thrombin-induced fibrin clotting has been investigated to an unusually high degree by hundreds of enthusiastic investigators. Many factors have been separated in this kind of clotting system and their roles in several mechanisms have been studied. The author trusts that fibrinogenin formation, a process occurring without participation of thrombin, will be investigated both extra vivum and in vivo with similar great enthusiasm as thrombin-induced fibrin clotting.

Numerous communications in the literature deal with the endothelium and, in particular, the endothelial cells without referring in any way to the in vivo situation, where the endoendothelial fibrin lining is that part of the luminal site of the vascular wall closest to the endothelium and to the flowing blood. The author considers the EEFL as the essential interface of the vessel-blood organ and of utmost significance in numerous physiological and pathological processes.

REFERENCES

1. COPLEY, A.L. The endoendothelial fibrin lining: A historical account. In: The Endoendothelial Fibrin Lining, A.L. Copley (Ed.), Oxford-New York, Pergamon Press, 1983, pp. 1-26; Thrombosis Res. 30 Suppl. V, 1-26, 1983.

2. COPLEY, A.L. Hemorheological aspects of the endoendothelial fibrin lining and of fibrinogen gel clotting. Their importance in physiology and pathological conditions. Clin. Hemorheology, 1, 9-72, 1981.

3. COPLEY, A.L. The clinical significance of the endoendothelial fibrin lining and of in vivo fibrinogen gel clotting. In: Verhandlungen der Deutschen Gesellschaft für Innere Medizin. 88. Kongress, 18-22 April 1982, Wiesbaden, B. Schlegel (Ed.) München, J.F. Bergmann Verlag, 1982, pp. 1385-1402.

4. COPLEY, A.L. Plenary lecture. The future of the science of biorheology. Biorheol. 19, 47-69, 1982.

5. COPLEY, A.L. Some problems in hemorheology. In: Proc. 5. Internat. Congr. on Rheology. S. Onogi (Ed.) Tokyo, Japan, Baltimore, MD, USA and Manchester, England, University of Tokyo Press and University Park Press, 1970, vol. 2, pp. 3-25.

6. BLOMBÄCK, B. and BLOMBÄCK, M. Purification of human and bovine fibrinogen. Arkiv. Kemi. 10, 415-443, 1956.

7. BLOMBÄCK, B. and OKADA, M. Fibrin gel structure and clotting time. Thrombosis Res. 25, 51-70, 1982.

8. BLOMBÄCK, B. and OKADA, M. On pores in fibrin gels. Thrombosis Res. 26, 141-142, 1982.

9. OKADA, M. and BLOMBÄCK, B. Factors influencing gel structure studied by flow measurement. Ann. N.Y. Acad. Sci. 408, 1983, in press.

10. PALADE, G.E., SIMIONESCU, M. and SIMIONESCU, N. Structural aspects of the permeability of the microvascular endothelium. Acta Physiol. Scand. 463, 11-32, 1979.

11. PALADE, G.E., SIMIONESCU, M. and SIMIONESCU, N. Differentiated microdomains on the luminal surface of the capillary endothelium. Biorheol. 17, 563-568, 1981.

12. COPLEY, A.L. The endoendothelial fibrin film and fibrinolysis. Proc. VIII. Internat. Congr. Hematol., Tokyo, 1960. Tokyo, Pan-Pacific Press, 1962, vol. 3, pp. 1648-1666.

13. COPLEY, A.L. Capillary permeability, capillary incontinence, compaction stasis and basement membrane breakdown. Bibl. Anat. 7, 148-155, 1965.

14. COPLEY, A.L. On cement fibrin, a proposed constituent of the

capillary basement membrane, and on lesions in the capillary wall in diabetes mellitus. In: Diabetes, Proc. 6. Congr. Internat. Diabetes Fed., Stockholm, Sweden, 1967. J. Östman and R.D.G. Milner (Eds.). Amsterdam: Excerpta Medica Foundation, 1969, pp. 606-609.

15. COPLEY, A.L. Vascular integrity and the endoendothelial fibrin film. Proc. 8. Congr. Europ. Soc. Haematol. Vienna 1961. Basel-New York: S. Karger, 1962, N° 357, pp. 1-20.

16. COPLEY, A.L. Vascular purpura in guinea-pigs and hamsters produced by guinea-pig antifibrin rabbit serum. Transact. 6. Congr. Europ. Soc. Haemat., Copenhagen, 1957. Basel-New York: S. Karger, 1958, pp. 518-522.

17. COPLEY, A.L. and CHAMBERS, R. Experimentally induced petechial hemorrhage and white embolization in the rabbit's nictitating membrane. Am. Heart J. 45, 237-251, 1953.

18. ZWEIFACH, B.W. The exchange of materials between blood vessels and lymph compartments. In: 5th Conf. on Connective Tissue and Lymph Compartments. Josiah Macy Jr. Foundation, New York, 1954. C. Ragan (Ed.), pp. 38-77.

19. ZWEIFACH, B.W. Structural makeup of capillary wall. Ann. N.Y. Acad. Sci. 61, 670-677, 1955.

20. SIMIONESCU, N., SIMIONESCU, M. and PALADE, G.E. Differentiated microdomains on the luminal surface of the capillary endothelium. I. Preferential distribution of anionic sites. J. Cell Biol. 90, 605-613, 1981.

21. SIMIONESCU, M., SIMIONESCU, N. and PALADE, G.E. Differential microdomains on the luminal surface of the capillary endothelium. II. Partial characterization of their anionic sites. J. Cell Biol. 90, 614-621, 1981.

22. COPLEY, A.L. and KING, R.G. The reducing action of highly purified γ-globulin and β-lipoprotein on the viscous resistance of surface layers of fibrinogen. Thrombosis Res. 4, 193-198, 1974.

23. COPLEY, A.L. Non-Newtonian behavior of surface layers of human plasma protein systems and a new concept of the initiation of thrombosis. Biorheology 8, 79-84, 1971.

24. COPLEY, A.L. and KING, R.G. Viscous resistance of thromboid (thrombus-like) surface layers in systems of plasma proteins including fibrinogen. Thrombosis Res. 1, 5-17, 1972.

25. WITTE, S. Vitalmikroskopische Untersuchungen über das Verhalten von Fibrinogen in der Mikrozirkulation. In: Fibrinogen, Fibrin and Fibrin Glue, Side Effects of Therapy with Clotting Factor Concentrates. K. Schimpf (Ed.), Stuttgart-New York: F. K. Schattauer Verlag, 1980, pp. 85-89.

26. WITTE, S. The endoendothelial lining as studied by a fluores-

cent labeling technique in situ. In: The Endoendothelial Fibrin Lining. A.L. Copley (Ed.), New York-Oxford, Pergamon Press, 1983, pp. 93-104; Thrombosis Res. 30, Suppl. V, 93-104, 1983.

27. PALADE, G.E. Personal communication, 1982.

28. RENKIN, E.M. and CURRY, F.E. Endothelial permeability: Pathways and modulations. Ann. N.Y. Acad. Sci. 401, 248-259, 1982.

29. MICHEL, C.. The investigation of capillary permeability in single vessels. Acta Physiol. Scand. Suppl. 463, 67-74, 1979.

30. CHAMBERS, R. and ZWEIFACH, B.W. Capillary endothelial cement in relation to permeability. J. Cell. Comp. Physiol. 15, 255-272, 1940.

31. COPLEY, A.L. Capillary permeability versus fragility and the significance of fibrin as a physiologic cement of the blood vessel wall. Bibl. Anat. 4, Basel-New York: S. Karger, 1964, pp. 3-19.

32. NOLF, P. Contribution à l'étude de la coagulation du sang. La fibrinolyse. Arch. internat. physiol. 6, 306-309, 1908.

33. MOROZ, L.A. Normal blood fibrinolytic activity: Its mandatory existence and role in intravascular homeostasis. Thrombosis Res. 18, 585-588, 1982.

34. COPLEY, A.L., HANIG, J.P., LUCHINI, B.W. and ALLEN, JR., R.L. On the capillary permeability enhancing activity of isolated fibrinopeptides and their role in the physiology of the blood capillary wall. Proc. IV Europ. Conf. Microcirculation. Cambridge, England, 1966. H. Harders (Ed). Basel-New York: S. Karger. pp. 475-481; Bibl. Anat. 9, 475-481, 1967.

35. COPLEY, A.L. and CAROL, B. Inhibiting action of epsilon aminocaproic acid on capillary permeability in guinea-pigs with new quantitative method based on Straus' peroxidase procedure. Variance and covariance analyses. Life Sciences 3, 65-76, 1964.

36. ASCHHEIM, E., TSULUCA, V. and COPLEY, A.L. Quantitative relation between certain fibrinolysis factors and inflammation. Proc. Soc. Exp. Biol. Med. 112, 434-436, 1963.

37. KARNOVSKY, M.J. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. 35, 213-236, 1967.

38. STEWART, G.J., LYNCH, P.R., REICHLE, F.A., RITCHIE, W.G.M., SMITH, A. and SCHAUB, R.G. The adhesion of leukocytes, erythrocytes, and non-cellular material to the luminal surface of natural and artificial blood vessels in vivo. Ann. N.Y. Acad. Sci. 283, 179-207, 1977.

39. COHEN, C., WEISEL, J.W., PHILLIPS, JR. G.N., STAUFFACHER, C.V., FILLERS, J. and DAUB, E. Structure of fibrinogen and fibrin: I. Electron microscopy and X-ray crystallography of fibrinogen. Ann. N.Y. Acad. Sci. 408, 1983, in press.

40. WEISEL, J.W., PHILLIPS, JR. G.N. and COHEN, C. Structure of fibrinogen:II. Architecture of the fibrin clot. Ann. N.Y. Acad. Sci. 408, 1983, in press.

41. GOLLWITZER, R., BODE, W. and KARGES, H.E. On the aggregation of fibrinogen molecules. In: The Endoendothelial Fibrin Lining. A.L. Copley (Ed.), New York-Oxford, Pergamon Press, 1983, pp. 41-53; Thrombosis Res. 30, Suppl. V, 41-53, 1983.

42. FRY, D.L. Acute vascular endothelial changes associated with increased blood velocity gradients. Circulation Res. 22, 165-197, 1968.

43. FRY, D.L. Responses of the arterial wall to certain physical factors. In: Atherogenesis: Initiating Factors. A Ciba Foundation Symposium. 1972. R. Porter and J. Knight (Eds.), Elsevier-Excerpta Medica North:Holland, Amsterdam, 1973, pp. 93-125.

44. OKA, S. Poiseuille award lecture: Present status of hemorheological theory. Biorheology, 12, 157-168, 1975.

45. OKA, S. Copley-Scott Blair phenomenon and electric double layer. Biorheology, 18, 347-353, 1981.

46. OKA, S. Cardiovascular Hemorheology. Cambridge-New York: Cambridge University Press, 1981, pp. 57-60.

47. COPLEY, A.L. Adherence and viscosity of blood contacting foreign surfaces and the plasmatic zone in blood circulation. Nature (London), 181, 551-552, 1958.

48. COPLEY, A.L. and SCOTT BLAIR, G.W. Comparative observations on adherence and consistency of various blood systems in living and artificial capillaries. Rheol. Acta. 1, 170-176, 1958: 665-668, 1961.

49. TAMAMUSHI, B. Surface chemical aspects of flow characteristics of blood. In: Theoretical and Clinical Hemorheology. H.H. Hartert and A.L. Copley (Eds.), Berlin: Springer Verlag, 1971, pp. 99-103.

50. MÜLLER, H.K. Functional coupling between endothelial layer and capillary flow. In: Theoretical and Clinical Hemorheology. H. H. Hartert and A.L. Copley (Eds.), Berlin: Springer-Verlag, 1971, pp. 206-212.

51. COPLEY, A.L. and KING, R.G. Polymolecular layers of fibrinogen systems and the genesis of thrombosis. In: Hemorheology and Thrombosis. A.L. Copley and S. Okamoto (Eds.), Oxford-New York: Pergamon Press, 1976, pp. 393-409; Thrombosis Res. 8, Suppl. II, 393-409, 1976.

52. WHITMORE, R.L. Rheology of the Circulation. Oxford-New York: Pergamon Press, 1968.

53. CHIEN, S. Present state of blood rheology. In: Hemodilution. Theoretical Basis and Clinical Application. K. Messmer and H. Schmid-Schönbein (Eds.). Basel: S. Karger, 1972, pp. 1-45.

54. CHARM, S.E. and KURLAND, G.S. Blood Flow and Microcirculation, New York: John Wiley & Sons, 1974.

55. KUDRYK, B.J., COLLEN, D., WOODS, K.R. and BLOMBÄCK, B. Evidence for localization of polymerization sites in fibrinogen. J. Biol. Chem. 249, 3322-3325, 1974.

56. BLOMBÄCK, B., HESSEL, B., HOGG, D. and THERKILDSEN, L. A two-step fibrinogen-fibrin transition in blood coagulation. Nature (London), 257, 501-505, 1978.

57. CHARM, S.E. and WONG, B.L. Shear degradation of fibrinogen in the circulation. Science, 170, 466-468, 1970.

58. MACFARLANE, R.G. Blood coagulation with particular reference to the early stages. Physiol. Rev. 36, 479-502, 1956.

59. SHUMAN, M.A., ISAACS, J.D., MAEROWITZ, T., SAVION, N., GOSPODAROWICZ, D., GLENN, K., CUNNINGHAM, D. and FENTON, J.W.O. Endothelial cell and platelet binding sites for thrombin. Ann. N.Y. Acad. Sci. 370, 57-66, 1981.

60. LOSKUTOFF, D.J. Effect of thrombin on the fibrinolytic activity of cultured bovine endothelial cells. J. Clin. Invest., 64, 329-336, 1979.

61. COPLEY, A.L. On the anticoagulant action of fibrin in the prevention of thrombosis. Proc. IX. Congr. Hematol. Mexico City, Mexico 1962. Mexico City: Universidad Nacional Autonoma de Mexico, 1964, pp. 367-378.

62. SCHMID-SCHÖNBEIN, G.W., SHIH, Y.Y. and CHIEN, S. Morphometry of human leukocytes. Blood, 56, 866-875, 1980.

63. SCHMID-SCHÖNBEIN, G.W., SUNG, K.-L.P. and TOZEREN, H. Passive mechanical properties of human leukocytes. Biophys. J. 36, 243-256, 1981.

64. CHIEN, S. Hemodynamics and rheology in the microcirculation. In: Symposium on Recent Developments in Microcirculation Research. E. Davis and G.A. Marcel (Eds.) Amsterdam-Oxford-Princeton: Excerpta Medica, 1981, pp. 54-69.

65. PLOW, E.F. and EDGINGTON, T.S. An alternative pathway for fibrinolysis. I. The cleavage of fibrinogen by leukocyte proteases at physiologic pH. J. Clin. Invest. 56, 30-38, 1975.

66. RIDDLE, J.M. and BARNHART, M.I. Ultrastructural study of fibrin dissolution via emigrated polymorphonuclear neutrophils. Am. J. Path. 45, 805-823, 1964.

67. BARNHART, M.I., RIDDLE, J.M., BLUHM, G.B. and QUINTANA, C. Fibrin promotion and lysis in arthritic joints. Ann. Rheum. Dis. 26, 206-218, 1967.

68. OPIE, E.L. Experimental pleurisy - Resolution of a fibrinous exudate. J. Exp. Med. 9, 391-413, 1907.

69. MENKIN, V. Biochemical Mechanisms in Inflammation. Springfield, Illinois, Charles C. Thomas, 1956.

70. JANCSO, N. Inflammation and the inflammatory mechanisms. J. Pharm. Pharmacol. 13, 577-594, 1961.

71. BARNHART, M.I. Role of blood coagulation in acute inflammation. Biochem. Pharmacol. 17, Suppl. 205-219, 1968.

72. CLARK, W.G. and JACOBS, E. Experimental nonthrombocytopenic vascular purpura: A Review of the Japanese literature, with preliminary confirmatory report. Blood, 5, 320-328, 1950.

73. COPLEY, A.L. and GÉLOT, B. Experimental production of vascular purpura in guinea-pigs by guinea-pig anti-fibrin serum of rabbits. Am. J. Physiol., 187, 593, 1956 (Abstr.).

74. COPLEY, A.L. and MARÉCHAL, J. Le taux de fibrinogène et la numération des plaquettes dans le purpura vasculaire produit chez le cobaye par du sérum de lapin anti-fibrine de cobaye. J. Physiologie, 49, 987-991, 1957.

75. SEAMAN, G.V.F. Plasma protein interactions at biological interfaces. In: The Endoendothelial Fibrin Lining. A.L. Copley (Ed.), New York-Oxford: Pergamon Press, 1983, pp. 83-91; Thrombosis Res. 30, Suppl. V, 83-91, 1983.

76. COPLEY, A.L. KING, R.G., KUDRYK, B. and BLOMBÄCK, B. Viscous resistance of surface layers of highly purified fibrinogen systems. Proc. VII. Internat. Congr. Rheology, Gothenburg, Sweden, August 1976, p. 95.

77. COPLEY, A.L. Hemorheological phenomena pertaining to thrombus formation and thromboembolization, their physiological role and clinical significance. Book of Abstracts. IX. World Conf. Europ. Soc. Microcirculation, Antwerp, Belgium, 1976, pp. 353-354.

78. COPLEY, A.L. New hemorheological findings on surface layers of fibrinogen systems and their relationship to thrombogenesis. Symposium on Hemorheological Aspects of Thrombosis. 16. Internat. Congr. Hematol. Kyoto, 1976. Abstracts, Main Topics and Symposia, p. 156.

79. COPLEY, A.L. On the endoendothelial fibrin lining and on fibrinogen clotting without thrombin action. Their significance in health and disease. In: European Symposium on Physiopathology of Veins and Capillaries, Rome, Italy, April 1978. Paris, Monographie Thérapeutique, Gazette Médicale de France, 1979, pp. 2-16.

80. COPLEY, A.L., KING, R.G., KUDRYK, B. and BLOMBÄCK, B. Visco-elasticity studies of surface layers of highly purified fibrinogen systems and the role of pH on the rigidity of such layers. Thrombosis Res. 18, 213-230, 1980.

81. KING, R.G. Personal communication, 1982.

82. SEAMAN, G.V.F. Electrokinetic behavior of red cells. In: The Red Blood Cell Vol. 2, 2nd ed., D. MacN. Surgenor (Ed.), New York: Academic Press, 1975, pp. 1135-1229 (See p. 1153).

83. SEAMAN, G.V.F. Personal communication, 1980.

84. COPLEY, A.L. Fibrin(ogen), platelets and a new theory of atherogenesis. Thrombosis Res. 14, 249-263, 1979.

85. MONCADA, S., HERMAN, A.G., HIGGS, E.A. and VANE, J.R. Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thrombosis Res. 11, 323-344, 1977.

86. MONCADA, S. and VANE, J.R. Prostacyclin, platelet aggregation and thrombosis. In: Platelets. A Multidisciplinary Approach, Florence, Italy, 1977. G. de Gaetano and S. Garattini (Eds.), New York: Raven Press, 1978, pp.239-258.

87. COPLEY, A.L. Platelets and physiological defense mechanisms. In: Internat. Symposium: Platelets. A Multidisciplinary Approach, Florence, Italy, 1977. G. de Gaetano and S. Garattini (Eds.), New York: Raven Press, 1978, pp. 161-197.

88. COPLEY, A.L. Roles of platelets in physiological defense mechanisms and pathological conditions. Folia Haematol. (Leipzig), 106, 732-764, 1979.

89. COPLEY, A.L. and WITTE, S. On physiological microthromboembolization as the primary platelet function. Elimination of invaded particles from the circulation and its pathogenic significance. Thrombosis Res. 8, 251-262, 1976.

90. COPLEY, A.L. and KING, R.G. Viscous resistance lowering action of highly purified gamma globulin on surface layers of fibrinogen and other plasma proteins. Abstracts, IV. Internat. Congr. on Thrombosis and Haemostasis, 1973, Vienna, Austria, p. 356.

91. COPLEY, A.L. and KING, R.G. Surface hemorheological studies on the inhibition of fibrinogenin formation employing surface layers of fibrinogen systems with heparins and other substances. A contribution on antithrombogenic action. Thrombosis Res. 31, 1983, in press.

92. LASKER, S.E. The heterogeneity of heparins. Federation Proc., 36, 92-97, 1977.

93. OCKELFORD, P.A., CARTER, C.J., MITCHELL, L. and HIRSH, J. Discordance between the anti-Xa activity and the antithrombo-

tic activity of an ultra-low molecular weight heparin fraction. Thrombosis Res. 28, 401-409, 1982.

94. COPLEY, A.L. Certain aspects of hemorheology in a near zero gravity environment. Biorheology 16, 37-49, 1979.

95. MORRISON, L.M., RUCKER, P.G. and ERSHOFF, B.H. Prolongation of thrombus-formation time in rabbits given chondroitin sulfate A. J. Atheroscler. Res. 8, 319-327, 1968.

96. BJORNSSON, T.D., NASH, P.V. and SCHATEN, R. The anticoagulant effect of chondroitin-4-sulfate. Thrombosis Res. 27, 15-21, 1982.

97. VROMAN, L., ADAMS, A., KLINGS, M. and FISCHER, G. Fibrinogen, globulins, albumin and plasma at interfaces. Adv. Chem. Series 145, 255-289, 1975.

98. VROMAN, L., ADAMS, A.L., KLINGS, M., FISCHER, G.C., MUNOZ, P. C. and SOLENSKY, R.P. Reactions of formed elements of blood with plasma proteins. Ann. N.Y. Acad. Sci. 283, 65-76, 1977.

99. ADAMS, A.L., FISCHER, G.C. and VROMAN, L. The complexity of blood at simple interfaces. J. Coll. Interface Sci. 65, 468-478, 1978.

100. VROMAN, L., ADAMS, A.L., FISCHER, G.C. and MUNOZ, P.C. Interaction of high molecular weight kininogen, factor XII and fibrinogen in plasma at interfaces. Blood 55, 156-159, 1980.

101. VROMAN, L., ADAMS, A.L., FISCHER, G.C. MUNOZ, P.C. and STANFORD, M. Proteins, plasma and blood in narrow spaces of clot-promoting surfaces. In: Advances in Chemistry. K.L. Mittal (Ed.) 199, 265-276, 1982.

102. ARDENNE, M.v., BÖHME, G. and KELL, E. On the optimization of local hyperthermy in tumors based on a new radiofrequency procedure. Local hyperthermy of large body areas using the CMT Selectotherm method. J. Cancer Res. Clin. Oncol. 94, 163-184, 1979.

103. COPLEY, A.L. Fibrinogen gel clotting, pH and cancer therapy. Thrombosis Res. 18, 1-6, 1980.

104. COPLEY, A.L. Hemorheology and tissue ischemia. 4. Kölner Symposium on the Disorders of Circulation of Brain and Heart (Brain and Heart Infarct), Max-Planck-Institut für Hirnforschung. Cologne, 22.-24. June, 1978, pp. 4-5. (Abstr.)

105. COPLEY, A.L. Hemorheology and tissue ischemia. In: Brain and Heart Infarcts II. K.J. Zülch, W. Kaufmann, K.A. Hossmann and V. Hossmann (Eds.), Heidelberg-Berlin-New York: Springer-Verlag, 1979, 72-87.

106. COPLEY, A.L. Fibrinolysis and atherosclerosis. Lancet (London) i, 102-103, 1957.

107. COPLEY, A.L. Atherosclerosis in a haemophiliac. Lancet (London) ii, 1119-1120, 1957.

108. OKA, S. Physical theory of permeability of vascular walls in relation to atherogenesis. Biorheology 15, 1978, p. 480. (Abstr.)

109. OKA, S. Theoretical approach to the effect of shear stress on the development of atheroma. In: Hemorheology and Thrombosis. A.L. Copley and S. Okamoto, (Eds.). New York-Oxford: Pergamon Press, 1976, pp. 305-313; Thrombosis Res. 8, Suppl. II, 305-313, 1976.

110. OKA, S. Physical theory of permeability of vascular walls in relation to atherogenesis. Biorheology 16, 203-209, 1979.

111. MILLER, I.R., GRAET, H. and FREI, Y.F. Cholesterol exchange between surface layers and plasma proteins in bulk. In: Atherogenesis: Initiating Factors, A Ciba Foundation Symposium, 1972. R. Porter and J. Knight, (Eds.). Amsterdam: Assoc. Sci. Publ., 1973, pp. 251-266.

112. COPLEY, A.L. and SEAMAN, G.V.F. The meaning of the terms rheology, biorheology and hemorheology. Clin. Hemorheology, 1, 117-119, 1981.

113. SHEN, L.L., HERMANS, J., McDONAGH, J., McDONAGH, R.P. and CARR, M. Effects of calcium ion and covalent cross-linking on formation and elasticity of fibrin gels. Thrombosis Res. 6, 255-265, 1975.

114. COPLEY, A.L. and KING, R.G. A note on the effect of Ca^{2+} on the viscous and elastic moduli of surface layers of fibrinogen. Colloid & Polymer Sci. 1983, in press.

115. CURRY, F.E. and MICHEL, C.C. A fiber matrix model of capillary permeability. Microvasc. Res. 20, 96-99, 1980.

116. COPLEY, A.L. and THORLEY, R.S. Fibrinization and coating with other materials of glass surface for haemorheological studies. Laboratory Practice (London) 11, 283-286, 1962.

117. OSSOFF, R. and CHARM, S.E. Blood viscosity reduction in negative charged capillary tubes. Biorheology, 11, 293-299, 1974.

118. OKA, S. Biorheological aspect in relation to endoendothelial fibrin lining. In: The Endoendothelial Fibrin Lining. A.L. Copley (Ed.), New York-Oxford: Pergamon Press, 1983, pp. 61-65; Thrombosis Res. 30, Suppl. V, 61-65, 1983.

119. COPLEY, A.L., KING, R.G. and CHIEN, S. On the antithrombogenic action of low molecular weight heparins and of chondroitins A, B and C. Biorheology 20, 1983, in press.

120. COPLEY, A.L. Apparent viscosity and wall adherence of blood

systems. In: Flow Properties of Blood and Other Biological Systems. Proceedings of an Informal Discussion Convened Jointly by the Faraday Society (Colloid and Biophysics Committee) and the British Society of Rheology, Oxford, September 1959. A.L. Copley and G. Stainsby (Eds.) Oxford-New York: Pergamon Press, 1960, pp. 97-121.

121. COPLEY, A.L. and THORLEY, R.S. Apparatus for measurement of viscosity and wall adherence. In: Flow Properties of Blood and Other Biological Systems. A.L. Copley and G. Stainsby (Eds.). New York - Oxford: Pergamon Press, 1960, pp. 361-368.

122. ANON. Appendix. A.L. Copley: Curriculum vitae and publications. In: Perspectives in Biorheology. Festschrift for A.L. Copley. A. Silberberg (Ed.). New York-Oxford: Pergamon Press, 1981, pp. 703-727; Biorheology 18, 703-727, 1981.

123. COPLEY, A.L., STEICHELE, D., SPRADAU, M. and THORLEY, R.S. Anticoagulant action of fibrin surfaces on mammalian blood. Nature (London) 183, 1683-1684, 1959.

124. COPLEY, A.L. and STEFKO, P.L. Coagulation thrombi in segments of artery and vein in dogs and the genesis of thromboembolism. Surg. Gyn. & Obstet. 84, 451-459, 1947.