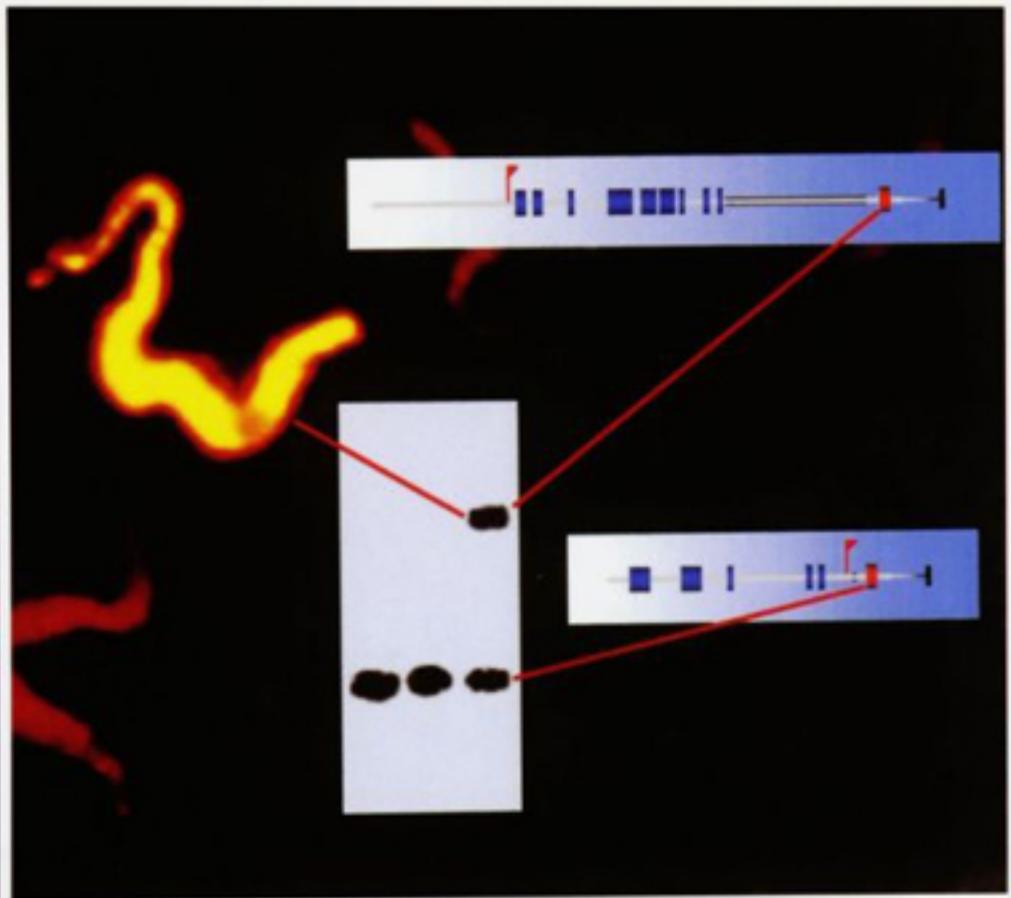


49

ADVANCES IN PARASITOLOGY



Edited by

J.R. BAKER, R. MULLER, D. ROLLINSON



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PREFACE

The volume starts with two contributions dealing with trypanosomes. The first suggestion that trypanosomes of the subgenus *Trypanozoon* could change immunologically during the course of an infection, and thus survive the onslaught of their host's antibodies, is generally credited to E. Franke in 1905 (*Münchener Medizinische Wochenschrift*, **52**, 2059–2060). In 1969 Keith Vickerman (*Journal of Cell Science*, **5**, 163–193) located the source of this change in the parasites' proteinaceous surface coat, and in the early 1970s George Cross and others isolated and characterized the glycoproteins involved (*Parasitology*, 1975, **71**, 393–417). The variation process was subsequently shown to be under genetic control, but it is only within the last few years that the details of the controlling process have become clear. The development of knowledge of this fascinating process is thoroughly reviewed by David Barry and Richard McCulloch, of the University of Glasgow in Scotland, and the authors go on to link this antigenic variation with the dynamics of the parasite population.

The second paper in this volume, by Jacques Pépin, of l'Université de Sherbrooke, Québec in Canada, and Honoré Méda, of Centre Muraz in Bobo-Dioulasso, Burkina Faso, reviews the recent developments in understanding the epidemiology of human African trypanosomiasis and its causative organisms, *Trypanosoma brucei rhodesiense* and *T. b. gambiense*. The two subspecies differ greatly in their relationships with their mammalian hosts, including humans, and the differences are vitally important in attempting to define suitable control measures for the two infections.

The next review, by George DosReis and Marcello Barcinski, of Universidade Federal do Rio de Janeiro and Universidade de São Paulo in Rio de Janeiro, Brazil, deals with the topic of apoptosis, or programmed cell death, and its occurrence in both parasites and the cells of their hosts. The interactions between these two events may profoundly affect the pathogenesis of a parasitic infection. The effect of these interactions is discussed in relation to American trypanosomiasis, schistosomiasis, malaria, toxoplasmosis and leishmaniasis. The authors suggest that future developments may permit the manipulation of host cell apoptosis in ways that benefit the host itself.

Finally, Bernard Fried of Lafayette College in Easton, Pennsylvania, USA, has reviewed the biology of the large group of echinostomes, except for the

genus *Echinostoma*, to supplement the reviews by himself and Jane Huffman in Volumes 29 (1990) and 38 (1996) of *Advances in Parasitology*. Four genera have been extensively investigated in the last few years, but available information on six other, less known, genera has also been collated. For the first four genera, the material reviewed includes descriptive, life cycle, experimental, biochemical and molecular studies. There has been a great deal of interest in the use of echinostomes as model systems, partly because they can be easily maintained in the laboratory. This has mainly involved species of *Echinostoma*, but recent research on *Echinochasmus*, *Echinoparyphium*, *Euparyphium* and *Hypoderaeum* in China, Spain and the United Kingdom is included in this contribution.

John Baker
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ABSTRACT

African trypanosomes are unicellular, eukaryotic parasites that live extracellularly in a wide range of mammals, including humans. They have a surface coat, composed of variant surface glycoprotein (VSG), which probably is essential and acts as a defence against general innate immunity and against acquired immunity directed at invariant surface antigens. In effect, the VSG is the only antigen that the host can target, and each trypanosome expresses only one VSG. To counter specific antibodies against the VSG, trypanosomes periodically undergo antigenic variation, the change to expression of another VSG. Antigenic variation belongs to the general survival strategy of enhanced phenotypic variation, where a subset of 'contingency' genes of viruses, bacteria and parasites hypermutate, allowing rapid adaptation to hostile or changing environments. A fundamental feature of antigenic variation is its link with the population dynamics of trypanosomes within the single host. Antigenic variants appear hierarchically within the mammalian host, with a mixture of order and randomness. The underlying mechanisms of this are not understood, although differential VSG gene activation may play a prominent part. Trypanosome antigenic variation has evolved a second arm in which the infective metacyclic population in the tsetse fly expresses a defined mixture of VSGs, although again each trypanosome expresses a single VSG. Differential VSG expression enhances transmission to new hosts, in the case of blood-stream trypanosomes by prolonging infection, and in the metacyclic population by generating diversity that may counter existing partial immunity in reservoir hosts. Antigenic variation employs a huge repertoire of VSG genes. Only one is expressed at a time in bloodstream trypanosomes, as a result of transcription being restricted to a set of about 20 bloodstream expression sites (*BESs*), which are at chromosome telomeres. Only one *BES* is active at a time, probably through transcriptional elongation being inhibited in the silent *BESs*. Although transcriptional switching between *BESs* can effect a VSG switch, the most prolific switch route involves homologous recombination of deoxyribonucleic acid, usually by the copying of a silent gene into a *BES*. Hierarchical expression of VSGs may be dictated in part by the different types of locus occupied by VSG genes. The VSG genes expressed in the metacyclic population also occupy telomeric sites, which appear to be derived from *BESs* but have a simpler structure. Their differential expression is achieved by random transcriptional activation; the detailed story requires direct study of the metacyclic stage itself. Available evidence suggests that the VSG originated as a surface receptor, and it can be proposed that a number of selective events have contributed to the evolution of the complex, multisystem phenomenon that antigenic variation has become.

1. INTRODUCTION

It is nearly 100 years since the study of antigenic variation in salivarian trypanosomes began and 20 years since the cloning of trypanosome variant surface glycoprotein (VSG) complementary deoxyribonucleic acid (cDNA) marked the beginning of molecular parasitology. In that time, we have gained much insight into the organismal biology of trypanosomes and amassed a large catalogue of information on gene organization and activation. It has become clear that antigenic variation is intimately linked with many other aspects of trypanosome growth and survival. Although it seems unlikely at present that antigenic variation can be used as a target for therapy, the trypanosome system continues to yield new insights and to act as a paradigm for antigenic variation in eukaryotic pathogens.

In this review, we have attempted to examine antigenic variation in the context of the complex organismal biology of trypanosomes: that is where the next generation of important questions lies. We have also drawn comparisons with related systems in simpler pathogens. It seems clear that, as a eukaryote, the trypanosome has evolved antigenic variation to a higher degree of sophistication than is apparent in prokaryotic pathogens. One example is that the VSG system is used in two distinct ways, depending on the pressures operating on different life cycle stages of the parasite. The organization of these two systems, and the control mechanisms that are applied to them, reveal much about how the apparatus for antigenic variation may have evolved in trypanosomes. Another aspect of trypanosome antigenic variation that sets it apart is the apparently bewildering number of molecular processes involved. We have also attempted to address why trypanosomes invest so many of their resources in antigenic variation and to assess the possible purposes, and relative contributions, of those different mechanisms.

2. THE COMPLETED TRYPANOSOME LIFE CYCLE?

In the many years since the first description of the most obvious stages in the life cycle of *Trypanosoma brucei*, it has become recognized more and more that they are components of a highly streamlined programme for development (Vickerman, 1985). Two main types of stage have been recognized (Figure 1). The replicative stages, including the long slender bloodstream form, the procyclic form and the epimastigote form, are responsible for establishing and maintaining infection in, respectively, the mammalian blood and tissue fluids, the gut of the tsetse fly vector (*Glossina* spp.) and the fly's salivary glands. They do so by repeated mitotic division. The transmission stages, on the other

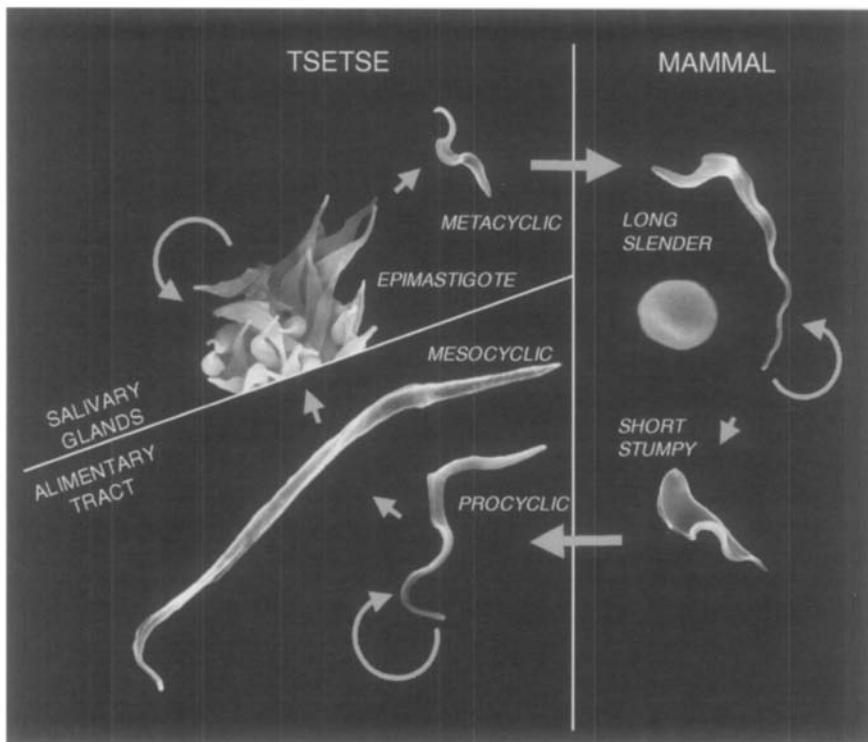


Figure 1 The life cycle of *Trypanosoma brucei*. The different life cycle stages shown are scanning electron micrographs, reproduced to scale. Curved arrows depict stages capable of replication. Epimastigote stage cells are shown as a cluster because they grow as a dense monolayer on the salivary gland epithelium. Recently described stages between the mesocyclic and epimastigote forms are not shown because they are transitory products of the intervening division process. Scanning electron micrographs provided by courtesy of Dr Laurence Tetley, Electron Microscopy Centre, Institute of Biomedical and Life Sciences, University of Glasgow, Scotland, UK.

hand, are pre-adapted for carrying infection to the next host, with the short stumpy form awaiting uptake by a tsetse fly and the metacyclic form going in the opposite direction, from fly to vertebrate. These transmission stages are quiescent, lying in the G₀ phase of the cell division cycle and having a finite half life if not transmitted (Shapiro, S.Z. *et al.*, 1984; Matthews and Gull, 1997). This interpretation of highly selected stages, each with a specialized role, has withstood a number of challenges over the years (e.g., Ormerod, 1979).

Until recently, our knowledge of the life cycle stages has remained incomplete. A significant step forward has been the description recently of what

may well be the final developmental stages in the tsetse fly. In the fly, there are two distinct phases of colonization, firstly in the gut and then in the salivary glands. What has been missing is a systematic description of the stage responsible for transmission between these phases. There has long been a debate whether trypanosomes migrate via the lumen of the alimentary system and thence into the salivary glands, or reach the glands by a series of invasions across tissue barriers (Ellis and Evans, 1977; Vickerman, 1985). It is clear that a bottle-neck in trypanosome numbers occurs in this part of the life cycle, which has important ramifications for the outcome of the genetic recombination that occurs in the salivary glands (Tait and Turner, 1990; Gibson, 1995), and for the kinetics of transmission. By detailed dissection and observation, a plausible account has now been obtained (Van Den Abbeele *et al.*, 1999). It transpires that the transition is achieved through luminal migration, intimately linked with developmental changes in the trypanosome, just as occurs in all other transitions in the life cycle. The procyclic stage develops to the very long 'mesocyclic' form, which yields an intermediate stage that has been observed in the foregut and proboscis, where a migrating stage would occur. Rather elegantly, by an asymmetrical division, a small daughter cell is produced that is the progenitor of the epimastigote stage, and the other, larger daughter cell is discarded. Asymmetrical divisions are a fundamental developmental strategy throughout evolution for the rapid generation of novel cell types and lineages (Horvitz and Herskowitz, 1992; Jacobs and Shapiro, 1998). We can therefore now add the mesocyclic stage to the standard life cycle diagram (see Figure 1) and admire the alternating pattern of replicative and transmission stages.

Although we now apparently have a description of the full life cycle, this is founded on morphological criteria. A more detailed description and dissection *in vitro* of the differentiation processes between stages would help to formalize this knowledge. In the past decade, many of these gaps have begun to be filled and the image of the life cycle as highly functionally organized has become strengthened. The transition between short stumpy and procyclic stages has been studied in detail, revealing a high degree of co-ordination between molecular events and at least some possible triggers (Overath *et al.*, 1986; Roditi *et al.*, 1989; Rolin *et al.*, 1993; Matthews and Gull, 1997; Matthews, 1999; Vassella *et al.*, 2000). Since most studies of bloodstream stage trypanosomes have used the highly convenient 'monomorphic' lines that are adapted to growth under laboratory conditions but, as signified by their name, yield only one form, the multiplicative, long slender stage, progress in studying the long slender to short stumpy transition has been slow. The recent achievement of growth *in vitro* of more natural, 'pleomorphic' lines has helped reveal that the long slender stage secretes a low molecular weight factor that induces development of the short stumpy stage through a cyclic adenosine monophosphate (cAMP) signal transduction pathway (Hamm *et al.*, 1990; Vassella *et al.*, 1997). This trigger is dependent upon the density of trypanosomes, in either the blood or

the culture dish (Vassella *et al.*, 1997), adding another item to the list of variables that contribute to the complex process of development of parasitaemia (Tyler *et al.*, 1997) and to transmission between hosts.

3. ENHANCED PHENOTYPIC VARIATION AS A GENERAL STRATEGY FOR PROPAGATION OF PATHOGENS

3.1. The Importance of Population Behaviour in the Single Host

Life is hazardous for unicellular organisms, especially those that dare to infect other organisms. Besides having to contend with the uncertainties of being transmitted between hosts, parasites face extremely potent immune defence systems. There are two contrasting ways in which the survival of parasite populations in the individual host can be achieved. Each individual parasite may be equipped to fend off assault by the host, gaining enough time to enhance transmission to new hosts. This is what helminths do, lasting long enough to generate progeny that are excreted and continue the life cycle (Maizels *et al.*, 1993). Alternatively, as happens with protozoan parasites, the pathogen can defend itself as a population, seeking safety in numbers, so that periodic death of the majority of the population within the host is not catastrophic, and indeed may enhance chronicity of infection. In a sense, this is co-operative behaviour at the population level.

Microbiologists have begun to develop such concepts further, questioning the basic genetic and reproductive structure of bacterial species. The importance of population behaviour in a species has been developed into two complementary theories, that some pathogenic bacteria are actually modular organisms (Andrews, 1998) and that they display interactive behavioural traits more usually identified between the cells of a multicellular organism (Shapiro, J.A., 1998). A modular organism is one that develops as an indeterminate structure composed of parts (modules) that are iterated with no definite limit. When applied to a pathogenic bacterium, some different modules would be the bacteria themselves, the population within a single host, and the population within a local host population (Andrews, 1998). There is a major genetic benefit in modularity. In effect, a single genetic individual is represented by the sum of all modules that have arisen since the last sexual event. Frequently, such modular organisms reproduce clonally, as with the bacteria *Escherichia coli* and *Salmonella* spp., and it has even been proposed that a pandemic can be caused by a single genetic individual, although obviously sublineages would arise during the considerable time it takes for a pandemic to develop (Ochman *et al.*, 2000). The fundamental ramification of the modularity theory is that a single, successful genotype continues propagating and does not die out, at the

same time sampling a huge range of different environments (host individuals). This sets conditions for the selection of even fitter variants. There is also a body of evidence for the idea that bacteria display aspects of multicellular behaviour within single hosts, where density-dependent molecular interactions lead to modulation of behaviour or developmental state, enhancing transmission between hosts (Shapiro, J.A., 1998).

Similar arguments can be applied easily to African (i.e., salivarian) trypanosomes. Several of the classical criteria for modularity, as refined for application to unicellular organisms (Andrews, 1998), are fulfilled, and elements of multicellular behaviour are displayed. Different trypanosome cells interact (Vassella *et al.*, 1997), differentiation to functionally different cell types occurs, and there is a specific stage for dispersal to new hosts (two, the short stumpy and the metacyclic stages). The main dispersal stage, the metacyclic population, provides immense adaptive value by its dispersal mode – the promiscuously biting tsetse fly – and the fact that it is the product of trypanosome mating, when mating does occur. As we shall see, antigenic variation makes a significant contribution to diversity of the metacyclic stage and hence to its dispersal potential. Finally, as with some bacteria, the human-infective *T. b. rhodesiense* expands clonally during epidemics (Hide *et al.*, 1994; Tibayrenc, 1999).

By undertaking extensive co-operation within the population it forms within its hosts, *T. brucei* strongly enhances the probability of its transmission and its fitness. The sacrifices – ‘trade-offs’ – it makes in achieving these goals are not fully understood, but include repeated bouts of antibody-mediated killing in the mammal, plateaux of growth in the tsetse fly, and absence of replicative growth in its transmission stages. Also important are the numerical bottle-necks it encounters throughout the life cycle, such as between growth peaks in the vertebrate host, in the transmissions between hosts and, most significantly, between the alimentary tract and the salivary glands in the fly. By having to overcome all these hurdles that are encountered throughout its life cycle, the trypanosome is under continuous, strong selection for fitness. There can be little doubt that these patterns of growth and behaviour are influential in the evolution and execution of antigenic variation.

There have been reports also that trypanosomes display apoptosis, the process by which multicellular organisms impose control on cell-based development and function in tissues (Welburn *et al.*, 1996, 1997). This would be an interesting and additional multicellular trait for the trypanosome, but the distinction between normal cell necrosis and apoptosis can be a fine one. The matter may be settled by demonstration in trypanosomes of some of the many well-known molecular mediators in apoptosis; the genome project should be of help in this quest (Budihardjo *et al.*, 1999). Natural death occurs in *T. brucei* at three stages. Both the short stumpy and metacyclic transmission stages expire if not taken up by a new host, and one product of the asymmetrical division in

the transition between tsetse gut and salivary glands appears to be a ‘dead end’ (Van Den Abbeele *et al.*, 1999). In multicellular organisms, unwanted cell division products can be disruptive to the body plan and can be subject to apoptosis (Metzstein *et al.*, 1998). It seems likely that it is simpler for the trypanosome to allow death of these stages by necrosis, but it might be interesting to ascertain exactly how they do expire. During growth in the mammal, the interplay of antigenic variation and antibody-mediated killing obviates any need for an apoptotic process.

3.2. Microbial Phase Variation and Antigenic Variation

Enhanced phenotypic variation, which includes the phenomenon of antigenic variation, is widespread in micropathogens. Basically, it is a general strategy that enhances propagation, and therefore transmission to new hosts. Viruses use it to avoid host immunity, the most famous cases being with influenza virus and human immunodeficiency virus 1 (HIV1). Influenza virus binds to host cell receptors via its capsid haemagglutinin protein, which undergoes antigenic drift and, less frequently, a more fundamental change known as antigenic shift (Fanning and Taubenberger, 1999). It is the latter change that leads to influenza pandemics. The HIV1 envelope glycoprotein gp160 consists of two subunits, gp41 and gp120 (Kwong *et al.*, 1998; Wyatt *et al.*, 1998). The main function of gp120 is to attach the virus to CD4+ host cells, as a prelude to membrane fusion and entry of the virus into the cells; gp120, which functions as a homotrimer, has two main conserved regions, responsible for binding to the CD4 and chemokine co-receptors, respectively. The CD4 receptor binding site on gp120, being in a deeply recessed groove, is inaccessible to antibodies, but the chemokine receptor binding site is not so. However, three variable loops guard the latter site, permitting binding to host cells but denying access by antibodies. The loops themselves undergo antigenic variation. In a sense, the use of variable, functionless sequences of this protein to hide more conserved, underlying sequences, is an intramolecular version of the intermolecular steric hindrance, described below (Section 4.1), that is mediated by the trypanosome’s surface molecular coat.

Bacteria have an impressive array of different systems and mechanisms for phenotypic variation. Some of these, referred to as phase variation, involve switching between alternative states that confer different fates on the bacterium. As an example, absence of the surface capsule from meningococci permits the bacterium to invade host cells, whereas switching to the alternative state, presence of the capsule, enables evasion of immunity (Bucci *et al.*, 1999). Phase variation can apply to various bacterial surface structures and molecules, such as the capsule, pili or fibrils, and can involve proteins or carbohydrates (Moxon *et al.*, 1994; Deitsch *et al.*, 1997; Henderson *et al.*, 1999).

The consequences for the pathogen can be critical, leading to binding to host surfaces, invasion of cells, tissue tropism or avoidance of phagocytosis, complement fixation or acquired immune responses. Where a species displays phase variation independently and simultaneously in a number of traits, the outcome is a population within the single host that is constantly varied and able to maximize its available opportunities.

Antigenic variation has a more specific role, evasion of acquired immunity, and hence differs from phase variation in fundamental ways. It cannot merely alternate between two states, because of its function of escaping repeatedly from new immune responses. As such responses persist, switching back to the previous state would be lethal; instead, continual forward switching is needed. Pathogenic *Borrelia* species use well-understood systems of antigenic variation, in which they repeatedly change their major surface proteins. *B. hermsii* (causing relapsing fever) regularly changes its variable major protein (VMP) coat, while *B. burgdorferi* (causing Lyme disease) changes its VMP-like sequence (vls) coat (Plasterk *et al.*, 1985; Barbour, 1990; Zhang *et al.*, 1997). In fact, the operational similarities with the trypanosome antigenic variation system are strikingly similar, from genetic to phenotypic levels.

What about parasites? For a long time, African trypanosomes, and to a lesser extent the malaria parasites *Plasmodium* spp., were seen as almost isolated examples of protozoan parasites undergoing antigenic variation. Trypanosomes do this by repeatedly changing the VSG that composes their surface coat. In recent years, other cases of antigenic variation have become better understood. Well-known examples are the gut parasite *Giardia duodenalis* (= *G. lamblia*), which possesses a system strongly reminiscent of the trypanosome, involving its variable surface protein (VSP) coat (Svard *et al.*, 1998). The opportunistic fungal pathogen *Pneumocystis carinii* displays antigenic variation of its major surface glycoprotein (MSG) coat (Nakamura and Wada, 1998). All these are extracellular pathogens whose surface must directly face host immunity, but the phenomenon extends to intracellular protozoan parasites such as *Plasmodium* and *Babesia*, which show antigenic variation of the proteins that they place on the surface of the red blood cells they infect. With these parasites, antigenic variation is more intricate, due to the fact that the proteins involved have functions in addition to the simple escape from antibodies employed by the extracellular parasites. In *Plasmodium falciparum*, the PfEMP1 protein family (*var* gene products) on the blood cell surface are associated with binding of the infected cell to host endothelial cells, thereby sequestering it from surveillance systems elsewhere, such as in the spleen (Gardner *et al.*, 1996; Wahlgren *et al.*, 1999). Other variable antigens on the infected red blood cell, including the rifins (Fernandez *et al.*, 1999; Kyes *et al.*, 1999), are thought to play similar roles, for example by concealing the infected cell amongst uninfected red cells.

So far, no case of phase variation has been reported within single life cycle

stages of parasites. It might be questioned whether *T. congolense*, which can be found attached to vascular endothelium within its mammalian host (Banks, 1978; Hemphill *et al.*, 1994), undergoes a phase variation between attachment-competent and incompetent states. As attachment to endothelium is unlikely to sequester the parasite from lytic antibodies, this is not equivalent to the sequestration of *Plasmodium*-infected red blood cells. There is a clearly adaptive advantage in being unattached – transmission to tsetse flies – but any advantage of attachment has not been studied.

3.3. Contingency Genes and the General Features of Enhanced Phenotypic Variation

The importance of enhanced phenotypic variation for the survival, transmission and evolution of pathogens is reflected in the prominence of the underlying genetic and biochemical systems over more conventional, housekeeping functions of the cell. This is seen clearly in the enormous proportion of the genome requisitioned for antigenic variation in trypanosomes, amounting to more than 20% of the nuclear DNA (see Section 7.4).

Based on studies of phenotypic variation in bacteria, Moxon and colleagues (1994) have been able to identify some generally applicable features of the underlying genetic mechanisms. Although a wide variety of genetic mechanisms for variation has evolved, ranging from the apparently purely accidental, such as small DNA deletions arising from mispairing within repetitive sequences (or even the standard errors arising during replication of viral ribonucleic acid (RNA) genomes), through to apparently highly ‘programmed’ systems for feeding different alleles of structural genes into a transcriptionally active site (Deitsch *et al.*, 1997; Henderson *et al.*, 1999), they all share an extremely important outcome: the ability to hypermutate a subset of genes and thereby adjust immediately in a highly unpredictable or hostile environment. In the face of such powerful predatory forces as the mammalian immune system, normal mutation rates are inadequate to produce a sufficiently rapid phenotypic change, so many pathogens in effect have partitioned their genomes into a set of housekeeping genes that undergo normal safe, low mutation rates and a smaller set of genes, known as contingency genes, that are subject to hypermutation. The contingency genes encode proteins that are directly involved in phenotypic variation. This principle, established for bacteria, applies to antigenic variation systems of parasites, where essentially random or stochastic events occur at a high frequency at specific loci and effect an antigen switch. Although the involvement of specific sequences can give the impression of ‘programming’, it has been pointed out that use of this term is incompatible with the randomness of switching (Moxon *et al.*, 1994).

The contingency gene theory has three central features that might be termed

'general features'. These are: the genes involved are subject to hypermutation; the resulting phenotypic changes are associated with coping with a hostile or unpredictable environment; and switching is pre-emptive of, rather than responsive to, environmental change (Moxon *et al.*, 1994). The hypermutation rate turns out to be markedly similar across all the known systems in bacteria and parasites, in a range around 10^{-3} switch/organism/population doubling (Moxon *et al.*, 1994; Deitsch *et al.*, 1997; Turner, 1999). As an example of pre-emptive switching, antigenic variation typically is not induced by antibodies, but occurs before the immune response arises, and the function of antibodies is to remove some variants, leaving others, including newly activated ones, to grow. We can question whether antigenic variation in trypanosomes complies with these principles. The answer clearly is affirmative, on all three counts. The structural genes of trypanosome switching are subject to hypermutation, usually by gene conversion (Barry, 1997a). Switching of the phenotype allows the parasite to cope with a hostile and changing environment, namely repeated waves of antibodies. Switching is certainly pre-emptive, occurring spontaneously in the absence of antibodies, as observed in culture *in vitro* (Doyle *et al.*, 1980).

Although it is encouraging to be able to draw parallels between these variability mechanisms in prokaryotic and eukaryotic pathogens, we should note that the latter have much more sophisticated systems. Phase variation, by presenting merely alternative states, provides bacteria with the least extensive possible degree of variation. Where bacteria benefit is in the coexistence, and simultaneous operation, of distinct variation systems in the same species. They also benefit from their great ability to generate diversity through a wide range of genetic interactions, including gene transfer, both within and between species (Ochman *et al.*, 2000). The parasitic protozoa, with their much larger genomes and more complex life cycles, have been able to evolve their antigenic variation systems to high degrees of sophistication. This is most evident in African trypanosomes, where the single antigenic variation system has developed into two distinct systems attuned to different life cycle stages. All the signs are that *Plasmodium* will also be found to have a high degree of sophistication in its antigenic variation systems.

4. THE MOLECULAR PHENOTYPE: THE TRYPANOSOME VSG COAT

What does *T. brucei* require to undergo its own particular brand of phenotypic variation? As an extracellular parasite inhabiting the vasculature, its greatest challenges are the innate and acquired immune systems. These are overcome by the presence of its surface coat, a densely packed layer of 5×10^6 dimers of the VSG. The coat is believed to thwart innate immune mechanisms (Ferrante

and Allison, 1983), possibly by concealing other molecules that would invoke them, and it also prevents antibodies gaining access to necessarily invariant, functionally conserved surface molecules (Overath *et al.*, 1994). This shielding strategy is highly effective, but has one weakness: the VSG itself is recognized as an antigen. Comprehensive antibody responses arise against a range of epitopes on the VSG, including those that are not exposed in the intact coat. To evade these antibodies, a complete change of exposed epitopes is called for, and the change must be rapid and complete. As random and background spontaneous point mutation could not satisfy such a demand, an easy way to achieve this is simply to change over to expression of an antigenically distinct VSG, and that is what trypanosome antigenic variation entails. The trypanosome's version of contingency loci is an enormous set of silent VSG genes that differ from each other in the epitopes they encode. Each silent gene has a chance to enter, and become transcribed from, a specific expression locus. The precise mechanisms for doing so, which we describe below, are immaterial as long as the required complete phenotypic change is achieved at each switch.

4.1. VSG Structure and Function

Although there is a demand for maximum diversity in VSG epitopes, there is a conflicting demand for conservation of those regions of the molecule that are important for function, assembly or final structure. The simplest way to maximize diversity would be to minimize those constraints, and loss of biochemical function would make a very significant contribution in that direction. Apparently this is what the trypanosome has evolved (we discuss in Section 7.1 some evidence that the VSG is a remnant of a cell surface receptor). The VSG comprises two domains, with the 350–400 residue amino terminal domain containing the variable epitopes and the remaining 50–100 residues of the carboxy terminal domain being more conserved and disposed towards the plasma membrane (Carrington *et al.*, 1991; Blum *et al.*, 1993). However, the surprise is that antigenically distinct VSGs, only 20% identical in peptide sequence over their entire length, fold to very similar structures in their variable amino terminal domains, dominated by the 'VSG fold' of two long, antiparallel α helices (Blum *et al.*, 1993). It has been proposed, based on the occurrence of cysteines and the α helical potential evident in their primary structure, that a range of trypanosome surface proteins with different functions share the VSG fold, which may be a structural unit that allows functional proteins to be incorporated efficiently on the trypanosome surface (Carrington and Boothroyd, 1996). Structural aspects of the surface coat have been reviewed in detail elsewhere (Overath *et al.*, 1994; Carrington and Boothroyd, 1996; Cross, 1996; Ferguson, 1999).

Even now, some 25 years after purification of VSG was achieved (Cross, 1975), complete definition of VSG epitopes has not been accomplished. The main problem is that the important epitopes are conformational, although another problem is that the improbability of exploiting them globally to generate vaccines has detracted from the impetus to understand them. Early work showed a number of variable epitopes, only some of which are exposed to antibodies on the living trypanosome (Clarke *et al.*, 1987; Miller *et al.*, 1984), but a precise mapping on to the known VSG structure has not been achieved. In a different technique, Boothroyd and colleagues (Hsia *et al.*, 1996) constructed chimaeric VSGs from two individual VSG sequences and examined them for addition or loss of a specific, surface-exposed epitope from one donor. This approach was successful, and it is hoped that it can be applied further. Identification of some important, individual residues (Baltz *et al.*, 1991), or perhaps of entire epitopes (Lu *et al.*, 1993), may also have been achieved in experiments investigating point mutations in expressed copies of VSG genes. Although point mutagenesis is unlikely to be a mechanism for VSG switching, as it is unlikely to effect the complete epitope change needed for escape from antibodies, it has been argued that the selection regime in some experiments on point mutation exerted pressure simultaneously for retention or loss of individual epitopes, and the observed 'hot-spot' regions of change may represent epitopes (Graham, V. S. and Barry, 1996).

In the absence of any designated, specific function other than passive protection, the VSG has long tantalized researchers into proposing, or even seeking, functions that might relate in other ways to countering immunity. The extremely high degree of variability around the whole surface of the variable domain, rather than just at the region actually exposed to antibodies, has led to speculation that cell-mediated immunity, and thus T cell epitopes, are involved in trypanosome infections (Blum *et al.*, 1993). However, a more recent analysis of the 117 VSG gene family in two distinct trypanosome stocks has shown that, at the protein level, the greatest drift has occurred in the hypervariable regions which contain epitopes associated with variant-specific immunity (J. C. Boothroyd, personal communication). The presence of many third base, silent substitutions suggests that constraints do exist on the exact amino acid sequence, perhaps to conserve overall VSG shape. There may be a broad similarity between the VSG and the haemagglutinin of influenza virus in the preferential occurrence of variation on the surface of the molecule. It has been noted that most sequence variation occurs on the surface of the trimeric haemagglutinin molecule, but not all variable sequences are readily recognizable as epitopes or receptor-binding regions (Fanning and Taubenberger, 1999).

4.2. The Importance of an Immune Response against the VSG Coat

A common concept in infection biology is that suppression of acquired immunity by a pathogen is a strongly selected trait that enables the pathogen to survive. There are many examples of this occurring, by different mechanisms. For example, ‘down-regulation’ (i.e., decreased expression) of major histocompatibility complex I molecules involved in antigen presentation is invoked by HIV1, the EBNA1 antigen of Epstein–Barr virus inhibits the function of the proteasome in antigen processing, and the expression by viruses of functionally altered, decoy mimics of cytokines is widespread (Tortorella *et al.*, 2000). For a considerable time, it has been known that trypanosome infection induces immunosuppression, and suppressor macrophages have been demonstrated (Askonas, 1985), which has sometimes led to the interpretation that this pathogen also indulges in such self protection. However, whether the immune response against trypanosomes, and specifically VSGs, is reduced to an ineffective level remains to be seen.

In the past few years, efforts have begun towards gaining an understanding of how these macrophages exert their suppressive effect, although the emerging knowledge is controversial. Trypanosomes produce a protein that triggers T lymphocytes to produce interferon γ (IFN γ), which stimulates macrophages into suppressor mode, producing nitric oxide (NO) and prostaglandins (Bakhiet *et al.*, 1993). These agents are immunosuppressive for T lymphocytes, and it has been proposed that this represents a means of depressing acquired, anti-trypanosome immune responses. However, experimental data are not entirely supportive. Absence of IFN γ or its receptor in transgenic mice does not lead to the predicted depression of parasitaemia, but rather has the opposite effect (Mabbott *et al.*, 1998; Hertz and Mansfield, 1999). It has also been proposed that NO has an indirect effect, increasing parasitaemia by suppressing lymphocyte responses against trypanosomes, but experiments in ‘knock-out’ mice incapable of generating NO gave conflicting results (Hertz and Mansfield, 1999; Millar *et al.*, 1999). Controversy extends to how trypanosomes actually are controlled by the host. Some researchers consider that general, non-specific mechanisms can be effective. NO has been proposed to have a second role by acting directly against trypanosomes, as it does against other pathogens, but the current view is that this is not the case (Mabbott *et al.*, 1994). Another cytokine, tumour necrosis factor α (TNF α), has been proposed to kill trypanosomes non-specifically (Magez *et al.*, 1999), but this also is controversial (Hertz and Mansfield, 1999).

What is clear is that a vigorous anti-VSG response is mounted. Upon growth of trypanosomes expressing distinct VSG coats (known as variable antigen types: VATs), specific immunoglobulin (Ig) M is elicited against each VSG. IgM antibodies are highly efficient in triggering complement-mediated lysis. In the mouse model, IgG subclasses are also produced early in

infection (Radwanska *et al.*, 2000). The specific response serves to clear circulating trypanosomes expressing the VSG to which it has specificity. During infection, wave after wave of IgM is produced, continually increasing the total IgM level. Although it has not been demonstrated unequivocally that this enormous production represents antibodies directed against many different VSGs, the only available data suggest that this is likely to be so (Nantulya *et al.*, 1986). It is notable that, throughout infection, there is not a continuous plateau of trypanosome growth, but rather a continued peaking effect or a merging of many peaks (see Figure 2, p. 19), indicative of continued antibody responses rather than effective suppression (Barry, 1986). By so maintaining the parasitaemia below levels that might induce severe pathogenesis, the IgM responses can ensure chronicity of infection. It would not matter if much of the IgM response were suppressed as an accidental consequence of infection; all that is required is a level adequate for lysis of circulating VATs. Whether incomplete suppression of the specific response occurs later in infection, and possible benefits that this might have for the parasite, have been discussed by Turner (1999).

5. ANTIGENIC VARIATION: POPULATION BEHAVIOUR WITHIN THE SINGLE HOST

Bearing in mind the fundamental importance of population behaviour in the development and persistence of pathogens when present in a single host, one can identify, during the life cycle of *T. brucei*, two discrete population behaviour traits that are based on differential VSG expression. Both contribute directly to transmission and both probably are tailored to the environmental pressures they face. The metacyclic population in the tsetse fly exists to enable transmission to new vertebrate hosts, whereas the bloodstream population must find ways of evading immunity sufficiently to present adequate numbers for transmission when a tsetse fly takes a blood meal. Differential VSG expression is a key feature in both populations, but in fundamentally different ways. In the metacyclic population it occurs independently of time, perhaps because all that matters is the instant of infection of a new host, whereas in the bloodstream it does occur as a function of time, to cope with continual waves of antibodies. Although there is considerable understanding of molecular events associated with differential expression, particularly in the bloodstream, relatively little is understood about the organismal and population biology in the mammal. In fact, more is known at that level about the metacyclic population.

5.1. The Metacyclic Population in the Tsetse Fly

The VSG coat is essential for survival in mammals, and it is first synthesized when the epimastigote stage in the fly salivary glands differentiates to the metacyclic stage, replacing the procyclin coat that covers all other trypanosome stages in the fly (Tetley and Vickerman, 1985; Tetley *et al.*, 1987; Roditi *et al.*, 1989). The trypanosome population at this stage is heterogeneous, displaying a number of variants (metacyclic variable antigen types; MVATs), with each individual trypanosome expressing just one VSG. On the principle that transmission stages of pathogens express phenotypes conducive to their transmission, the most readily imagined VAT expression pattern is that the metacyclic population is as unpredictable and diverse as possible. This is not the case. The population expresses a large set of MVATs that is, quite remarkably, very predictable in content. In a population that can number from tens to about one hundred thousand, two estimates have shown, respectively, more than 14 MVATs and a maximum of 27 MVATs. The former study, based on immunofluorescence, revealed that collectively >95% of the population could be labelled by 14 distinct monoclonal antibodies (Lenardo *et al.*, 1984). It has been shown, however, that at least five MVATs are expressed at very low levels and, for four of these, only sporadically (Barry *et al.*, 1983), so an assay covering all individuals in the metacyclic population is required for full enumeration. This has been achieved in the second study, which was based on two approaches. By immunofluorescence, 27 monoclonal antibodies labelled 99–100% of coated trypanosomes from fly saliva. Using viability neutralization, which can detect single surviving cells, it was found that 27 distinct antibodies could eliminate the entire population (Turner *et al.*, 1988). A very striking feature is that this MVAT set appears to be activated regardless of which particular VAT was being expressed by the trypanosomes that originally infected the fly (Hajduk *et al.*, 1981), demonstrating that metacyclic and bloodstream VSGs are likely to be under distinct control systems. Although such predictability sometimes raises hopes for development of a vaccine (Capecci, 1990), there is a gradual turnover in the content of the repertoire (Barry *et al.*, 1983), presumably preventing the long-term onset of herd immunity in the wild.

Arguments have been presented before that, in the trypanosome's strategy of reliably generating diversity, the predictability is a trade-off that can counter the immediate problem of antibodies already existing in reservoir hosts in the field (Barry *et al.*, 1990, 1998). In essence, it is thought that the existence of a molecular mechanism guaranteeing the differential activation of VSGs in the fly requires a distinct gene activation mechanism. This in turn requires that a subset of the VSG gene repertoire be partitioned. It is the reservation of this subset for activation in the fly that unavoidably results in predictability of expression. Examination of expression patterns has shown

that, in the metacyclic population, the activation of metacyclic VSGs (MVSGs) takes precedence over that of bloodstream VSGs (BVSGs), adding further evidence for the existence of distinct systems (Turner *et al.*, 1986a). By exploiting the fact that trypanosomes remain attached to the salivary gland epithelium when they begin to synthesize VSG, it has been possible to show that the decision to activate *MVSG* genes differentially coincides with the initiation of VSG synthesis, and that this decision selects a gene randomly, resulting in polyclonal activation of any MVSG (Tetley *et al.*, 1987). All these observations give clues as to the underlying genetic mechanism, as we discuss in Section 6.3.

On entry into the mammalian host via a fly bite, infection initially becomes established in a subcutaneous depot at the site of biting, known as the chancre. Although the trypanosomes initiate division there, probably after several hours (Brun *et al.*, 1984), and a colony grows, they are rapidly disseminated to the vascular system via lymphatic channels (Emery *et al.*, 1980; Barry and Emery, 1984) or more directly through lymph nodes (Theis and Bolton, 1980). In one study, the kinetics of antigenic variation were studied during early establishment of infection (Barry and Emery, 1984). In the first several days (up to day 7 in the lymph, day 10 in the blood), MVSGs continue to be expressed by the proliferating population, and a broad anti-MVSG antibody response ensues, with consequent removal of MVATs. Clearly, commencement of BVSG expression in the population is necessary before removal of all the MVSG expressors. The earliest observed BVSG expressor was after 4 days of infection in the lymph and 6 days (the earliest day on which parasites were detectable) in the blood, and the development of each VAT differed between the two niches (Barry and Emery, 1984). The mouse is unusual in that it does not develop a skin chancre and may have different kinetics of initial trypanosome growth in the blood, but the same general pattern of a peak of MVATs leading to a larger BVSG parasitaemic peak occurs (Hajduk and Vickerman, 1981). Little is known about the change-over between the metacyclic and bloodstream VSG systems. In immunosuppressed mice, where the change can be observed independently of the effects of antibodies, there is a natural drop in the incidence of MVATs after 3–5 days (Hajduk and Vickerman, 1981), and a low percentage of trypanosomes that apparently are in transition between MVSG and BVSG expression has been observed in immunocompetent mice on days 4–6 (Esser and Schoenbechler, 1985). A detailed clonal analysis should help to reveal the kinetics of the transition between the two VSG gene systems. This might help us to understand the intriguing fact that, despite the two systems being so distinct, the metacyclic one persists for so long after differentiation to the long slender bloodstream stage. That fact emphasizes the fundamental importance of the initial selection of a specific VSG gene for transcription in the fly.

5.2. The Population in the Mammalian Host

The time-dependent differential expression of VSGs in the bloodstream is intimately linked to the complex biology of the parasite. Although *T. brucei* is primarily a parasite of extravascular tissue fluids, in the literature frequently it is considered wholly as bloodstream-borne. In extravascular niches, the long slender, multiplicative form often appears to be the only stage present, at least in rats (Ssenyonga and Adam, 1975; Tanner *et al.*, 1980), but the short stumpy stage can also predominate in other host species (Barry and Emery, 1984), whereas in the blood the full range of forms develops. Antigenic variation is not synchronous in these different niches (Seed and Effron, 1973), although there is ready exchange of parasites between them (Barry and Emery, 1984; Turner *et al.*, 1986b). Apart from these and similar studies, knowledge of antigenic variation in the mammal is based exclusively on what happens in the blood. In general, some patterns have been described that reveal a wondrous system that appears to follow, somewhat liberally, a set of basic rules, generating considerable diversity as it goes. The epithet 'semi-predictable' is often applied. What can be distilled is that the population in the mammal continually switches with time, yielding a steady trickle of variants. Several host species have been used to dissect aspects of antigenic variation, ranging from small laboratory rodents, which permit detailed analysis of the switch from one peak to another, to more chronic infections in rabbits and larger mammals. In cattle, carefully maintained under laboratory conditions, self-cure can occur, with *T. brucei*, *T. congolense* and *T. vivax* (see Nantulya *et al.*, 1984, 1986; Barry, 1986), although subsequent reinvasion from immunoprivileged sites (Jennings *et al.*, 1979) can cause remission. One interpretation of the self-cure is that the trypanosome has exhausted its VSG repertoire and the host has antibodies against all VATs. Evidence for this comes from the comprehensive anti-VAT antibody responses and resistance to reinfection with the same, but not with a different, trypanosome stock (Nantulya *et al.*, 1984, 1986; Barry, 1986). If VSG exhaustion does account for it, the entire course of antigenic variation can be encapsulated in such single infections, which form a barely tapped experimental resource. One such infection, of *T. vivax* in a cow, is depicted in Figure 2 (Barry, 1986). It is worth noting that the potential to self-cure depends on rapid development of immune responses against the different VATs and a sufficiently robust rate of trypanosome growth to create the opportunities for the appearance of many switch products. In the cow infection depicted in Figure 2, it can be calculated that there were approximately 3×10^{11} trypanosomes present in the entire bloodstream on day 10, creating formidable potential for the generation of many VATs. The ensuing account describes several features of antigenic variation, some of which are illustrated in Figure 2.

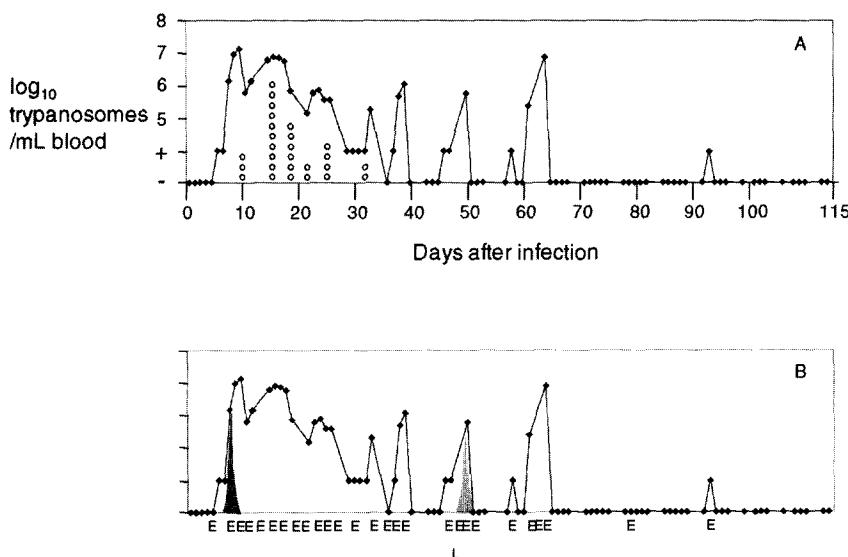


Figure 2 Chronic infection with *Trypanosoma vivax*. A. Parasitaemia in a cow infected by syringe inoculation with a clone of bloodstream stage trypanosomes. This cow typically recovered from infection, possibly due to development of humoral immunity against the whole VSG repertoire. The cow was monitored daily for presence of antibodies against 27 different VATs, and the day of onset of the specific response against each is depicted as an open circle. This demonstrates the antigenic diversity present in each peak of parasitaemia. The parasitaemia scale (abscissa) is not linear below the quantifiable level, as numbers of trypanosomes are recorded merely as +; – indicates undetectable. All data are redrawn from Barry (1986). B. The same graph with a scheme superimposed indicating one way in which it is believed that hierarchical VAT expression is achieved. E represents activation events of an 'early' VSG gene, and the dark grey subpeak indicates the growth of the corresponding VAT. Frequent activations of the same VSG later in the infection do not give rise to further VAT E subpeaks, because host antibodies directed against the VSG coat kill the switched trypanosomes. L represents the much rarer activation of a 'late' VSG gene, and the light grey subpeak depicts growth of that VAT. The actual numbers of activations are under-represented and are intended merely for comparison between the two VAT classes.

- (a) *Switching is spontaneous.* Antigenic variation occurs in culture *in vitro*, in the absence of host antibodies (Doyle *et al.*, 1980), whose function *in vivo* is to select against variants. In this regard, it has the pre-emptive nature of the contingency gene system described in bacteria, setting new variants in place before antibodies begin to remove those variants already growing.
- (b) *Switching is rapid.* In common with the high rate of enhanced phenotypic variation systems in other pathogens, trypanosome antigenic variation occurs at an overall rate of about 10^{-2} switch/cell/population doubling

(Turner and Barry, 1989; Moxon *et al.*, 1994; Deitsch *et al.*, 1997; Turner, 1999). Trypanosome lines that have been adapted to perpetual division display a background rate of 10^{-6} to 10^{-7} switch/cell/population doubling (Lamont *et al.*, 1986; McCulloch and Barry, 1999) and a similar attenuation has been reported for *Borrelia* (Koomey, 1997). There are two sets of evidence that this lower rate is an artefact: decrease of the rate occurs routinely during adaptation of clonal lines from different trypanosome stocks (Le Ray *et al.*, 1977; Barry *et al.*, 1979a, 1985; Graham, S. V. *et al.*, 1990; Turner *et al.*, 1991; Turner, 1997), and adapted lines appear to have a greatly diminished use of the main VSG switch mechanism (Robinson *et al.*, 1999).

- (c) *Switching is finite.* As described above, there is some evidence that the VSG repertoire is finite, possibly resulting in self-cure. This reflects the nature of the main genetic resource of antigenic variation, a large set of VSG genes. Nevertheless, it is also known that the trypanosome can develop novel VSG genes during infection by splicing together segments of different VSG genes and pseudogenes. This has the potential greatly to increase the size of the repertoire, in a transient way. We describe the gene repertoire and these events in Section 6.
- (d) *Switching is divergent.* A clonal population expressing mainly one VAT switches to about five or six VATs in the ensuing peak (Van Meirvenne *et al.*, 1975; Miller and Turner, 1981; Robinson *et al.*, 1999). Contrary to a common view that each peak comprises just one VAT, peaks of parasitaemia usually contain complex mixtures of VATs. This is apparent, as shown in Figure 2, in the irregular shape of peaks and the timing of development of antibody responses against many VSGs within the course of individual peaks (Barry, 1986). Nevertheless, it is possible sometimes for one VAT to dominate a peak.
- (e) *Switching is hierarchical.* Some VATs tend to appear early in infection, some appear only late, and intervening there may be a spectrum of the timing of appearance (Gray, 1965; Van Meirvenne *et al.*, 1975; Capbern *et al.*, 1977; Barry, 1986). It is thought that VATs appearing early are in fact being activated continually, with high frequency, throughout infection, but their first appearance induces antibodies that eliminate them as soon as they subsequently appear. Such a rolling system, involving the continual addition of antibodies to the pool, progressively selects against more VATs that are being reactivated and progressively allows less frequently activated VATs to grow, as proposed in Figure 2. Two main consequences of hierarchical development of VATs are that (i) there is a degree of predictability in their order of appearance and (ii) the infection is chronic, as new VATs trickle through. A third consequence is that, following syringe transfer of trypanosomes from any stage of infection into a new host, the order is reset. The transferred VATs continue to be expressed until

removed by an immune response, and the usual hierarchy is then followed. Fly-transmitted infection results in an initial growth of MVATs, with the hierarchy ensuing.

5.3. Modelling Infection

Trypanosome growth, antigenic variation and the immune system are inextricably linked. Growth by cell division generates each new rise in parasitaemia, acquired immune responses produce the opposite effect, and antigenic switching provides the escape link that allows iteration of this pattern. It may be the case that these effects exist in equilibrium, not only allowing prolonged growth but also maintaining parasite numbers within limits, minimizing pathogenesis and enhancing transmission. Amongst these basic features of population behaviour lie some of the subtleties of antigenic variation, outlined above, that are important contributors to chronicity of infection. Foremost amongst these, the semi-predictability and hierarchical nature of expression have been seen as critical for transforming what might be overwhelming early growth of many VATs into a phased, chronic infection. The tantalizing coexistence of order and randomness has stimulated a number of attempts to provide mathematical descriptions of the underlying processes.

There have been several theories to explain the partially ordered appearance of VATs. One, that there is growth competition between newly emerged VATs allowing them to proliferate according to their place in a growth hierarchy (Seed, 1978), has foundered after experimental analysis. Comparison of growth rates of trypanosomes expressing different VATs has shown that, although there can be some variation in growth rates among clones isolated at the same time from a single infection, there is little or no correlation with the VAT being expressed (Seed, 1978; Barry *et al.*, 1979b; Aslam and Turner, 1992).

Another theory was that presence of two VSG types on the surface of individual trypanosomes undergoing switching might be lethal, for example due to the onset of antibodies against the departing VSG or due to a mixed coat failing in its ability to protect the parasite (Agur *et al.*, 1989). Different VSG combinations could be lethal in different ways or at different rates, leading to the ordered appearance of VATs. This has effectively been ruled out by the observation that trypanosomes artificially engineered for simultaneous expression of different pairs of VSGs are not deficient in either rate of growth or the ability to produce relapsing growth *in vivo* (Muñoz-Jordán *et al.*, 1996).

A third main theory, long favoured by trypanosome biologists, is that order is imposed on the system basically through differences in the probability with which different VSG genes become activated: a hierarchy of gene expression. Disorder arises because these probabilities are imprecise, so that each gene has

a tendency, rather than a certainty, for activation at a given rate. The hierarchical appearance of VATs within the host ensues because of selection by the steadily growing pool of antibodies against different VSGs, as we described above (Section 5.2). Although this view has long been espoused by researchers studying the molecular biology (Laurent *et al.*, 1984; Liu *et al.*, 1985) or the population dynamics of antigenic variation (Barry, 1986, 1997a; Barry and Turner, 1991; Turner, 1999), it has only very recently gained support from modelling of infection. Frank (1999) has formulated a model demonstrating that small variations in the switching rate of each VAT yield the combination of order and disorder that features in antigenic variation. Such small variations in fact have been demonstrated in clones isolated from pleomorphic trypanosome populations (Turner, 1997). The attraction of the Frank model is not only this basis in experimental observation, but also that it has a testable mechanistic foundation, imprecise activation frequencies for VSG genes. There is considerable evidence that, at least at a global level, VSG genes are indeed subject to different activation frequencies according to where they are located in the genome (Laurent *et al.*, 1984; Liu *et al.*, 1985; Robinson *et al.*, 1999). We discuss this evidence below (Section 6).

In another theory (Antia *et al.*, 1996), aimed at explaining parasitaemia profiles rather than hierarchical VAT expression, VAT-specific immunity predominates in infection, but immunity against invariable antigens assumes importance later, effecting a more general suppression of trypanosomes. Although this model can reproduce the observed pattern of growth, there is no tenable evidence that immunity against invariable antigens is active against living trypanosomes. In fact, all evidence suggests otherwise, presumably due to the steric blocking action of the VSG coat. It has also been suggested that the immunosuppression in trypanosomiasis may serve to depress partially the anti-trypanosomal responses, enabling competition between trypanosomes of different serodemes during mixed infections (Turner, 1999).

5.4. Adaptations to Artificial, Laboratory Conditions

As with many unicellular organisms, trypanosome gene expression patterns have evolved to cope rapidly with changing environments. This can lead to complications during study in defined laboratory conditions. Microorganisms commonly adapt to novel, or even invariant, conditions by transcriptionally activating some genes and deactivating others, by using control systems downstream of transcription, or through the selection of mutants that arise randomly during prolonged growth. It is often the case that the action of subtly linked control systems, such as kinase–phosphatase activities, can lead to improved growth or survival, accompanied by an altered phenotype. Changes of this kind can occur through the absence, in laboratory conditions, of specific host

processes or molecules that demand a corresponding specific parasite phenotype. Knock-on effects can ensue, so that the relaxation of regulation can affect expression of other genes not subject to direct selection. Such changes certainly occur in trypanosomes, and there are now several important cases of alteration in phenotype arising during prolonged growth as a single stage in the laboratory.

A newly emerging example is in the pattern of expression of procyclins as a series of surface coats on all stages of *T. brucei* in the fly before the metacyclic stage. Different culture lines express different procyclin proteins depending, at least in part, on specific culture medium components, but *in vivo* the expression pattern appears to be much more tightly controlled (Butikofer *et al.*, 1997; Ruepp *et al.*, 1997; Mehlert *et al.*, 1999; Vassella *et al.*, 2000) and it is clear that an accurate picture of expression requires study *in vivo*. An analogous alteration in phenotype associated with laboratory adaptation occurs with antigenic variation. Prolonged syringe passaging between mammalian hosts results in loss of the capacity to differentiate beyond the long slender bloodstream stage and selection of 'monomorphic' lines. This occurs routinely, as evidenced from a number of lines that have been developed in trypanosome stocks of different genetic backgrounds (Le Ray *et al.*, 1977; Barry *et al.*, 1979a, 1985; Graham *et al.*, 1990; Turner *et al.*, 1991; Turner, 1997). The alteration may result from loss of a signal transduction pathway that causes trypanosomes to respond to a low molecular weight factor that induces differentiation of the short stumpy stage (Vassella *et al.*, 1997). It has long been known that monomorphic lines have a greatly depressed rate of antigenic variation (Le Ray *et al.*, 1977) and there is now evidence that they are altered in their usage of different genetic pathways for this variation (Barry, 1997a; Robinson *et al.*, 1999). It has even been proposed that selection conditions imposed during prolonged growth in the laboratory have led to the misidentification of one mechanism for antigenic variation (Graham and Barry, 1996). When the subject of study is a volatile phenotypic trait, such as that involved with antigenic variation, it cannot be overemphasized that due attention should be paid to what actually happens *in vivo*, with both gross phenotype and molecular pathways. This will be particularly important as the study of antigenic variation moves further towards amalgamating trypanosome organismal and population biology with studies of molecular processes.

6. GENETIC MECHANISMS OF ANTIGENIC VARIATION

The molecular mechanisms underlying trypanosome antigenic variation have been discussed in many previous reviews (see below), but these have focused primarily on those mechanisms that are used during growth of the parasite in

the mammalian host. Antigenic variation ultimately is a means of regulating the expression of the VSG coat to enhance the transmission of the parasite and, as noted above, differential VSG expression preventing immune destruction and enhancing transmission in fact occurs in both the metacyclic and blood-stream stages of the life cycle. We shall compare and contrast the current understanding of the molecular mechanisms involved in VSG expression in both life cycle stages. Perhaps surprisingly, given the contrasting biological requirements of the two systems, there are significant parallels to be drawn, and the available evidence suggests a common evolutionary origin for the two processes.

6.1. VSG Gene Expression Sites

One feature of VSG expression is invariant. VSG genes are expressed only when present in specialized telomeric locations, a set of transcription units called the VSG expression sites (Figure 3). As outlined below, there are substantial differences in genetic organization between the metacyclic expression sites (*MESs*) and bloodstream expression sites (*BESs*), but there are also striking similarities. In both sites, the VSG is located adjacent to the telomere. In addition, although a large number of *MESs* and *BESs* are present in the trypanosome genome, normally only one is expressed at a time, thereby encoding a homogeneous VSG coat on the cell surface. Finally, both expression sites are transcribed by an RNA polymerase that is resistant to treatment with the mushroom toxin α -amanitin. This characteristic, allied with other evidence (Chung *et al.*, 1992; Lee and Van der Ploeg, 1997), has suggested that the *BESs* and *MESs*, despite containing protein-coding genes, are transcribed by RNA polymerase I (pol I), as are the ribosomal RNA (*rRNA*) and procyclin loci (Laufer *et al.*, 1999). The *BES* promoter has an unusual structure, which can be aligned to match the *T. brucei rRNA* and procyclin gene promoters; no RNA polymerase II promoter has yet been described reliably in this organism. It is unusual to find similarity between promoters for *rRNA* genes and protein-coding genes. There are reported to be some minor differences in the requirements for active transcription of these different sets of genes, but at least the Mn^{++} effect can be explained by the experimental conditions used (Laufer *et al.*, 1999).

The known *MESs* have a similar structural organization (Figure 3). Based on studies on other life cycle stages (it is extremely difficult to study the metacyclic stage directly), they are all about 4–6 kb in size (measured from the presumed transcription start site to the telomere tract repeat sequences at the end of the chromosome). Only the VSG is expressed within each transcription unit, making them the only known monocistrons in trypanosomes. Other than the regulatory elements needed to direct *MVSG* RNA expression

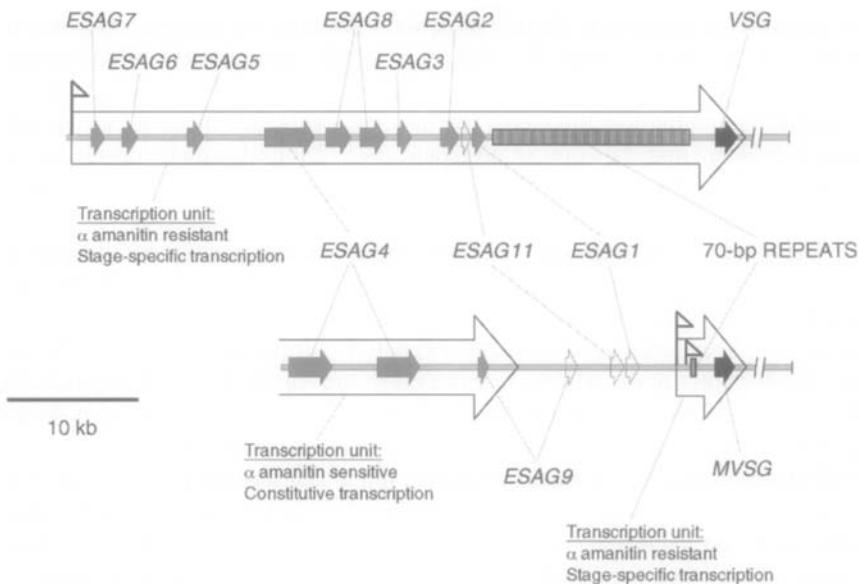


Figure 3 Expression sites for VSG genes in trypanosomes. The upper diagram shows a telomere containing a bloodstream expression site (*BES*), with the chromosome end to the right. Genes are depicted directionally, as filled arrows, and pseudogenes are shown as empty arrows. The 70 bp repeat regions are vertically hatched. The lower diagram is a typical metacyclic expression site (*MES*). The transcription units on both chromosomes are indicated by the large open arrows, running from the known *BES* promoter (flag) or two proposed *MES* promoters (flags). The upstream transcription unit of the *MES* begins further upstream, although the promoter directing this transcription has not been characterized. Most of the *BES* is an accurate graphic derived from the sequence of the AnTat 1.3A *BES* (Genbank accessions L20156, AJ239060), generated by Vector nti (Informax) software. The entire *MES*, containing the ILTat 1.61 *MVSG* gene and associated sequences, was generated similarly (accession AJ012199).

and processing, the only other DNA sequence of known function in the *MESs* is a short series of imperfect repeats termed the 70 bp repeats. A small number of repeats (typically 1–2) are found upstream of the *MVSG*, although some *MESs* have been described that do not contain any (Lenardo *et al.*, 1984). The 70 bp repeats probably have a function, by providing sequence homology during VSG switching by recombination (see below). Their small number in *MESs* may contribute to the relative invariability of the *MVSG* repertoire, by minimizing recombination with other VSG loci, leading to the predictability of *MVSG* expression. However, rearrangements of the *MVSGs* do occur occasionally, including deletions that are associated with the instability of the *MES* repertoire (Barry *et al.*, 1983). All *MVSG* genes appear to

be capable of activation during infections initiated by syringe injection of bloodstream trypanosomes (Le Ray *et al.*, 1978; Esser *et al.*, 1982), which means they can use bloodstream activation mechanisms in addition to the system that operates in the tsetse fly. In some cases, this bloodstream activation is frequent and uses the 70 bp repeats as 5' flanks during recombinational switching (Matthews *et al.*, 1990). Where the repeats are lacking, activation is rare and seems to involve unusual events (Donelson *et al.*, 1998).

The bloodstream-stage expression sites are both larger and more complex in structure than the *MESs* (see Figure 3). Current estimates suggest that there are approximately 20 *BESs* within the trypanosome genome, of which two to three have been sequenced or mapped in detail (Alexandre *et al.*, 1988; Pays *et al.*, 1989; Xong *et al.*, 1998), in contrast to the four to five *MESs* (Graham, S. V. *et al.*, 1999). The continuing *T. brucei* genome sequencing project should provide a more complete catalogue of the number of functional *BESs*, and a definitive comparison of their structures. It appears that the *BESs* are approximately 10 times larger than their metacyclic counterparts, usually being approximately 40–60 kb in size (see Figure 3), although they can be considerably shorter than that (Xong *et al.*, 1998). Conservation of the positioning of the VSG gene proximal to the telomere makes it attractive to speculate that this genomic location has functional ramifications for VSG expression or switch regulation. Upstream of the VSG are the same set of 70 bp repeats seen in the *MESs*, although in the *BESs* these take the form of an array, many kilobase pairs long, comprising up to hundreds of copies (Liu *et al.*, 1983; Campbell *et al.*, 1984; Shah *et al.*, 1987). The primary distinction between the two types of transcription unit lies upstream of the 70 bp repeats: here the *BES* contains a repertoire of expression site-associated genes (*ESAGs*) (Cully *et al.*, 1985; Pays *et al.*, 1989), which appear largely to be conserved between all *BESs*, and are absent from the *MESs*. The *ESAGs* are co-expressed with the VSG from a single promoter, leading to a multigene transcript that is co-transcriptionally trans-spliced (which leads to capping of the messenger (m) RNA) and polyadenylated, to yield individual mRNAs (Matthews *et al.*, 1994; Schurch *et al.*, 1994).

The number of distinct *ESAG* genes in the *BESs* is estimated currently as at least eight, but in some cases up to ten (Gottesdiener, 1994; Borst and Fairlamb, 1998). The functions encoded by some of the *ESAGs* are beginning to be understood. For example, *ESAG6* and *ESAG7*, found most proximal to the *BES* promoter, together encode a receptor for the uptake of host transferrin (Schell *et al.*, 1991; Steverding *et al.*, 1995), and *ESAG4* encodes an adenylate cyclase (Paindavoine *et al.*, 1992; Alexandre *et al.*, 1996). The functions encoded by other *ESAGs*, such as *ESAG2* and *ESAG5* (Alexandre *et al.*, 1988; Pays *et al.*, 1989), are unknown. Some others (e.g. *ESAG3* and *ESAG1*) are not limited to the *BESs*, since related gene copies have been described elsewhere in the genome (Carruthers *et al.*, 1996; Morgan *et al.*, 1996). Why it

should be necessary to co-express such a range of gene products with the *VSG* is not clear. This is not used to ensure high level expression for *ESAGs*, since the mRNA steady-state level of *ESAG1* is only a small fraction of that of the *VSG* mRNA (Cully *et al.*, 1985). In the case of *ESAG6* and *ESAG7*, presence in the *BESs* ensures expression of the transferrin receptor in the mammalian stages. For others, this might not be the case, since the non-*BES* copies of the genes cannot be subject to the same expression controls. Furthermore, a recent study has shown that the absence from the active *BES* of all but *ESAG5*, *ESAG6* and *ESAG7*, and absence of transcripts of the missing *ESAGs* that might emanate from other alleles, apparently had no adverse effect on trypanosome growth (Xong *et al.*, 1998). Another intriguing question is why the *MVSGs* are expressed from radically shorter transcription units. This may be associated with the distinct modes of *VSG* expression regulation operating in the two life-cycle stages, or with different requirements for the *ESAG* products.

6.2. Differential *VSG* Gene Expression in the Mammal

The strategy of antigenic variation in the mammalian stage, consisting of periodic changes in the *VSG* coat and evasion of the host immune system, requires a large repertoire of distinct *VSG* genes. It has been estimated, based somewhat indirectly on a calculation of the proportion of clones in a genomic DNA library that hybridized with a probe for the 70 bp repeats, that the trypanosome genome contains approximately 1000 silent *VSGs* (Van der Ploeg *et al.*, 1982). Whether or not this is an accurate figure for the number of *VSGs* that can be functionally employed will soon be answered by genome sequencing. The size of the *VSG* repertoire that is composed of intact genes (i.e. not pseudogenes with open reading frame mutations or lacking in processing signals) and can be immediately expressed is not known; nor is the number of *VSGs* that possess all the DNA sequence elements (e.g. 70 bp repeats) associated with efficient activation. The majority (perhaps 80–90%) of the silent *VSGs* are present in the interior of the large chromosomes (Figure 4). A substantial minority (perhaps 10–20%) are present at telomeric locations, primarily in the 100 or so minichromosomes (Figure 4) (Gull *et al.*, 1998; Ersfeld *et al.*, 1999), where they are not expressed. The remainder occupy the *BESs* and *MESs*.

Multiple mechanisms have been detailed by which a switch from one expressed *VSG* to another is achieved, and have been reviewed extensively (Borst and Cross, 1982; Pays and Steinert, 1988; Van der Ploeg *et al.*, 1992; Borst and Rudenko, 1994; Pays *et al.*, 1994; Borst *et al.*, 1996, 1998; Cross, 1996; Barry, 1997a; Cross *et al.*, 1998a; Donelson *et al.*, 1998; Pays and Nolan, 1998). Here, we will consider the switching mechanisms as either transcription-based or recombination-based events. Although convenient, this distinction may yet prove unfounded, as we still have much to learn about the

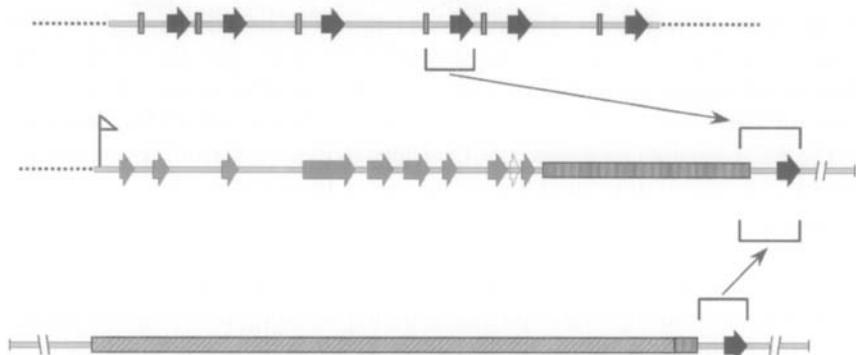


Figure 4 Activation of VSG genes in trypanosomes by recombination. Three chromosomal loci are shown: an array of chromosome-internal VSG genes (top), a BES (middle) and a minichromosome (bottom). The extent of sequence typically duplicated by gene conversion from silent VSG genes, replacing equivalent sequence in the BES, is boxed, and the arrows show the direction of sequence transfer. With minichromosomal VSGs, the downstream boundary of gene conversion can extend into the common repeats found at all telomeres (not shown). The upstream boundary of gene conversion for both types of silent gene is normally a set of 70 bp repeats (vertical hatching), and the minichromosome-specific 177 bp repeats (slanted hatching) have never been described as being involved in VSG switching. Silent BESs and MESs can also be used as VSG donors during switching (not shown).

genetic and biochemical mechanisms involved, and the potential regulatory processes that control switching.

A third class of switch mechanism has been proposed in which multiple point mutations arise during the generation of a copy of a VSG gene (Donelson, 1995). This is by analogy with antibody affinity maturation, where the spontaneous and rapid appearance of point mutations during transcription yields a series of cell lines synthesizing mutated antibodies from which are selected those lines that happen to have greater affinity for the corresponding antigen (Neuberger *et al.*, 1999). Notwithstanding that the trypanosome experiments are thought to have created conditions exerting a strong selective pressure for point mutations (as discussed by Graham, V. S. and Barry, 1996), it is difficult to conceive how only a partial change of epitopes could allow trypanosomes to reuse a gene whose product had already elicited a specific immune response. It is unlikely that a high rate of point mutagenesis is an authentic VSG switch mechanism.

6.2.1. *Transcriptional Switching*

Transcriptional (or *in situ*) switching occurs by inactivating the expression of the single actively transcribed *BES*, and activating expression from one of the silent *BESs*. In terms of direct contribution to the activation of all members of the *VSG* gene repertoire, this route is not of great significance, because it can occur only between expression sites, and is therefore able to execute a switch between only the small number of *VSG* genes that happen to occupy the *BESs*. Only recently have attempts begun to address why the trypanosome requires more than one site for *VSG* transcription (see Section 7.3). Furthermore, the mechanisms that act to control *BES* expression and switching remain unclear. Whether exclusive expression of a single *BES* is achieved at the level of transcription initiation or at a downstream control point is still being tested, as is the question of whether transcriptional switching is a regulated or a stochastic reaction.

Many of our advances in understanding transcriptional switching and *BES* regulation have come from recent work that has 'tagged' individual sites with unique marker genes, normally encoding antibiotic resistance. This appears to have ruled out mechanisms involving DNA rearrangements around the promoter (Zomerdijk *et al.*, 1990, 1991; Navarro and Cross, 1996; Horn and Cross, 1997; Rudenko *et al.*, 1998). One other potential control mechanism has also been ruled out: exclusive occupation of the nucleolus by the active *BES*. Because the *BESs* (and *MESs*) appear to be transcribed by RNA polymerase I (or a closely related enzyme), it was possible that the same nucleolar recruitment used by the *rRNA* genes (Trumtel *et al.*, 2000) was needed for expression site transcription. However, fluorescence *in situ* hybridization analysis with probes corresponding to the actively transcribed *BES* showed no co-localization with the nucleolus (Chaves *et al.*, 1998). This does not, however, rule out the possibility that a specific, but uncharacterized, sub-nuclear compartment is necessary for *BES* expression.

It has been held, until recently, that silencing of the inactive *BESs* in the mammalian-stage trypanosomes occurs primarily at the point of transcription initiation, with one *BES* promoter directing high level transcription and the others either being untranscribed or expressing very low levels of transcripts (Blundell and Borst, 1998; Navarro and Cross, 1998; Rudenko *et al.*, 1995). However, the extent to which the 'inactive' *BESs* are transcriptionally silent is now under scrutiny, with a number of reports of transcription extending a short distance down these expression sites (Rudenko *et al.*, 1994; Ansorge *et al.*, 1999; Vanhamme *et al.*, 2000). For antigenic variation to be successful, only a single *VSG* species should normally be expressed at one time in the trypanosome surface coat. It is important, therefore, that the *VSG* gene is silent in 'inactive' *BESs*, but this does not preclude the possibility that the promoter can be active and transcription terminates before it reaches the *VSG* gene.

Vanhamme and colleagues (2000) have shown that transcription from the silent expression sites is not incompatible with antigenic variation, since transcript elongation as far as the VSG occurs only in the single, fully active *BES*. (This work also casts doubts upon the interpretation in one report (Alarcon *et al.*, 1999) that appeared to detect VSG transcripts from silent expression sites.) It is interesting that a similar picture of one active site and many other partially active sites has emerged recently for the *var* genes that are responsible for antigenic variation in *Plasmodium falciparum* (see Taylor *et al.*, 2000). Why different *T. brucei* lines or individual *BESs* should display different basal levels of 'inactive' transcription is not clear, but the fact that some transcription can be found suggests that the inactive *BESs* are primed for expression, perhaps already containing some components of the transcription machinery (Navarro *et al.*, 1999; Vanhamme *et al.*, 2000). One note of caution is worth recording. The old concept that there is a dominant *BES* in each trypanosome strain has received some support in a recent survey of the transcriptional status of many of the *BESs*; the active AnTat 1.3 *BES* appears to assume a much greater dominance than any other active *BES* (Vanhamme *et al.*, 2000). It may be that there are qualitative differences amongst the *BES* set, complicating the control systems and the comparison of data from different studies. As a result of this continuing debate, several models have been proposed to explain the regulation and switching of *BES* transcription: telomeric silencing, transcriptional attenuation, monoallelic exclusion, and DNA modification. These are discussed below.

The first model is a modified version of the telomeric silencing phenomenon first described by Gottschling and colleagues (1990). In *Saccharomyces cerevisiae*, a specific and reversible higher order chromatin state that is capable of preventing expression of genes positioned proximal to chromosome ends can spread from telomeres. The cellular factors and mechanisms that govern this process are still being unravelled (reviewed by Grunstein, 1997; Sherman and Pillus, 1997). Because of the telomeric location of VSG genes, this seemed an attractive hypothesis to explain *BES* control, with the reversible nature of the chromatin-mediated silencing explaining transcriptional activation and inactivation. Two reports lent some support to this theory. Rudenko *et al.* (1995) demonstrated that the *BES* promoter could be replaced by a partially distinct promoter sequence (from the *rRNA* genes) and could still be activated and inactivated, and Horn and Cross (1995) showed that a number of promoters inserted within the *BES* could be silenced when the *BES* promoter is silenced. The fact that the *BES* promoter, despite considerable sequence conservation, is not the primary source of switching is in keeping with epigenetic mechanisms of transcriptional control. The observation of some transcription in silent *BESs* is not incompatible with telomeric silencing, and may be explained by variations in the extent of the *BES* sequence covered by chromatin, or alterations in the state of nucleosome packing around the

promoter. However, modifications of the yeast telomeric silencing model must be made to account for the unusual requirements of VSG expression, at least in *BESs*. The distances over which this putative silencing process would operate in bloodstream trypanosomes (40–60 kb) are much greater than normally seen around yeast telomeres (about 5 kb), and the reaction must act to silence a pol I promoter (only pol II and pol III silencing have been experimentally demonstrated in yeast; Borst *et al.*, 1998).

Recent observations have undermined the predictions made by the simple telomeric silencing model. Probing chromatin structure by micrococcal nuclease digestion could not detect differences between silent and active *BESs* around the promoter (Navarro and Cross, 1998), and Greaves and Borst (1987) had earlier found the same to apply around the VSG. Furthermore, Navarro *et al.* (1999) showed that bacteriophage T7 RNA polymerase was able to direct transcription within an inactive *BES* in bloodstream trypanosomes, but not when differentiated to the procyclic stage, suggesting that the chromatin structure in the former stage is actually in a state allowing some transcription when VSG switching is required. The same work (and others; Davies *et al.*, 1997) showed that disruption of sequences within the active *BES* leads rapidly to a transcriptional switch to another *BES*. This effect may be connected to the major deletions of chromosome ends that have been observed during analysis *in vitro* of transcriptional switching (Cross *et al.*, 1998b; Rudenko *et al.*, 1998), and suggests that specific *BES* sequences are necessary to maintain expression of the active *BES*. All this work is in keeping with a model presented by Vanhamme *et al.* (2000), who suggested that exclusive, maximal activity of a single *BES* is due to the selective recruitment of RNA elongation or processing factors to one expression site. Those authors suggested also that the inactive *BESs* do not possess these factors, and therefore express, from the vicinity of the promoter, only short transcripts that are transported inefficiently from the nucleus. Disruption of *BES* sequences that bind these factors would release them to interact with a silent *BES*, leading to its activation and a switch in expressed VSG. Furthermore, the incomplete transcription of all *BESs* in the procyclic stage (Rudenko *et al.*, 1994) can be explained by a global down-regulation of *BES*-specific elongation or processing factors, or perhaps both.

Attractive as this model is, it relies on the existence of *BES*-specific factors that must be identified before its validity can be tested. Although little progress has been made towards this end, some information has become available about the *BES* promoter. Mutational analysis has revealed that the most important regions of this promoter are the initiation site, consisting of CA, and two boxes, known as A and B, lying at, respectively, about -60 and -37 bases from the initiation base. The A box consists of the sequence CCCTAT, and the B box is nearly identical (CCCTGT), but in reverse orientation (Berberof *et al.*, 2000). Attempts to define transcription factors have

yielded the initial information that some factors bind in a double-strand specific fashion over the entire 70 bp of the promoter (Pham *et al.*, 1997) but, unusually, only single-strand specific factors bind to shorter segments. In particular, there is now evidence that a 40 kDa protein binds to the A and B boxes (Berberof *et al.*, 2000), with a consensus binding sequence CCCTNN. Intriguingly, this consensus sequence also corresponds to the telomere tract sequence of *T. brucei* – CCCTAA – and part of a transcriptional terminator that lies downstream of procyclin genes, and it appears that a similar 40 kDa protein, possibly the same one, binds to these too (Berberof *et al.*, 2000). The single strand specificity of binding raises the question of whether or not this protein binds at all to the DNA *in vivo*, or if local unwinding of the duplex is a physiological process important for either or both function and structure in these motifs. Generally, it is only the complementary sequence, GGGTTA, that exists single stranded at telomeres.

Chaves *et al.* (1999) tested the stochastic nature of *BES* transcription inactivation and activation predicted by yeast telomeric silencing. They found that two expression sites cannot be transcribed maximally at the same time. This is not because trypanosomes cannot accommodate two VSG species on their surface simultaneously (Muñoz-Jordán *et al.*, 1996), and so the authors concluded that activation of a silent *BES* and inactivation of the transcribed *BES* do not occur randomly, but are coupled reactions. This appears to rule out simple models of telomeric silencing. In contrast to Vanhamme *et al.* (2000), these authors favoured the idea that control of *BES* expression is exerted at the level of transcription initiation, and proposed a model that was comparable with monoallelic expression mechanisms found in higher eukaryotes. In such models, epigenetic control mechanisms ensure that only one copy of a multi-gene family is transcribed (Chess, 1998; Hollander *et al.*, 1998). How this might act in trypanosomes is not known, but competition for a limited amount of putative activating factor(s), or limited access to a sub-nuclear transcription domain, have been suggested (Navarro and Cross, 1998; Chaves *et al.*, 1999). In one important feature the current models of eukaryotic monoallelic expression must be modified to account for the requirements of *BES* expression during antigenic variation. The mechanisms that down-regulate expression of all the inactive *BESs* must be capable of being reversed to allow their reactivation, which contrasts with the stable inheritance of silencing/activation so far described in other eukaryotes (Chess, 1998).

Whether or not a common control mechanism acts upon both the metacyclic and bloodstream expression sites, and throughout the trypanosome developmental programme, is not yet clear. Subtle changes in the activities predicted by both the telomeric silencing and transcriptional attenuation models could account for the changing patterns of *BES* and *MES* activity in the different life cycle stages. In the former model, alterations in the extent of chromatin spread from the telomere could act, whereas in the latter the

putative elongation factors might recognize common motifs in the *BESs* and *MESs* and might simply become down-regulated in the procyclic stage. The Chaves model suggests that a distinct mechanism acts in the bloodstream stage compared with the procyclic stage, where all *BESs* are thought to become partially transcribed (Rudenko *et al.*, 1994). This must mean that the bloodstream form controls that limit transcription initiation to only the active *BES* are alleviated in the procyclic stage, but the purpose of such complex regulation is not obvious. Furthermore, whether or not the bloodstream control process is reactivated in the metacyclic stage, where again a single expression site is activated (although here the *MES* activation/inactivation decision is thought to be irreversible), awaits further examination.

Another factor complicates this emerging picture of *BES* transcriptional control mechanisms. This is a modified base called β -D-glucosyl(hydroxy-methyl)uracil, or J, which replaces a fraction of thymine in trypanosome DNA (Gommers-Ampt *et al.*, 1993). J is present in the DNA of only mammalian-stage *T. brucei* (Gommers-Ampt *et al.*, 1991; van Leeuwen *et al.*, 1998a) and its presence explains the observed modification of certain restriction endonuclease activities in these cells (Bernards *et al.*, 1984b; Pays *et al.*, 1984). Most J is found in the telomere repeats (van Leeuwen *et al.*, 1996), and it may generally be localized to repeat sequences, as suggested by its presence in the 50 bp repeats found upstream of the *BESs*, and in the minichromosomal 177 bp repeats (van Leeuwen *et al.*, 1997). Significantly, J is also found throughout the *BES* sequences (including the *ESAGs*, 70 bp repeats and *VSG*), but only when the expression sites are inactive. In the single active *BES*, J is excluded from the transcribed region (promoter to *VSG*), but is still present in the flanking, and presumably untranscribed, repeats. This is all in keeping with the hypothesis that J is an unusual form of DNA modification that is responsible for silencing, with competition between transcription and DNA modification determining the activity or silence of each *BES* (Bernards *et al.*, 1984b). However, some observations suggest that J is not exclusively involved in transcriptional silencing of *VSGs*. It is present also in the rDNA repeats of *T. brucei*, and can be detected in species of Kinetoplastida and *Diplonema* that do not undergo antigenic variation (van Leeuwen *et al.*, 1998b). Experimentally increasing or decreasing its amount in trypanosomes causes a slight de-repression of silenced transcription around the *BES* promoters (van Leeuwen *et al.*, 1998c). Together, these data suggest that J has been co-opted into helping control *BES* transcription in trypanosome antigenic variation, where it most probably has an indirect role in the formation or stabilization of silencing, and that it may also have other cellular functions (Borst *et al.*, 1998; van Leeuwen *et al.*, 1998c). Interestingly, a nuclear factor has now been identified from *T. brucei* and other kinetoplastids that binds specifically to J (Cross *et al.*, 1999). Whether or not this protein also functions in *BES* silencing, confirming that J has an indirect effect on the process, can now be tested. Despite some

apparent similarities in VSG expression control exerted on the bloodstream and metacyclic expression sites, J is undetectable in metacyclic-stage trypanosomes (van Leeuwen *et al.*, 1998a). This may indicate a difference in the control mechanisms that act in the bloodstream and metacyclic stages, or it might be a consequence of the metacyclic cells being non-dividing and therefore not requiring a replication-linked function of J, such as stabilization of silencing during DNA replication.

6.2.2. Recombinational Switching

DNA recombination is the most common route for VSG switching. As much as 98% of the VSG repertoire is in non-transcribable loci and can be activated only by recombination into *BESs*. Recently, it has been demonstrated that recombination is also the most actively used switching mechanism (Robinson *et al.*, 1999). Three different recombination reactions have been described: gene conversion-like reactions using chromosome-internal or telomeric VSG genes as sequence donors (often referred to, respectively, as duplicative transposition or telomere conversions); reciprocal exchanges between telomeric VSGs; and mosaic gene formation, where segments of VSG genes or pseudogenes are shuffled to create new VSGs that are subsequently expressed from *BESs*. Much is known about the relative contribution of the different reactions to VSG switching and the DNA sequences involved, but still very little is known about the detailed biochemical pathways and genetic controls. For example, no recombination intermediate has been described and, until recently, no protein involved in controlling or catalysing the reaction had been characterized (see below). Moreover, a debate is still running on whether this is a truly random process or is biochemically driven.

From extensive analysis, gene conversion has been shown to be the major recombination route in VSG switching, in terms of both the proportion of genes that use it and the frequency with which it operates during switching between peaks (Bernards *et al.*, 1981, 1984a; Pays *et al.*, 1981, 1983b; Liu *et al.*, 1985; Timmers *et al.*, 1987; Robinson *et al.*, 1999). In this process, the VSG within the expression site is deleted and replaced by a copy generated from a silent VSG (see Figure 4). The length of VSG sequence replaced must encompass all the exposed VSG epitopes encoded within the gene, and in fact normally extends to blocks of homology upstream and downstream of the gene (Liu *et al.*, 1983; Michels *et al.*, 1984). For chromosome-internal VSG genes, 70 bp repeats (also found within the *BESs*) normally demarcate the upstream conversion boundary (Liu *et al.*, 1983; Campbell *et al.*, 1984; Aline *et al.*, 1985; Shah *et al.*, 1987; Matthews *et al.*, 1990), whereas short blocks of homology in the C-terminal coding region or 3' untranslated region act as the downstream boundary. While this has the look of a convenient 'cassette'

for *VSG* recombination, the extent of gene conversion can be greater when the silent *VSG* is telomeric. In this case, the downstream boundary has been shown occasionally to extend into the chromosome's telomere repeats; this could imply either a resolution of the gene conversion event within the common telomere repeat sequences, or that the entire *VSG* and telomere are copied into a *BES* (de Lange *et al.*, 1983; Aline and Stuart, 1989; Scholler *et al.*, 1989). The upstream boundary of gene conversion can extend beyond the 70 bp repeats (Laurent *et al.*, 1983; Pays *et al.*, 1983a; Myler *et al.*, 1984), but only when the donor, silent *VSG* gene is present within an *ES* and upstream sequence homology is present. This normally limits such a reaction to silent *VSGs* in *BESs*, but one case of an entire *MES* (*VSG* and promoter) gene conversion has been detailed (Kim and Donelson, 1997).

The predominance of the 70 bp repeats as a sequence element during *VSG* switching has suggested that they act as initiating elements in the conversion process, either by acting as substrate for an endonuclease or by adopting unusual structures conducive to general recombination activities (Liu *et al.*, 1983; Pays, 1985; Matthews *et al.*, 1990; Weiden *et al.*, 1991; Barry, 1997a). The possibility has been raised, based on a survey of all the switch events in the literature, that switches normally involve the 70 bp repeats, except in low-switching, laboratory-adapted lines. It has been proposed that these lines are deficient in the ability to involve the repeats, relying instead on the use of other homologous sequences in *VSG* gene flanks (Barry, 1997a). Deleting the 70 bp repeats from the active *BES* in monomorphic cells did not prevent *BES* gene conversion reactions (McCulloch *et al.*, 1997), showing they are not indispensable in *VSG* switching, but the same experiment is yet to be performed in high-switching trypanosome lines not adapted to growth in the laboratory. Even if they have no active role, the repeats are composed primarily of triplet TTA.TAA repeats, which have biochemical properties distinct from all other characterized triplet repeats in that they predispose the DNA helix to melting *in vitro* (Ohshima *et al.*, 1996). These unusual characteristics may facilitate *VSG* interactions during recombination.

The flexibility in the extent of sequence copied during *VSG* gene conversion is reminiscent of reactions that rely upon homologous recombination (see, e.g., Shinohara and Ogawa, 1995; Kanaar *et al.*, 1998; Paques and Haber, 1999), rather than being a site-specific transposition process (see, e.g., Grivell, 1996; Belfort and Roberts, 1997). This has now been critically assessed, by inactivating, in monomorphic trypanosomes, the gene that encodes RAD51 (McCulloch and Barry, 1999), an enzyme central to much of DNA strand exchange in eukaryotes. RAD51 is a eukaryotic homologue of bacterial RecA and catalyses the transfer of single-strand DNA ends into intact duplex DNA, where it searches for sequence homology and generates strand exchange intermediates (Shinohara *et al.*, 1993; Baumann and West, 1998; Thacker, 1999). Disruption of *RAD51* substantially impairs the ability

of trypanosomes to switch their VSG coat, suggesting that homologous recombination is used. Switching is still possible, however, and apparent VSG gene conversions can be detected. This is compatible with work in other eukaryotes that suggests that multiple conserved pathways of homologous recombination can lead to genetic exchange (Paques and Haber, 1999). For trypanosome VSG switching, many of these pathways appear to involve exchange catalysed by *RAD51*, but other routes are also present (this is discussed more fully below). Two factors complicate this analysis. The first is that the same *RAD51* disruption is yet to be analysed in high-switching cells. These use primarily gene conversion for VSG switching (Robinson *et al.*, 1999), but it is not yet known whether this is due to greater activity of a discrete recombination pathway (perhaps involving *RAD51*) or all types of homologous exchange. Secondly, the *RAD51* mutant cells also appeared to have a reduced rate of transcriptional switching. It is not obvious why *RAD51* should influence this reaction, since no recombination is known to be involved (it is interesting to note, however, that when two *BES*s undergo transcriptional switching they appear to be in close proximity in the nucleus; Chaves *et al.*, 1999). One possibility is that there is a DNA modification of *BES*s involved in switching that also stalls DNA replication, necessitating the use of recombination activities to trigger resumption of replication (Cox *et al.*, 2000). This is not likely, however, as quantitative alteration of the only detectable modification in trypanosomes (*J*) appears not to influence the level of switching in the ways predicted for stalling (van Leeuwen *et al.*, 1998b, 1998c). Another possible way in which *RAD51* might be involved in *in situ* switching is through a regulatory function, but no such activity has been detected in numerous studies in other organisms.

Telomeric VSG reciprocal exchange was first described by Pays *et al.* (1985a). More examples have been found more recently (Rudenko *et al.*, 1996; Chaves *et al.*, 1999), and a switching event that could be explained by an aberrant telomeric exchange has also been observed (Robinson *et al.*, 1999). Although these VSG switching reactions are readily explained by general homologous exchange, they occur less commonly than gene conversion reactions, which is in keeping with the general observation in eukaryotic mitotic recombination that intermolecular cross-overs, even between homologous chromosomes, are rarer than intramolecular exchanges and non-reciprocal gene conversions (Richardson *et al.*, 1998; Burgess and Kleckner, 1999; Paques and Haber, 1999). Directing homologous recombination in high-switching trypanosomes towards gene conversion, in preference to reciprocal exchanges, would facilitate genetic exchanges between VSG sequences without the potentially deleterious consequences of up-regulating all forms of genetic exchange across the whole genome. In fact, despite considerable variation in the size of individual chromosomes and chromosome homologues between, and within, trypanosome strains (Melville *et al.*, 1998), gene order

appears to be constant, suggesting that large-scale genetic rearrangements and translocations are relatively rare.

Mosaic VSG formation (Figure 5) was discovered during analysis of VSGs expressed late in infection, which has been taken to mean that it is a relatively rare event that is unmasked when circulating antibodies select against intact, readily expressed VSGs (Longacre and Eisen, 1986; Roth *et al.*, 1986; Pays, 1989; Thon *et al.*, 1989, 1990; Kamper and Barbet, 1992). The formation of mosaic genes depends on there being families of VSGs, the members of which can employ sequence similarities to interact in partial gene conversion events. Transfer of trypanosomes into fresh hosts, and therefore away from antibodies against related VSGs that have already been expressed, can yield mosaic products that share extensive epitope-encoding regions with their intact progenitor genes (Pays *et al.*, 1985b). When the mosaic product arises within the course of a single infection, and there is likely to be antibody pressure against shared epitopes, the mosaic patterns can be much more intricate, involving many small patches of conversion. Barbet and Kamper (1993) have argued that the mosaic phenomenon may in fact be more extensive than suspected. As we note below, steps along the pathway of construction of a complex mosaic

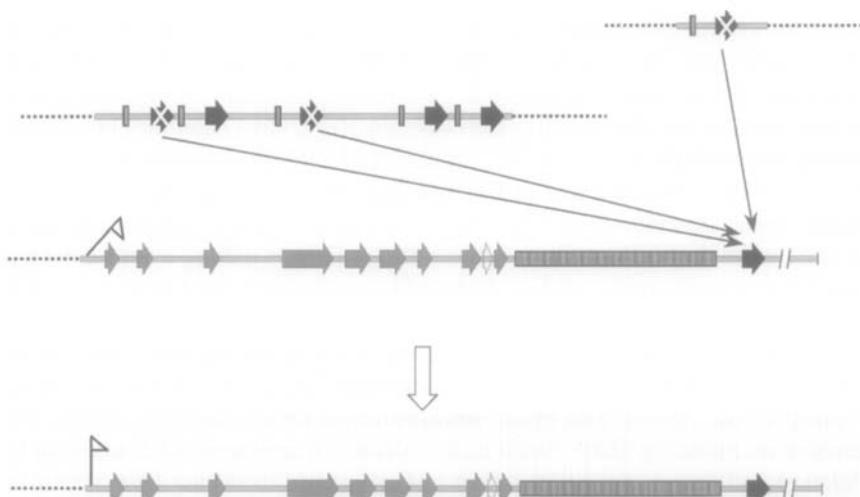


Figure 5 Mosaic VSG gene formation in trypanosomes. Two chromosome-internal VSG gene loci are depicted at the top, and a transcriptionally silent BES is shown below them, with an inactive promoter. Three VSG pseudogenes, marked with white crosses, each contribute sequences to the VSG gene in this silent BES. Following subsequent onset of transcription, the BES becomes active, yielding mRNA encoding the mosaic gene.

gene cannot occur in the active *BES*, because an incomplete product would be immunologically lethal to that individual trypanosome. It may be the case that trypanosomes have substantial numbers of incomplete VSGs, or VSGs with processing or translational mutations, which cannot be transposed as they are, but require shuffling and reassortment. The infrequency with which these novel VSGs would be generated may be capable of extending the length of an infection, thus enhancing transmissibility. It will be interesting to see from genome sequencing what proportion of the estimated 1000 silent VSGs fall into this class. The use of this means of generating diversity has the potential greatly to expand the extent of antigenic variation beyond the coding capacity of the genome. It also may be a key to the constant diversification of the silent VSG repertoire; there is a very large range of distinct VSG repertoires in circulation in the field (Van Meirvenne *et al.*, 1977).

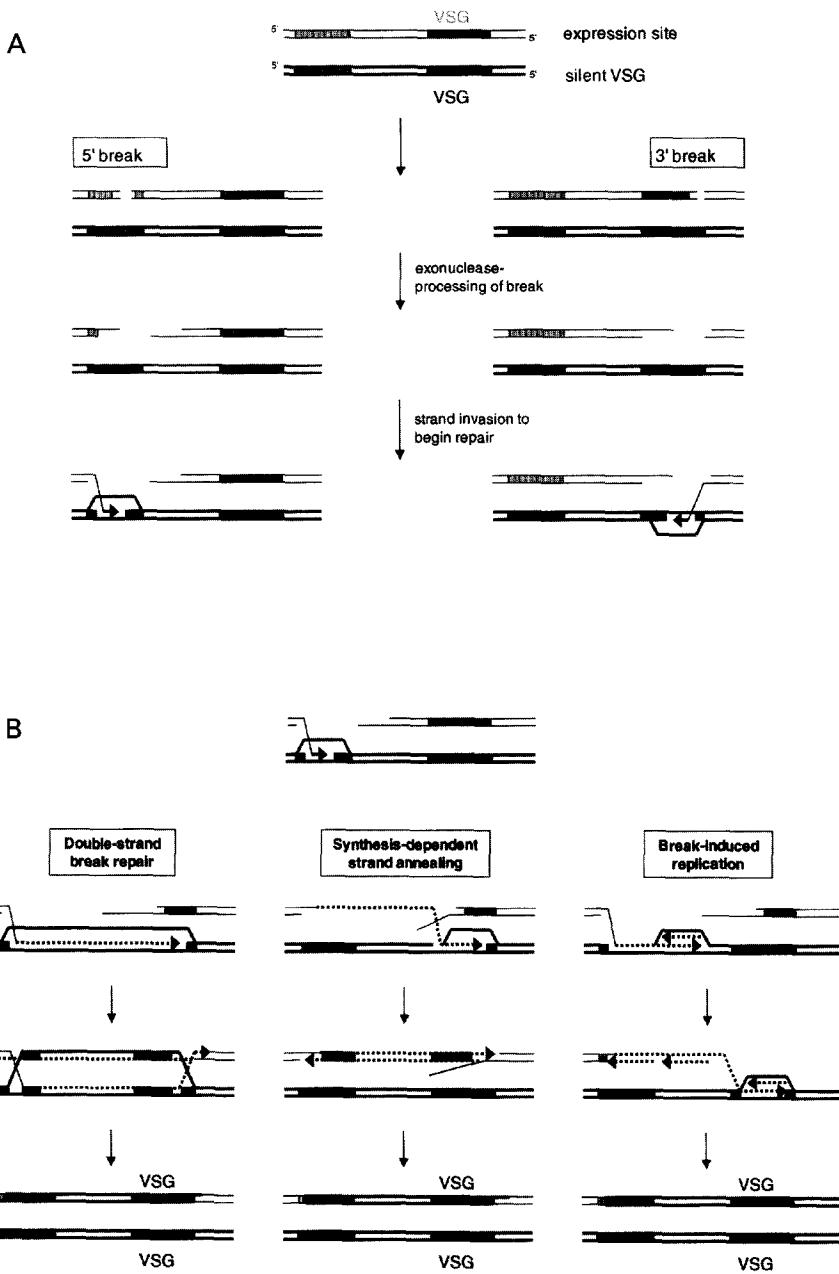
In the absence of characterized intermediates of VSG switching, especially in high-switching trypanosome lines, we should be careful about predicting the homologous recombination pathways that are involved. It remains possible that forms of non-homologous recombination, such as non-LTR retrotransposon copying mechanisms (Kazazian, 1998), could occur that look like homologous recombination-mediated gene conversions. Indeed, we know very little about trypanosome recombination pathways in general. Nevertheless, the phenotypes observed in *RAD51* mutant cells, and the descriptions of the DNA sequences implicated in VSG gene conversions (and reciprocal recombination), are compatible with homologous recombination reactions described in other eukaryotes (Shinohara and Ogawa, 1995; Paques and Haber, 1999). It is now clear that the enzymes responsible for homologous (and non-homologous) recombination, and the reaction pathways involved, have been well conserved throughout evolution (Cox, 1997; Kanaar *et al.*, 1998). Recombination reactions first described in yeast or bacteria have now been clearly identified in higher eukaryotes (and vice versa). Below, we describe three putative pathways of VSG switching. All are compatible with the VSG switching events detected thus far in trypanosomes, and are forms of homologous recombination detailed by experimentation in a number of organisms.

The three putative recombination pathways are termed double strand break repair (after Szostak *et al.*, 1983), synthesis-dependent strand annealing (Nassif *et al.*, 1994), and break-induced replication (Kogoma, 1996). All involve an initiating DNA break in the actively transcribed *BES* and lead to deletion and then replacement of the VSG sequence, resulting from repair of the break by copying the sequence of a silent VSG (Figure 6). They are distinguished by the types of copying mechanism involved and by the way copying intermediates are resolved into separate DNA molecules. Versions of synthesis-dependent strand annealing have been proposed previously for VSG gene conversion (Borst *et al.*, 1996; Barry, 1997a) but, in the absence of substantial genetic and biochemical characterization of the reactions, the other two models

must also be considered. Moreover, the fact that VSG gene conversion reactions can persist in the absence of RAD51 (see above) suggests that multiple pathways do act, and it remains to be seen which (if any) predominate. The advantage of considering these reaction schemes is that we can predict at least some of the likely conserved recombination factors that act at each step, and thereby critically test them.

Where and how a double strand break occurs to initiate VSG switching remains controversial. Barry (1997a) has proposed that an endonuclease acts upon the 70 bp repeat sequences, and that the absence or decrease of this activity in laboratory-adapted trypanosomes has led to their lower switch rate. Such an endonuclease remains to be isolated, if it exists. Furthermore, cleavage around the 3' conserved sequences, with a reversal of the direction of sequence copying during recombination (Figure 6A), is also possible, although it cannot explain gene conversions that involve the entire telomere (e.g. by break-induced replication). A more generalized role for the 70 bp repeats, assuming they are intrinsically prone to creating breaks (Liu *et al.*, 1983; Weiden *et al.*, 1991), seems unlikely, since laboratory-adapted trypanosomes lacking the repeats in a specific *BES* still undergo gene conversion into that *BES*, with no change in efficiency (McCulloch *et al.*, 1997). Random DNA breaks within the *BES* may be sufficient to initiate switching, although it is difficult to envisage how these could occur more frequently in high-switching cells, unless the trypanosomes are subject to greater environmental stresses (Torkelson *et al.*, 1997; Ochman, 1999; Radman, 1999).

Double strand break repair (Figure 6B) involves the creation and resolution of the standard DNA strand exchange structures known as Holliday junction recombination intermediates. In prokaryotic organisms, at least, specific Holliday junction-resolving enzymes complete the recombination reaction (Eggleston and West, 1996; Shinagawa and Iwasaki, 1996) and, depending on which of the alternative resolution routes is used, the recombination or conversion event can be accompanied by exchange of flanking chromosomal sequences. In reactions involving silent, chromosome-internal VSGs, flank exchange of this sort could create potentially lethal translocations into the *BESs*. For this reason, two previous models (Borst *et al.*, 1996; Barry, 1997a) have favoured the synthesis-dependent strand annealing reaction, in which flanking cross-overs are rare, because it incurs no such specialized Holliday junction recombination intermediates (Figure 6B). Although this reasoning has merit, it is not necessarily correct. It appears that the VSG genes most frequently used as conversion donors are telomeric (Liu *et al.*, 1985; McCulloch *et al.*, 1997; Robinson *et al.*, 1999). In these reactions, cross-over of sequences downstream of the VSG would have no deleterious effect, since only telomeric sequences would be exchanged. In fact, we cannot exclude the possibility that those reactions resembling gene conversions of the whole telomere (de Lange *et al.*, 1983) are actually resolved by a cross-over in the telomere repeats. It is



also not clear how important a catalytic role RAD51 plays in synthesis-dependent strand annealing. At least in yeast, another recombination enzyme, RAD52, seems to play a more central role in strand annealing repair pathways (Mortensen *et al.*, 1996), perhaps the most relevant model being *Saccharomyces cerevisiae* mating type switching (Haber, 1998). Although conserved in eukaryotes (Haber, 1999), including perhaps the primitive *Giardia duodenalis* (J.D. Barry and R. McCulloch, unpublished observations), RAD52-homologous sequences have not yet been identified in trypanosomes, *Drosophila melanogaster* or *Caenorhabditis elegans*. For this reason, we cannot readily address whether or not the reduction in VSG switching in RAD51 mutants is incompatible with the use of a synthesis-dependent strand annealing reaction mechanism.

Break-induced replication, or recombination-dependent replication, is an alternative strategy for repair of DNA breaks that arise primarily during DNA replication (Kogoma, 1996; Bosco and Haber, 1998; Haber, 1999; Marians, 2000; Rothstein *et al.*, 2000). (In fact, re-establishment of stalled replication forks may be the main function of bacterial DNA homologous recombination

Figure 6 A hypothetical scheme for the reaction steps in VSG switching in trypanosomes by gene conversion. A (top). Only the 70 bp repeats (vertically hatched box) and VSG sequences (shaded box) are shown; for the bloodstream expression site (BES; thin lines) this corresponds to the telomere-proximal region, whilst the silent VSG (thick lines) can be telomeric or chromosome-internal. The putative double-strand break which initiates switching is shown at the boundaries of the chromosome-internal VSG transposition 'cassette' (the 70 bp repeats and 3' end of the VSG), but this is theoretical and a break anywhere between these sites could in fact initiate gene conversion of chromosome-internal VSGs. 5' to 3' nuclease resection of the broken DNA ends exposes 3' single-strands, which are acted upon by recombination enzymes (e.g. RAD51) and transferred into homologous duplex DNA to initiate repair DNA synthesis. For simplicity, strand invasion is shown to involve only one end, but both 3' ends could act together (except during break-induced replication). B (bottom). Three pathways to repair the broken BES. Left: double-strand break repair. Newly synthesized DNA (broken line) is copied from the silent VSG sequence and remains associated with the intact duplex template. Annealing of homologous sequences between the displaced bubble and the broken BES yields two Holliday junctions, which can be resolved to produce gene conversion products with (not shown) or without (shown) crossover of flanking sequences. Centre: synthesis-dependent strand annealing. The newly synthesized strand is displaced from the duplex template. DNA synthesis continues until homology is reached at the broken BES, where re-annealing takes place followed by synthesis of the other strand (some trimming of non-homologous sequence is normally necessary for this to proceed). More complex models (not shown) can be envisaged that involve leading and lagging strand synthesis from the strand exchange intermediate. Right: break-induced replication. The strand exchange intermediate is acted upon by replication enzymes to establish an origin-independent, replication-dependent replication fork capable of traversing large distances. The reaction is terminated by the chromosome end (shown), by sites of replication termination (not shown), or perhaps by recombination recapture using homologous sequences (not shown).

systems; Cox *et al.*, 2000.) As before, repair of the double strand break begins by strand invasion of intact duplex DNA by one end of the break. Rather than this intermediate being acted upon by recombination enzymes, an origin-independent replication fork is established (involving both leading and lagging-strand DNA synthesis). As shown in Figure 6B, the replication fork simply progresses until the end of the chromosome. If this were to act during VSG switching, it could involve only silent telomeric VSGs as donors, although recently it has been suggested that the same pathway could perform more limited gene conversion reactions (Paques and Haber, 1999). This is potentially interesting, because the reaction appears not to require RAD51 (Malkova *et al.*, 1996), and is therefore a viable mechanism to explain the apparent VSG gene conversions in trypanosomes lacking this enzyme. Moreover, replication-mediated repair seems the most likely candidate mechanism to explain gene conversion events that apparently copy very large sequence tracts, such as the many thousands of bases around the VSG that can extend into the ESAGs (Pays *et al.*, 1983b; Liu *et al.*, 1985; Bernards *et al.*, 1986; McCulloch *et al.*, 1997), and in some cases the entire BES (Cross *et al.*, 1998b).

6.2.3. Is there a Genetic Basis for Hierarchical VSG Switching?

To be able to conclude whether or not there is a genetic basis for the hierarchy of VSG expression, it is important to determine the relative contribution of the various described VSG gene-switching pathways. These mechanisms for differential VSG gene activation are mere servants of the phenotypic requirements of antigenic variation. Why are there so many of them? Although it is possible that there is an existential reason for their abundance, it seems more likely that they have evolved to enable the system to operate efficiently to the benefit of the trypanosome. Other enhanced phenotypic variation systems do not display this great mechanistic diversity, making do with fewer, usually single, mechanisms. In most cases where different mechanisms are used, this has resulted from the simultaneous operation of distinct phenotypic variation systems, generating diversity in more than one trait at a time; in these cases, each variation system still uses a single switch mechanism.

It appears that *T. brucei* probably is not very different from other micropathogens. All of the VSG switch mechanisms were described originally in monomorphic trypanosome lines, which are well recorded as switching at an overall rate of about 10^{-6} switch/cell/generation (Lamont *et al.*, 1986; McCulloch and Barry, 1999), which is equivalent to the background (point) mutation rate (Valdes *et al.*, 1996). Pleomorphic trypanosomes, on the other hand, switch at an overall rate of 10^{-2} switch/cell/generation, with a measured range of 3×10^{-2} to 1×10^{-5} (Turner and Barry, 1989; Turner, 1997). It has now been shown that, even at the low end of this range, duplication is by far the

most common switch mechanism, occurring with a frequency of at least 80% (Robinson *et al.*, 1999). In contrast, monomorphic trypanosomes switch mainly transcriptionally, at a frequency of about 60% both *in vivo* and *in vitro*, and then less commonly by duplication (20–33%) (Liu *et al.*, 1985; McCulloch and Barry, 1999) (although it should be noted that experiments *in vitro* could not distinguish between long-range interchromosomal exchanges and switches *in situ*). It has also been noted that pleomorphic, but not monomorphic, trypanosomes appear to have a preference for using 70 bp repeats as the upstream limit of duplication (summarized by Barry, 1997a and tested experimentally in a monomorphic line by McCulloch *et al.*, 1997). It is not difficult to imagine that there is a default mechanism in trypanosome antigenic variation, VSG gene duplication, and that this default has been lost, or greatly diminished, in monomorphic lines. At a very low level, well in the background, are a variety of spontaneous mutational events that are relatively more prominent in the literature than in the trypanosome. Although the less active mechanisms usually will be swamped by the default mechanism, they might well gain prominence when the host has become immune to the products of normal switching, providing rare survivors. This certainly appears to be the case for the formation of multiply mosaic genes that, by the nature of their multistep activation and randomness in yielding open reading frames, must be rare.

With this background, we can question whether there might be a genetic basis for the all-important hierarchical appearance of VSG expressors. There probably is. This question has been posed before, and it has been shown in monomorphic trypanosomes that the same VSG gene is activated more frequently when telomeric than when it occupies a chromosome-internal locus (Laurent *et al.*, 1984). Telomeric genes in general have been shown to be activated by duplication more frequently than internal genes (Young *et al.*, 1983; Myler *et al.*, 1984; Liu *et al.*, 1985; Van der Werf *et al.*, 1990; Robinson *et al.*, 1999). In pleomorphic trypanosomes, it is also the case that telomeric genes are activated preferentially, using the duplication process (Matthews *et al.*, 1990; Robinson *et al.*, 1999). A genetic basis for hierarchical activation therefore might be expressed in this order of frequency of use:

- duplication of telomeric genes (high-efficiency homologous recombination, utilizing not just gene flanks but also the high interactivity of telomeres);
- duplication of internal genes (homologous recombination using gene flanks);
- transcriptional switching;
- other homologous recombinational events;
- rare, multistep homologous recombinational events.

There would be an overlap between these members of the hierarchy,

because the initiating events involve an element of randomness. In what is essentially a naturally derived monomorphic line, *T. equiperdum*, Capbern *et al.* (1977) have reported that VSGs are expressed in three hierarchical groups, 'précoce', 'semi-précoce' (or 'semi-tardif') and 'tardif'. In *T. vivax*, however, such groups are not readily discernible (Barry, 1986). One reason could be that *T. vivax* has very few, if any, minichromosomes (Van der Ploeg *et al.*, 1984; Dickin and Gibson, 1989) and therefore a small pool of telomeric genes forming the head of the hierarchy.

6.3. Differential VSG Gene Expression in the Tsetse Fly

There are many basic similarities between the metacyclic and bloodstream VSG gene repertoires; it is likely that the few known differences are related directly to the biological differences between the two systems. Nucleotide sequence analysis suggests that MVSGs have all the features of BVSGs, and indeed they naturally form coats on bloodstream trypanosomes. The MVSG repertoire is encoded by an *MVSG* gene repertoire, which is distinguished by one main feature, the specialized type of locus its members occupy. That locus type confers life cycle stage-specific expression on *MVSG* genes (Graham *et al.*, 1998a).

Of about ten characterized *MVSG* genes, all are located at the telomeres of megabase chromosomes, and none is on a minichromosome (Lenardo *et al.*, 1984; Cornelissen *et al.*, 1985; Delauw *et al.*, 1987; Graham *et al.*, 1999). All are single copy genes, except where two telomeric copies exist (Cornelissen *et al.*, 1985). In those cases, there appears to be a dosage effect, with the fixed proportion of the MVAT in the metacyclic population being doubled in those trypanosome stocks where the gene number is doubled (Barry *et al.*, 1983). It is not certain, however, that all *MVSG* genes have this arrangement. The ILTat 1.3 MVAT, which is expressed at a very low level (GUTat 7.14 of Barry *et al.*, 1983), is encoded within a large gene family, only some members of which are telomeric (Young *et al.*, 1983). It may be that many, but not all, *MVSGs* are controlled one way, with others becoming expressed in a less programmed way. There are several possible explanations for those *MVSGs* that appear at very low levels and only sporadically (Barry *et al.*, 1983). They may represent barely used, conventional *MESs*, they may occur in other loci that become activated by rare accident, or they may result from rare gene conversion events within the epimastigote or premetacyclic population, yielding occasional metacyclic trypanosomes with a new *VSG* gene in the *MES* repertoire.

The activation and expression of *MVSGs* have been studied only indirectly, because of the low numbers of the metacyclic stage cells produced in flies and the lack of a culture system. As it is known that activation of individual *MVSG*

genes in the developing metacyclic population is polyclonal (Tetley *et al.*, 1987), distinguishing individual activation events requires study of individual metacyclic cells or clones derived therefrom. Analysis of metacyclic clones growing in mice as bloodstream-stage trypanosomes but continuing to express the MVSG has revealed that these genes are activated apparently without duplication or other rearrangements, indicating that they probably use differential transcriptional activation (Graham *et al.*, 1990). The same interpretation has been reached from analysis of uncloned populations following fly transmission (Lenardo *et al.*, 1986). Amalgamating these limited data with what can be deduced from the pattern of coating observed *in situ* in the nascent metacyclic population in the tsetse salivary glands (Tetley *et al.*, 1987), it seems that the MVSG system is based on regulation of transcriptional initiation through the life cycle, with selection of a gene for transcription being possible only at the metacyclic stage. At that point, when faced with the developmental instruction to begin synthesizing the VSG coat, the cell selects at random from the pool of MVSG promoters. The chosen promoter then drives transcription from a monocistronic expression site. There are clear differences not only from the bloodstream VSG system, but also from how gene expression in general proceeds in the trypanosome. In an organism that appears to be committed to polycistronic transcription and post-transcriptional regulation of gene expression, why and how does the MVSG differ in these ways? To answer these, it is necessary to understand how transcription is controlled.

Our knowledge of MVSG gene promoters is limited by the scarcity of the metacyclic stage and, to some extent, the need to study them clonally. Work so far has involved merely mapping transcription sites and testing the activity of putative promoters in life cycle stages other than the metacyclic stage. Two types of approach have been used to identify putative promoters, namely indirect study of other life cycle stages, or less indirect study of early bloodstream populations initiated by metacyclic trypanosomes; neither is ideal. The more indirect approach has been to use trypanosome stages that are more manipulable than the metacyclic stage. The most widely used promoter screen has been to subclone fragments lying upstream of MVSG genes and test their ability, in transient transfection assays in the procyclic stage, to drive expression of a plasmid-borne reporter gene. In two studies with different trypanosome stocks, this screen has yielded the same putative promoter, which is similar in sequence to the bloodstream VSG gene promoter (Nagoshi *et al.*, 1995; Vanhamme *et al.*, 1995). Application of the same screen, in a genome-wide search for any promoter of protein-coding genes, also yielded this element (McAndrew *et al.*, 1998). Another approach, applied to the bloodstream stage, has been the extensive selection of trypanosomes that express MVSG genes and then examination of whether these genes are being transcribed *in situ*, rather than having moved into a BES. The same putative promoter sequence resembling a BES promoter was obtained (Alarcon *et al.*,

1994; Kim and Donelson, 1997). Mutational analysis of this putative promoter revealed that its activity depends on residues distinct from the functional A and B boxes identified in the *BES* promoter (Kim and Donelson, 1997). None of this set of putative promoters has been tested, however, in tsetse transmission experiments.

The less indirect approach of studying transcription in metacyclic trypanosome clones has yielded different data. Initially by transcriptional run-on assay and then by primer extension on pre-mRNA, an initiation point was identified, the immediately upstream region of which resembles no known trypanosome promoter region (Graham *et al.*, 1998b, 1999). Alignment of these potential start sites for the two genes studied, encoding the ILTat 1.22 and 1.61 MVSGs, revealed very limited sequence similarity with each other (Graham *et al.*, 1999), although upstream of each lie sequences closely resembling the putative metacyclic promoter element suggested from study of other life cycle stages, as described above. Indeed, more recent primer extension studies directly on trypanosomes dissected from tsetse salivary glands have indicated that transcription may start at the latter putative promoter element (M.L. Ginger, P.A. Blundell and J.D. Barry, unpublished observations). It is now important that functional studies be undertaken in metacyclic trypanosomes.

6.4. Comparison of the Bloodstream and Metacyclic Systems

The contrasting phenotypic demands in the bloodstream and metacyclic life cycle phases have yielded two rather distinct genetic strategies for differential expression of VSGs. Nevertheless, both strategies operate within the general rules for VSG expression, such as having a shared gene structure and using only one gene at a time. A summary of the main features of the system (Table 1) reveals the similarities and differences.

Considering these features together, time is a major driving force in the bloodstream, whereas it is irrelevant for the metacyclic population, in terms of both when differential activation occurs – at the developmental decision to synthesize VSG – and when diversity is needed – the instant of transfer to a new mammalian host. The molecular processes for differential gene activation match this feature. Homologous recombination in the bloodstream stage is ideal, as it is linked intimately to DNA replication during the cell cycle (Cox *et al.*, 2000). The metacyclic stage, conversely, cannot indulge in replication-repair because it is cell cycle-arrested, so transcriptional initiation control is the best option available.

Table 1 Comparison of bloodstream and metacyclic systems for differential VSG expression.

	Bloodstream	Metacyclic
Silent VSG information	Extensive (1000 genes?)	Extensive for a small population (27 genes?)
Use of silent information	One VSG/trypansome	One VSG/trypansome
Mechanism of variation		
Differential VSG expression	Gradual	Immediate
Differential gene activation	Homologous recombination	Transcriptional
Maximizing diversity	Hierarchical expression	Random promoter activation
Evolution of silent repertoire	? Mosaicism of short segments ? Point mutation ? Movement between telomeres and internal loci	? Transfer of genes from BVSG repertoire
Growth status of stage	Mitotically active	Cell cycle arrested

7. EVOLUTION OF ANTIGENIC VARIATION

7.1. Was the VSG Ancestor a Receptor?

It has become apparent that the ancestor of the VSG might have been a receptor. Sequence analysis has revealed that some VSGs have peptide sequence similarity with the trypanosome's transferrin receptor (TfR), in the most notable case the variable domain of one VSG having 23% identity and 76% similarity with the *ESAG6* polypeptide (Hobbs and Boothroyd, 1990; Salmon *et al.*, 1997). Analysis of secondary structure potential indicates also a sharing of the α helical 'VSG fold' structure (Carrington and Boothroyd, 1996). Directed mutagenesis of the TfR, modelled on the known VSG structure, provided reasonable evidence that the two molecules do indeed share at least some structural elements (Salmon *et al.*, 1997). One major inference to be

drawn is that the VSG may have originated as a receptor and retained its general structure and dimensions, while losing its function. Dimerism is a feature of many receptors that is allied to function, and this structural feature has been retained by the VSG. Carrington and Boothroyd (1996) also noted that the sequences of other kinetoplastid surface proteins display a tendency to form the VSG fold. It would be interesting to seek, in free-living relatives, a common ancestor of the VSG and TfR, both of which presumably arose as a requirement of parasitism.

7.2. The Metacyclic VSG Repertoire Evolved from the Bloodstream System

Since the VSG coat is essential for survival in the mammalian host, it is likely that, earlier in evolution, the metacyclic population may have expressed a single coat type. However, as discussed in Section 5.1, it is plausible that there arose strong selective pressure for the evolution of differential VSG expression in the metacyclic stage. This is the presence of anti-VSG antibodies in partially immune hosts in the field. A consequent prediction is that the bloodstream VSG system evolved first and is likely to have become adapted for different use in the metacyclic stage. Strong clues that this is so exist in the sequence of the telomere regions containing *MESs*. Without exception, all genes and pseudogenes detected in the characterized telomeres are related to what occurs in *BESs*. Besides some VSG (pseudo)genes, (pseudo)genes corresponding to *ESAG1*, *ESAG4*, *ESAG9* and *ESAG11*, have been detected (see Figure 3). Some of these belong to upstream transcription units that are active in bloodstream and procyclic stages, and so possibly have genuine functions. The remaining *ESAG* homologous sequences, lying immediately upstream of the *MES*, are prominent in their lack of function; almost all are pseudogenes. This is a strong indicator that *MESs* arose from *BESs*, gaining a new promoter and losing the function of other genes along the way.

7.3. Why Is There More Than One Bloodstream Expression Site?

Why are there many *BESs*, when only one is required at any time for expression of a VSG coat? This question has received some attention in the past few years and there are two main theories that could provide rather different answers. Although there is as yet no definitive enumeration of *BESs* in the diploid genome, the presence of conserved sequences around the *BES* promoter has provided a small set of probes that have been applied to Southern blot analysis of trypanosome chromosomes fractionated by pulsed field gel

electrophoresis. Each of the 11 main chromosomes in the nuclear genome of *T. brucei* provides two ends and, as they are diploid, a total of 44 telomeres. Use of probes corresponding to the conserved *BES* promoter, the 50 bp repeats upstream of the promoter and some of the *ESAGs*, has revealed that there may be as many as 20 *BESs* in *T. brucei* S427 (S. E. Melville, personal communication) and that at least 12 individual chromosomes hybridize for *T. brucei* EATRO 795 (P. A. Blundell, V. Leech and S. E. Melville, personal communication), although it is not known whether some or all of these have a *BES* at both telomeres. If all *MVSGs*, which number up to 27, are located in expression sites at telomeres, it might be the case that all the telomeres of the megabase chromosomes are occupied by one or other type of expression site. It is known that telomeres of intermediate-size chromosomes can also harbour *BESs*, so the pool of available telomeres may be greater. By analogy with the yeast *Saccharomyces cerevisiae*, in which it is clear that the frequent genetic interactions among telomeres has led to a homogenization of their sequences (Britten, 1998), a very simple explanation for the abundance of *BESs* might be that telomeric sequences will inevitably spread, with no functional consequence. We do not favour this explanation, as the *BES* usually extends much further into the chromosome than the conserved sequence range seen in yeast. The theory that has gained most attention is that qualitative differences between *BESs* confer a potential for a wide host range. This is based on observed differences between different alleles of *ESAG6* and *ESAG7*, encoding the two TFR sub-units. There is ample evidence that TFRs encoded in distinct *BESs* have different affinities for transferrin (Tf) from different host species (Bitter *et al.*, 1998). Furthermore, based on the differential expression of two *BESs*, a relationship has been shown between the affinity for Tf of individual species and the ability of bloodstream trypanosomes to grow *in vitro*. As the high concentration of Tf *in vivo* would lead to occupancy of even low affinity TFRs and therefore might still fulfil the trypanosome's iron requirements, it has been proposed further that host range may be influenced by competition between antibodies and Tf for the receptor (Bitter *et al.*, 1998; Borst and Fairlamb, 1998). Thus, if high affinity antibodies can displace Tf, the infection might be controlled, but expression of a high affinity receptor would prevent that. A number of questions remains to be answered, including whether antibodies of sufficiently high affinity do actually appear in infection, whether these can exert an effect *in vivo*, and whether expression of an appropriate *BES* is selected in a given host species.

The other main theory for the existence of so many *BESs* (Barry, 1997b) is much simpler and is well supported from observation. As discussed above, there is considerable evidence that mosaic *VSG* genes are selected late in infection. As their assembly can involve more than two donor sequences, more than one recombination event is required and these are likely to occur in a *BES*,

the major recipient site of VSG gene conversions. Use of an active *BES* would be fatal, yielding an incomplete VSG or incompletely replacing already experienced epitopes. Hence, late in infection there is probably a very strong requirement for silent *BESs* that foster the assembly of mosaic genes and then become active. It could even be argued that transmission to tsetse flies at that stage absolutely depends on their presence, and transmission would fix that extra *BES* in the genome. It is thus easy to imagine how a duplication of the original *BES* could become selected, and subsequently spread over many telomeres.

An early theory was that the mysterious hypervariable regions of the TfR subunits might represent exposed regions of the receptor undergoing antigenic variation, so that the existence of many *BESs* represented the repertoire of TfR variant genes (Borst, 1991). One problem with this theory was that the range of TfR variants would be greatly in deficit of the range of variation displayed by the VSG, TfR's neighbour in the surface coat. Subsequent theoretical modelling of the TfR, based on sequence similarity with VSGs, suggested the hypervariable region would not be exposed to the host (Salmon *et al.*, 1997), apparently ruling out this hypothesis.

7.4. What is the Defence Budget of the Trypanosome?

Survival, especially against an enormously adaptable immune system, can call for an enormous investment by the pathogen. *T. brucei* has made a very large investment in its defence system. The silent VSG repertoire has been estimated crudely at about one-tenth of all the genes, and often these VSG genes are organized in cassettes about 4 kb long. Perhaps all chromosome ends are devoted to the system, with perhaps half of these being *MESs* that occupy 5 kb each and the rest being *BESs* that occupy about 45 kb each. Then there are the minichromosomes, which can be thought of as being wholly devoted to antigenic variation in the sense that the only coding sequences they contain are VSG genes. They number about 100 and total about 10 Mb of sequence. Combining these simple calculations, which are underestimates that ignore intergenic regions and extensive silent regions upstream of *ESs*, it becomes apparent that more than 15 Mb are associated with antigenic variation. In a diploid genome of 70 Mb, this amounts to more than 20% of the nuclear DNA. In *T. vivax*, the size of whose genome is unknown, absence of minichromosomes may mean that relatively less of the DNA is involved. This illustrates the plasticity of the genome of simple eukaryotes, and the speed at, and extent to, which they can evolve.

7.5. Key Events in the Evolution of Antigenic Variation in the Trypanosome

The multifarious ways in which *T. brucei* effects its antigenic variation are indicative of a cumulative recruitment of a number of different molecular processes in the cell. By considering the evolution of antigenic variation in this organism and the possible order of recruitment, it may be possible to discern what was the original system and which of these processes are the most important. The following points are worth discussing. All Kinetoplastida execute gene transcription polycistrónically, so few genes have their own promoter. It was only much later in evolution, after divergence of the salivarian trypanosomes, that antigenic variation evolved (Haag *et al.*, 1998; Overath *et al.*, 1999). This means that the variation system, which depends on differential gene expression, was built initially on a background not geared to differential promoter use. Perhaps it was easier to base differential expression on use of recombination to move sequences into transcription units. There appears to be considerable support for this argument from consideration of the final point, which centres on the antigenic variation system of the bacterial genus *Borrelia*.

There are striking similarities between trypanosomal and borrelial antigenic variation, at all levels, from pathogen population behaviour within the single infection to the molecular coat and to the strategy for differential gene expression. As outlined above (Section 3.2), *B. hermsii* has a densely packed surface coat composed of the VMP (variable major protein), which is encoded by a large gene family. Like the trypanosomal VSG, the VMP is post-translationally modified by addition of what appears to be a lipid membrane anchor and, at least theoretically, the VMP folds in much the same way as does the VSG (Burman *et al.*, 1990). Related proteins are encoded in other members of the genus, including *B. burgdorferi* and *B. miyamotoi* (see Hamase *et al.*, 1996). In *B. hermsii*, the silent VMP-encoding genes are carried on linear plasmids and are activated by being copied into a transcriptionally active, terminal locus in a linear plasmid (Plasterk *et al.*, 1985). In slight contrast, in *B. burgdorferi* the *vls* (vmp-like sequences) use segmental gene conversion, creating variation by mosaic gene formation (Zhang *et al.*, 1997; Zhang and Norris, 1998). It is believed that the unusual linear plasmids of *Borrelia* arose from more conventional circular plasmids (Casjens, 1999) and it is possible that the selective force for evolution of these strange replicons was antigenic variation. All these features bear remarkable similarity to the VSG system. There appears to be little question that an organism that lives extracellularly in mammalian blood is well served by an antigenic variation system based on recombinational means of differential gene expression. Perhaps the polycistronic organization of the genomes of these pro- and eukaryotes exerted strong pressure in this direction. With its

more complex genome, the trypanosome has since evolved further, adding transcriptional activation as a consequence of evolving more than one expression site. (Interestingly, *Borrelia* has also evolved a rosetting system whereby the bacterium apparently can hide amongst a cluster of blood cells (Burman *et al.*, 1998); so has *Plasmodium*, the malaria parasite, during its sojourn within erythrocytes.)

On this background, we present in Figure 7 a scheme outlining how the trypanosome antigenic variation system may have evolved. As is normal, it is likely that this evolutionary process was punctuated, with certain key events proving to be critical in the perfection of the antigenic variation function. Following crucial early steps, namely the overexpression of a receptor to form a coat and the loss of function that liberated the molecule for extensive variation, numerous alleles arose. It is likely that exclusive expression was important from the start, so it is possible that a locus akin to the *BES* was already in use; the gene for the related TfR resides there also. At about this time, the location of the active site became important, providing an enhanced rate of recombination. It is not possible to say whether the telomeric location existed previously, arose as an accident, or was a later adaptation. Once one variant allele became located within a chromosome, the strong selective pressure for expansion of the *VSG* repertoire allowed persistence of tandem duplication events arising as errors of DNA replication, leading to the expansion of internal arrays of silent genes. A mechanism for copying silent information into the active site was implemented and refined through the recruitment of a recombinogenic upstream flank, the 70 bp repeat. Adding a new dimension to antigenic variation, expression sites began to multiply, providing a haven for the assembly of mosaic genes and creating a tendency for geometric, combinatorial use of silent information. The phenomenon of silencing *in situ* was necessary in this situation, so silencing rather than activation became the new default for these sites, and switching between *BESs* has remained as a minor process in antigenic variation. The high rate of interaction amongst telomeres created a new élite of genes at the head of the expression hierarchy, and this process became extended by the development of a new, large set of telomeres: the minichromosomes are no more than a pool of telomeres with a necessary backbone made of repetitive DNA. With the success of this system, reservoir hosts co-evolved robust antibody responsiveness to *VSGs*, leading to new pressure on transmission that was ameliorated by the development of the metacyclic system that guarantees *VSG* diversity.

It is usually difficult to test such schemes, but there are some clues that are not inconsistent with this theory. The evidence for the exploitation of a receptor and for the evolution of *MVSG* expression sites has come from DNA sequence determination. Less strong evidence for the reason underlying selection of development of minichromosomes comes from the observation that

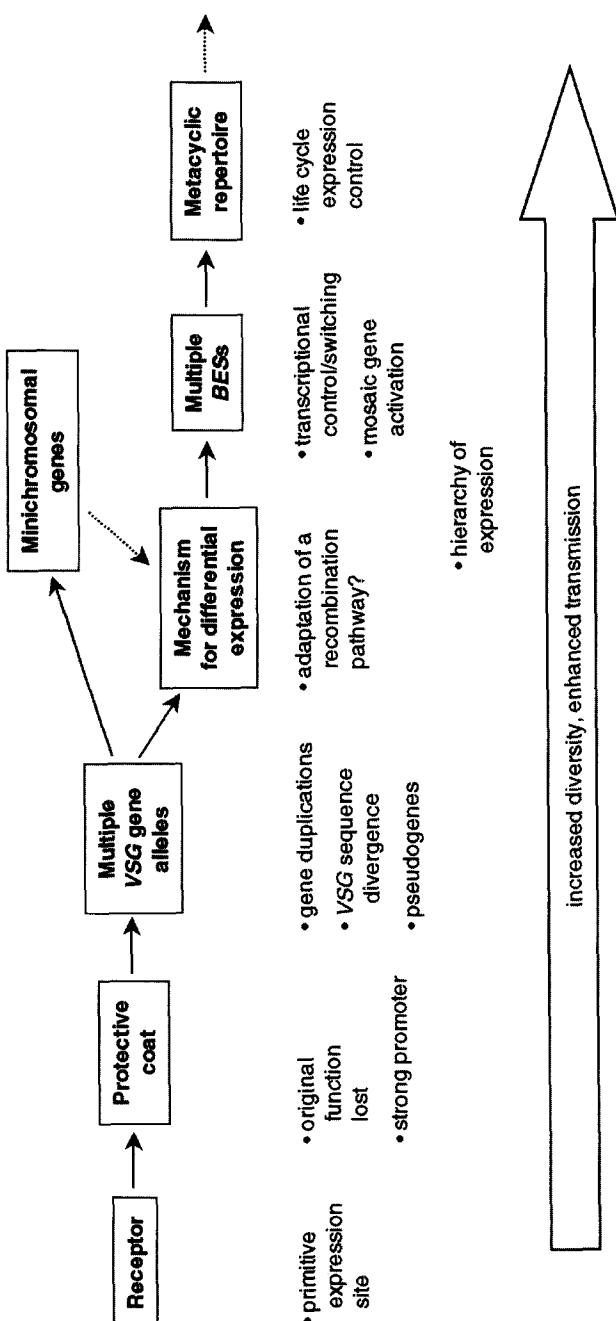


Figure 7 Possible evolutionary scheme for the *Trypanosoma brucei* antigenic variation system. A hypothetical evolutionary progression from a gene encoding a surface receptor of unknown function to the current, sophisticated VSG system of antigenic variation is shown. Several evolutionary events are envisaged, and the changes in VSG gene function and expression that underlie them are indicated. Not all *Trypanosoma* species contain the same number of minichromosomes as *T. brucei*, shown as an offshoot to indicate that this VSG class was a specialized adaptation to increase the repertoire of available silent VSGs and not a prerequisite event in the evolution of antigenic variation. The selection that directs this evolution is enhancement of the ability of trypanosomes to be transmitted from host to host, and the key component is increasing the diversity of the VSG system, at both a phenotypic and a molecular level.

they are only minimally present in, or absent from, *T. vivax*, which is regarded as the most primitive trypanosome known to have a VSG system. Perhaps the emerging *T. brucei* genome sequence will provide further clues pertaining to this evolutionary scheme.

8. PROSPECTS

The VSG system is too large and diversifies too rapidly for us to countenance control of trypanosomiasis by vaccination. This applies to both the blood-stream and metacyclic repertoires. An alternative approach has been to propose blocking a key step in the switching process, so that the infection would terminate when antibodies killed existing VATs. This now also seems remote. An important legacy of the study of monomorphic trypanosomes is that, like the *RAD51* null mutants, they probably demonstrate that it will not be possible to develop a single inhibitor capable of eradicating VSG switching. Suppression of one pathway simply opens the way for products of switching achieved by background genetic pathways. The observed rates appear to be sufficient to allow persistence of infection.

The future should see the emergence of an understanding of how antigenic variation and trypanosome growth are linked and how this pathogen contrives the balanced growth important for development of chronic infection. On the molecular side, study of the remarkable VSG genes and their expression should continue to reveal much that is fundamental to our understanding of genome flexibility, protein structure, and different levels of regulation of gene expression. One example is the factors controlling transcriptional switching. Molecular study should also contribute basic information on the interplay of separate recombinational pathways. Although it is only relatively recently that large scale study of immunity against trypanosomes has begun, it is envisaged that our understanding of this important side of antigenic variation will increase greatly in the coming years, yielding also insights into innate immune mechanisms.

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REFERENCES

- Agur, Z., Abiri, D. and Van der Ploeg, L.H.T. (1989). Ordered appearance of antigenic variants of African trypanosomes explained in a mathematical model based on a stochastic switch process and immune selection against putative switch intermediates. *Proceedings of the National Academy of Sciences of the USA* **86**, 9626–9630.
- Alarcon, C.M., Son, H.J., Hall, T. and Donelson, J.E. (1994). A monocistronic transcript for a trypanosome variant surface glycoprotein. *Molecular and Cellular Biology* **14**, 5579–5591.
- Alarcon, C.M., Pedram, M. and Donelson, J.E. (1999). Leaky transcription of variant surface glycoprotein gene expression sites in bloodstream African trypanosomes. *Journal of Biological Chemistry* **274**, 16884–16893.
- Alexandre, S., Guyaux, M., Murphy, N.B., Coquelet, H., Pays, A., Steinert, M. and Pays, E. (1988). Putative genes of a variant-specific antigen gene-transcription unit in *Trypanosoma brucei*. *Molecular and Cellular Biology* **8**, 2367–2378.
- Alexandre, S., Paindavoine, P., Hanocq-Quertier, J., Paturiaux-Hanocq, F., Tebabi, P. and Pays, E. (1996). Families of adenylate cyclase genes in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **77**, 173–182.
- Aline, R.F. and Stuart, K. (1989). *Trypanosoma brucei* – conserved sequence organization 3' to telomeric variant surface glycoprotein genes. *Experimental Parasitology* **68**, 57–66.
- Aline, R., Macdonald, G., Brown, E., Allison, J., Myler, P., Rothwell, V. and Stuart, K. (1985). (TAA)_n within sequences flanking several intrachromosomal variant surface glycoprotein genes in *Trypanosoma brucei*. *Nucleic Acids Research* **13**, 3161–3177.
- Andrews, J.H. (1998). Bacteria as modular organisms. *Annual Review of Microbiology* **52**, 105–126.
- Ansorge, I., Steverding, D., Melville, S., Hartmann, C. and Clayton, C. (1999). Transcription of 'inactive' expression sites in African trypanosomes leads to expression of multiple transferrin receptor RNAs in bloodstream forms. *Molecular and Biochemical Parasitology* **101**, 81–94.
- Antia, R., Nowak, M.A. and Anderson, R. M. (1996). Antigenic variation and the within-host dynamics of parasites. *Proceedings of the National Academy of Sciences of the USA* **93**, 985–989.
- Askonas, B.A. (1985). Macrophages as mediators of immunosuppression in murine African trypanosomiasis. *Current Topics in Microbiology and Immunology* **117**, 119–127.
- Aslam, N. and Turner, C.M.R. (1992). The relationship of variable antigen expression and population growth rates in *Trypanosoma brucei*. *Parasitology Research* **78**, 661–664.
- Bakhiet, M., Olsson, T., Edlund, C., Hojeberg, B., Holmberg, K., Lorentzen, J. and Kristensson, K. (1993). A *Trypanosoma brucei brucei*-derived factor that triggers CD8+ lymphocytes to interferon gamma secretion – purification, characterization and protective effects *in vivo* by treatment with a monoclonal antibody against the factor. *Scandinavian Journal of Immunology* **37**, 165–178.
- Baltz, T., Giroud, C., Bringaud, F., Eisen, H., Jacquemot, C. and Roth, C.W. (1991). Exposed epitopes on a *Trypanosoma equiperdum* variant surface glycoprotein altered by point mutations. *EMBO Journal* **10**, 1653–1659.
- Banks, K.L. (1978). Binding of *Trypanosoma congolense* to the walls of small blood vessels. *Journal of Protozoology* **25**, 241–245.
- Barbet, A.F. and Kamper, S.M. (1993). The importance of mosaic genes to trypanosome survival. *Parasitology Today* **9**, 63–66.
- Barbour, A.G. (1990). Antigenic variation of a relapsing fever *Borrelia* species. *Annual Review of Microbiology* **44**, 155–171.

- Barry, J.D. (1986). Antigenic variation during *Trypanosoma vivax* infections of different host species. *Parasitology* **92**, 51–65.
- Barry, J.D. (1997a). The relative significance of mechanisms of antigenic variation in African trypanosomes. *Parasitology Today* **13**, 212–218.
- Barry, J.D. (1997b). The biology of antigenic variation in African trypanosomes. In: *Trypanosomiasis and Leishmaniasis: Biology and Control* (G. Hide, J.C. Mottram, G.H. Coombs and P. H. Holmes, eds), pp. 89–107. Wallingford: CAB International.
- Barry, J.D. and Emery, D.L. (1984). Parasite development and host responses during the establishment of *Trypanosoma brucei* infection transmitted by tsetse fly. *Parasitology* **88**, 67–84.
- Barry, J.D. and Turner, C.M.R. (1991). The dynamics of antigenic variation and growth of African trypanosomes. *Parasitology Today* **7**, 207–211.
- Barry, J.D., Hajduk, S.L., Vickerman, K. and Le Ray, D. (1979a). Detection of multiple variable antigen types in metacyclic populations of *Trypanosoma brucei*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 205–208.
- Barry, J.D., Le Ray, D. and Herbert, W. J. (1979b). Infectivity and virulence of *Trypanosoma (Trypanozoon) brucei* for mice. IV. Dissociation of virulence and variable antigen type in relation to pleomorphism. *Journal of Comparative Pathology* **89**, 465–470.
- Barry, J.D., Crowe, J.S. and Vickerman, K. (1983). Instability of the *Trypanosoma brucei rhodesiense* metacyclic variable antigen repertoire. *Nature* **306**, 699–701.
- Barry, J.D., Crowe, J.S. and Vickerman, K. (1985). Neutralization of individual variable antigen types in metacyclic populations of *Trypanosoma brucei* does not prevent their subsequent expression in mice. *Parasitology* **90**, 79–88.
- Barry, J.D., Graham, S.V., Matthews, K.R., Shiels, P.G. and Shonekan, O.A. (1990). Stage-specific mechanisms for activation and expression of variant surface glycoprotein genes in *Trypanosoma brucei*. *Biochemical Society Transactions* **18**, 708–710.
- Barry, J.D., Graham, S.V., Fotheringham, M., Graham, V.S., Kobryn, K. and Wymer, B. (1998). VSG gene control and infectivity strategy of metacyclic stage *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **91**, 93–105.
- Baumann, P. and West, S. C. (1998). Role of the human RAD51 protein in homologous recombination and double-stranded break repair. *Trends in Biochemical Sciences* **23**, 247–251.
- Belfort, M. and Roberts, R. J. (1997). Homing endonucleases: keeping the house in order. *Nucleic Acids Research* **25**, 3379–3388.
- Berberof, M., Vanhamme, L., Alexandre, S., Lips, S., Tebabi, P. and Pays, E. (2000). A single-stranded DNA-binding protein shared by telomeric repeats, the variant surface glycoprotein transcription promoter and the procyclin transcription terminator of *Trypanosoma brucei*. *Nucleic Acids Research* **28**, 597–604.
- Bernards, A., Van der Ploeg, L.H.T., Frasch, A.C.C., Borst, P., Boothroyd, J.C., Coleman, S. and Cross, G.A.M. (1981). Activation of trypanosome surface glycoprotein genes involves a duplication-transposition leading to an altered 3' end. *Cell* **27**, 497–505.
- Bernards, A., de Lange, T., Michels, P.A.M., Liu, A.Y.C., Huisman, M.J. and Borst, P. (1984a). Two modes of activation of a single surface antigen gene of *Trypanosoma brucei*. *Cell* **36**, 163–170.
- Bernards, A., Van Hartenloosbroek, N. and Borst, P. (1984b). Modification of telomeric DNA in *Trypanosoma brucei* – a role in antigenic variation? *Nucleic Acids Research* **12**, 4153–4170.
- Bernards, A., Kooter, J.M., Michels, P.A.M., Moberts, R.M.P. and Borst, P. (1986). Pulsed field gradient electrophoresis of DNA digested in agarose allows the sizing of the large duplication unit of a surface antigen gene in trypanosomes. *Gene* **42**, 313–322.

- Bitter, W., Gerrits, H., Kieft, R. and Borst, P. (1998). The role of transferrin-receptor variation in the host range of *Trypanosoma brucei*. *Nature* **391**, 499–502.
- Blum, M.L., Down, J.A., Gurnett, A.M., Carrington, M., Turner, M.J. and Wiley, D.C. (1993). A structural motif in the variant surface glycoproteins of *Trypanosoma brucei*. *Nature* **362**, 603–609.
- Blundell, P.A. and Borst, P. (1998). Analysis of a variant surface glycoprotein gene expression site promoter of *Trypanosoma brucei* by remodelling the promoter region. *Molecular and Biochemical Parasitology* **94**, 67–85.
- Borst, P. (1991). Transferrin receptor, antigenic variation and the prospect of a trypanosome vaccine. *Trends in Genetics* **7**, 307–309.
- Borst, P. and Cross, G.A.M. (1982). Molecular basis for trypanosome antigenic variation. *Cell* **29**, 291–303.
- Borst, P. and Fairlamb, A.H. (1998). Surface receptors and transporters of *Trypanosoma brucei*. *Annual Review of Microbiology* **52**, 745–778.
- Borst, P. and Rudenko, G. (1994). Antigenic variation in African trypanosomes. *Science* **264**, 1872–1873.
- Borst, P., Rudenko, G., Taylor, M.C., Blundell, P.A., van Leeuwen, F., Bitter, W., Cross, M. and McCulloch, R. (1996). Antigenic variation in trypanosomes. *Archives of Medical Research* **27**, 379–388.
- Borst, P., Bitter, W., Blundell, P.A., Chaves, I., Cross, M., Gerrits, H., van Leeuwen, F., McCulloch, R., Taylor, M. and Rudenko, G. (1998). Control of VSG gene expression sites in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **91**, 67–76.
- Bosco, G. and Haber, J.E. (1998). Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture. *Genetics* **150**, 1037–1047.
- Britten, R.J. (1998). Precise sequence complementarity between yeast chromosome ends and two classes of just-subtelomeric sequences. *Proceedings of the National Academy of Sciences of the USA* **95**, 5906–5912.
- Brun, R., Hecker, H., Jenni, L. and Moloo, S.K. (1984). A quantitative ultrastructural study on the transformation of *Trypanosoma brucei brucei* metacyclic to bloodstream forms *in vitro*. *Acta Tropica* **41**, 117–129.
- Bucci, C., Lavitola, A., Salvatore, P., DelGiudice, L., Massardo, D.R., Bruni, C.B. and Alifano, P. (1999). Hypermutation in pathogenic bacteria: frequent phase variation in meningococci is a phenotypic trait of a specialized mutator biotype. *Molecular Cell* **3**, 435–445.
- Budihardjo, I., Oliver, H., Lutter, M., Luo, X. and Wang, X.D. (1999). Biochemical pathways of caspase activation during apoptosis. *Annual Review of Cell and Developmental Biology* **15**, 269–290.
- Burgess, S.M. and Kleckner, N. (1999). Collisions between yeast chromosomal loci *in vivo* are governed by three layers of organization. *Genes and Development* **13**, 1871–1883.
- Burman, N., Bergstrom, S., Restrepo, B.I. and Barbour, A.G. (1990). The variable antigens VMP7 and VMP21 of the relapsing fever bacterium *Borrelia hermsii* are structurally analogous to the VSG proteins of the african trypanosome. *Molecular Microbiology* **4**, 1715–1726.
- Burman, N., Shamaei-Tousi, A. and Bergstrom, S. (1998). The spirochete *Borrelia crocidurae* causes erythrocyte rosetting during relapsing fever. *Infection and Immunity* **66**, 815–819.
- Butikofer, P., Ruepp, S., Boschung, M. and Roditi, I. (1997). ‘GPEET’ procyclin is the major surface protein of procyclic culture forms of *Trypanosoma brucei brucei* strain 427. *Biochemical Journal* **326**, 415–423.
- Campbell, D.A., Vanbree, M.P. and Boothroyd, J.C. (1984). The 5'-limit of transposition

- and upstream barren region of a trypanosome VSG gene – tandem 76 base-pair repeats flanking (TAA)₉₀. *Nucleic Acids Research* **12**, 2759–2774.
- Capbern, A., Giroud, C., Baltz, T. and Mattern, P. (1977). *Trypanosoma equiperdum*: étude des variations antigéniques au cours de la trypanosomose expérimentale du lapin. *Experimental Parasitology* **42**, 6–13.
- Capecci, M. (1990). Gene targeting – how efficient can you get? *Nature* **348**, 109.
- Carrington, M. and Boothroyd, J. (1996). Implications of conserved structural motifs in disparate trypanosome surface proteins. *Molecular and Biochemical Parasitology* **81**, 119–126.
- Carrington, M., Miller, N., Blum, M., Roditi, I., Wiley, D. and Turner, M. (1991). Variant specific glycoprotein of *Trypanosoma brucei* consists of 2 domains each having an independently conserved pattern of cysteine residues. *Journal of Molecular Biology* **221**, 823–835.
- Carruthers, V.B., Navarro, M. and Cross, G.A.M. (1996). Targeted disruption of Expression Site Associated Gene 1 in bloodstream form *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **81**, 65–79.
- Casjens, S. (1999). Evolution of the linear DNA replicons of the *Borrelia* spirochetes. *Current Opinion in Microbiology* **2**, 529–534.
- Chaves, I., Zomerdijk, J., Dirksmulder, A., Dirks, R.W., Raap, A.K. and Borst, P. (1998). Subnuclear localization of the active variant surface glycoprotein gene expression site in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the USA* **95**, 12328–12333.
- Chaves, I., Rudenko, G., Dirks-Mulder, A., Cross, M. and Borst, P. (1999). Control of variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *EMBO Journal* **18**, 4846–4855.
- Chess, A. (1998). Immunology – expansion of the allelic exclusion principle? *Science* **279**, 2067–2068.
- Chung, H.M., Lee, M.G.S. and Van der Ploeg, L. H. T. (1992). RNA polymerase I-mediated protein-coding gene-expression in *Trypanosoma brucei*. *Parasitology Today* **8**, 414–418.
- Clarke, M.W., Barbet, A.F. and Pearson, T.W. (1987). Structural features of antigenic determinants on variant surface glycoproteins from *Trypanosoma brucei*. *Molecular Immunology* **24**, 707–713.
- Cornelissen, A.W.C.A., Bakkeren, G.A.M., Barry, J.D., Michels, P.A.M. and Borst, P. (1985). Characteristics of trypanosome variant antigen genes active in the tsetse fly. *Nucleic Acids Research* **13**, 4661–4676.
- Cox, M.M. (1997). Recombinational crossroads: eukaryotic enzymes and the limits of bacterial precedents. *Proceedings of the National Academy of Sciences of the USA* **94**, 11764–11766.
- Cox, M.M., Goodman, M.F., Kreuzer, K.N., Sherratt, D.J., Sandler, S.J. and Marians, K.J. (2000). The importance of repairing stalled replication forks. *Nature* **404**, 37–41.
- Cross, G.A.M. (1975). Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitology* **71**, 393–417.
- Cross, G.A.M. (1996). Antigenic variation in trypanosomes – secrets surface slowly. *Bioessays* **18**, 283–291.
- Cross, G.A.M., Wirtz, L.E. and Navarro, M. (1998a). Regulation of vsg expression site transcription and switching in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **91**, 77–91.
- Cross, M., Taylor, M.C. and Borst, P. (1998b). Frequent loss of the active site during variant surface glycoprotein expression site switching *in vitro* in *Trypanosoma brucei*. *Molecular and Cellular Biology* **18**, 198–205.

- Cross, M., Kieft, R., Sabatini, R., Wilm, M., de Kort, M., van der Marel, G.A., Vanboom, J.H., van Leeuwen, F. and Borst, P. (1999). The modified base J is the target for a novel DNA-binding protein in kinetoplastid protozoans. *EMBO Journal* **18**, 6573–6581.
- Cully, D.F., Ip, H.S. and Cross, G.A.M. (1985). Coordinate transcription of variant surface glycoprotein genes and an expression site associated gene family in *Trypanosoma brucei*. *Cell* **42**, 173–182.
- Davies, K.P., Carruthers, V.B. and Cross, G.M. (1997). Manipulation of the vsg co-transposed region increases expression site switching in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **86**, 163–177.
- Deitsch, K.W., Moxon, E.R. and Wellem, T.E. (1997). Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. *Microbiology and Molecular Biology Reviews* **61**, 281–294.
- De Lange, T., Kooter, J.M., Michels, P.A.M. and Borst, P. (1983). Telomere conversion in trypanosomes. *Nucleic Acids Research* **11**, 8149–8165.
- Delauw, M.F., Laurent, M., Paindavoine, P., Aerts, D., Pays, E., Le Ray, D. and Steinert, M. (1987). Characterization of genes coding for 2 major metacyclic surface antigens in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **23**, 9–17.
- Dickin, S.K. and Gibson, W.C. (1989). Hybridization with a repetitive DNA probe reveals the presence of small chromosomes in *Trypanosoma vivax*. *Molecular and Biochemical Parasitology* **33**, 135–142.
- Donelson, J.E. (1995). Mechanisms of antigenic variation in *Borrelia hermsii* and African trypanosomes. *Journal of Biological Chemistry* **270**, 7783–7786.
- Donelson, J.E., Hill, K.L. and Elsayed, N.A. (1998). Multiple mechanisms of immune evasion by African trypanosomes. *Molecular and Biochemical Parasitology* **91**, 51–66.
- Doyle, J.J., Hirumi, H., Hirumi, K., Lupton, E.N. and Cross, G.A.M. (1980). Antigenic variation in clones of animal-infective *Trypanosoma brucei* derived and maintained in vitro. *Parasitology* **80**, 359–369.
- Eggleston, A.K. and West, S.C. (1996). Exchanging partners – recombination in *Escherichia coli*. *Trends in Genetics* **12**, 20–26.
- Ellis, D.S. and Evans, D.A. (1977). Passage of *Trypanosoma brucei rhodesiense* through the peritrophic membrane of *Glossina morsitans morsitans*. *Nature* **267**, 834–835.
- Emery, D.L., Barry, J.D. and Moloo, S.K. (1980). The appearance of *Trypanosoma (Duttonella) vivax* in lymph following challenge of goats with infected *Glossina morsitans morsitans*. *Acta Tropica* **37**, 375–379.
- Ersfeld, K., Melville, S.E. and Gull, K. (1999). Nuclear and genome organization of *Trypanosoma brucei*. *Parasitology Today* **15**, 58–63.
- Esser, K.M. and Schoenbechler, M.J. (1985). Expression of two variant surface glycoproteins on individual African trypanosomes during antigen switching. *Science* **229**, 190–193.
- Esser, K.M., Schoenbechler, M.J. and Gingrich, J.B. (1982). *Trypanosoma rhodesiense* blood forms express all antigen specificities relevant to protection against metacyclic (insect form) challenge. *Journal of Immunology* **129**, 1715–1718.
- Fanning, T.G. and Taubenberger, J.K. (1999). Phylogenetically important regions of the influenza A H1 hemagglutinin protein. *Virus Research* **65**, 33–42.
- Ferguson, M.A.J. (1999). The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research. *Journal of Cell Science* **112**, 2799–2809.
- Fernandez, V., Hommel, M., Chen, Q.J., Hagblom, P. and Wahlgren, M. (1999). Small, clonally variant antigens expressed on the surface of the *Plasmodium falciparum*-infected erythrocyte are encoded by the rif gene family and are the target of human immune responses. *Journal of Experimental Medicine* **190**, 1393–1403.

- Ferrante, A. and Allison, A.C. (1983). Alternative pathway activation of complement by African trypanosomes lacking a glycoprotein coat. *Parasite Immunology* **5**, 491–498.
- Frank, S.A. (1999). A model for the sequential dominance of antigenic variants in African trypanosome infections. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **266**, 1397–1401.
- Gardner, J.P., Pinches, R.A., Roberts, D.J. and Newbold, C.I. (1996). Variant antigens and endothelial receptor adhesion in *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences of the USA* **93**, 3503–3508.
- Gibson, W.C. (1995). The significance of genetic exchange in trypanosomes. *Parasitology Today* **11**, 465–468.
- Gommers-Ampt, J., Lutgerink, J. and Borst, P. (1991). A novel DNA nucleotide in *Trypanosoma brucei* only present in the mammalian phase of the life cycle. *Nucleic Acids Research* **19**, 1745–1751.
- Gommers-Ampt, J.H., van Leeuwen, F., Debeer, A.L.J., Vliegenthart, J.F.G., Dizdaroglu, M., Kowalak, J.A., Crain, P.F. and Borst, P. (1993). β-d-glucosyl-hydroxymethyluracil – a novel modified base present in the DNA of the parasitic protozoan *Trypanosoma brucei*. *Cell* **75**, 1129–1136.
- Gottesdiner, K.M. (1994). A new VSG expression site-associated gene (ESAG) in the promoter region of *Trypanosoma brucei* encodes a protein with 10 potential transmembrane domains. *Molecular and Biochemical Parasitology* **63**, 143–151.
- Gottschling, D.E., Aparicio, O.M., Billington, B.L. and Zakian, V.A. (1990). Position effect at *Saccharomyces cerevisiae* telomeres – reversible repression of pol II transcription. *Cell* **63**, 751–762.
- Graham, S.V., Matthews, K.R., Shiels, P.G. and Barry, J.D. (1990). Distinct, developmental stage-specific activation mechanisms of trypanosome VSG genes. *Parasitology* **101**, 361–367.
- Graham, S.V., Wymer, B. and Barry, J.D. (1998a). Activity of a trypanosome metacyclic variant surface glycoprotein gene promoter is dependent upon life cycle stage and chromosomal context. *Molecular and Cellular Biology* **18**, 1137–1146.
- Graham, S.V., Wymer, B. and Barry, J.D. (1998b). A trypanosome metacyclic VSG gene promoter with two functionally distinct, life cycle stage-specific activities. *Nucleic Acids Research* **26**, 1985–1990.
- Graham, S.V., Terry, S. and Barry, J.D. (1999). A structural and transcription pattern for variant surface glycoprotein gene expression sites used in metacyclic stage *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **103**, 141–154.
- Graham, V.S. and Barry, J.D. (1996). Is point mutagenesis a mechanism for antigenic variation in *Trypanosoma brucei*? *Molecular and Biochemical Parasitology* **79**, 35–45.
- Gray, A.R. (1965). Antigenic variation in a strain of *Trypanosoma brucei* transmitted by *Glossina morsitans* and *G. palpalis*. *Journal of General Microbiology* **41**, 195–214.
- Greaves, D.R. and Borst, P. (1987). *Trypanosoma brucei* variant-specific glycoprotein gene chromatin is sensitive to single-strand-specific endonuclease digestion. *Journal of Molecular Biology* **197**, 471–483.
- Grivell, L.A. (1996). Transposition: mobile introns get into line. *Current Biology* **6**, 48–51.
- Grunstein, M. (1997). Molecular model for telomeric heterochromatin in yeast. *Current Opinion in Cell Biology* **9**, 383–387.
- Gull, K., Alsfeld, S. and Ersfeld, K. (1998). Segregation of minichromosomes in trypanosomes: implications for mitotic mechanisms. *Trends in Microbiology* **6**, 319–323.
- Haag, J., OhUigin, C. and Overath, P. (1998). The molecular phylogeny of trypanosomes: evidence for an early divergence of the Salivaria. *Molecular and Biochemical Parasitology* **91**, 37–49.

- Haber, J.E. (1998). Mating-type gene switching in *Saccharomyces cerevisiae*. *Annual Review of Genetics* **32**, 561–599.
- Haber, J.E. (1999). DNA recombination: the replication connection. *Trends in Biochemical Sciences* **24**, 271–275.
- Hajduk, S.L. and Vickerman, K. (1981). Antigenic variation in cyclically transmitted *Trypanosoma brucei*. Variable antigen type composition of the first parasitaemia in mice bitten by trypanosome-infected *Glossina morsitans*. *Parasitology* **83**, 609–621.
- Hajduk, S.L., Cameron, C.R., Barry, J.D. and Vickerman, K. (1981). Antigenic variation in cyclically transmitted *Trypanosoma brucei*. Variable antigen type composition of metacyclic trypanosome populations from the salivary glands of *Glossina morsitans*. *Parasitology* **83**, 595–607.
- Hamase, A., Takahashi, Y., Nohgi, K. and Fukunaga, M. (1996). Homology of variable major protein genes between *Borrelia hermsii* and *Borrelia miyamotoi*. *FEMS Microbiology Letters* **140**, 131–137.
- Hamm, B., Schindler, A., Mecke, D. and Duszenko, M. (1990). Differentiation of *Trypanosoma brucei* bloodstream trypomastigotes from long slender to short stumpy-like forms in axenic culture. *Molecular and Biochemical Parasitology* **40**, 13–22.
- Hemphill, A., Frame, I. and Ross, C. A. (1994). The interaction of *Trypanosoma congoense* with endothelial cells. *Parasitology* **109**, 631–641.
- Henderson, I.R., Owen, P. and Nataro, J.P. (1999). Molecular switches – the ON and OFF of bacterial phase variation. *Molecular Microbiology* **33**, 919–932.
- Hertz, C.J. and Mansfield, J.M. (1999). IFN-gamma-dependent nitric oxide production is not linked to resistance in experimental African trypanosomiasis. *Cellular Immunology* **192**, 24–32.
- Hide, G., Welburn, S.C., Tait, A. and Maudlin, I. (1994). Epidemiological relationships of *Trypanosoma brucei* stocks from south east Uganda: evidence for different population structures in human infective and non-human infective isolates. *Parasitology* **109**, 95–111.
- Hobbs, M.R. and Boothroyd, J.C. (1990). An expression-site-associated gene family of trypanosomes is expressed *in vivo* and shows homology to a variant surface glycoprotein gene. *Molecular and Biochemical Parasitology* **43**, 1–16.
- Hollander, G.A., Zuklys, S., Morel, C., Mizoguchi, E., Mobiisson, K., Simpson, S., Terhorst, C., Wishart, W., Golan, D.E., Bhan, A.K. and Burakoff, S.J. (1998). Monoallelic expression of the interleukin-2 locus. *Science* **279**, 2118–2121.
- Horn, D. and Cross, G.A.M. (1995). A developmentally-regulated position effect at a telomeric locus in *Trypanosoma brucei*. *Cell* **83**, 555–561.
- Horn, D. and Cross, G.A.M. (1997). Analysis of *Trypanosoma brucei* VSG expression site switching *in vitro*. *Molecular and Biochemical Parasitology* **84**, 189–201.
- Horvitz, H.R. and Herskowitz, I. (1992). Mechanisms of asymmetric cell division – 2 Bs or not 2 Bs, that is the question. *Cell* **68**, 237–255.
- Hsia, R.C., Beals, T. and Boothroyd, J.C. (1996). Use of chimeric recombinant polypeptides to analyze conformational, surface epitopes on trypanosome variant surface glycoproteins. *Molecular Microbiology* **19**, 53–63.
- Jacobs, C. and Shapiro, L. (1998). Microbial asymmetric cell division: localization of cell fate determinants. *Current Opinion in Genetics and Development* **8**, 386–391.
- Jennings, F.W., Whitelaw, D.D., Holmes, P.H., Chizyuka, H.G. and Urquhart, G.M. (1979). The brain as a source of relapsing *Trypanosoma brucei* infection in mice after chemotherapy. *International Journal for Parasitology* **9**, 381–384.
- Kamper, S.M. and Barbet, A.F. (1992). Surface epitope variation via mosaic gene formation is potential key to long-term survival of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **53**, 33–44.

- Kanaar, R., Hoeijmakers, J.H.J. and Van Gent, D.C. (1998). Molecular mechanisms of DNA double-strand break repair. *Trends in Cell Biology* **8**, 483–489.
- Kazazian, H.H. (1998). Mobile elements and disease. *Current Opinion in Genetics and Development* **8**, 343–350.
- Kim, K.S. and Donelson, J.E. (1997). Co-duplication of a variant surface glycoprotein gene and its promoter to an expression site in African trypanosomes. *Journal of Biological Chemistry* **272**, 24637–24645.
- Kogoma, T. (1996). Recombination by replication. *Cell* **85**, 625–627.
- Koomey, M. (1997). Bacterial pathogenesis: a variation on variation in Lyme disease. *Current Biology* **7**, R538–R540.
- Kwong, P.D., Wyatt, R., Robinson, J., Sweet, R.W., Sodroski, J. and Hendrickson, W.A. (1998). Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**, 648–659.
- Kyes, S.A., Rowe, J.A., Kriek, N. and Newbold, C.I. (1999). Rifins: a second family of clonally variant proteins expressed on the surface of red cells infected with *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences of the USA* **96**, 9333–9338.
- Lamont, G.S., Tucker, R.S. and Cross, G.A.M. (1986). Analysis of antigen switching rates in *Trypanosoma brucei*. *Parasitology* **92**, 355–367.
- Laufer, G., Schaaf, G., Bollgonn, S. and Gunzl, A. (1999). *In vitro* analysis of alpha-amanitin-resistant transcription from the rRNA, procyclic acidic repetitive protein, and variant surface glycoprotein gene promoters in *Trypanosoma brucei*. *Molecular and Cellular Biology* **19**, 5466–5473.
- Laurent, M., Pays, E., Magnus, E., Van Meirvenne, N., MatthysSENS, G., Williams, R.O. and Steinert, M. (1983). DNA rearrangements linked to expression of a predominant surface antigen gene of trypanosomes. *Nature* **302**, 263–266.
- Laurent, M., Pays, E., Van der Werf, A., Aerts, D., Magnus, E., Van Meirvenne, N. and Steinert, M. (1984). Translocation alters the activation rate of a trypanosome surface-antigen gene. *Nucleic Acids Research* **12**, 8319–8328.
- Lee, M.S. and Van der Ploeg, L.H.T. (1997). Transcription of protein-coding genes in trypanosomes by RNA polymerase I. *Annual Review of Microbiology* **51**, 463–489.
- Lenardo, M.J., Rice-Ficht, A.C., Kelly, G., Esser, K.M. and Donelson, J.E. (1984). Characterization of the genes specifying two metacyclic variable antigen types in *Trypanosoma brucei rhodesiense*. *Proceedings of the National Academy of Sciences of the USA* **81**, 6642–6646.
- Lenardo, M.J., Esser, K.M., Moon, A.M., Van der Ploeg, L.H.T. and Donelson, J.E. (1986). Metacyclic variant surface glycoprotein genes of *Trypanosoma brucei* subsp. *rhodesiense* are activated *in situ*, and their expression is transcriptionally regulated. *Molecular and Cellular Biology* **6**, 1991–1997.
- Le Ray, D., Barry, J.D., Easton, C. and Vickerman, K. (1977). First tsetse fly transmission of the 'AnTat' serodeme of *Trypanosoma brucei*. *Annales de la Société Belge de Médecine Tropicale* **57**, 369–381.
- Le Ray, D., Barry, J.D. and Vickerman, K. (1978). Antigenic heterogeneity of metacyclic forms of *Trypanosoma brucei*. *Nature* **273**, 300–302.
- Liu, A.Y.C., Van der Ploeg, L.H.T., Rijsewijk, F.A.M. and Borst, P. (1983). The transposition unit of variant surface glycoprotein gene 118 of *Trypanosoma brucei* – presence of repeated elements at its border and absence of promoter-associated sequences. *Journal of Molecular Biology* **167**, 57–75.
- Liu, A.Y.C., Michels, P.A.M., Bernards, A. and Borst, P. (1985). Trypanosome variant surface glycoprotein genes expressed early in infection. *Journal of Molecular Biology* **182**, 383–396.

- Longacre, S. and Eisen, H. (1986). Expression of whole and hybrid genes in *Trypanosoma equiperdum* antigenic variation. *EMBO Journal* **5**, 1057–1063.
- Lu, Y., Hall, T., Gay, L.S. and Donelson, J.E. (1993). Point mutations are associated with a gene duplication leading to the bloodstream re-expression of a trypanosome metacyclic VSG. *Cell* **72**, 397–406.
- Mabbott, N.A., Sutherland, I.A. and Sternberg, J.M. (1994). *Trypanosoma brucei* is protected from the cytostatic effects of nitric oxide under *in vivo* conditions. *Parasitology Research* **80**, 687–690.
- Mabbott, N.A., Coulson, P.S., Smythies, L.E., Wilson, R.A. and Sternberg, J.M. (1998). African trypanosome infections in mice that lack the interferon-gamma receptor gene: nitric oxide-dependent and -independent suppression of T-cell proliferative responses and the development of anaemia. *Immunology* **94**, 476–480.
- Magez, S., Radwanska, M., Beschin, A., Sekikawa, K. and De Baetselier, P. (1999). Tumor necrosis factor alpha is a key mediator in the regulation of experimental *Trypanosoma brucei* infections. *Infection and Immunity* **67**, 3128–3132.
- Maizels, R.M., Bundy, D.A.P., Selkirk, M.E., Smith, D.F. and Anderson, R.M. (1993). Immunological modulation and evasion by helminth parasites in human populations. *Nature* **365**, 797–805.
- Malkova, A., Ivanov, E.L. and Haber, J.E. (1996). Double-strand break repair in the absence of RAD51 in yeast – a possible role for break-induced DNA-replication. *Proceedings of the National Academy of Sciences of the USA* **93**, 7131–7136.
- Marians, K.J. (2000). Replication and recombination intersect. *Current Opinion in Genetics and Development* **10**, 151–156.
- Matthews, K.R. (1999). Developments in the differentiation of *Trypanosoma brucei*. *Parasitology Today* **15**, 76–80.
- Matthews, K.R. and Gull, K. (1997). Commitment to differentiation and cell cycle re-entry are coincident but separable events in the transformation of African trypanosomes from their bloodstream to their insect form. *Journal of Cell Science* **110**, 2609–2618.
- Matthews, K.R., Shiels, P.G., Graham, S.V., Cowan, C. and Barry, J.D. (1990). Duplicative activation mechanisms of two trypanosome telomeric VSG genes with structurally simple 5' flanks. *Nucleic Acids Research* **18**, 7219–7227.
- Matthews, K.R., Tschudi, C. and Ullu, E. (1994). A common pyrimidine-rich motif governs transsplicing and polyadenylation of tubulin polycistronic pre-mRNA in trypanosomes. *Genes and Development* **8**, 491–501.
- McAndrew, M., Graham, S., Hartmann, C. and Clayton, C. (1998). Testing promoter activity in the trypanosome genome: isolation of a metacyclic-type VSG promoter, and unexpected insights into RNA polymerase II transcription. *Experimental Parasitology* **90**, 65–76.
- McCulloch, R. and Barry, J.D. (1999). A role for RAD51 and homologous recombination in *Trypanosoma brucei* antigenic variation. *Genes and Development* **13**, 2875–2888.
- McCulloch, R., Rudenko, G. and Borst, P. (1997). Gene conversions mediating antigenic variation in *Trypanosoma brucei* can occur in variant surface glycoprotein expression sites lacking 70 base-pair repeat sequences. *Molecular and Cellular Biology* **17**, 833–843.
- Mehlert, A., Treumann, A. and Ferguson, M.A. (1999). *Trypanosoma brucei* GPEET–PARP is phosphorylated on six out of seven threonine residues. *Molecular and Biochemical Parasitology* **98**, 291–296.
- Melville, S.E., Leech, V., Gerrard, C.S., Tait, A. and Blackwell, J.M. (1998). The molecular karyotype of the megabase chromosomes of *Trypanosoma brucei* and the assignment of chromosome markers. *Molecular and Biochemical Parasitology* **94**, 155–173.

- Metzstein, M.M., Stanfield, G.M. and Horvitz, H.R. (1998). Genetics of programmed cell death in *C. elegans*: past, present and future. *Trends in Genetics* **14**, 410–416.
- Michels, P.A.M., Van der Ploeg, L.H.T., Liu, A.Y.C. and Borst, P. (1984). The inactivation and reactivation of an expression-linked gene copy for a variant surface glycoprotein in *Trypanosoma brucei*. *EMBO Journal* **3**, 1345–1351.
- Millar, A.E., Sternberg, J., McSharry, C., Wei, X.Q., Liew, F.Y. and Turner, C.M.R. (1999). T-cell responses during *Trypanosoma brucei* infections in mice deficient in inducible nitric oxide synthase. *Infection and Immunity* **67**, 3334–3338.
- Miller, E.N. and Turner, M.J. (1981). Analysis of antigenic types appearing in first relapse populations of clones of *Trypanosoma brucei*. *Parasitology* **82**, 63–80.
- Miller, E.N., Allan, L.M. and Turner, M.J. (1984). Topological analysis of antigenic determinants on a variant surface glycoprotein of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **13**, 67–81.
- Morgan, R.W., Elsaied, N.M.A., Kepa, J.K., Pedram, M. and Donelson, J.E. (1996). Differential expression of the Expression Site Associated Gene I family in African trypanosomes. *Journal of Biological Chemistry* **271**, 9771–9777.
- Mortensen, U.H., Bendixen, C., Sunjevaric, I. and Rothstein, R. (1996). DNA strand annealing is promoted by the yeast Rad52 protein. *Proceedings of the National Academy of Sciences of the USA* **93**, 10729–10734.
- Moxon, E.R., Rainey, P.B., Nowak, M.A. and Lenski, R.E. (1994). Adaptive evolution of highly mutable loci in pathogenic bacteria. *Current Biology* **4**, 24–33.
- Muñoz-Jordán, J.L., Davies, K.P. and Cross, G.A.M. (1996). Stable expression of mosaic coats of variant surface glycoproteins in *Trypanosoma brucei*. *Science* **272**, 1795–1797.
- Myler, P.J., Allison, J., Agabian, N. and Stuart, K.D. (1984). Antigenic variation in African trypanosomes by gene replacement or activation of alternate telomeres. *Cell* **39**, 203–211.
- Nagoshi, Y.L., Alarcon, C.M. and Donelson, J.E. (1995). The putative promoter for a metacyclic VSG gene in African trypanosomes. *Molecular and Biochemical Parasitology* **72**, 33–45.
- Nakamura, Y. and Wada, M. (1998). Molecular pathobiology and antigenic variation of *Pneumocystis carinii*. *Advances in Parasitology* **41**, 63–107.
- Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R. and Moloo, S.K. (1984). Resistance of cattle to tsetse-transmitted challenge with *Trypanosoma brucei* and *Trypanosoma congoense* after spontaneous recovery from syringe-passaged infections. *Infection and Immunity* **43**, 735–738.
- Nantulya, V.M., Musoke, A.J. and Moloo, S.K. (1986). Apparent exhaustion of the variable antigen repertoires of *Trypanosoma vivax* in infected cattle. *Infection and Immunity* **54**, 444–447.
- Nassif, N., Penney, J., Pal, S., Engels, W.R. and Gloor, G.B. (1994). Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Molecular and Cellular Biology* **14**, 1613–1625.
- Navarro, M. and Cross, G. A. M. (1996). DNA rearrangements associated with multiple consecutive directed antigenic switches in *Trypanosoma brucei*. *Molecular and Cellular Biology* **16**, 3615–3625.
- Navarro, M. and Cross, G.M. (1998). *In situ* analysis of a variant surface glycoprotein expression site promoter region in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **94**, 53–66.
- Navarro, M., Cross, G.A.M. and Wirtz, E. (1999). *Trypanosoma brucei* variant surface glycoprotein regulation involves coupled activation/inactivation and chromatin remodeling of expression sites. *EMBO Journal* **18**, 2265–2272.

- Neuberger, M.S., Lanoue, A., Ehrenstein, M.R., Batista, F.D., Sale, J.E. and Williams, G.T. (1999). Antibody diversification and selection in the mature B-cell compartment. *Cold Spring Harbor Symposia on Quantitative Biology* **64**, 211–216.
- Ochman, H. (1999). Bacterial evolution: jittery genomes. *Current Biology* **9**, R485–R486.
- Ochman, H., Lawrence, J.G. and Groisman, E.A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**, 299–304.
- Ohshima, K., Kang, S., Larson, J.E. and Wells, R.D. (1996). TTA.TAA triplet repeats in plasmids form a non-H bonded structure. *Journal of Biological Chemistry* **271**, 16784–16791.
- Ormerod, W.E. (1979). Development of *Trypanosoma brucei* in the mammalian host. In: *Biology of the Kinetoplastida* (W.H.R. Lumsden and D.A. Evans, eds). Vol. 2, pp. 339–393. London: Academic Press.
- Overath, P., Czichos, J. and Haas, C. (1986). The effect of citrate/cis-aconitate on oxidative metabolism during transformation of *Trypanosoma brucei*. *European Journal of Biochemistry* **160**, 175–182.
- Overath, P., Chaudhri, M., Steverding, D. and Ziegelbauer, K. (1994). Invariant surface proteins in bloodstream forms of *Trypanosoma brucei*. *Parasitology Today* **10**, 53–58.
- Overath, P., Haag, J., Mameza, M.G. and Lischke, A. (1999). Freshwater fish trypanosomes: definition of two types, host control by antibodies and lack of antigenic variation. *Parasitology* **119**, 591–601.
- Paindavoine, P., Rolin, S., Van Assel, S., Geuskens, M., Jauniaux, J.C., Dinsart, C., Huet, G. and Pays, E. (1992). A gene from the variant surface glycoprotein expression site encodes one of several transmembrane adenylate cyclases located on the flagellum of *Trypanosoma brucei*. *Molecular and Cellular Biology* **12**, 1218–1225.
- Paques, F. and Haber, J. E. (1999). Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews* **63**, 349.
- Pays, E. (1985). Gene conversion in trypanosome antigenic variation. *Progress in Nucleic Acid Research and Molecular Biology* **32**, 1–26.
- Pays, E. (1989). Pseudogenes, chimaeric genes and the timing of antigen variation in African trypanosomes. *Trends in Genetics* **5**, 389–391.
- Pays, E. and Nolan, D.P. (1998). Expression and function of surface proteins in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **91**, 3–36.
- Pays, E. and Steinert, M. (1988). Control of antigen gene expression in African trypanosomes. *Annual Review of Genetics* **22**, 107–126.
- Pays, E., Van Meirvenne, N., Le Ray, D. and Steinert, M. (1981). Gene duplication and transposition linked to antigenic variation in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the USA* **78**, 2673–2677.
- Pays, E., Van Assel, S., Laurent, M., Dero, B., Michiels, F., Kronenberger, P., MatthysSENS, G., Van Meirvenne, N., Le Ray, D. and Steinert, M. (1983a). At least two transposed sequences are associated in the expression site of a surface antigen gene in different trypanosome clones. *Cell* **34**, 359–369.
- Pays, E., Van Assel, S., Laurent, M., Darville, M., Vervoort, T., Van Meirvenne, N. and Steinert, M. (1983b). Gene conversion as a mechanism for antigenic variation in trypanosomes. *Cell* **34**, 371–381.
- Pays, E., Delauw, M.F., Laurent, M. and Steinert, M. (1984). Possible DNA modification in GC dinucleotides of *Trypanosoma brucei* telomeric sequences – relationship with antigen gene transcription. *Nucleic Acids Research* **12**, 5235–5247.
- Pays, E., Guyaux, M., Aerts, D., Van Meirvenne, N. and Steinert (1985a). Telomeric reciprocal recombination as a possible mechanism for antigenic variation in trypanosomes. *Nature* **316**, 562–564.

- Pays, E., Houard, S., Pays, A., Van Assel, S., Dupont, F., Aerts, D., Huetduvillier, G., Gomes, V., Richet, C., Degand, P., Van Meirvenne, N. and Steinert, M. (1985b). *Trypanosoma brucei* – the extent of conversion in antigen genes may be related to the DNA coding specificity. *Cell* **42**, 821–829.
- Pays, E., Tebabi, P., Pays, A., Coquelet, H., Revelard, P., Salmon, D. and Steinert, M. (1989). The genes and transcripts of an antigen gene expression site from *T. brucei*. *Cell* **57**, 835–845.
- Pays, E., Vanhamme, L. and Berberof, M. (1994). Genetic controls for the expression of surface antigens in African trypanosomes. *Annual Review of Microbiology* **48**, 25–52.
- Pham, V.P., Rothman, P.B. and Gottesdiener, K.M. (1997). Binding of trans-acting factors to the double-stranded variant surface glycoprotein (VSG) expression site promoter of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **89**, 11–23.
- Plasterk, R.H.A., Simon, M.I. and Barbour, A.G. (1985). Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*. *Nature* **318**, 257–263.
- Radman, M. (1999). Mutation – enzymes of evolutionary change. *Nature* **401**, 866.
- Radwanska, M., Magez, S., Michel, A., Stijlemans, B., Geuskens, M. and Pays, E. (2000). Comparative analysis of antibody responses against HSP60, invariant surface glycoprotein 70, and variant surface glycoprotein reveals a complex antigen-specific pattern of immunoglobulin isotype switching during infection by *Trypanosoma brucei*. *Infection and Immunity* **68**, 848–860.
- Richardson, C., Moynahan, M.E. and Jasin, M. (1998). Double-strand break repair by inter-chromosomal recombination: suppression of chromosomal translocations. *Genes and Development* **12**, 3831–3842.
- Robinson, N.P., Burman, N., Melville, S.E. and Barry, J.D. (1999). Predominance of duplicative VSG gene conversion in antigenic variation in African trypanosomes. *Molecular and Cellular Biology* **19**, 5839–5846.
- Roditi, I., Schwarz, H., Pearson, T.W., Beecroft, R.P., Liu, M.K., Richardson, J.P., Buhring, H.J., Pleiss, J., Bulow, R., Williams, R.O. and Overath, P. (1989). Procyclin gene expression and loss of the variant surface glycoprotein during differentiation of *Trypanosoma brucei*. *Journal of Cell Biology* **108**, 737–746.
- Rolin, S., Paindavoine, P., Hanocq-Quertier, J., Hanocq, F., Claes, Y., Le Ray, D., Overath, P. and Pays, E. (1993). Transient adenylate cyclase activation accompanies differentiation of *Trypanosoma brucei* from bloodstream to procyclic forms. *Molecular and Biochemical Parasitology* **61**, 115–126.
- Roth, C.W., Longacre, S., Raibaud, A., Baltz, T. and Eisen, H. (1986). The use of incomplete genes for the construction of a *Trypanosoma equiperdum* variant surface glycoprotein gene. *EMBO Journal* **5**, 1065–1070.
- Rothstein, R., Michel, B. and Gangloff, S. (2000). Replication fork pausing and recombination or ‘gimme a break’. *Genes and Development* **14**, 1–10.
- Rudenko, G., Blundell, P.A., Taylor, M.C., Kieft, R. and Borst, P. (1994). VSG gene expression site control in insect form *Trypanosoma brucei*. *EMBO Journal* **13**, 5470–5482.
- Rudenko, G., Blundell, P.A., Dirksmulder, A., Kieft, R. and Borst, P. (1995). A ribosomal DNA promoter replacing the promoter of a telomeric VSG gene expression site can be efficiently switched on and off in *Trypanosoma brucei*. *Cell* **83**, 547–553.
- Rudenko, G., McCulloch, R., Dirksmulder, A. and Borst, P. (1996). Telomere exchange can be an important mechanism of variant surface glycoprotein gene switching in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **80**, 65–75.
- Rudenko, G., Chaves, I., Dirksmulder, A. and Borst, P. (1998). Selection for activation of a new variant surface glycoprotein gene expression site in *Trypanosoma brucei* can result in deletion of the old one. *Molecular and Biochemical Parasitology* **95**, 97–109.

- Ruepp, S., Furger, A., Kurath, U., Renggli, C.K., Hemphill, A., Brun, R. and Roditi, I. (1997). Survival of *Trypanosoma brucei* in the tsetse fly is enhanced by the expression of specific forms of procyclin. *Journal of Cell Biology* **137**, 1369–1379.
- Salmon, D., Hanocq-Quertier, J., Paturiaux-Hanocq, F., Pays, A., Tebabi, P., Nolan, D.P., Michel, A. and Pays, E. (1997). Characterization of the ligand-binding site of the transferrin receptor in *Trypanosoma brucei* demonstrates a structural relationship with the N-terminal domain of the variant surface glycoprotein. *EMBO Journal* **16**, 7272–7278.
- Schell, D., Evers, R., Preis, D., Ziegelbauer, K., Kiefer, H., Lottspeich, F., Cornelissen, A.W.C.A. and Overath, P. (1991). A transferrin-binding protein of *Trypanosoma brucei* is encoded by one of the genes in the variant surface glycoprotein gene expression site. *EMBO Journal* **10**, 1061–1066.
- Scholler, J.K., Myler, P.J. and Stuart, K.D. (1989). A novel telomeric gene conversion in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **35**, 11–20.
- Schurch, N., Hehl, A., Vassella, E., Braun, R. and Roditi, I. (1994). Accurate polyadenylation of procyclin mRNAs in *Trypanosoma brucei* is determined by pyrimidine-rich elements in the intergenic regions. *Molecular and Cellular Biology* **14**, 3668–3675.
- Seed, J.R. (1978). Competition among serologically different clones of *Trypanosoma brucei gambiense* in vivo. *Journal of Protozoology* **25**, 526–529.
- Seed, J.R. and Effron, H.G. (1973). Simultaneous presence of different antigenic populations of *Trypanosoma brucei gambiense* in *Microtus montanus*. *Parasitology* **66**, 269–278.
- Shah, J.S., Young, J.R., Kimmel, B.E., Iams, K.P. and Williams, R.O. (1987). The 5' flanking sequence of a *Trypanosoma brucei* variable surface glycoprotein gene. *Molecular and Biochemical Parasitology* **24**, 163–174.
- Shapiro, J.A. (1998). Thinking about bacterial populations as multicellular organisms. *Annual Review of Microbiology* **52**, 81–104.
- Shapiro, S.Z., Naessens, J., Liesegang, B., Moloo, S.K. and Magondi, J. (1984). Analysis by flow cytometry of DNA synthesis during the life cycle of African trypanosomes. *Acta Tropica* **41**, 313–323.
- Sherman, J.M. and Pillus, L. (1997). An uncertain silence. *Trends in Genetics* **13**, 308–313.
- Shinagawa, H. and Iwasaki, H. (1996). Processing the Holliday junction in homologous recombination. *Trends in Biochemical Sciences* **21**, 107–111.
- Shinohara, A. and Ogawa, T. (1995). Homologous recombination and the roles of double-strand breaks. *Trends in Biochemical Sciences* **20**, 387–391.
- Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K. and Ogawa, T. (1993). Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and RecA. *Nature Genetics* **4**, 239–243.
- Ssenyonyga, G.S.Z. and Adam, K.M.G. (1975). The number and morphology of trypanosomes in the blood and lymph of rats infected with *Trypanosoma brucei* and *T. congolense*. *Parasitology* **70**, 255–261.
- Steverding, D., Stierhof, Y.D., Fuchs, H., Tauber, R. and Overath, P. (1995). Transferrin-binding protein complex is the receptor for transferrin uptake in *Trypanosoma brucei*. *Journal of Cell Biology* **131**, 1173–1182.
- Svard, S.G., Meng, T.C., Hetsko, M.L., McCaffery, J.M. and Gillin, F.D. (1998). Differentiation-associated surface antigen variation in the ancient eukaryote *Giardia lamblia*. *Molecular Microbiology* **30**, 979–989.
- Szostak, J.W., Orr-Weaver, T.L. and Rothstein, R.J. (1983). The double-strand-break repair model for recombination. *Cell* **33**, 25–35.
- Tait, A. and Turner, C.M.R. (1990). Genetic exchange in *Trypanosoma brucei*. *Parasitology Today* **6**, 70–75.

- Tanner, M., Jenni, L., Hecker, H. and Brun, R. (1980). Characterization of *Trypanosoma brucei* isolated from lymph nodes of rats. *Parasitology* **80**, 383–391.
- Taylor, H.M., Kyes, S.A., Harris, D., Kriek, N. and Newbold, C.I. (2000). A study of *var* gene transcription *in vitro* using universal *var* gene primers. *Molecular and Biochemical Parasitology* **105**, 13–23.
- Tetley, L. and Vickerman, K. (1985). Differentiation in *Trypanosoma brucei*: host–parasite cell junctions and their persistence during acquisition of the variable antigen coat. *Journal of Cell Science* **74**, 1–19.
- Tetley, L., Turner, C.M.R., Barry, J.D., Crowe, J.S. and Vickerman, K. (1987). Onset of expression of the variant surface glycoproteins of *Trypanosoma brucei* in the tsetse fly studied using immunoelectron microscopy. *Journal of Cell Science* **87**, 363–372.
- Thacker, J. (1999). A surfeit of RAD51-like genes? *Trends in Genetics* **15**, 166–168.
- Theis, J.H. and Bolton, V. (1980). *Trypanosoma equiperdum*: movement from the dermis. *Experimental Parasitology* **50**, 317–330.
- Thon, G., Baltz, T. and Eisen, H. (1989). Antigenic diversity by the recombination of pseudogenes. *Genes and Development* **3**, 1247–1254.
- Thon, G., Baltz, T., Giroud, C. and Eisen, H. (1990). Trypanosome variable surface glycoproteins: composite genes and order of expression. *Genes and Development* **9**, 1374–1383.
- Tibayrenc, M. (1999). Toward an integrated genetic epidemiology of parasitic protozoa and other pathogens. *Annual Review of Genetics* **33**, 449–477.
- Timmers, H.T.M., de Lange, T., Kooter, J.M. and Borst, P. (1987). Coincident multiple activations of the same surface antigen gene in *Trypanosoma brucei*. *Journal of Molecular Biology* **194**, 81–90.
- Torkelson, J., Harris, R.S., Lombardo, M.J., Nagendran, J., Thulin, C. and Rosenberg, S.M. (1997). Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO Journal* **16**, 3303–3311.
- Tortorella, D., Gewurz, B.E., Furman, M.H., Schust, D.J. and Ploegh, H.L. (2000). Viral subversion of the immune system. *Annual Review of Immunology* **18**, 861–926.
- Trumtel, S., Leger-Silvestre, I., Gleizes, P.E., Teulieres, F. and Gas, N. (2000). Assembly and functional organization of the nucleolus: ultrastructural analysis of *Saccharomyces cerevisiae* mutants. *Molecular Biology of the Cell* **11**, 2175–2189.
- Turner, C.M.R. (1997). The rate of antigenic variation in fly-transmitted and syringe-passaged infections of *Trypanosoma brucei*. *FEMS Microbiology Letters* **153**, 227–231.
- Turner, C.M.R. (1999). Antigenic variation in *Trypanosoma brucei* infections: an holistic view. *Journal of Cell Science* **112**, 3187–3192.
- Turner, C.M.R. and Barry, J.D. (1989). High frequency of antigenic variation in *Trypanosoma brucei rhodesiense* infections. *Parasitology* **99**, 67–75.
- Turner, C.M.R., Barry, J.D. and Vickerman, K. (1986a). Independent expression of the metacyclic and bloodstream variable antigen repertoires of *Trypanosoma brucei rhodesiense*. *Parasitology* **92**, 67–73.
- Turner, C.M.R., Hunter, C.A., Barry, J.D. and Vickerman, K. (1986b). Similarity in variable antigen type composition of *Trypanosoma brucei rhodesiense* populations in different sites within the mouse host. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 824–830.
- Turner, C.M.R., Barry, J.D., Maudlin, I. and Vickerman, K. (1988). An estimate of the size of the metacyclic variable antigen repertoire of *Trypanosoma brucei rhodesiense*. *Parasitology* **97**, 269–276.
- Turner, C.M.R., Aslam, N., Smith, E., Buchanan, N. and Tait, A. (1991). The effects of genetic exchange on variable antigen expression in *Trypanosoma brucei*. *Parasitology* **103**, 379–386.

- Tyler, K.M., Matthews, K.R. and Gull, K. (1997). The bloodstream differentiation–division of *Trypanosoma brucei* studied using mitochondrial markers. *Proceedings of the Royal Society of London Series B: Biological Sciences* **264**, 1481–1490.
- Valdes, J., Taylor, M.C., Cross, M.A., Ligtenberg, M.J.L., Rudenko, G. and Borst, P. (1996). The viral thymidine kinase gene as a tool for the study of mutagenesis in *Trypanosoma brucei*. *Nucleic Acids Research* **24**, 1809–1815.
- Van Den Abbeele, J., Claes, Y., Van Bockstaale, D., Le Ray, D. and Coosemans, M. (1999). *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. *Parasitology* **118**, 469–478.
- Van der Ploeg, L.H.T., Valerio, D., de Lange, T., Bernards, A., Borst, P. and Grosveld, F.G. (1982). An analysis of cosmid clones of nuclear DNA from *Trypanosoma brucei* shows that the genes for variant surface glycoproteins are clustered in the genome. *Nucleic Acids Research* **10**, 5905–5923.
- Van der Ploeg, L.H.T., Cornelissen, A.W.C.A., Barry, J.D. and Borst, P. (1984). Chromosomes of Kinetoplastida. *EMBO Journal* **3**, 3109–3115.
- Van der Ploeg, L.H.T., Gottesdiener, K. and Lee, M.G.S. (1992). Antigenic variation in African trypanosomes. *Trends in Genetics* **8**, 452–457.
- Van der Werf, A., Van Assel, S., Aerts, D., Steinert, M. and Pays, E. (1990). Telomere interactions may condition the programming of antigen expression in *Trypanosoma brucei*. *EMBO Journal* **9**, 1035–1040.
- Vanhamme, L., Pays, A., Tebabi, P., Alexandre, S. and Pays, E. (1995). Specific binding of proteins to the noncoding strand of a crucial element of the variant surface glycoprotein, procyclin, and ribosomal promoters of *Trypanosoma brucei*. *Molecular and Cellular Biology* **15**, 5598–5606.
- Vanhamme, L., Poelvoorde, P., Pays, A., Tebabi, P., Xong, H.V. and Pays, E. (2000). Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei*. *Molecular Microbiology* **36**, 328–340.
- Van Leeuwen, F., Wijsman, E.R., Kuyl-Yeheskiely, E., van der Marel, G.A., Van Boom, J.H. and Borst, P. (1996). The telomeric GGGTAA repeats of *Trypanosoma brucei* contain the hypermodified base J in both strands. *Nucleic Acids Research* **24**, 2476–2482.
- Van Leeuwen, F., Wijsman, E.R., Kieft, R., van der Marel, G.A., Vanboom, J.H. and Borst, P. (1997). Localization of the modified base J in telomeric VSG gene expression sites of *Trypanosoma brucei*. *Genes and Development* **11**, 3232–3241.
- Van Leeuwen, F., Dirksmulder, A., Dirks, R.W., Borst, P. and Gibson, W. (1998a). The modified DNA base beta-D-glucosyl-hydroxymethyluracil is not found in the tsetse fly stages of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **94**, 127–130.
- Van Leeuwen, F., Taylor, M.C., Mondragon, A., Moreau, H., Gibson, W., Kieft, R. and Borst, P. (1998b). β -D-glucosyl-hydroxymethyluracil is a conserved DNA modification in kinetoplastid protozoans and is abundant in their telomeres. *Proceedings of the National Academy of Sciences of the USA* **95**, 2366–2371.
- Van Leeuwen, F., Kieft, R., Cross, M. and Borst, P. (1998c). Biosynthesis and function of the modified DNA base beta-D-glucosyl-hydroxymethyluracil in *Trypanosoma brucei*. *Molecular and Cellular Biology* **18**, 5643–5651.
- Van Meirvenne, N., Janssens, P.G. and Magnus, E. (1975). Antigenic variation in syringe passaged populations of *Trypanosoma (Trypanozoon) brucei*. I. Rationalization of the experimental approach. *Annales de la Société Belge de Médecine Tropicale* **55**, 1–23.
- Van Meirvenne, N., Magnus, E. and Vervoort, T. (1977). Comparisons of variable antigenic types produced by trypanosome strains of the subgenus *Trypanozoon*. *Annales de la Société Belge de Médecine Tropicale* **57**, 409–423.
- Vassella, E., Reuner, B., Yutzy, B. and Boshart, M. (1997). Differentiation of African

- trypanosomes is controlled by a density sensing mechanism which signals cell cycle arrest via the cAMP pathway. *Journal of Cell Science* **110**, 2661–2671.
- Vassella, E., Van Den Abbeele, J., Butikofer, P., Renggli, C.K., Furger, A., Brun, R. and Roditi, I. (2000). A major surface glycoprotein of *Trypanosoma brucei* is expressed transiently during development and can be regulated post-transcriptionally by glycerol or hypoxia. *Genes and Development* **14**, 615–626.
- Vickerman, K. (1985). Developmental cycles and biology of pathogenic trypanosomes. *British Medical Bulletin* **41**, 105–114.
- Wahlgren, M., Fernandez, V., Chen, Q.J., Svard, S. and Hagblom, P. (1999). Waves of malarial variations. *Cell* **96**, 603–606.
- Weiden, M., Osheim, Y.N., Beyer, A.L. and Van der Ploeg, L.H.T. (1991). Chromosome structure – DNA nucleotide sequence elements of a subset of the minichromosomes of the protozoan *Trypanosoma brucei*. *Molecular and Cellular Biology* **11**, 3823–3834.
- Welburn, S.C., Dale, C., Ellis, D., Beecroft, R.P. and Pearson, T.W. (1996). Apoptosis in procyclic *Trypanosoma brucei rhodesiense* *in vitro*. *Cell Death and Differentiation* **3**, 229–236.
- Welburn, S.C., Barcinski, M.A. and Williams, G.T. (1997). Programmed cell death in trypanosomatids. *Parasitology Today* **13**, 22–26.
- Wyatt, R., Kwong, P.D., Desjardins, E., Sweet, R.W., Robinson, J., Hendrickson, W.A. and Sodroski, J.G. (1998). The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* **393**, 705–711.
- Xong, H.V., Vanhamme, L., Chamekh, M., Chimfwembe, C.E., Van Den Abbeele, J., Pays, A., Van Meirvenne, N., Hamers, R., Debaetselier, P. and Pays, E. (1998). A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* **95**, 839–846.
- Young, J.R., Shah, J.S., MatthysSENS, G. and Williams, R.O. (1983). Relationship between multiple copies of a *T. brucei* variable surface glycoprotein gene whose expression is not controlled by duplication. *Cell* **32**, 1149–1159.
- Zhang, J.R. and Norris, S.J. (1998). Genetic variation of the *Borrelia burgdorferi* gene *vlsE* involves cassette-specific, segmental gene conversion. *Infection and Immunity* **66**, 3698–3704.
- Zhang, J.R., Hardham, J.M., Barbour, A.G. and Norris, S.J. (1997). Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* **89**, 275–285.
- Zomerdijk, J.C.B.M., Ouellette, M., ten Asbroek, A.L.M.A., Kieft, R., Bommer, A.M.M., Clayton, C.E. and Borst, P. (1990). The promoter for a variant surface glycoprotein gene expression site in *Trypanosoma brucei*. *EMBO Journal* **9**, 2791–2801.
- Zomerdijk, J.C.B.M., Kieft, R., Shiels, P.G. and Borst, P. (1991). Alpha-amanitin-resistant transcription units in trypanosomes – a comparison of promoter sequences for a VSG gene expression site and for the ribosomal RNA genes. *Nucleic Acids Research* **19**, 5153–5158.

The Epidemiology and Control of Human African Trypanosomiasis

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ABSTRACT

Human African trypanosomiasis is caused by *Trypanosoma brucei gambiense* in West and Central Africa, and by *Trypanosoma brucei rhodesiense* in East and southern Africa. In recent years there has been a dramatic resurgence of Gambian trypanosomiasis in Central Africa, especially in the Democratic Republic of Congo, Angola and Sudan. The disease is quiescent in most of West Africa, as is Rhodesian trypanosomiasis the other side of the continent. The epidemiology of Gambian trypanosomiasis is reviewed in detail. The long duration of infection in human hosts with cycles of intermittent parasitaemia, the vectors' feeding habits and the intensity of human–fly contact are the major determinants of the dynamics of transmission of this parasite. The development of immunity may lead to a reduction in the fraction of the population that is susceptible to infection and the burning out of epidemics after 20 to 30 years. So far, the acquired immune deficiency syndrome pandemic has had no impact on the epidemiology of Gambian trypanosomiasis. A brief review of the epidemiology of Rhodesian trypanosomiasis highlights the differences from Gambian trypanosomiasis that, to some extent, explain its lower propensity to cause epidemics: it is a more aggressive disease that rapidly kills its human host, and its transmission involves mostly domestic and game animals, humans being in most circumstances an accidental host. The various methods and strategies for the surveillance and control of both diseases are reviewed.

1. INTRODUCTION

Human African trypanosomiasis (HAT), also known as sleeping sickness, is caused by haemoflagellates of the genus *Trypanosoma*, subgenus *Trypanozoon*, which classically includes three subspecies: *T. brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense*. The three subspecies are morphologically identical but differ in their ability to infect various hosts. *T. b. brucei* is essentially a parasite of domestic animals (Bovidae, Suidae and Canidae) and game animals (antelopes) and is not pathogenic to humans because it is lysed by a haptoglobin-like molecule (Smith, A.B. *et al.*, 1995). Only *T. b. rhodesiense* and *T. b. gambiense* are considered to be human pathogens. Two clinical variants of HAT are encountered: an acute syndrome, attributed to *T. b. rhodesiense* (Rhodesian trypanosomiasis), and a chronic syndrome caused by *T. b. gambiense* (Gambian trypanosomiasis). In both instances, the disease results from complex interactions between vertebrate hosts (humans and animals), the parasite and its tsetse fly vector (*Glossina* spp.).

HAT is the only vector-borne parasitic disease whose geographical distribution is limited to the African continent. *T. b. gambiense* is seen in West and Central Africa and in parts of East Africa, and *T. b. rhodesiense* only in East and southern Africa (Figure 1). *T. b. gambiense* has, however, been eliminated from a number of West African countries, for reasons which probably have more to do with ecological changes than with extremely vigorous disease control. Within each country, current endemic foci are generally the same as those identified by the first mobile teams in the 1920s. Uganda is the only country where both subspecies are found: Gambian trypanosomiasis is prevalent in the north-west of the country, and Rhodesian trypanosomiasis in the south-east. The distribution of the two subspecies has probably remained constant over the last century and, in retrospect, apparent historical exceptions to this distribution corresponded to misidentification of the parasite. For instance, the devastating epidemic in south-east Uganda at the beginning of the 20th century was then thought to be caused by *T. b. gambiense*, but is now considered to have been caused rather by *T. b. rhodesiense*, the etiological agent of the epidemics seen in the same location in the 1940s and in the 1970s (Koerner *et al.*, 1995).

2. HISTORICAL BACKGROUND

The history of HAT is intimately linked to the history of the African continent, and several authors have reviewed various episodes of this long story (Prothero, 1963; Burke, 1971; McKelvey, 1973; de Raadt, 1976; Janssens and Burke, 1992; Williams, 1996; Hide, 1999). Human trypanosomiasis has been endemic in West Africa at least since the 14th century. The Arab historian Ibn Khaldun described the 'sleeping illness' of an emperor of Mali who died around 1374. The disease has been known to slave traders and naval surgeons at least since the beginning of the 18th century (Williams, 1996). In 1803, Thomas Winterbottom described what he called the 'Negro lethargy' and the enlarged cervical lymph nodes, which still bear his name. The magnitude of this problem was, however, unknown, as the colonial powers did not venture inland until the second half of the 19th century. Huge epidemics of African trypanosomiasis devastated the continent at the end of the 19th and the beginning of the 20th century. According to Burke (1971), Winston Churchill, then colonial under-secretary, reported to the House of Commons in 1906 that the population of Uganda had been reduced from 6.5 to 2.5 million due to this epidemic. A disaster of a similar order of magnitude had been observed in the Free State of Congo, along the Congo River (Burke, 1971). Although the reliability of such estimates is very doubtful (other estimates of the Uganda epidemic mention half a million infected individuals [Hide, 1999]), African trypanosomiasis clearly represented a massive public health problem, and the

dissemination of the disease was to some extent precipitated by Africa's exploitation by European colonial powers. Britain, France, Belgium and Germany invested in medical research and significant progress was rapidly made. Forde and Dutton discovered the first human trypanosome, which they called *Trypanosoma gambiense*, at the end of 1901 and less than two years later Castellani observed the same trypanosome in the cerebrospinal fluid (CSF) of sleeping sickness cases in Uganda. In 1910, Stephens and Fantham identified a new, more virulent, form in a patient infected in Rhodesia, which they called *Trypanosoma rhodesiense* (see Williams, 1996).

Colonial administrations tried to control the disease through restriction of population movements, case detection by examination of lymph node aspirates and blood films, treatment of cases with toxic drugs such as Atoxyl™, and various methods of vector control. The concept of specialized mobile teams was developed by Jamot in 1922 in Cameroon and rapidly adopted by most countries in which the disease was endemic. During these massive military-style operations, millions of individuals were screened once or twice per year through cervical lymph node palpation and aspiration, and treated with newer drugs that were more effective, if not less toxic. Twenty-five years later, pentamidine chemoprophylaxis complemented this traditional approach. Lapeyssonnie (1992) reported that, between 1939 and 1960, mobile teams in French colonies of West and Central Africa had examined 73 000 000 individuals and detected 250 000 cases.

Such efforts were extremely successful, and the incidence of African trypanosomiasis had dramatically decreased in most countries by the time they became independent around 1960. Prevalence rates were in the order of 0·1 per 1000 (Lapeyssonnie, 1992). This regular surveillance, the cornerstone of trypanosomiasis control, was abandoned, as it was difficult to justify substantial expenditure for what had become a relatively rare disease. In several countries, the lack of systematic surveillance, the progressive shortage of qualified and experienced personnel, the refusal by a substantial proportion of inhabitants of endemic foci to participate voluntarily in screening activities and the intensification of population movement following political disturbances or changes in the economy, all contributed to a resurgence of African trypanosomiasis in the 1970s. In some countries, control efforts were resumed and the incidence of trypanosomiasis kept under control. In others, where war or civil strife hampered control activities, massive epidemics developed in the 1980s and 1990s.

3. CURRENT DISEASE BURDEN

According to the World Health Organization (1998), there remain within the 'tsetse belt' more than 200 active foci of African trypanosomiasis, located

between latitudes 15° north and 15° south (Figure 1). Within this geographical area, approximately 60 millions individuals living in 36 countries are exposed to the infection. Less than 4 million benefit from an adequate surveillance and case-finding programme or from vector control activities. All endemic countries are characterized by shortages of the financial and human resources necessary to implement or sustain a comprehensive control programme.

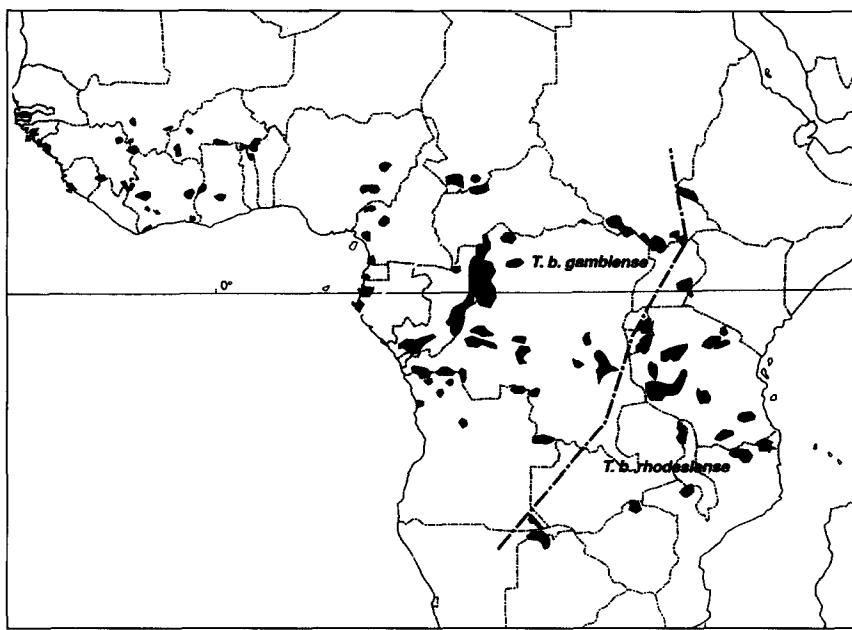


Figure 1 Major endemic foci of *T. b. gambiense* and *T. b. rhodesiense* African trypanosomiasis, 1995. Reproduced with permission from the World Health Organization (1998).

Reports from trypanosomiasis control programmes can give only a rough idea of the burden of disease in each country. Because of the difficult security situation and the decay of communication systems in many parts of high-incidence countries, case finding and passive diagnosis may be impossible in several foci during various periods of time (from months to years). There is certainly now, in the Democratic Republic of Congo (DRC), Angola and Sudan, substantial underdiagnosis, a sizeable fraction of cases dying at home without any access to diagnostic or therapeutic facilities. Underdiagnosis is also exacerbated by the imperfect sensitivity of diagnostic methods. Under-reporting is less of a problem, as health institutions usually have to report

cases in order to obtain trypanocidal drugs, but the strength of the national control programme, and its capacity to collect meaningful national statistics, vary between countries.

The overall epidemiological situation for the 36 endemic countries, subdivided into four levels of incidence according to the average annual number of cases reported during 1977–1997, is shown in Figure 2.

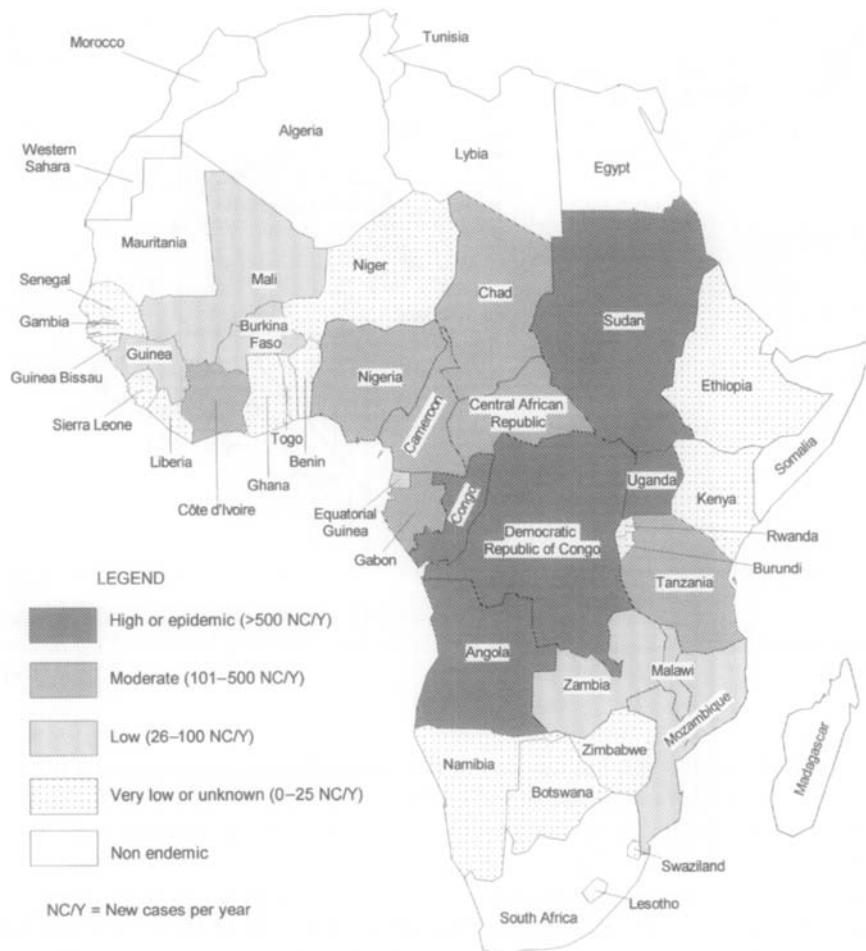


Figure 2 Map of Africa showing the average number of new cases of human African trypanomiasis per year in each endemic country during the period 1977–1997.

T. b. gambiense trypanosomiasis is now a major public health problem in Central Africa, especially in DRC, Angola and Sudan. In DRC, where better epidemiological information is available, the number of new cases reported each year has now reached, despite substantial underdiagnosis due to inadequate coverage of endemic regions, levels comparable to those seen in the early 1930s, and may result in the death of as many adults as the acquired immune deficiency syndrome (AIDS) (Ekwanza et al., 1996). This is a public health disaster, considering that the disease had been nearly eliminated in the years preceding independence. The situation is also catastrophic in Angola, where the continuing civil war hampers control efforts to such an extent that national statistics give only a very incomplete view of the problem. Similarly, the true extent of the burden of Gambian trypanosomiasis in southern Sudan remains impossible to delineate. In Uganda, there has been some improvement recently, after many years of effort by national authorities with substantial external support. In contrast, in West Africa the disease has regressed or disappeared from several countries as ecological changes reduced the intensity of human-fly contact, and only Côte d'Ivoire and Guinea still report a significant number of cases. In East and southern Africa, the incidence of *T. b. rhodesiense* trypanosomiasis remains low and the disease can hardly be considered a public health priority. However, Rhodesian trypanosomiasis is a zoonosis and new epidemics, similar to that seen in Uganda 20 years ago, could be triggered by unpredictable ecological changes.

We will later describe the distribution of disease at the country level, with an emphasis on a few high-incidence countries. Year to year incidence for the period 1977–1994 in all endemic countries can be found in a recent World Health Organization report (1998). It is difficult to estimate the overall burden of African trypanosomiasis because of underdiagnosis in the most heavily infected countries, but we believe that it must be in the vicinity of 100 000 new cases per year, with between one-third and one-half of cases remaining undetected and untreated. Certainly the figure of 300 000 to 500 000 new cases per year recently proposed by the World Health Organization (1997), based on fewer than 10% of the cases in DRC and Angola being detected and reported, seems exaggerated. Rhodesian trypanosomiasis represents only around 2% of the overall burden of African trypanosomiasis.

The World Bank estimated that, in 1990, there were 55 000 deaths due to HAT (World Bank, 1993). This was probably then an overestimate, but this figure must be close to reality now, considering that virtually all untreated cases die as well as 5–10% of detected cases, either of drug toxicity or drug-resistant trypanosomiasis. The same study estimated that African trypanosomiasis resulted in the loss of 1.8 million disability-adjusted life years (DALY) each year. More recent estimates (World Health Organization, 2000) are rather similar, with 2.05 million DALYs lost and 66 000 deaths in 1999 due to HAT. As a comparison, the number of millions of DALYs

lost is estimated at 45·0 for malaria, 4·9 for lymphatic filariasis, 2·0 for leishmaniasis, 1·9 for schistosomiasis, 1·1 for onchocerciasis and 0·7 for Chagas disease.

4. MEASURES OF INCIDENCE AND PREVALENCE

Year to year changes in incidence have to be interpreted with caution. Gambian trypanosomiasis progresses slowly, after an asymptomatic stage that might last months or years. Thus, the first year or two of an active case-finding initiative in a given focus usually reveal a large number of prevalent cases which had been accumulating for some time, while data for subsequent years correspond mostly to incident cases. Transient interruption of a control programme, because of lack of funding or security problems, leads to an apparent reduction in the number of cases, and a large increase when case finding is resumed. These facts have implications on the basic epidemiological concepts of incidence and prevalence.

Prevalence, the proportion of individuals in a population who have the disease at a specific instant, is measured, for African trypanosomiasis, during case-finding surveys. Using a serological test to identify 'suspects', on whom parasitological assays are carried out, roughly doubles the number of parasitologically proven cases that are ultimately identified compared with surveys relying only on classical parasitological methods. Thus, such surveys yield estimates of prevalence that are higher, and much closer to the true prevalence of trypanosomal infection, than traditional surveys. These measures are relatively reliable, with the caveat that prevalence is estimated for the fraction of the population available for the survey. If children are over-represented during the survey (schoolchildren generally have no choice but to be present, while the participation of adults, especially males, is problematic), prevalence is underestimated since they are at lower risk of acquiring HAT than adults. For instance, in two large surveys in Cameroon, 70% of participants belonged to the 0–14 years age group (Asonganyi and Ade, 1994).

Incidence, the number of new cases of a disease that develop in a specific population during a defined period of time (usually one year), corresponds for African trypanosomiasis to the sum of cases diagnosed passively by health centres or hospitals and cases detected actively during case-finding surveys. Measures of incidence of African trypanosomiasis are thus largely influenced, especially in epidemic situations, by the quality of case finding and should always be interpreted with caution.

5. GEOGRAPHICAL DISTRIBUTION

5.1. West Africa

5.1.1. *Côte d'Ivoire*

For many years, Côte d'Ivoire has been the country reporting the highest number of new cases (300 to 500 per year) in West Africa (Cattand, 1994). The disease is endemic in the forest areas of the centre-west and the south-east. The most active foci are those of Daloa, Vavoua, Zoukougbeu, Bouaflé, Sinfra and Aboisso, where prevalence in recent years has varied between 0·2% and 6·1% (Laveissière *et al.*, 1994a; Médá *et al.*, 1995).

5.1.2. *Guinea*

In Guinea, surveillance by specialized teams was abandoned in the 1960s as the incidence had then reached a very low level. This led to a recrudescence a decade later, and 200 to 250 cases are now detected passively each year, mostly from the Dubréka district, only 15% of cases being in the early stage. Small numbers of cases are seen in the Siguiri, Koubia, Lola and N'Zérékoré foci (in the forest area of the country), and even in the surroundings of Conakry.

5.1.3. *Other Countries of West Africa*

Elsewhere in West Africa, few cases are reported, reflecting a very low incidence in some countries and underdiagnosis and under-reporting in others (see Figure 2). The disease has been eliminated from Senegal, The Gambia, Guinea-Bissau, Niger and maybe Ghana. The massive seasonal migrations between Côte d'Ivoire and neighbouring countries facilitate the circulation of the parasite, but only a few dozen cases are seen each year in Burkina Faso and Mali, corresponding both to importation of cases and local transmission. Very low numbers of cases are observed in the northern part of Togo and Bénin. No information is available from Liberia and Sierra Leone following their long and devastating civil wars. There remain active foci in Nigeria (Delta State and others) with a few hundred cases per year, but reporting has been abandoned since the early 1980s.

5.2. Central Africa

5.2.1. Democratic Republic of Congo

The DRC is the country facing by far the most dramatic epidemic and probably bears more than half of the global burden of African trypanosomiasis. The disease had been nearly eliminated at the end of the 1950s (slightly over 1000 cases in 1959), but cessation of control activities during the civil war that followed independence led to a resurgence, with 4000 to 6000 cases documented annually when case finding was resumed at the end of the 1960s. Despite sustained efforts by dedicated staff and continuous external funding to support up to 25 mobile teams, the annual incidence gradually increased to about 10 000 cases at the end of the 1980s (Ekwanzala *et al.*, 1996). Control activities were then hampered by the deteriorating sociopolitical situation, and the incidence reached 19 340 cases in 1994, 18 182 cases in 1995 and 19 485 cases in 1996 (unpublished data from the Bureau Central de la Trypanosomiase). Incidence increased further in 1997 and 1998 with, respectively, 25 094 and 27 044 reported cases.

There is substantial underdiagnosis, as mobile teams are able to reach only a fraction of the endemic villages. Furthermore, until recently, case finding relied on the traditional parasitological methods, which have limited sensitivity. It has been estimated that the real number of cases is probably twice as high as the reported figures (Ekwanzala *et al.*, 1996). In 1996, just under one-third of cases (6058) were identified by active case finding, despite 28 mobile teams, some of which used serological assays on a large scale, and a similar number (5775) were in the early stage (defined as a CSF white cell count below 5 mm^{-3}). This reflects the tremendous operational difficulties encountered by the case-finding teams, and control of the disease will not be achieved until a higher proportion of cases is identified actively in the early stage. The war that resumed in the middle of 1998, with some endemic areas under government control while others are split between various rebel factions and foreign troops, further hampered case finding and will undoubtedly lead to additional increases in incidence and in the proportion of people with the disease who die without ever having been diagnosed.

Distribution of Gambian trypanosomiasis in DRC is very patchy and corresponds closely to what was reported earlier in the 20th century (Kabeya *et al.*, 1988; Janssens and Burke, 1992). The worst affected region is Equateur, in the north-west of the country, where almost two-thirds of the total caseload are found (14 913 cases in 1997), followed by Bandundu (centre-west of the country, 5624 cases) and Kasai (centre-east, 2919 cases). Incidence is much lower in Bas-Congo, Haut-Congo, Kivu and Katanga regions. In Equateur region, the worst affected districts are Karawa, Gomena, Tandala and Bwamanda, all located between the Congo and the Oubangui rivers, while in Bandundu

region, Kenge and Masi-Manimba districts have more than half of the cases. In these two regions, many communities have been found to have prevalences over 10% during recent case-finding surveys. An extraordinary prevalence of 72% was documented in a small village of Bandundu region in 1994 (Ekwanzala *et al.*, 1996), suggesting that some isolated rural communities might have disappeared altogether due to HAT. The epidemic is more recent in Equateur than in Bandundu (Arbyn *et al.*, 1995), and the explosive spread of the disease in the former region might have resulted from the general lack of immunity among its population (see Section 6.2.5).

The total population at risk is estimated at 12 500 000 individuals by the national control programme, 10% of whom at most are covered by mobile teams, which obviously concentrate their efforts on the areas of highest incidence. The incidence in non-covered regions is probably at least an order of magnitude lower than in those covered. Most undiagnosed cases presumably occur in villages of Equateur, Bandundu and Kasai, regions that are not covered by mobile teams and have little access to passive diagnosis, rather than in the other regions of the country.

5.2.2. *Angola*

After DRC, Angola is the country with the second highest incidence of HAT. Respectively, 8275 and 6610 new cases were reported in 1997 and 1998 by the national control programme (unpublished reports). The disease is found mostly in seven provinces in the north-west of the country: Zaire, Uige, Bengo, Kuanza Norte, Luanda, Malange and Kuanza Sul. The prevalence rates reported from recent mass surveys conducted in the provinces of Kuanza Norte, Malange and Zaire varied between 1.3% and 9.7%. The situation in other provinces is less well known but certainly less severe.

Year to year variations in the number of reported cases must be interpreted with caution because of the impact of war on case-finding. Again, substantial efforts to improve case-finding and more widespread use of serological screening have been made in recent years by the national control programme and several non-governmental organizations (NGOs) (Smith, D.H. *et al.*, 1998). The large-scale fighting during 1999 has led to not only a famine but also the destruction of parts of the health system and flare-ups of many endemic diseases, including trypanosomiasis.

5.2.3. *Other Countries of Central Africa*

In 1998, 1069 cases of Gambian trypanosomiasis were reported from the Central African Republic (CAR), where the disease is limited to three foci in

the districts of Nola-Bilolo (south-west of the country), Ouham (north) and Haut-Mbomou (Sudanese border). The incidence in CAR has increased in recent years, following civil disturbances.

In Congo-Brazzaville, between 500 and 800 cases were reported each year in the early 1990s. Some endemic foci are located west of Brazzaville while others are situated on the Congo River or its tributaries, adjacent to foci in DRC. The Sangha focus has been thoroughly described, with prevalence up to 10% in some villages in the late 1980s (Jannin *et al.*, 1992; Penchenier *et al.*, 1993). Control activities stopped with the civil war in 1996 and no reliable information has been available recently, but there must have been an increase in incidence.

Only residual endemicity (with around 20 reported cases per year) is present in the Cameroon, mostly in the Fontem-Santchou and Campo foci (Penchenier *et al.*, 1998). The former is located north of Douala, toward the Nigerian border, while the latter is adjacent to the border with Equatorial Guinea. The situation is unknown in the other previously described foci of Mamfe, Wouri (near Douala), Bafia, Bipindi and Yokadouma (near the Nola focus of CAR), as there was very little case finding in the 1990s (Asonganyi *et al.*, 1991; Asonganyi and Ade, 1994).

In Tchad, a reduction of incidence was observed after the independence of the country. Case finding was reduced or stopped completely. The classical scenario occurred and a recrudescence was documented from the mid-1970s into the 1980s, with up to 357 cases in 1989 (Stanghellini *et al.*, 1989; World Health Organization, 1998). The most active foci are those of Tapol, Timbéri, Ranga and Moïssala, all in the southern extremity of the country, toward CAR and Cameroon (Stanghellini *et al.*, 1989). Effective control programmes were reintroduced and the incidence fell to less than 100 cases per year. Similar numbers of cases are reported each year from Gabon and Equatorial Guinea (on Bioko Island). In Equatorial Guinea, this low incidence is also a consequence of vigorous control efforts (Simarro *et al.*, 1991).

5.3. East and Southern Africa

5.3.1. Uganda

Uganda is the only country where both *T. b. gambiense* and *T. b. rhodesiense* are found, the former in the north-west of the country, near the Sudanese border, and the latter in the south-east, without overlap. Incidence of Gambian trypanosomiasis in north-west Uganda increased progressively during the 1980s, as Ugandans fleeing their own civil war became infected in neighbouring countries and returned with the disease, followed a few years later by an influx of infected refugees from southern Sudan (Paquet *et al.*, 1995).

Between 1000 and 2000 cases were reported annually in 1990–1994, but this has at least stabilized with 978 cases reported in 1998, more than half of them from Arua district. The situation is under better control in Moyo and Adjumani districts, which were the most severely involved in the 1980s and early 1990s respectively.

A major epidemic of Rhodesian HAT devastated south-east Uganda from the mid-1970s. It started in 1976 in Luuka and Kigulu counties and spread throughout the Busoga region at the northern end of Lake Victoria, from where it extended into Tororo district (Mbulamberi, 1989a, 1989b, 1990; Lancien, 1991; Okoth *et al.*, 1991; Smith, D.H. *et al.*, 1998; Hide, 1999). Up to 8465 cases were recorded in 1981. The incidence decreased considerably from 1988 onwards, as a result of active case-finding by sleeping sickness orderlies and, to a lesser extent, vector control (Okiria, 1985; Lancien, 1991; Smith, D.H. *et al.*, 1998). Only 271 cases were reported in 1998, mostly from Iganga and Kamuli districts, near Busoga. This outbreak resulted in a cumulative total of about 40 000 new cases over 15 years.

5.3.2. Sudan

Foci are located in the southern part of Equatoria region, west of the Nile, within 100 km of the borders with CAR, DRC and Uganda. Trypanosomiasis is found in Kako-Keji, Yei, Maridi, Yambio and Tambura counties. Reliable national statistics are not available, as cases are treated locally by several independent NGOs, others in neighbouring countries, and the apparent incidence varies according to the intensity of case finding, which itself depends on the current security situation. Extrapolations suggest that there must be at least a few thousand cases per year.

5.3.3. Other Countries of East and Southern Africa

Reporting from most countries endemic for *T. b. rhodesiense* trypanosomiasis is somewhat irregular, as the disease is not considered a priority. Tanzania reports between 200 and 400 cases per year, and there are several foci throughout the country. Even lower numbers of cases are reported in Zambia and in Kenya, where the disease is seen essentially near the Ugandan border (Busia and Teso districts) and in the Lambwe valley, near Lake Victoria (Wellde *et al.*, 1989a). There is also a small number of cases (fewer than 20) seen each year in Rwanda, Mozambique, Malawi, Botswana and Zimbabwe. This is not much different from what was described 30 years ago (Apted, 1970). A detailed map of these foci is available elsewhere (Hide, 1999).

6. EPIDEMIOLOGICAL PRINCIPLES OF GAMBIAN TRYPANOSOMIASIS

6.1. The Parasite and its Life Cycle

The cellular and molecular characteristics of human trypanosomes have been reviewed elsewhere (Barry, 1997; El-Sayed and Donelson, 1997; Barry and McCulloch, 2001). Their life cycle can be summarized as follows. When a tsetse fly takes a blood meal from an infected host, it ingests bloodstream trypanosomes which then move to the fly's midgut, where over a few days they transform into the procyclic stage. The variant surface glycoprotein (VSG), the dominant constituent of the surface of bloodstream trypanosomes, is replaced by an invariant surface protein. After two to three weeks, trypanosomes migrate to the salivary gland, where other transformations lead to their development into metacyclic trypanosomes, which reacquire VSG, ready to be injected into a susceptible host during the next blood meal. Once infected, a tsetse fly remains so for the rest of its life. The possibility of mechanical, non-cyclical, transmission by other insects has been suggested for a long time (Scott, D., 1970). If it does happen, it is probably too rare to have any impact on the epidemiology of the disease, and the distribution of HAT mirrors that of the tsetse fly.

The parasite's life cycle within the vector will be reviewed further in Section 6.4.2. Within the human host, the parasites multiply at the site of inoculation, where a chancre may develop. Inoculation chancres are rarely recognized in Africans because of their skin pigmentation and the numerous insect bites of all kinds from which they suffer all year long. From this inoculation site the parasites get into the bloodstream and eventually into lymph nodes. The parasite multiplies through binary fission in the peripheral blood where three morphological forms of trypomastigotes are found: short stumpy forms, intermediate forms, and long slender forms.

Antigenic variation, a unique feature of trypanosomes (Barry, 1997; Barry and McCulloch, 2001), has direct consequences for the epidemiology of the disease. After infection of a human host, there is a switch from the expression of metacyclic VSG to bloodstream VSG. To evade the host's immune response, trypanosomes can successively express different VSGs, but only one at a time. Up to a thousand different VSGs are genetically encoded, and it is thought that the VSG currently expressed protects invariant constituents from the host's immune response. The mechanisms through which trypanosomes switch to expressing a different VSG are complex, but allow the parasite to escape from antibodies directed against the previous VSG. This sophisticated process explains the intermittent parasitaemia and the very long, largely asymptomatic, incubation period. Thus, there is an alternation between periods of higher parasitaemia following expression of a new VSG, during which the human host might be more

infectious to tsetse flies, and periods of lower or undetectable parasitaemia, during which infectivity must be lower.

Variations in the virulence of *T. b. gambiense* strains have been noted by clinicians (Van Hoof, 1947). The pace of progression of symptoms is far from uniform and there are marked differences between foci in the proportion of patients in whom trypanosomes are found in the blood rather than in lymph node aspirate. A less virulent strain should theoretically be able to disseminate more easily than a strain that rapidly kills its host, as the total duration of infection will be longer. Low virulence strains are typically found in new foci or in epidemic extensions from old areas (Van Hoof, 1947). On the other hand, less virulent strains might be associated with periods of parasitaemia that are shorter or of lower intensity. *T. b. rhodesiense* is much more virulent than *T. b. gambiense* and less prone to cause large epidemics, but there are other major differences between the two subspecies that might affect their propensity to cause epidemics (see Section 7).

Several modern methods have been developed for the identification and investigation of trypanosomes. The analysis of several isoenzymes allows the classification of trypanosomes into zymodemes. More recently, molecular methods, including the polymerase chain reaction, have been used to detect or identify trypanosomes by examining their genetic material (Gibson, 1994). By and large, these sophisticated assays have been little used in epidemiological investigations, with the exception of studies on the animal reservoirs of *T. b. gambiense* and *T. b. rhodesiense*.

6.2. The human reservoir

Humans are the main reservoir of *T. b. gambiense*. Four factors may influence the potential of infected persons to transmit *T. b. gambiense*: the duration of infection, the degree of parasitaemia, the number and distribution of individuals who are infected, and the intensity of contact with the vectors. Human–fly contact will be reviewed in Section 6.4.7.

6.2.1. The Natural History of Infection in Humans

Duration of infection in *T. b. gambiense* trypanosomiasis is thought to range from a few months to several years, from the infective bite to the moment an individual is either diagnosed with HAT (and treated) or dies. In the pre-treatment era (which lasted only a few years as Atoxyl™, a pentavalent arsenical drug, was discovered shortly after human trypanosomiasis was recognized), the natural history of *T. b. gambiense* infection had already been documented. Mr Kelly, the British captain of a steamboat that plied the River Gambia, in whose

blood human trypanosomes were first seen by Dutton and Forde in December 1901, remained intermittently parasitaemic until his death in January 1903 (Williams, 1996). Ross and Thomson (1910, 1911) elegantly described the cycles of parasitaemia in a patient with *T. b. rhodesiense* infection, with peaks occurring approximately every 7 days separated by intervals of much lower (but never absent) parasitaemia. They referred to a similar study by Koch and collaborators on the parasitaemia of patients with *T. b. gambiense* infection, in which they showed the parasites to be cyclically present in the blood for 2 to 5 days and absent for 2 to 3 weeks. In the 1940s, Van Hoof (1947) kept a dozen patients, whom he thought were infected with low-virulence *T. b. gambiense* strains, under observation without treatment for 6 months: during this period they showed no evidence of disease progression, and trypanosomes were seen in the blood on average once per week, with negative periods lasting up to 3 weeks. This long asymptomatic stage allows the infected host to carry on normal activities and to be regularly exposed to the vector.

At one extreme of this spectrum, but of little epidemiological importance because of their rarity, are anecdotal observations of healthy carriers who remain persistently well (Frézil and Carnevale, 1976). For instance, a Congolese woman living in Britain delivered a child who was later found to have congenital trypanosomiasis; 3 years and 6 months after leaving Africa, the mother was asymptomatic and persistently aparasitaemic (but had anti-trypanosomal antibodies) (Woodruff *et al.*, 1982). In Congo, three patients found to have trypanosomes did not attend for treatment and were perfectly healthy when seen again 4 years later (Ginoux and Frézil, 1981), but it is impossible to guess what would have been their ultimate fate if left untreated again. These cases are thought to be exceptional: in Côte d'Ivoire, five patients diagnosed with HAT hid to avoid the toxic treatment; when seen again 3 to 6 years later, they were all in a moribund condition (H. Méda and F. Doua, unpublished data).

Given that the tsetse fly is a relatively ineffective vector, the very long duration of infection during which the host can maintain normal activities, and provide blood meals for the vectors, must be the key determinant behind the endemic features and epidemic potential of Gambian HAT (Baker, J.R., 1974). At the same time, this long duration of infection offers a golden opportunity for intervention. Control programmes focusing on the identification and treatment of asymptotically infected humans, and thus on shortening the duration of infection, are extremely effective if an overwhelming majority of the population is examined during case-finding surveys and if sensitive diagnostic methods are used.

Individuals infected with *T. b. gambiense* usually harbour a modest degree of parasitaemia, below 50 trypanosomes mL⁻¹ (Evans, 1981), in contrast with the much higher parasitaemia seen with *T. b. rhodesiense*. During a blood meal, a tsetse ingests minute amounts of blood and a few trypanosomes at

most. However, tsetse flies can become infected from individuals whose parasitaemia is below the limit of detection with classical parasitological methods. Some researchers think that the short stumpy forms are more infectious to tsetse flies than other trypanosomes. Experiments on tsetse flies fed on pig blood through an artificial membrane showed that a single trypanosome is sufficient to infect a tsetse and that, above a threshold of approximately seven trypanosomes per meal, the probability of an infection developing in the tsetse is independent of the number of trypanosomes taken during the meal (Maudlin and Welburn, 1989). Thus the duration of parasitaemia must be a much more important determinant of transmission than the degree of parasitaemia.

In the absence of treatment, the infection eventually progresses to involve the central nervous system. The CSF becomes abnormal, the white cell count (WCC) goes up and trypanosomes can be found. The more advanced the neurological involvement and the higher the CSF WCC, the lower is the probability of finding trypanosomes in the blood or in the lymph nodes. Such patients become progressively bedridden and are little exposed to the vector. Thus, in the last few months of the natural history of Gambian trypanosomiasis, the patient becomes less available to tsetse.

6.2.2. The Distribution of Infection in Human Populations

Prevalence of trypanosome-infected individuals in human populations varies tremendously. In foci where a substantial number of new cases are seen, case-finding surveys generally document a prevalence between 1% and 5%. In very active foci, prevalences over 10% can be found, but the extreme prevalence of 72% recently documented in an isolated village of DRC (Ekwanzala *et al.*, 1996) is exceptional and incompatible with the long-term survival of such a community. In West Africa, where there is only residual endemicity, the prevalence documented during surveys is generally under 1%. Prevalence in a community slowly builds up over several years until the problem is recognized and case-finding initiated. During the first case-finding survey, a higher prevalence is found as it reflects the accumulation of cases over a long period. Prevalence will be two- or three-fold lower during the next survey, but the decline will be slower during subsequent years, especially if vector control activities are not implemented.

Trypanosomiasis is not found equally in males and females. In some foci, the incidence and prevalence are higher in males if the tsetse population is denser in areas of typically male activities. In other foci, the disease is more common in females, if tsetse densities are higher in, for example, rice or cassava fields or in the village itself (Willett, 1963; Stanghellini and Duvallet, 1981). Differences in sex distribution can be misleading if estimated only by the number of cases. In the Nioki focus of DRC, 60% of adult cases treated in

the district hospital occurred in females, while no such imbalance between sexes was observed among paediatric cases. Although higher exposure of women cannot be completely ruled out, migration seems to be the major explanation: in highly endemic villages, there is a clear preponderance of females in the population (Khonde *et al.*, 1995), as many young males migrate to regional urban centres, where exposure to infective tsetse flies is less intense. Similar findings were noted in another focus of central DRC (Mentens *et al.*, 1988). This apparent preponderance of cases among females would not correspond to substantial differences in incidence or prevalence if reliable denominators were available (which is rarely so). Females often participate more regularly in case-finding sessions, which can also lead to the detection of more cases (Asonganyi and Ade, 1994).

Children under the age of 10 years usually have a three- to five-fold lower incidence than adults (Scott, D., 1970; Stanghellini and Duvallet, 1981; Henry *et al.*, 1982; Mentens *et al.*, 1988), and this reflects differential exposure, as tsetse populations are often more numerous in fishing or farming sites than in the villages. Children over 10 years of age tend to frequent more regularly sites of potential exposure to the vectors and incidence rates progressively increase toward those of adults. Trypanosomiasis is less frequent in individuals over 50 to 60 years of age than in middle-aged adults (Stanghellini and Duvallet, 1981), possibly as a result of immunity but also due to lower exposure of these less active subjects.

Occupation is also related to exposure and to incidence. For instance, in Côte d'Ivoire HAT is more frequent in coffee and cocoa plantation workers or in people who fetch water than in other inhabitants (Laveissière *et al.*, 1986b; Méda *et al.*, 1993). More than 80% of cases occur in people who not only work but live in small plantation settlements; a case-control study showed that such people were five times more likely to develop trypanosomiasis than their counterparts who lived in villages (Méda *et al.*, 1993). In some foci of DRC, higher prevalence was found in fishermen, while elsewhere farmers were more likely to be infected (Henry *et al.*, 1982; Mentens *et al.*, 1988).

Variations in incidence and prevalence between ethnic groups have also been noted. This probably relates to differences in occupational exposure and in other determinants of human-fly contact rather than to genetically determined susceptibility (Stanghellini and Duvallet, 1981; Laveissière *et al.*, 1986b; Hervouet and Laveissière, 1987; Méda *et al.*, 1993). Other risk factors have been described, which are probably all markers of exposure to infective tsetse flies: lack of formal education, absence of pigs (an alternative source of blood meals for tsetse) in the habitat, etc. (Méda *et al.*, 1993, 1995).

Human population density is thought to influence the risk of epidemics of Gambian trypanosomiasis (Scott, D., 1970). If population density is very light, tsetse flies take most blood meals from non-human sources and transmission to humans is unlikely. At the other extreme, high human population density

results in modifications of the habitat that reduce the tsetse population. Thus, an intermediate population density is optimal for Gambian trypanosomiasis to prosper. A recent study in Côte d'Ivoire showed a strong correlation between the epidemiological risk and settlement density (Laveissière and Médà, 1999).

Given the long and quite variable duration of the asymptomatic stage, there is no seasonal variation in the incidence of the disease, apart from artefactual variations due to seasonal changes in the activity of case-finding teams. Whether or not there is seasonal variation in the frequency with which humans become infected with *T. b. gambiense* is unknown, but it is plausible because of changes in tsetse densities, human–fly contact, the presence of alternative sources of blood meals, etc.

6.2.3. *Familial Aggregation*

Familial clustering of trypanosomiasis has been recognized since the beginning of the 20th century (Martin *et al.*, 1909). Contradictory results have been obtained in studies using different methods (Henry, 1981; Mentens *et al.*, 1988; Gouteux *et al.*, 1989), but case-finding teams have often observed that more than one member of a given family are found with the disease at the same time. In a case–control study in Côte d'Ivoire, cases were three times more likely than controls to have had at least one relative in their family who had developed HAT (Médà *et al.*, 1993). In three adjacent villages of DRC, neither spatial nor familial aggregation was detected when looking at the distribution of the disease in the whole community using various statistical models (Khonde *et al.*, 1997). However, clustering was documented when examining specific familial relationships. Compared with children of mothers without a past history of HAT, the risk of a child having trypanosomiasis was approximately four times higher if the mother had had the disease. A past history of HAT in the father had no influence on the child's cumulative risk of trypanosomiasis. The risk of HAT was twice as high in brothers and sisters of a case than in their half-brothers and half-sisters. Such clustering could be due to either genetic susceptibility or shared exposure to the vector, but several arguments, reviewed elsewhere (Khonde *et al.*, 1997), suggest that the latter is the more plausible explanation. Shared exposure could result from simultaneous contact with an infective tsetse whose blood meal on a first individual was interrupted and resumed on a nearby relative, or from members of the same family sharing an ecological microcosm and being similarly, but not simultaneously, exposed to the vector (Gouteux *et al.*, 1989).

6.2.4. *The Influence of Human Behaviour*

Human behaviour plays an important role in the epidemiology of Gambian trypanosomiasis. Its impact on human–fly contact will be discussed later (Section 6.4.7). Health seeking behaviour might delay the recognition of an epidemic and enhance transmission of the parasite if symptomatic individuals wait for months before reaching a health facility where trypanosomes can be detected, maybe because they first attribute the disease to other, supranatural, causes and seek traditional treatment. Participation in case-finding surveys varies from place to place and over time, and is a key determinant in the success or failure of such programmes.

Migrations have contributed to trypanosomiasis epidemics, as they favour the circulation of trypanosomes from high-incidence to low-incidence areas where the population is more susceptible (Prothero, 1963). Although this remains controversial, the explosive epidemics in Uganda and Congo a century ago have been attributed by many authors to large-scale circulation of manpower organized by the colonizers to suit their needs (Janssens and Burke, 1992; Leak, 1999). A recent example of the impact of migrations is the epidemic in north-west Uganda, resulting from the exodus of Ugandan refugees to Sudan and Zaire where they became infected, followed a few years later by the migration to Uganda of infected Sudanese refugees and the return of the Ugandans (Paquet *et al.*, 1995).

6.2.5. *Immunity*

It was generally thought that no immunity followed a first episode of HAT, because of the parasite's antigenic variation and its repertoire of VSGs, which includes hundreds of different antigens. However, observational and experimental studies in animal models showed these animals to be more resistant to homologous trypanosomes when rechallenged after adequate treatment of a previous infection. This protection resulted from exposure to metacyclic trypanosomes rather than to bloodstream forms (Nantulya *et al.*, 1984; Akol and Murray, 1985; Vos *et al.*, 1988).

The existence of protective immunity in humans was investigated in a very high incidence community in DRC, where 38% of adults had a past history of trypanosomiasis (Khonde *et al.*, 1995). The incidence rate of trypanosomiasis after a previous episode in individuals previously diagnosed and treated was compared with that during the same period of time in individuals never diagnosed with the disease. When taking into consideration a 24 months' refractory period after the initial diagnosis (during which most second episodes of the disease would be expected to correspond to relapses rather than reinfections), the incidence rate in previously diagnosed adults was only 15% of that in their

counterparts who had never previously had HAT (Khonde *et al.*, 1995). If this refractory period was not taken into consideration, the rate ratio became 0.30. Several arguments, reviewed by Khonde *et al.* (1995), suggest that the true value of the protective immunity is closer to the first estimate than to the second. In other words, a first episode of trypanosomiasis confers 85% protection against subsequent reinfection. No immunity was observed among children less than 16 years old, but this observation lacked precision because of the lower incidence of disease in this age group.

This immunity could explain why the disease tends to reappear in foci that were endemic decades previously. Given stable ecological conditions and the persistence of the vectors, the disease might disappear, even in the absence of an effective control programme, if, after many years of high incidence, the number of adults who developed protective immunity was sufficient to drive the net case reproduction rate below unity. Conditions would be favourable for resurgence of the disease when, decades later and after the birth of one or two new generations, the number of susceptible individuals had increased enough for the net case reproduction rate to become greater than one. This immunity, or lack thereof, would also explain why refugees or other migrants who move from a region without trypanosomiasis to a region endemic for the disease are often found to experience a higher incidence than local populations, even when the exposure seems similar (Burke, 1971). It might also explain the explosive epidemic in Uganda 100 years ago, if the disease had hitherto been absent until introduced by travellers from Central Africa.

6.2.6. The Impact of Human Immunodeficiency Virus

Three studies have examined possible interactions between human immunodeficiency virus (HIV) infection and Gambian trypanosomiasis. In Nioki, DRC, the prevalence of HIV infection among 220 new cases of trypanosomiasis did not differ significantly from that seen in 388 blood donors (3.2% versus 2.1%) (Pépin *et al.*, 1992). In a case-control study in Central Africa, no significant difference in prevalence was seen between cases of HAT and controls recruited during the same case-finding surveys. For instance, in the Bouenza focus of Congo-Brazzaville, 4.3% of 163 cases were HIV-infected compared with 2.4% of 326 controls (Louis *et al.*, 1991). A more recent case-control study in Côte d'Ivoire, which had higher power than the previous one, also failed to reveal an association: 4.3% of 301 cases were HIV-infected compared with 3.5% of 896 controls (odds ratio 1.28, 95% confidence interval 0.65–2.50) (Méda *et al.*, 1995). Although these estimates might be biased because of control selection in the first study or, in the two others, because HIV-infected subjects with HAT might have been less likely to survive until a case-finding session in their villages, overall they suggest that HIV infection has so far had little impact on the

epidemiology of Gambian trypanosomiasis, to some extent because the prevalence of HIV remains relatively low in rural communities where HAT is endemic. HIV co-infected HAT patients respond less well to eflornithine treatment than HIV-seronegative patients (Milord *et al.*, 1992), but this is unlikely to have any impact on transmission as eflornithine is little used and relapsing cases of HAT are often not parasitaemic.

6.2.7. Drug Resistance

Drug resistance has been relatively uncommon in Gambian trypanosomiasis, despite suramin, pentamidine and melarsoprol having been used for five decades (Pépin and Milord, 1994) and, as a consequence, it has had no impact on the epidemiology of the disease. Suramin is little used in the treatment of Gambian trypanosomiasis. Pentamidine is given throughout Africa to patients with early-stage disease, and there is no evidence of an increased failure rate, which remains around 7% (Pépin and Khonde, 1996). There are at least two foci where melarsoprol resistance is more frequent than elsewhere, but clearly this is an issue that will need to be better investigated and monitored over the next few years. One is the Mbanza Kongo focus of northern Angola, close to the border with Bas-Congo: a 40% failure rate was reported nearly 25 years ago (Ruppel and Burke, 1977) and similar failure rates have been observed in recent years. Limited epidemiological data suggest that there has been little spread of resistant strains over time. In the Arua focus of northern Uganda, a 27% failure rate has recently been reported among new cases treated with melarsoprol (Legros *et al.*, 1999), but it is unclear for how long this rate of melarsoprol failure has been present. Again, this seems geographically limited as a ten-fold lower failure rate was documented in the adjacent focus of Adjumani.

In a hospital in DRC where melarsoprol failures were not especially frequent, statistically significant differences in failure rates were found when comparing patients from foci located within 50 km of each other, even after adjusting for pre-treatment characteristics (Pépin *et al.*, 1994). All this suggests that circulation of trypanosomes might be geographically quite limited, and that the distribution of the disease within a given region results from the superposition of relatively independent mini-epidemics. A factor limiting the spread of resistant strains is that patients who fail melarsoprol treatment are not very infectious to tsetse, even if eflornithine is not available for salvage therapy. These most unfortunate patients live a miserable life for a year or two until they die in a comatose condition but, typically, trypanosomes can be found only in their CSF and not in their blood. Their contact with the vectors is also limited. Thus, even though the duration of their disease is much longer than that of patients who respond to melarsoprol, the duration of infectivity might be rather similar.

6.3. The Animal Reservoir

The possible existence of an animal reservoir of *T. b. gambiense* has been investigated for a long time (Kageruka, 1989; Leak, 1999). Earlier studies showed that many species of domestic animals could be experimentally infected with *T. b. gambiense*: pigs, dogs, goats, sheep and even chickens (Van Hoof, 1947; Mehlitz *et al.*, 1981). These infections generally resulted in low-level parasitaemia that lasted less than a year.

The study of naturally occurring infections in animals was facilitated by the development of appropriate laboratory methods: the blood incubation infectivity test (BIIT), isoenzyme electrophoresis and analysis of deoxyribonucleic acid. Domestic animals were found to be naturally infected with parasites enzymatically identical to *T. b. gambiense*. Pigs have generated more interest because they are a frequent source of blood meals for tsetse flies, and have been found infected with *T. b. gambiense* in Liberia (a country with little human disease), Côte d'Ivoire, Congo and DRC (Gibson *et al.*, 1978; Mehlitz *et al.*, 1981, 1982; Noireau *et al.*, 1989; Truc *et al.*, 1991). In Côte d'Ivoire, 52 sympatric *T. brucei* strains were characterized by isoenzyme electrophoresis: among 12 zymodemes encountered, the most frequent was found in both humans and pigs (Penchenier *et al.*, 1997). In Congo, sheep were also found to be infected with *T. b. gambiense*, and the prevalence of *Trypanozoon* infection in domestic animals was estimated at 0·5% (Scott, C.M. *et al.*, 1983; Noireau *et al.*, 1986, 1989; Truc *et al.*, 1991). A dog was found infected with *T. b. gambiense* in Liberia (Zillmann *et al.*, 1984). A more recent study using the polymerase chain reaction documented the simultaneous presence of *T. b. gambiense* in humans and animals (a dog and a pig) from the Bas-Congo province in DRC (Schares and Mehlitz, 1996) but, by and large, the presence of *T. b. gambiense* in animals has been better documented in West than in Central Africa.

Other investigators have looked for serological evidence of *T. b. gambiense* infections in animals. In Congo, a high rate (81·8%) of seropositivity in the card agglutination test for trypanosomiasis (CATT) was found in domestic animals (pigs, sheep, goats) living close to humans in an endemic focus, but none of these samples was positive when using an immune lysis test (Noireau *et al.*, 1991). This demonstrates the lack of specificity of the CATT in detecting antibodies against *T. b. gambiense* in animals, due to cross-reactivity with other trypanosomes.

These studies have not clearly established that animals are an epidemiologically significant reservoir of *T. b. gambiense* trypanosomiasis. Firstly, only a few strains have been characterized, and the prevalence of *T. b. gambiense* parasitaemia in domestic animals seems to be rather low, although it might be underestimated due to the intermittent character of the parasitaemia and by the occurrence of dual *T. b. gambiense* and *T. b. brucei* infections. Secondly, the

degree of parasitaemia has generally been found to be low, as previously reported in experimentally infected animals (Van Hoof, 1947; Kageruka, 1989) – although tsetse flies can become infected from blood meals taken from such animals. Thirdly, in none of these studies have infected animals been followed longitudinally. The duration of parasitaemia is unknown but it is thought that animals generally spontaneously clear their *T. b. gambiense* infection.

The elimination of Gambian trypanosomiasis, or at least a drastic reduction in incidence, was achieved in several countries through control efforts that focused exclusively on the human reservoir, without significant vector control activities. It is thus difficult to believe that animal hosts play a significant role in the dynamics of *T. b. gambiense* transmission where there is a large human reservoir. It seems plausible that transmission from animals to humans occurs, but too rarely to have an impact on prevalence rates among humans in high incidence foci. If anything, some animals, especially pigs, seem to reduce the risk of human trypanosomiasis by providing tsetse flies with alternative sources of blood meals (Méda *et al.*, 1993). Indeed, increases in incidence of human trypanosomiasis have been noted when epidemics have drastically reduced the porcine population (Janssens and Burke, 1992).

However, the situation might differ when the human reservoir has dwindled. Whether or not animals may become a risk for reintroduction or persistence of the parasite in communities where near elimination of the human disease has been achieved remains an important research question (Molyneux, 1980b), especially in West Africa where a low incidence is seen in several countries and where *T. b. gambiense* infections in animals have been better documented. It has been speculated that the pig might also play such a role in Cameroon (Penchenier *et al.*, 1998).

6.4. The Vectors

6.4.1. Vector Species and Subspecies

Glossinids (tsetse flies) are Diptera of the family Glossinidae. They are vectors of trypanosomes that infect humans as well as wild animals (antelopes, giraffes [*Giraffa camelopardalis*], etc.) and domestic stock (pigs, cattle, sheep, goats, canids, horses, etc.). Understanding of their vector role dates back to the work of Bruce (Bruce and Nabarro, 1903), which was followed by Roubaud's demonstration in 1906 of human trypanosomes in the salivary glands of these insects. Tsetse flies belong to the genus *Glossina*, made up of about 30 known species divided into three groups: the subgenus *Glossina*, which includes species of the *G. morsitans* group; the subgenus *Nemorhina* (*G. palpalis* group) and the subgenus *Austenina* (*G. fusca* group). These three groups differ from one another by the morphology of male and female genitalia and by the

karyotype. Their identification used to be based on Pollock's scheme (1982), after dissection and microscopical examination. Recently, software has been developed for the specific and subspecific identification of glossinids and the determination of their epidemiological importance (Brunhes, 1994).

The vectorial capacity of a species of *Glossina* is determined by its ability to become infected while feeding on a vertebrate host, and subsequently to support the development of the infection and to transmit the trypanosome to another vertebrate host (Challier, 1982). According to these criteria, only the *G. palpalis* and *G. morsitans* groups contain species and subspecies that are vectors of *T. b. gambiense* (Table 1). The *G. palpalis* group contains two excellent vector species of *T. b. gambiense* and of animal trypanosomiases in West and Central Africa: *G. palpalis palpalis* in forest areas and *G. p. gambiensis* in savannah areas. In West Africa, biometric studies of the male and female genitalia of *G. p. palpalis* and *G. p. gambiensis*, carried out on samples captured along four north-south lines, has delineated the geographical boundary between the two subspecies: the former inhabits the forest areas and the moist savannah, whereas the latter is found in the semi-arid savannah (Nekpeni *et al.*, 1989). In the forest belt of Côte d'Ivoire, where several species cohabit, *G. p. palpalis* is the only vector of the human disease and outnumbers other species

Table 1 Species and subspecies of the two subgenera of *Glossina* which are vectors of *T. b. gambiense* and *T. b. rhodesiense*.

Subgenus (group)	Species	Subspecies
<i>Nemorhina (palpalis)</i>	<i>G. palpalis</i> ^a	<i>G. p. palpalis</i> ^a
	<i>G. fuscipes</i> ^{a,b}	<i>G. p. gambiensis</i> ^a
		<i>G. f. fuscipes</i> ^{a,b}
		<i>G. f. martinii</i>
		<i>G. f. quanzensis</i>
	<i>G. pallicera</i>	<i>G. p. pallicera</i>
	<i>G. tachinoides</i> ^a	<i>G. p. newsteadi</i>
<i>Glossina (morsitans)</i>	<i>G. caliginea</i>	
	<i>G. morsitans</i> ^b	<i>G. m. morsitans</i> ^b
		<i>G. m. submorsitans</i>
		<i>G. m. centralis</i> ^b
	<i>G. swynnertonii</i> ^b	
	<i>G. longipalpis</i>	
	<i>G. pallidipes</i> ^b	
<i>G. austeni</i>		

^a Vector of *T. b. gambiense*.

^b Vector of *T. b. rhodesiense*.

(Dagnogo *et al.*, 1977). *G. tachinoides* and *G. fuscipes fuscipes* are related to the *G. palpalis* group and are vectors of HAT in the savannahs of West and Central Africa. *G. morsitans* also transmits *T. b. gambiense* in West and Central Africa, as does *G. pallidipes* in the savannah of Central Africa. The other species of the *G. palpalis* group (*G. pallicera* and *G. caliginea*) and the species of the *G. fusca* group (*G. fusca*, *G. longipalpalis*, *G. brevipalpalis*, *G. negro-fusca*, etc.) are involved in the transmission of animal trypanosomes but are not vectors of the human disease.

6.4.2. *The Life Cycle of Glossina*

Even before its first blood meal, the female tsetse fly is impregnated during mating (in theory, a single act) as the male deposits a spermatophore containing the seminal fluid and spermatozoa deep in the uterus. The spermatozoa are stored in the spermathecae where they can survive up to 200 days, which is much longer than the average life span of the insect. The first egg is fertilized as it passes through the spermathecal duct and it descends into the uterus between the eighth and eleventh days of the female's life. In less than 10 days, the larva developing from the egg goes through two metamorphoses and three larval stages while still inside the female. During this period, the survival of the larva is extremely dependent on the female's nutritional status (itself dependent on adequate blood meals) and on environmental conditions. Abortion rates can be high, hence the female's need to feed frequently (Langley and Stafford, 1990).

Approximately every 10 days, the female produces a third-stage larva which is deposited on the ground or in any other site for the larva to develop (between the roots or at the foot of trees, under banana trees, in bushes, etc.), close to the female's diurnal resting site and feeding ground. As indicated in Leak's review (1999), reproduction sites vary between species. After it is deposited, the larva buries itself in the soil to avoid dessication and sunlight. In the soil, the larva undergoes a third and final metamorphosis to the pupal stage where it changes into a pupa inside a shell called a puparium, which protects it as it goes through the metamorphoses required to reach the pre-imaginal and then the adult stage. The duration of this pupal period is quite variable (from 20 to 80 days) and depends mainly on the ambient temperature that is dependent on the site chosen by the *Glossina* to deposit her larva. The optimum temperature for the female tsetse's reproduction cycle is about 25°C. Below 18°C and above 37°C the larva cannot develop. Usually, the site chosen by the female to deposit a larva has the right temperature and humidity conditions for the survival of the larva and pupa. Floods, changes in vegetation, bush fires and trampling by animals may be fatal for the pupae.

After its pupal development is completed, the adult *Glossina* emerges from

the puparium and the soil. The time at which it hatches also depends on temperature, but not on light intensity, as is the case with most Diptera (Denlinger and Zdárek, 1994). The peak time for emergence is during the afternoon (15:00–18:00). The sex ratio when the adults emerge is close to one but, since females live longer, they are more numerous in representative samples of the adult population (70–80%).

6.4.3. *Distribution and Ecology of Glossina*

A large variety of traps to collect samples and to control tsetse flies has been tested, a dozen of which were reviewed by Leak (1999). The favourite version currently used to study *Glossina* habitats, densities and ecodistribution is the biconical trap developed by Challier and Laveissière (1973). Turner (1980) proposed a modification of the ‘marking–release–recapture’ technique to study *Glossina* habitats, behaviour and dynamics, population size, densities, dispersal and survival rates, daytime and diurnal resting sites. A random sample of tsetse flies is collected, marked with a radioactive compound (e.g. ^{59}Fe) and then released in their natural environment so that they disperse randomly with respect to the unmarked flies. Later on, a scintillometer is used to detect the marked flies in their resting sites.

(a) *Geographical distribution and habitats.* The classical study on geographical distribution of glossinids was by Ford and Katondo (1973), but more recent data are available (Katondo, 1984; Gouteux, 1990; Moloo, 1993). In West and Central Africa, *G. palpalis palpalis* is found mainly in mesophile, gallery and mangrove forests, while *G. fuscipes* is found in the mesophile forest of Central Africa. Ombrophile forest is not suitable for vector species. Savannah is the exclusive habitat of *G. morsitans* in various regions of Africa; it is limited to gallery forests bordering streams, where optimal survival conditions are found.

(b) *Ecodistribution.* In a given geographical area, the distribution of tsetse flies varies depending on the species and is determined mainly by the climate, presence of water, vegetation and availability of sources of blood meals (humans and animals). Climate influences the number of tsetse through its effect on reproduction, mortality and movement. Numerous species have succeeded in colonizing certain ecological zones created by humans. Studies in the forest zone of West Africa have shown that *G. p. palpalis* is a very mobile vector found in different biotopes and its distribution is closely linked to human occupation patterns (Baldry, 1980; Challier and Gouteux, 1980; Gouteux and Laveissière, 1982). The highest apparent densities per trap (ADT) per day were observed on the edges of villages where pigs provide an

abundant, easily accessible food source. High densities have also been found near sources of potable water, in coffee and cocoa plantations, especially those located on the edge of forests or gallery forests, and along paths separating plantations from the remaining forest used by humans and some wild animals, especially bushbuck (*Tragelaphus scriptus*), which are food sources for *Glossina*.

(c) *Activity, dispersal and resting sites.* Tsetse flies are active for only a small part of the day (35 minutes on average) and move around in sudden small bursts lasting a few seconds (Bursell and Taylor, 1980). Cuisance and Février (1985) found that *G. palpalis* suddenly moves over very long distances in the hot season, up to 22 km in 5 days for the female. Most of the activity of tsetse is motivated by the search for food and for a suitable resting site and, for males, by the quest for females. Most species are diurnal, with the exception of *G. pallidipes* and *G. morsitans* which are occasionally active and can bite at night. The amount of activity varies depending on climatic factors (temperature, humidity, amount of light, wind and rain), olfactory and visual stimuli (smelling and seeing a potential feeding host) and on intrinsic factors (physiological age, nutritional status, gravidity).

Tsetse flies spend most of the time resting, to digest or gestate. They choose different diurnal and nocturnal resting sites. They are guided by negative phototaxis and shelter in the darkest, which are usually the coolest, corners of resting sites (holes in tree trunks or between roots). They generally prefer dark, shady, cool places at different heights depending on the species and whether it is day or night (between 1 and 9 m above ground floor level) (Laveissière, 1988). In forest galleries of savannah, *G. palpalis gambiensis* has a marked preference for resting sites located less than 1 m from the ground (in decreasing order: branches, roots, trunks, leaves) and very close to water (less than 4 m from the edge of a stream) (Bois *et al.*, 1977). Resting sites differ during daytime and at night: flies are observed mostly on leaves at night, while they prefer the woody parts of plants during the day. The location of these sites makes this subspecies vulnerable to selective spraying of persistent insecticides.

6.4.4. Vectorial Capacity of *Glossinids*

(a) *Biological cycle of the parasite in Glossina.* *Glossina* become infected by ingesting trypomastigote forms during the blood meal. The complex life cycle undergone by *T. brucei* in tsetse flies has been described in various standard textbooks (e.g. that by Hoare, 1972). It culminates in the development in the salivary glands of metacyclic trypomastigotes, the only stage infective to vertebrates. The whole cycle lasts about 30 days on average, but its duration

varies, according to species and the ambient temperature, between 1 and 8 weeks. An infected *Glossina* remains so, in principle, throughout its life span of between 3 and 4 months and, while taking subsequent blood meals, it can inoculate another vertebrate host with the metacyclic trypanosomes.

(b) *Factors affecting the development of trypanosomes in Glossina.* The ability of a tsetse to become infected while feeding on a parasitaemic host depends on several poorly understood factors. A priori, it is thought that the number of trypanosomes ingested by the fly during its blood meal could be one such factor. However, there are few data supporting the concept that a blood meal taken from an individual with low parasitaemia is less infectious for the vector than one taken from a host with high parasitaemia. Maudlin and Welburn (1989) have shown experimentally that a single *T. congolense* was sufficient to infect *G. m. morsitans* and that, above a threshold (somewhere about seven trypanosomes per meal), infection rates did not depend on the number of trypanosomes ingested by the fly. According to Baker's probabilistic model (Baker, R.D., 1991), a tsetse fly has at least a one in ten chance of becoming infected if it ingests a blood meal that contains a single trypanosome.

Under natural conditions, the infection rate among tsetse flies is quite low. On average, fewer than 1% of tsetse flies are infected with *T. brucei* spp. (Molyneux, 1980a). Teneral flies (those aged less than 48 hours) are more susceptible to infection by trypanosomes. However, old male *G. morsitans* (21–25 days old), kept in the laboratory without food for 3 or 4 days, can become infected in the same proportions as teneral males (Gingrich *et al.*, 1982). Harmsen (1973) reported that there is a physical and chemical barrier in the gut of *G. pallidipes* that affects the capacity of the fly to become infected. Welburn and Maudlin (1992) recently elucidated the role of the peritrophic membrane in the acquisition of trypanosome infection in the teneral fly. In teneral *Glossina*, the peritrophic membrane is shorter and cannot contain the entire blood meal. Therefore, the trypanosomes remain in the crop for 1–3 hours, where they undergo enzymatic transformations that protect them from the hostile intestinal environment. In older flies the peritrophic membrane can contain the entire meal, so the trypanosomes pass rapidly into the intestine, where they are destroyed.

Lectin systems have been described in the haemolymph (Croft *et al.*, 1982) and midgut (Grubhoffer *et al.*, 1994) of *Glossina*. Susceptibility of tsetse to trypanosome infection is probably mediated through midgut lectins responsible for destroying the parasites in the midgut, and experiments with tsetse flies susceptible or refractory to trypanosome infection showed that susceptible flies secrete less lectin (Welburn *et al.*, 1989; Welburn and Maudlin, 1992). Also, in susceptible flies, the action of an enzyme (endochitinase) produced by rickettsia-like organisms, present in the midgut, leads to the accumulation of a protein that blocks the lectin-mediated trypanocidal effect (Leak, 1999).

As reviewed by Leak (1999), it has been suggested that lectins are responsible for the increasing resistance with age of tsetse flies to infection with *T. brucei* and *T. congolense*. Lectins are absent or blocked in unfed (teneral) flies, thus permitting infection, while in older flies lectin production seems to be stimulated by blood meals, preventing subsequent infections (Maudlin and Welburn, 1988).

(c) *Effects of trypanosome infection on Glossina.* Some authors have shown that infected tsetse have a tendency to bite more often and feed more voraciously (Jenni *et al.*, 1980; Roberts, 1981), which can have a substantial impact on transmission potential. In the infected fly, the flow of blood is markedly reduced in the proximal one-third of the labrum by the presence of the trypanosomes, which cluster in the mechanoreceptors.

Initial studies suggested that tsetse flies infected with trypanosomes of the subgenus *Trypanozoon* survived longer than non-infected flies (Baker, J.R. and Robertson, 1957). This contrasts with recent observations by Maudlin *et al.* (1998), according to which the risk of death increases with age, but much more quickly in infected than in uninfected flies. Most authors reported that infected flies are much more susceptible to insecticides than non-infected flies (Golder *et al.*, 1984; Nitcheman, 1990).

6.4.5. Feeding Behaviour and Trophic Preferences

(a) *Host location and feeding grounds.* The first concern of a teneral *Glossina* is to find a host from which a blood meal may be taken. As soon as it emerges from the puparium, the young adult needs a blood meal to complete the maturation of its wing muscles and its endocuticle (Hargrove, 1975). This first meal takes place between 24 and 72 hours after emergence, or even later, depending on factors intrinsic to the vector (diurnal rhythm, sex, gravidity, species, etc.) and environmental conditions (climatic conditions, visual, mechanical and olfactory stimuli) (Colvin and Gibson, 1992).

The feeding ground of tsetse may be restricted by the relatively limited dispersion of potential sources of blood meals. For example, in forest zones, *G. palpalis palpalis* is confined to the outskirts of villages where pigs congregate, along paths through plantations, near sources of potable water, and around farm camps and other places used by humans (Laveissière and Hervouët, 1981; Gouteux, 1987).

(b) *Host preferences.* Almost all studies on the feeding preferences of *Glossina* have been conducted in West Africa and were based on analyses of blood meals obtained by dissecting the insects. They provided information on the epidemiology of the disease and guided the choice of control strategies.

Methods with differing degrees of sensitivity are available and have been reviewed by Leak (1999), including the precipitin test, the agglutination inhibition test, the complement fixation test, direct and indirect enzyme-linked immunosorbent assays, tests based on the latex agglutination technique and, more recently, one based on electrophoresis of superoxide dismutase (Diallo *et al.*, 1997), which can distinguish only between meals of human and animal origin. Glossinids of the *G. palpalis* group are notable for their eclecticism and opportunism: they feed indiscriminately on many species and are therefore very dangerous to humans. In the forest zone of Côte d'Ivoire, Gouteux *et al.* (1982) found that at least 75% of the blood meals were from Suidae living around the villages. In plantations in the same zone, however, Laveissière *et al.* (1985) found that 46% of the blood meals were from humans. This observation has been confirmed by the recent results of Sané *et al.* (2000).

Comparison of the results of analyses of blood meals collected in the five main foci in Côte d'Ivoire shows that the proportion of human blood meals varies significantly from one focus to the other, although the sociogeographic conditions in these foci appear to be identical: a human origin was ascribed to 42% of blood meals of *G. p. palpalis* at Vavoua, 73% at Daniafla, 55% at Zoukougbeu, 91% at Gagnoa and 27% at Sinfra. Humans, therefore, seem to be the favourite host, in differing degrees, of *G. palpalis palpalis* in the plantation zones of Côte d'Ivoire. This preference for humans was greater in the foci with low transmission rates (Daniafla, Gagnoa) than in the more active foci (Sintra, Vavoua and Zoukougbeu). Domestic animals appear to play an important role in providing blood meals for tsetse flies at Vavoua and Zoukougbeu, the most active foci, whereas in the low-incidence foci, the percentage of animal blood meals was insignificant. At Zoukougbeu, apart from humans, *G. p. palpalis* feeds freely on domestic swine (30% of meals), whereas at Vavoua it prefers bushbuck, which provide the same percentage of its food meals as humans do (42%). The diversity of the food sources of *G. p. palpalis* might explain to some extent the variations in levels of incidence of human disease. In foci where transmission is more intense, *G. p. palpalis* feeds on both humans and animals and case-finding and treatment of infected humans is not sufficient to control the disease: with humans and animals both acting as sources of blood meals, tsetse flies are able not only to transmit trypanosomes to humans, but also to maintain infection in the putative animal reservoir. In contrast, in low-transmission foci, the tsetse fly depends on humans, transmission to and from animals is rare, and case-finding prevents the accumulation of human cases. This West African paradigm should probably not be extrapolated to high-incidence foci of Central Africa, where the role of animals is less clear.

If one of its usual mammal hosts is not available, *G. tachinoides* replaces it with reptiles, as observed in the gallery forest of the savannah: snakes or lizards account for between 54% and 67% of the blood meals of *G. tachinoides*, while

only 8% of its meals are from humans (Laveissière and Boreham, 1976). The feeding preference of this vector also varies seasonally because of changes in the availability of hosts. In the hot season, 30% to 55% of its meals come from mammal hosts (mainly humans and bushbuck), whereas in the cold season the only wild animals available are reptiles, which provide over 50% of the meals (Laveissière and Boreham, 1976).

6.4.6. *Transmission Cycles*

In the forest zone of Côte d'Ivoire, human–vector contacts occur in almost all biotopes but especially in plantations and peridomestic areas, where it is easier for the fly to find hosts (Challier and Gouteux, 1980; Laveissière *et al.*, 1985). Some authors attribute the persistence of residual foci of HAT to the existence of other cycles adjacent or parallel to the common human–fly–human cycle (Allsopp, 1972; Molyneux, 1980b; World Health Organization, 1986), i.e., cycles in which the parasite is passed between domestic and/or wild animals (pigs, sheep, goats, bushbuck, etc.) and humans. Two such transmission cycles have been proposed: (a) a domestic cycle in which the *Glossina* transmits the parasite between human hosts and domestic animals; and (b) a sylvatic cycle in which the vector transmits the parasite mainly between wild animals, with humans or domestic animals sometimes entering the cycle, resulting in sporadic human cases or even epidemic outbreaks. The domestic cycle hypothesis is supported by the similarities observed between parasites isolated from humans, domestic animals and vectors (Gibson *et al.*, 1978; Mehltz *et al.*, 1982), whereas there is little evidence that wild animals are infected with *T. b. gambiense*.

6.4.7. *Tsetse Flies and the Epidemiology of African Trypanosomiasis*

The transmission of infectious trypanosomes to humans depends on many factors: the density of *Glossina* populations, the longevity and susceptibility of the tsetse to infection, their infection rate and the factors that influence this, and human behaviour and activities in the biotopes of the flies that determine the frequency of human–fly contacts (Leak, 1999). As described in previous sections, the vector's biology, ecology and feeding behaviour directly affect transmission of the parasite. Thus *Glossina* is involved in the development, multiplication, maturation, transmission and dissemination of the parasite. The feeding behaviour of various species of *Glossina* determines their epidemiological contribution to the transmission of *T. b. gambiense* to the humans and, to some extent, the animals, that constitute the parasite's reservoir. Female *G. palpalis palpalis*, because of their more aggressive feeding

behaviour and longer life span, play an essential role in the transmission of the parasite.

The number of trypanosomes inoculated into a mammal host during feeding is probably one of the factors that determine the probability of transmission. Laboratory studies on *G. m. centralis* by Southon and Cunningham (1966) and field studies on *G. pallidipes* infected with *T. b. brucei* by Otieno and Darji (1979) indicated that the number of trypanosomes extruded in the saliva of tsetse during each feed is very high, in the order of 23 000. It has been estimated that, to infect humans, the inoculum must contain between 300 and 500 trypanosomes (Challier, 1982). Transmission is also influenced by factors intrinsic to the vector (physiological age, nutritional status, gravidity, etc.) and by climatic and other environmental conditions. The low infection rate naturally observed among tsetse flies limits the devastating epidemic potential of African trypanosomiasis, and explains the apparent paradox between the abundance of *G. palpalis palpalis* and *G. morsitans* and the relative rarity of human disease. There is no direct correlation between the density of *G.p. palpalis*, the major vector living in close contact with humans, and the incidence of sleeping sickness.

In endemic foci, the nature, frequency and intensity of human-fly contacts are the major determinants of the risk of African trypanosomiasis. In the forest zone of Côte d'Ivoire, there is virtually no ecological zone where humans are safe from being bitten by *G. palpalis palpalis*. Human-fly contacts can occur in all botanical zones and are affected not only by the vegetational environment but also, and especially, by the human hosts. Multidisciplinary studies have shown that human activity and behaviour have an important impact on the epidemiology of HAT by affecting the frequency and intensity of contact with the flies (Laveissière *et al.*, 1985, 1986b, 1986c; Hervouët and Laveissière, 1987; Méda *et al.*, 1993). For example, coffee growing, which requires the planter to spend more time in the plantation than he would if growing cocoa or food crops, is associated with a high risk of infection due to the increased contact time with the vector. This risk is heightened by living in farm camps and by procuring water from natural water sources located near edges of plantations and gallery forests. Many other activities, such as collecting water and firewood, washing and fishing, bring human hosts into contact with tsetse flies. The availability of other sources of blood meals (e.g., pigs) reduces the chances of transmission.

Some trypanosomal infections acquired by tsetse flies abort, because of lectins or other mechanisms, which explains to some extent the low prevalence rates observed among the vectors. Tsetse flies are probably more efficient at transmitting trypanosomes to uninfected human hosts than in becoming infected themselves during a blood meal from an infected human. A female tsetse fly produces a third stage larva every 10 days, and probably does not need to take blood meals more frequently than that throughout her

3–4 months' life span, during which the total number of blood meals might be a dozen or so. The number of humans that the fly might infect depends on the proportion of its life span during which it is infected by trypanosomes, and on the number of blood meals taken on humans after trypanosomes have appeared in its salivary glands. Interrupted meals might allow a fly to infect two or more humans within a short period of time. But clearly, for major epidemics to build up, human–fly contact must be intense and sustained to compensate for the low, albeit progressively increasing, infection rates among tsetse flies.

7. EPIDEMIOLOGY OF RHODESIAN TRYPANOSOMIASIS

It is beyond the scope of this paper to review in detail the epidemiology of *T. b. rhodesiense* trypanosomiasis. However, we will examine briefly some characteristics of this disease that differ from those of Gambian trypanosomiasis. There has been little research on the epidemiology of Rhodesian trypanosomiasis over the last three decades as, with the exception of the epidemic in Busoga, Uganda, it has become a rare disease.

Whether or not *T. b. rhodesiense* deserves its current status as a distinct subspecies or if it is merely a more virulent variant of *T. b. gambiense* has been discussed for a long time. What is clear, however, is that *T. b. rhodesiense* is less well adapted to humans than *T. b. gambiense*. It causes a disease that progresses over months rather than years, characterized by high parasitaemia. Even though this higher and more constant parasitaemia might, with regard to transmissibility, compensate to some extent for the much shorter duration of infection in humans, *T. b. rhodesiense* has shown less potential for causing large epidemics than *T. b. gambiense* over the last few decades.

The vectors differ from those of *T. b. gambiense*. *T. b. rhodesiense* is transmitted mostly by three savannah species of the *G. morsitans* group: *G.m. morsitans* and *G.m. centralis* in East Africa (except Kenya and Uganda), *G. pallidipes* in East and southern Africa and *G. swynnertoni* in Kenya and Tanzania. *G. fuscipes fuscipes*, which belongs to the *G. palpalis* group, is a vector of human and animal trypanosomiases in Central and East Africa (including Kenya and Uganda) (World Health Organization, 1998; Leak, 1999). *T. b. rhodesiense* trypanosomiasis is essentially a zoonosis, sporadically transmitted to humans in a wild animal–fly–human cycle. In such circumstances, *G. morsitans*, *G. swynnertoni* and *G. pallidipes* accidentally transmit the parasite to humans when they venture into bush areas infested by these vectors, whose blood meals are normally taken on game animals. Except during epidemics, human–fly–human or domestic animal–fly–human transmission is thought to be rare. During epidemics, *G. fuscipes fuscipes*, a peridomestic fly, is often the

main vector, and transmission occurs around the house rather than in the bush, from cattle, sheep or other humans.

The existence of an animal reservoir was established in the late 1950s (Leak, 1999). Cattle are now thought to be the major reservoir (Hide *et al.*, 1996; Hide, 1997) and have been implicated in the south-east Uganda epidemic (Okoth, 1999). In Tororo district, Uganda, it was estimated that at the height of the HAT epidemic a tsetse was five times more likely to acquire *T. b. rhodesiense* from cattle than from humans, as 23% of cattle in those villages carried human-infective strains (Hide *et al.*, 1996; Hide, 1999). Other domestic animals (dogs, sheep, maybe pigs) and various game animals can also harbour the parasite: warthogs, bushbuck, hartebeest, waterbuck, impala, lions, zebras and hyaenas* (Leak, 1999). Wild antelopes and hyaenas are well adapted to the parasite and can remain infected for over 2 years without overt disease. Such strains are thought to lose their infectivity over time. In some animals, such as the warthog, *T. b. rhodesiense* infection results in only short-lived parasitaemia while other species (dogs, Thomson's gazelle [*Gazella thomsonii*]) rapidly succumb from this infection, transmission to humans being then unlikely. Paradoxically, the presence of domestic animals near water collection points and farming areas has been found to decrease the risk of *T. b. rhodesiense* trypanosomiasis in south-east Uganda, presumably by decreasing the proportion of tsetse blood meals taken from humans (Okia *et al.*, 1994).

In contrast to Gambian trypanosomiasis, which to a large extent results from peridomestic infection in Central Africa (less so in West Africa), Rhodesian trypanosomiasis is, in endemic situations, acquired at some distance from home, where the animal reservoir lives (Baker, J.R., 1974). It is mainly an occupational disease of adult men (Robertson and Baker, 1958; Baker, J.R., 1974; Apted, 1970; Welldé *et al.*, 1989b), and hunters, fishermen, railway workers, honey gatherers and firewood collectors have been found to be at higher risk. Tourists visiting game parks in East and southern Africa are also exposed to Rhodesian trypanosomiasis. In endemic situations, adults are more likely to get the disease than children less than 10 years old (Welldé *et al.*, 1989b). In epidemic situations, however, where transmission becomes peridomestic, all age groups and both males and females are at a similar risk. Indeed, it has been observed that an increase in the number of cases in children and women is an indication that an outbreak is developing (Apted, 1970). Familial aggregation has been noted for Rhodesian trypanosomiasis (Welldé *et al.*, 1989b; Okia *et al.*, 1994). There does not seem to be any difference in incidence between ethnic groups apart from what could be expected as a result of varying degrees of exposure (Wyatt *et al.*, 1985). In contrast to

**Phacochoerus aethiopicus*, *Tragelaphus scriptus*, *Alcelaphus buselaphus*, *Kobus defassa*, *Aepyceros melampus*, *Felis leo*, *Equus burchelli* and *Crocuta crocuta*.

Gambian trypanosomiasis, there is marked seasonality of disease occurrence, with a higher incidence during warmer months when tsetse are more active – a consequence of the much more rapid progression of disease. Seasonal variations in incidence have been reported in Kenya and Uganda, both in the recent epidemic and in earlier reports (Robertson and Baker, 1958; Welde *et al.*, 1989b; Smith *et al.*, 1998).

Political upheavals played a role in the development of the recent Busoga epidemic by enhancing population movement and reducing the ability of health institutions to detect cases early (Mbulamperi, 1989a; Smith *et al.*, 1998). Changes in agricultural practice also resulted in a more favourable habitat for the *G. fuscipes* vector close to human habitation so that peridomestic infection became common (Smith *et al.*, 1998; Okoth, 1999). This epidemic was brought under control by surveillance and early diagnosis, after introducing sleeping sickness orderlies and upgrading the local health infrastructures, and by vector control (Smith *et al.*, 1998).

8. CONTROL OF AFRICAN TRYPANOSOMIASIS: PRINCIPLES, METHODS AND STRATEGIES

Over the last two decades, new and much more sophisticated tools for African trypanosomiasis control have been developed compared with those that existed some 40 years ago, when sleeping sickness was almost eliminated. These technical advances contrast with the present epidemiological situation in which the disease is increasing because of a number of socioeconomic and political factors.

8.1. Principles of Control

Control of HAT relies on two principles: reduction of the parasite reservoir through case detection and treatment, and reduction of human-fly contact through vector control. In the case of *T. b. gambiense* trypanosomiasis, reduction of the (mostly human) reservoir can be achieved through case-finding and treatment. The limiting factor is the relatively low sensitivity of the standard parasitological techniques. More frequent use of more sensitive serological and parasitological techniques could lead to a rapid and sustainable reduction of the human reservoir. In order to reduce human exposure to infectious bites, the tsetse flies must be destroyed. This must be carried out with the active involvement of members of the community, who can undertake trap laying, spraying, bush clearing, etc. There is a variety of vector control techniques, the choice of which depends on financial and human resources, the epidemiological situation

and the duration of the programme. Within the African context, vector control must be done with cheap, cost-effective and easy-to-use methods. Vector control for animal trypanosomiases has sometimes resulted in the reduction of the number of glossinids that transmit the disease to humans, as in Nigeria where massive spraying of insecticides to combat animal trypanosomiasis has been instrumental in controlling the human disease (World Health Organization, 1986).

8.2. Case Detection and Treatment

Case detection has been the corner-stone of Gambian trypanosomiasis control since the beginning of the 20th century. As infected individuals may remain asymptomatic for many months, if not years, before they develop complaints that will lead to a consultation in a health centre or district hospital, case-finding activities have been set up to detect them actively while they are still healthy. For many years, specialized case-finding teams have relied essentially on the presence of cervical lymphadenopathy among some of the asymptomatic patients. These teams examine the whole population of endemic villages, palpate the neck of every woman, man and child, identify those who have enlarged lymph nodes and aspirate such lymph nodes to examine the lymph node fluid for trypanosomes. The few patients complaining of symptoms compatible with trypanosomiasis are also investigated during surveys, as they would have been otherwise in their district hospital. All those with trypanosomes detected have a lumbar puncture to determine whether they are in the early or late stage of disease and to decide about treatment. These patients are generally referred to nearby hospitals or treatment centres where they are given pentamidine or melarsoprol. A single injection of pentamidine is often given at once by the mobile team, to clear the parasitaemia, because it might take a few weeks before the asymptomatic patient reaches hospital. In some very active foci, the mobile team also carries out examination of blood (using wet films or Giemsa-stained thick films) from either the whole population or those with enlarged lymph nodes but with negative lymph node aspirate. This is rarely possible, however, as it is extremely time-consuming. Better tools need to be developed.

8.2.1. Tools for case-detection

(a) *Serological methods.* Diagnostic problems are more important in the chronic, *T. b. gambiense*, disease in which the parasitaemia usually comprises cryptic periods during which it is difficult to document the presence of the parasite (Molyneux and Ashford, 1983). Various serological assays were

developed to help case-finding teams identify a small number of antibody carriers on whom they could concentrate their efforts for trypanosome detection by parasitological methods. In the 1970s, the indirect fluorescent antibody test (IFAT) was deemed the most reliable technique for epidemiological surveillance of *T. b. gambiense* trypanosomiasis. However, it required relatively expensive equipment and qualified staff, and its implementation was possible only in a fixed or mobile laboratory. Delays in obtaining results were rather long and some of the seropositive suspects could not be located again to undergo parasitological tests to check the diagnosis.

From the 1980s onwards, the IFAT was supplanted by the card agglutination test for trypanosomiasis (CATT) (Magnus *et al.*, 1978), the advent of which considerably enhanced detection of cases of Gambian trypanosomiasis. The CATT, a latex agglutination test relying on the detection of antibodies using the variable antigenic type (VAT) LiTat 1.3 antigen, is the only serological assay currently used in control programmes. It can be performed in the field, without electricity and without specialized staff, and results are available within 10 minutes. It is cheap, approximately US \$0.40 per test, and is generally performed on whole blood for the sake of simplicity. Its sensitivity varies between 92% and 100% when assessed in patients parasitologically confirmed in hospital or other settings and its specificity is thought to be 94–97% (Noireau *et al.*, 1988; Miézan *et al.*, 1991). The CATT proved highly specific during testing in non-endemic areas (Bafort *et al.*, 1986), but the validity of such estimates is doubtful as false positive results probably arise from antibodies developed against animal trypanosomes. Some strains of *T. b. gambiense* do not express the LiTat 1.3 antigen, but their distribution seems fairly limited (Dukes *et al.*, 1992). The positive predictive value of the CATT depends on the prevalence of disease in the population. This prevalence ranges between 1% and 5% in most endemic foci and the positive predictive value generally varies between 14% and 40% (Miézan *et al.*, 1991; Truc *et al.*, 1994). The apparent positive predictive value depends on the quality of the parasitological assays used to elicit trypanosomes among the CATT-positive subjects. Apart from the classical lymph node aspirate and examination of wet and thick blood films, more sensitive methods can be used such as the miniature anion-exchange centrifugation technique (mAECT) and the quantitative buffy coat technique (QBCTM) (Bailey and Smith, 1992), and a higher proportion of CATT-positive individuals will then be parasitologically confirmed. However, the latter methods are expensive and time consuming and can hardly be used on a large scale in high-incidence countries.

Variations on the same theme have been proposed. The CATT can be performed with filter paper, using smaller volumes of reagents and thus reducing costs (Miézan *et al.*, 1991). However, the reliability of the micro-CATT is less satisfactory under field conditions than in a research setting. The CATT can also be used on diluted serum rather than whole blood, resulting in higher

specificity at the expense of lower sensitivity (World Health Organization, 1998). Thus some researchers have proposed screening with the CATT on whole blood, followed by a CATT on diluted serum for individuals positive with whole blood, or performing the CATT simultaneously on both whole blood and diluted serum, before carrying out the parasitological assays (Penchenier *et al.*, 1991). Although this may be, on paper, a very rational approach, it is not practical for mobile teams having to screen large numbers of individuals day after day.

By and large, use of the CATT on whole blood results in doubling the number of parasitologically confirmed cases that are identified, compared with surveys relying on traditional methods. In other words, at least half of the individuals who carry trypanosomes in their blood have no cervical lymphadenopathy or have a negative aspirate when such lymph nodes are tapped.

Other screening methods, based on the detection of antigens rather than antibodies, have been proposed recently (Nantulya, 1997). However, the very high prevalence of positive results in areas of low endemicity (Asonganyi *et al.*, 1998) raises serious doubts about the specificity of the assays which are unlikely to become popular with national control programmes.

(b) *Parasitological methods.* Confirmation of the diagnosis depends on demonstrating trypanosomes in biological fluids. This confirmation can be obtained through the examination of wet or Giemsa-stained blood films, or of fresh lymph node aspirate when typical lymphadenopathy is palpable (Cattand and de Raadt, 1991; Miézan *et al.*, 1994). These classical methods have a rather low sensitivity in *T. b. gambiense* trypanosomiasis. Demonstrating the presence of trypanosomes in blood and CSF has been considerably facilitated by the development of concentration methods. The micro-haematocrit centrifugation technique (Woo's test) is much more effective in *T. b. rhodesiense* disease (and in veterinary medicine) than in *T. b. gambiense* HAT. The mAECT is deemed at present the most sensitive parasitological method for the detection of blood parasites (Miézan *et al.*, 1994). It is relatively costly and its implementation calls for adequately trained staff. Therefore, the mAECT is used selectively to test CATT-positive suspects in whom the diagnosis could not be confirmed by classical methods. Two new parasitological techniques have been recently added to the diagnostic arsenal: the QBC™ (Bailey and Smith, 1992) and the kit for *in vitro* isolation of trypanosomes (KIVI) (Aerts *et al.*, 1992). The QBC™, which requires relatively expensive equipment, and the KIVI, which is a parasite isolation method rather than a screening test, have not proved to be superior to the mAECT (Truc *et al.*, 1994).

With CSF, the most sensitive technique is double centrifugation (Cattand *et al.*, 1988; Miézan *et al.*, 1994). A variation of the latter, single centrifugation of CSF in a sealed Pasteur pipette, has been developed recently (Miézan *et al.*, 2000). It makes detection of trypanosomes in the CSF simpler, quicker and

more sensitive and it is specially suitable for passive diagnosis in suspects who present at health facilities with symptoms suggestive of HAT.

The combination of all these seroparasitological methods ensures increased sensitivity, but falls short of a perfectly reliable parasitological diagnostic technique for all seropositive persons; hence the need to pursue efforts to develop more sensitive parasitological assays and more specific serodiagnostic techniques. The combination of CATT and mAECT is increasingly replacing the traditional methods based on the direct examination of lymph node aspirate. Generally, these more sensitive but more laborious techniques are used more widely in the relatively modest foci of West Africa than in the high-incidence foci of Central Africa. Although the conventional methods lack sensitivity, they are still widely used by most health institutions for passive diagnosis and case finding.

8.2.2. *Treatment*

The treatment of HAT is determined by first establishing the stage of infection. The diagnosis of late-stage trypanosomiasis is based on at least one of the following criteria (World Health Organization, 1998): CSF WCC > 5 mm⁻³ or CSF proteins > 37 mg (100 mL)⁻¹ (as measured by dye-binding protein assay), or both criteria, with or without the presence of trypanosomes in CSF. Miézan *et al.* (1998) reported that the CSF WCC is, by itself, as sensitive for the diagnosis of central nervous involvement as the combination of the above criteria. Therefore, the WCC was recommended in patients with confirmed infection, especially in poorly equipped facilities. This is already the *de facto* approach in most of Central Africa, where little more than a cell count and trypanosome detection can be carried out on CSF samples.

Advances have been made in the treatment of HAT. A new drug, eflornithine, has been developed (Pépin and Milord, 1994), but pentamidine remains the standard treatment for early-stage patients and melarsoprol for the late-stage cases. Between 5% and 10% of late-stage patients treated with melarsoprol, an arsenical derivative, succumb to its undesirable effects. Until recently, melarsoprol was the only available treatment for late-stage patients. Results obtained with eflornithine are excellent, but its future availability remains doubtful. Although they remain too toxic, HAT treatments are very effective for 'sterilizing' the human reservoir.

Individuals found to have trypanosomes during case-finding surveys are rapidly treated; they are then no longer parasitaemic and most of them will ultimately be cured. Thus the dynamics of trypanosome transmission are substantially altered: the duration of infection is dramatically reduced in a significant fraction of the human reservoir. Tsetse flies are then less likely to take their meals from an infected human, so infection rates in flies are reduced

and the flies will be less likely to transmit the parasite during a subsequent blood meal.

8.2.3. *Efficacy of Case Detection and Treatment*

The traditional approach yielded remarkable results in the first half of the 20th century. The inadequate sensitivity of the classical parasitological methods was compensated by an overwhelming participation of the population of endemic foci, as absentees were punished by colonial administrations. Furthermore, resources were sufficient for surveys to be carried out every 6 months. However, in the late 1940s the prevalence of trypanosomiasis stabilized, despite repeated surveys. At this time, pentamidine prophylaxis was added and resulted in further drops in prevalence. In retrospect, considering what is now known of pentamidine pharmacokinetics (Pépin and Milord, 1994), the administration of intramuscular pentamidine to everybody except pregnant women probably corresponded more to mass treatment than to prophylaxis. In recently infected individuals, a single injection of pentamidine was probably sufficient to result in cure. Thus pentamidine further reduced the duration of infection in some individuals and the size of the human reservoir.

Nowadays, participation in case-finding surveys is less massive, generally in the order of 50%. Thankfully, during repeated surveys the participants are not always the same individuals, so that after three or four visits by the mobile team a high proportion of the population has been seen at least once. The use of the CATT, which increases the efficacy of case finding, compensates to some extent for the lower participation.

8.3. Vector Control

The tsetse fly is one of the rare insects for which several control methods have been developed, based on bioecological and epidemiological studies. Before the advent of insecticides, vector control depended primarily on the elimination of the wooded vegetation which constitutes the habitat of *Glossina*. Nowadays, insecticides are applied to various types of traps and screens to destroy the vector.

8.3.1. *Destruction of Glossina Habitat*

Tsetse flies are sensitive to extremes of temperature and humidity, and changing the vegetation of their habitat creates unfavourable conditions for them. Total destruction of the vegetation can be used to eradicate particular species

or protect a region where tsetse flies have been eliminated by another vector control method (physical barrier). The inconveniences are obvious: high cost, harmfulness to the soil, maintenance, etc. This approach is ineffective in forest areas, as the vegetation quickly grows again.

Bioecological studies have shown that close relations exist between *Glossina* and certain plant species or groups of species that are its favourite resting places. Partial clearing has therefore been used in the past to limit human-fly contact through the elimination of vegetation in epidemiologically dangerous savannah areas, such as around bridges and the undergrowth or edges of forests, but this is now ecologically unacceptable. Controlled bush fires have only limited effects on riverine tsetse flies.

Agronomic prophylaxis is a type of bush clearing that consists of destroying vegetation in risky areas so as to eliminate human-fly contact; in place of the destroyed vegetation, crops such as rice, cereals, sugar cane, etc. are planted. This practice, commonly used in the past in the whole of French West Africa, has helped, in conjunction with case-finding surveys and chemoprophylaxis, to reduce prevalence considerably. It has, however, major inconveniences. It is labour-intensive and the staff must be well motivated. It involves the creation of forest edges and contributes to enlarging the flight lines of the tsetse fly which may, paradoxically, increase human-fly contact.

8.3.2. *Destruction of the Vector with Insecticides, Traps and Screens*

(a) *Insecticide sprays.* Ground spraying consists of dispersing a persistent insecticide (DDT, dieldrin, endosulfan or synthetic pyrethroids) in the resting places of the tsetse flies, which are killed when they settle on the treated vegetation. Spraying is done from ground level up to between 1 and 4 m above ground level with back sprayers, portable sprayers or tractor-mounted sprayers (with preset pressure or power-driven). In Nigeria, it has been possible to eliminate *Glossina* and animal trypanosomiases over an area of about 200 000 km²; this area included a number of old human trypanosomiasis foci which have since remained inactive.

Aerial spraying is rarely used in human trypanosomiasis control. In emergencies, helicopters have been used in West Africa to spray organochlorinated insecticides and, more recently, synthetic pyrethroids, on riverside vegetation to destroy certain species of the *G. morsitans* group. This very expensive method involves substantial risk for non-target fauna, especially aquatic species. It demands a lot of insecticide, suitable equipment and qualified staff. It also requires suitable weather conditions, which further complicates programme planning.

(b) *Insecticide-impregnated traps or screens.* Traps are used in control

campaigns, but also to capture *Glossina* specimens for epidemiological studies or control programme evaluation. Because of its shape and colour or contrast between colours, the trap is able to attract the tsetse and hold them captive. Traps do not necessarily need insecticide impregnation: tsetse flies that are caught are killed by exposure to the sun (effect of temperature or dessication) (Gouteux and Lancien, 1986). However, most of the flies attracted do not enter the trap. Thus, an impregnated trap can significantly reduce initial fly densities when urgent intervention is needed to halt transmission (Küpper *et al.*, 1982). The early traps were barely effective. For some years now, better designed models, some of which are insecticide-impregnated, have been in use. Vector control is in more widespread use, thanks also to the development of simple screens that can be impregnated with insecticides and which then kill the tsetse flies that come into contact with them.

Several improved biconical, monoconical and pyramidal traps, inspired by the Challier-Laveissière biconical trap (Challier and Laveissière, 1973), have been tested for the control of *G. p. palpalis*, *G. morsitans* and *G. f. quanzensis* in West and Central Africa. Lancien designed a monoconical trap (Lancien, 1981), which was followed by a pyramidal trap (Gouteux and Lancien, 1986) and, later, by the Vavoua trap (Laveissière and Grébaut, 1990). To enhance trap efficacy, especially against *G. morsitans*, olfactory attractive baits are combined with them (carbon dioxide, acetone, urine phenols and host skin secretions) (Leak, 1999). These are effective at a longer distance than the purely visual attraction.

Pilot studies conducted in various epidemiological settings showed that trapping is effective. Efficacy is measured in terms of the ADT per day. The efficacy varies according to the type of trap, the species or sub-species of *Glossina*, the environmental and climatic conditions, etc. (Dagnogo *et al.*, 1985; Laveissière, 1988). In the forest areas of Côte d'Ivoire, it was demonstrated that a black/blue/black screen was about twice as efficient as a simple blue one (Laveissière *et al.*, 1987). In the West African savannah, the ADTs of *G. tachinoides* and *G. p. gambiensis* populations were reduced by 88–92% using blue screens (Mérot *et al.*, 1984). The same screens reduced the ADT of *G. tachinoides* by 98% in only 15 days (Laveissière and Couret, 1981). In contrast, in the forest areas of Congo, they did not yield satisfactory results. The ADT was more drastically reduced (by 99%) after 5 months, in the forest areas of Côte d'Ivoire, using biconical traps (Laveissière *et al.*, 1981). Later, in the same areas, about 16 000 blue screens sited in coffee and cocoa plantations reduced the ADT of *G. p. palpalis* by 90% in 1 week, and by 98% at the end of 5 months (Laveissière *et al.*, 1986a).

Traps and screens have thus replaced insecticide spraying. These vector control methods have generated much interest: they are effective, simple, environmentally friendly and suitable for use by the communities themselves. Pilot projects in Burkina Faso (Mérot *et al.*, 1984), Côte d'Ivoire (Laveissière *et al.*,

1994b), Uganda (Lancien, 1991) and Congo (Gouteux and Sinda, 1990) have demonstrated the feasibility and efficiency of traps and screens used with the participation of rural communities under the supervision of specialized teams.

The number of traps and/or screens required for a particular location depends on the type of vegetation in the tsetse habitats, and on the presence or number of forest edges, water sources, paths and encampments that determine the frequency and intensity of human–fly contact. The communities are in a position to provide information on locations where the villagers are most often bitten. The life span of a trap depends on the quality of materials and of maintenance, and on the environmental conditions. In experiments in West Africa, it was estimated that about 10% and 20% of the traps or screens needed to be renewed each year in the forest and savannah zones, respectively (Laveissière, 1988). It is not possible to give the precise number of traps or screens to install per hectare, since each microcosm will have its own features (Laveissière, 1988). The number of traps or screens to be installed does not depend on the density of the human population to be covered. In the Vavoua pilot study, traps and screens were installed as follows: one screen every 100 m, one or two screens per water-point or encampment, one screen at each working place in the plantation, one trap every 300 m in forest areas, and one every 100 m around villages (Laveissière *et al.*, 1994b).

Traps are preferable to screens if reimpregnation and surveillance cannot be carried out by the population. Traps need to be reimpregnated once a year, at the end of the rainy season. Screens have to be reimpregnated once during the first year of the campaign, and twice a year later on. Traps should be installed as far beyond the endemic area as possible, so as to provide an effective barrier. In the gallery forest of savannah areas, open and sunny places frequently visited by people, such as washing and bathing places, water collection points, bridges and banks of rivers, are the preferable sites. In forest areas, the tsetse must be intercepted at the interfaces between different ecological patterns, i.e. the ‘edges’ that are considered as epidemiologically dangerous areas (Laveissière *et al.*, 1986c): areas around villages, paths separating wooded areas and other types of ecological patterns, especially cocoa or coffee plantations, etc.

Vector control with community involvement, as part of a comprehensive HAT control programme, was organized in the forest areas of Vavoua (Côte d'Ivoire), where Laveissière *et al.* (1985) recorded a 90% reduction of ADT in the villages and farms one week after setting up the traps and screens, and over 99% after three months. These results remained stable for the first six months. Subsequently, ADTs have increased. This phenomenon was linked to the gradual abandonment of equipment maintenance, especially during the farming season, and to lower efficacy due to the insecticide being washed away by rain and the screens being concealed by weeds which grew quickly during the rainy season. Two years later, the impact of the control programme

on the incidence of human disease was nevertheless obvious: no new patient was detected during a case-finding survey carried out in the study area, where the overall prevalence was initially higher than 1%. Overall, this experiment showed that about one year's vector control was needed to reduce transmission substantially. Similar results were recorded in Congo (Gouteux and Sinda, 1990) and Uganda (Lancien, 1991). Clearly, efficient case finding must be conducted simultaneously with vector control, otherwise the persistence of the human reservoir will lead to a rapid resurgence of disease when, sooner or later, vector control is pursued less vigorously.

The costs of a screen and a trap (Vavoua type) were estimated to be US \$3 and 6, respectively (Laveissière and Méda, 1992). The cost of one hectare protected was estimated at US \$1 in the first year, and much less in the second year. Costs vary depending on the type of trap or screen used and on whether or not the equipment is insecticide-impregnated.

(c) *Other vector control methods.* The other control methods have only very limited potential: biological control with the help of predators, arthropod parasites (dipteron and hymenopteran parasites) and pathogenic agents (*Serratia*, fungi, etc.). Infection of *G. tachinoides* adults or puparia with *Bacillus thuringiensis*, which is used for the biological control of mosquitoes and Simuliidae, was not pathogenic to tsetse flies (Leak, 1999). Genetic control by the release of sterile males consists of introducing into the wild population physically or chemically sterilized male insects of the same species to reduce breeding and, in the long run, to eliminate the population. This technique poses a series of problems: mass breeding of the *Glossina* species against which control measures are to be implemented, the competitiveness of laboratory-manipulated males, the sheer number of males to be sterilized and released, etc. It requires considerable logistic and financial support. It is unsuitable for human trypanosomiasis control since the objective, in the case of an epidemic, is quickly to stop disease transmission. Destruction or removal of mammalian hosts is not applicable to species of the subgenus *Nemorhina* of *Glossina* because of their eclectic nature (they feed on whatever hosts are easily accessible to them) and the need to protect wild life. It is effective solely for the control of savannah tsetse of the *G. morsitans* group, which are vectors of Rhodesian and animal trypanosomiases.

8.4. Control Strategies

8.4.1. Control Strategies Applied by Countries

HAT control has been, and will continue to be, based on the passive and active surveillance of populations at risk and the treatment of cases detected, coupled

with vector control in epidemic or hyperendemic circumstances (World Health Organization, 1986). This basic principle has prevailed in the development of surveillance and control models that have been tested in different geographical and epidemiological contexts.

(a) *Democratic Republic of Congo*. The DRC is the country most severely affected by the current trypanosomiasis epidemic. Owing to the high incidence and all kinds of logistical difficulties, control rests at the moment on 30 or so mobile teams that use conventional screening methods. CATT is now used on quite a large scale in the highest incidence regions, but there is little if any vector control. This country has witnessed several experiments on HAT control which we shall now summarize.

The operational integration of trypanosomiasis control within a network of health centres was tried out in the Nioki district of Bandundu region between 1983 and 1987 (Pépin *et al.*, 1989). Following the training and supervision of some non-specialist nurses in charge of the health centres, the percentage of new cases in the district that had been diagnosed passively by the health centres increased from zero to 31%. However, three-quarters of these new cases were already in the late stage when diagnosed compared with only one-third of the cases actively identified by the mobile teams. It was concluded that these two approaches were complementary but that the gains were relatively modest since, for the cases diagnosed by the health centres, the symptomatic period (and the total duration of infection) had probably been reduced by only a few weeks on average. This was beneficial for the patients but was likely to have had little impact on transmission. It appeared difficult to delegate to these nurses active case finding among their catchment populations. In parallel with the subsequent deterioration of the entire Congolese health system, the number of cases diagnosed passively by the health centres in Nioki district decreased progressively.

Elsewhere in DRC, it was shown that the use of modern seroparasitological techniques by mobile teams, during one to three successive diagnostic surveys, reduced transmission from a hyperendemic level to a residual level in just three years (Van Nieuwenhove, 1992). Compared with conventional screening methods based on lymph node aspirate, this strategy reportedly helped to diagnose two to five times as many patients. This experiment was repeated successfully in Equatorial Guinea (Simarro *et al.*, 1991).

A novel *T. b. gambiense* control method based on the treatment of serological suspects, who are CATT-positive but parasite-negative, with a single dose of diminazene, together with the conventional treatment of trypanosome carriers, was proposed (Bruneel *et al.*, 1994). The method was evaluated in a hyperendemic area, where the traditional diagnostic strategy, based on lymph node aspirate, did not manage to reduce transmission sufficiently, in spite of massive participation in case-finding surveys. The combination of case-finding

through CATT serological testing and the presumptive treatment of serological suspects led to a rapid and sustained reduction in the incidence of the disease.

(b) *Uganda*. Uganda is generally thought of as a pioneer in the control of Rhodesian trypanosomiasis by primary health care (PHC) workers. The control programme was based on case finding and passive diagnosis by specialized, motivated (by provision of a salary and a bicycle, and by supervision, etc.) village health workers (VHWs), known as sleeping sickness orderlies (Mbulamperi, 1989b), and on trapping with substantial community participation (Lancien, 1991). These strategies were developed to control the serious *T. b. rhodesiense* trypanosomiasis epidemic that developed in Busoga region from 1976 onwards. A control programme using deltamethrine-impregnated traps was implemented and produced excellent entomological results, with 95% reduction in the ADT (Lancien, 1991). However, there had already been a reduction in the incidence before trapping began, so it was difficult to assess its real impact on the disease.

The experience of Médecins sans Frontières (MSF) in north-west Uganda and southern Sudan, where sociopolitical disturbances led to a serious increase in *T. b. gambiense* HAT, was reported by Paquet *et al.* (1995). Given the epidemic nature of the situation, the MSF strategy was mainly the vertical type (see Section 8.4.2), based on a combination of passive diagnosis and annual case-finding surveys with modern seroparasitological methods (CATT and mAECT). The strategy did not include any vector control. Analysis of the first 5 years of activity (1987–1991) indicated that the mobile teams had identified 40% of the 4822 patients, 60% of whom were then in the early stage, compared with only 30% of cases passively diagnosed at the district hospital. This classical strategy led to a considerable reduction in prevalence and it was then deemed necessary to move towards integration of control activities into PHC.

(c) *Côte d'Ivoire*. In endemic areas of Côte d'Ivoire, HAT control is integrated into the activities of the health system at the intermediate level. The district medical officer is the head of the control programme within the district. The activities (diagnosis and treatment of cases) are carried out by a multi-functional mobile team, also responsible for immunization, the control of leprosy, malaria, schistosomiasis, etc. This is reinforced in Daloa district (the country's most highly endemic area) by a specialized mobile team based at a research station. The surveillance strategy rests mainly on passive diagnosis with traditional methods, coupled with systematic seroparasitological screening using more sensitive techniques in the most active foci. The health districts have neither the qualified staff nor the financial resources to use the most sensitive diagnostic methods available (Laveissière and Médá, 1993). The persistence of the disease can be attributed, to a certain extent, to the absence

of surveillance or at least to the poor sensitivity of the standard diagnostic methods used in the health districts.

In 1985, vector control was introduced in Vavoua, where there was an upsurge of the disease. A vector control programme, together with 'sterilization' of the human reservoir by treatment following mass seroparasitological screening, was applied in the epicentre of the focus (Laveissière *et al.*, 1986a), with the participation of the local communities. This strategy proved effective and easy to apply. It gave rapid results at a reasonable cost and was environmentally friendly. The success of this pilot study justified the introduction of a similar programme beyond the epicentre of the focus (Laveissière and Méda, 1993; Laveissière *et al.*, 1994b). On such a large scale, this strategy remained effective. However, certain constraints limited its widespread use in Côte d'Ivoire or elsewhere, in comparable epidemiological situations: (a) although the cost is reduced due to community participation, it is beyond the financial capability of endemic countries faced with multiple health priorities; (b) a rational use of trapping methods cannot be contemplated without first conducting an epidemiological study to characterize the environment, identify the sites of transmission, and so on; (c) it is necessary to train and supervise the communities in the implementation of the various activities; and (d), finally, one of the major obstacles to trapping by the community is some resistance to the concept among certain sociocultural, religious or ethnic groups (Laveissière and Méda, 1992).

These obstacles have prompted us to ponder over the choice of the best control strategy and to look for more rational and more efficient alternative strategies (Laveissière and Méda, 1993). This is why a surveillance system based on the use of VHWs and local health services, both involved in case-finding using the micro-CATT technique, was tested in Issia, a low-incidence area in the central western part of Côte d'Ivoire. The populations living in the zones at risk, identified by this surveillance system, were visited annually by specialized teams, with the participation of VHWs who were in charge of vector control activities. Since the programme tested at Issia proved feasible, the strategy was applied on a larger scale in Sinfra, a more highly endemic area in the same region. The VHWs, who were supervised and supported by the local health services, carried out control activities (blood collection on filter paper for diagnosis, vector control). Results indicated that the VHWs were capable of curbing transmission and maintaining surveillance in the medium term at a cost at least four times lower than that of the mobile teams (Laveissière *et al.*, 1998).

(d) *Other African countries.* In Angola, the conditions created by the civil war have led to an epidemic situation (Smith *et al.*, 1998), and a vertical control programme was put in place. Specialized mobile teams carry out case finding and passive diagnosis with modern techniques. Treatment of cases is

done in health centres and hospitals. A classical control model imposed by a specific situation (epidemic upsurge in a country torn by civil war), including a vertical surveillance programme and a treatment policy of integration into the regular health services, has been implemented. Several NGOs are involved in case-finding activities, and the voluntary sector has obviously become a main player in trypanosomiasis control in Angola and other countries.

In Congo-Brazzaville, two types of approach have been tested. The first was based on seroparasitological surveillance, combined with the treatment of parasitologically proven cases and of serological suspects, in order to reduce the human reservoir (Noireau *et al.*, 1988). The other, mainly entomological, was based on the elimination of the vector using non-impregnated traps (Gouteux and Sinda, 1990), with the participation of local communities. The efficacy and impact of the latter intervention were evaluated by periodically measuring the ADT and the seroprevalence through surveys using IFAT. Trapping carried out by the villagers considerably reduced the population of *G. palpalis palpalis*, the only vector found locally, as well as the seroprevalence of fluorescent antibodies, which decreased from 4% to 0·4% over 3 years.

In southern Sudan, the use of serological tests for screening by a mobile team, followed by the treatment of cases diagnosed parasitologically, led to a drastic reduction in transmission in a hyperendemic zone (Van Nieuwenhove, 1983). On the basis of these results, serological screening and systematic treatment of seropositive subjects were recommended as the best strategy for the rapid control of the disease in high incidence areas, even though this presumptive treatment of seropositive persons had not been specifically tested.

8.4.2. *Vertical and Horizontal Strategies*

Two fundamentally different strategies for trypanosomiasis control exist and have long been debated by health planners: (a) the vertical strategy, applied most often in hyperendemic or epidemic situations; and (b) the integrated horizontal strategy, used essentially in low-incidence situations in view of its advantageous cost-efficacy ratio.

(a) *Vertical strategy.* Vertical strategy targets a single disease. It is usually applied in epidemic or hyperendemic situations, to a region or a whole country. Control activities are carried out by specialized or multifunctional mobile teams. This strategy, which is tried and tested, is not outmoded; it is still current at least in its principles. It remains the most rational approach in the high-incidence, politically unstable, countries of Central Africa, but serious obstacles limit its application. The main disadvantages of the vertical strategy are its costs, lack of sustainability and dependence on substantial external

assistance. Its advantages are important, however: (a) the vertical strategy can be carried out even if the health system has to a large extent collapsed; (b) it guarantees more reliably active case-finding surveys being implemented at predetermined intervals; (c) it enables more technically demanding techniques (CATT, mAECT, QBCTM) to be used in high-incidence foci and thus will lead, in such settings, to a much more rapid reduction in incidence.

(b) *Horizontal strategy.* In the low-incidence countries of West Africa, the health authorities (and even the affected populations) feel increasingly less concerned about a disease they consider a low priority compared with other health problems. Most of these countries cannot continue to bear the increasing cost of classical vertical surveillance, which could absorb a considerable proportion of their health budget. In such countries, the application of the vertical strategy based on mobile teams seems to be posing many more problems than it can solve. This is attested by the multiple studies conducted with the aim of developing alternative strategies more suited to the local epidemiological and socioeconomic circumstances, as financial and human resources earmarked for the control of trypanosomiasis are dwindling daily. In the face of these difficulties, an alternative model of integration of sleeping sickness control into PHC has been proposed (De Brouwere and Pangu, 1989). In the horizontal strategy, the control programme is usually integrated in the health system at the district level, which offers the most favourable framework for the development of health programmes through PHC. Two different levels of integration have been described (De Brouwere and Pangu, 1989).

- (i) Integration at the intermediate (district) level (administrative integration). This is the policy applied in Côte d'Ivoire, where HAT control is under the responsibility of the district medical officer and the activities are carried out by the multifunctional mobile teams.
- (ii) Integration both at the district level and at the peripheral (health centres) level (administrative and operational integration). The health centres carry out screening activities under the supervision of the district medical officer. The integration is more or less complete, depending on the level at which the surveillance activities are conducted and whether the staff involved are specialized or not. The operational integration is incomplete if, in the health centres, a specialized technician carries out passive diagnosis and case-finding activities, in order not to overburden the other nurses. It is complete if the regular staff of the health centres are trained and assume routinely the control activities, with possible recourse to the support of the district mobile team, when necessary, for the organization of mass screening surveys among higher-risk populations.

An alternative model has been proposed and tested at Issia (Laveissière and

Méda, 1993) and Sinfra (Laveissière *et al.*, 1998), Côte d'Ivoire, and involves VHWS. A further adaptation to the epidemiological characteristics and the priorities of the affected population was applied in the Zoukougbeu focus (Méda, 1997), where a sleeping sickness surveillance system integrated into PHC was put in place and monitored for 18 months, to determine to what extent the local communities could carry out trypanosomiasis control activities in a low incidence area through village health committees and VHWS. The trial showed that control integrated into PHC and targeted on groups at risk (plantation workers and residents, families of trypanosomiasis patients) is feasible provided that the VHWS are adequately motivated, that the beneficiary populations participate fully, and that the health problem to be tackled is considered by the communities themselves as a priority. This having been said, such approaches are difficult to develop or maintain outside dedicated research projects, as there is an inherent contradiction between, on the one hand, the low incidence and modest burden of trypanosomiasis in such communities and, on the other hand, the substantial motivation required for the integration of trypanosomiasis control into the activities of largely unpaid VHWS.

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REFERENCES

- Aerts, D., Truc, P., Penchenier, L., Claes, Y. and Le Ray, D. (1992). A kit for *in vitro* isolation of trypanosomes in the field: first trial with sleeping sickness patients in the Congo Republic. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 394–395.
- Akol, G.W. and Murray, M. (1985). Induction of protective immunity in cattle by tsetse-transmitted cloned isolates of *Trypanosoma congolense*. *Annals of Tropical Medicine and Parasitology* **79**, 617–627.
- Allsopp, R. (1972). The role of game animals in the maintenance of endemic and enzootic trypanosomiases in the Lambwe Valley, South Nyanza District, Kenya. *Bulletin of the World Health Organization* **47**, 737–746.
- Apted, F.I.C. (1970). The epidemiology of Rhodesian sleeping sickness. In: *The African Trypanosomiases* (H.W. Mulligan and W.H. Potts, eds), pp. 645–660. London: George Allen and Unwin.
- Arbyn, M., Bruneel, H., Molisho, S. and Ekwanzala, M. (1995). Human trypanosomiasis in

- Zaire: a return to the situation at the beginning of the century? *Archives of Public Health* **53**, 365–371.
- Asonganyi, T. and Ade, X. (1994). Sleeping sickness in Cameroon. *Journal Camerounais de Médecine* **3**, 30–37.
- Asonganyi, T., Hengy, C., Louis, J.P. and Ghogomou, N.A. (1991). Reactivation of an old sleeping sickness focus in Mamfe (Cameroon): epidemiological, immunological and parasitological findings. *Revue d'Épidémiologie et de Santé Publique* **39**, 55–62.
- Asonganyi, T., Doua, F., Kibona, S.N., Nyasulu, Y.M.Z., Masake, R. and Kuzoe, F. (1998). A multi-centre evaluation of the card indirect agglutination test for trypanosomiasis. (*TrypTest CIATT*). *Annals of Tropical Medicine and Parasitology* **92**, 837–844.
- Bafort, J.M., Schutte, C.H.J. and Gathiram, V. (1986). Specificity of the CATT – card agglutination test in a non-sleeping sickness area of Africa. *South African Medical Journal* **69**, 541–542.
- Bailey, J.W. and Smith, D.H. (1992). The use of the acridine orange QBC® technique in the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 630.
- Baker, J.R. (1974). Epidemiology of African sleeping sickness. In: *Trypanosomiasis and Leishmaniasis, with special reference to Chagas disease*, Ciba Foundation Symposium no. 20, pp. 29–50. Amsterdam: Associated Scientific Publishers.
- Baker, J.R. and Robertson, D.H.H. (1957). Experiment on the infectivity to *Glossina morsitans* of a strain of *Trypanosoma rhodesiense* and of a strain of *T. brucei*, with some observations on the longevity of infected flies. *Annals of Tropical Medicine and Parasitology* **51**, 121–135.
- Baker, R.D. (1991). Modelling the probability of a single trypanosome infecting a tsetse fly. *Annals of Tropical Medicine and Parasitology* **85**, 413–415.
- Baldry, D.A.T. (1980). Local distribution and ecology of *Glossina palpalis* and *G. tachinoides* in the forest foci of West African human trypanosomiasis, with special reference to association between peri-domestic tsetse and their hosts. *Insect Science and its Application* **1**, 85–93.
- Barry, J.D. (1997). The biology of antigenic variation in African trypanosomes. In: *Trypanosomiasis and Leishmaniasis. Biology and Control* (G. Hide, J.C. Mottram, G.H. Coombs and P.H. Holmes, eds), pp. 89–107. Wallingford: CAB International.
- Barry, J.D. and McCulloch, R. (2001). Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. *Advances in Parasitology* **49**, 1–70.
- Bois, J.F., Challier, A., Laveissière, C. and Ouedraogo, V. (1977). Recherche des lieux de repos diurnes des glossines (*Glossina palpalis gambiensis* Vanderplank, 1949: Diptera, Glossinidae) par détection de spécimens marqués au ⁵⁹Fe. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologique* **15**, 3–13.
- Bruce, D. and Nabarro, D. (1903). Progress report on sleeping sickness in Uganda. *Proceedings of the Royal Society of London* **1**, 11–88.
- Bruneel, H., Van den Eeckhout, A., Molisho, D., Burke, J., Degroof, D. and Pépin, J. (1994). Contrôle de la trypanosomiase à *T. b. gambiense*: évaluation d'une stratégie basée sur le traitement des suspects sérologiques par une dose unique de diminazène. *Annales de la Société Belge de Médecine Tropicale* **74**, 203–215.
- Brunhes, J. (1994). *Logiciel d'identification: Glossine expert. Manuel illustré d'utilisation. [Identification software: Glossina expert. Illustrated user's manual]*. Paris: Centre for International Cooperation in Agronomic Research for Development in Cooperation (CIRAD)/French Scientific Research Institute for Development in Cooperation (ORSTOM)/University of Paris VI.
- Burke, J. (1971). Historique de la lutte contre la maladie du sommeil au Congo. *Annales de la Société Belge de Médecine Tropicale* **51**, 465–477.

- Bursell, E. and Taylor, P. (1980). An energy budget for *Glossina* (Diptera, Glossinidae). *Bulletin of Entomological Research* **70**, 187–196.
- Cattand, P. (1994). Trypanosomiase humaine africaine: situation épidémiologique actuelle, une recrudescence alarmante de la maladie. *Bulletin de la Société de Pathologie Exotique* **87**, 307–310.
- Cattand, P. and de Raadt, P. (1991). Laboratory diagnosis of trypanosomiasis. *Clinics in Laboratory Medicine* **11**, 899–908.
- Cattand, P., Miézan, T. and de Raadt, P. (1988). Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. *Bulletin of the World Health Organization* **66**, 83–86.
- Challier, A. (1982). The ecology of tsetse (*Glossina* spp.) (Diptera, Glossinidae): a review (1970–1981). *Insect Science and its Application* **3**, 97–143.
- Challier, A. and Gouteux, J.P. (1980). Ecology and epidemiological importance of *Glossina palpalis* in the Ivory Coast forest zone. *Insect Science and its Application* **1**, 77–83.
- Challier, A. and Laveissière, C. (1973). Un nouveau piège pour la capture des glossines (Diptera, Muscidae: *Glossina*): description et essai sur le terrain. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **11**, 251–262.
- Colvin, J. and Gibson, G. (1992). Host seeking behaviour and management of tsetse. *Annual Review of Entomology* **37**, 21–40.
- Croft, S., East, J.S. and Molyneux, D.H. (1982). Anti-trypanosomal factor in the haemolymph of *Glossina*. *Acta Tropica* **39**, 293–302.
- Cuisance, D. and Février, J. (1985). Dispersion linéaire de *Glossina palpalis gambiensis* et de *Glossina tachinoides* dans une galerie forestière de zone soudano-guinéenne (Burkina Faso). Étude sur le pouvoir de dispersion des glossines. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* **37**, 84–98.
- Dagnogo, M., Lohuirignon, K. and Traoré, G. (1977). Diversity of *Glossina* in the forest belt of Côte d'Ivoire. *Acta Tropica* **65**, 149–153.
- Dagnogo, M., Eouzan, J.P. and Lohuirignon, K. (1985). Comparaison de différents pièges à tsésé (Diptera, Glossinidae) en Côte d'Ivoire et au Congo. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux* **38**, 371–378.
- De Brouwere, V. and Pangu, K.A. (1989). Réflexions sur la flexibilité d'un service de santé intégré dans la lutte contre la trypanosomiase à *Trypanosoma brucei gambiense*. *Annales de la Société Belge de Médecine Tropicale* **69**, supplément **1**, 221–229.
- Denlinger, D.L. and Zdárek, J. (1994). Metamorphosis behaviour of flies. *Annual Review of Entomology* **17**, 127–130.
- De Raadt, P. (1976). African sleeping sickness today. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **70**, 114–116.
- Diallo, B.P., Truc, P. and Laveissière, C. (1997). A new method for identifying blood meals of human origin in tsetse flies. *Acta Tropica* **63**, 61–64.
- Dukes, P., Gibson, W.C., Gashumba, J.K., Hudson, K.M., Bromidge, T.J., Kaukus, A., Asonganyi, T. and Magnus, E. (1992). Absence of the LiTAT 1.3 (CATT antigen) gene in *Trypanosoma brucei gambiense* stocks from Cameroon. *Acta Tropica* **51**, 123–134.
- Ekwanzala, M., Pépin, J., Khonde, N., Molisho, S., Brunel, H. and De Wals, P. (1996). In the heart of darkness: sleeping sickness in Zaire. *Lancet* **348**, 1427–1430.
- El-Sayed, N.M.A. and Donelson, J. (1997). Sequencing and mapping the African trypanosome genome. In: *Trypanosomiasis and Leishmaniasis. Biology and Control*. (G. Hide, J.C. Mottram, G.H. Coombs, P.H. Holmes, eds), pp. 51–63. Wallingford: CAB International.
- Evans, D. (1981). African trypanosomes. *Antibiotics and Chemotherapy* **30**, 272–287.
- Ford, J. and Katondo, K.M. (1973). Maps of tsetse fly (*Glossina*) distribution in Africa, 1973, according to sub-generic groups on scale of 1: 5 000 000 (plus a set of 9 maps in colour). *Bulletin of Animal Health Production* **25**, 187–193.

- Frézil, J.L. and Carnevale, P. (1976). Le problème du réservoir de virus et du maintien des foyers en Afrique Centrale. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **14**, 307–313.
- Gibson, W. (1994). Identification of trypanosomes in animals, humans and *Glossina*. *Bulletin de la Société de Pathologie Exotique* **87**, 315–318.
- Gibson, W., Mehlitz, D., Lanham, S.M. and Godfrey, D.G. (1978). The identification of *Trypanosoma brucei gambiense* in Liberian pigs and dogs by isoenzymes and by resistance to human plasma. *Tropical Medicine and Parasitology* **29**, 335–345.
- Gingrich, J.B., Ward, R.A., Macken, L.M. and Esser, K.M. (1982). African sleeping sickness: new evidence that mature tsetse flies (*Glossina morsitans*) can become potent vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 479–481.
- Ginoux, P.Y. and Frézil, J.L. (1981). Recherches sur la latence clinique et la trypano-tolérance humaine dans le foyer du couloir du fleuve Congo. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **19**, 33–40.
- Golder, T.K., Otieno, L.H., Patel, N.Y. and Onyango, P. (1984). Increased sensitivity to endosulfan of *Trypanosoma*-infected *Glossina morsitans*. *Annals of Tropical Medicine and Parasitology* **76**, 483–484.
- Gouteux, J.P. (1987). Écodistribution de *Glossina palpalis palpalis* (Rob.- Desv.) en secteur préforestier de Côte d'Ivoire. Sexe, rythmes ovario-utérins et utilisation de l'espace. *Acta Oecologica* **8**, 27–38.
- Gouteux, J.P. (1990). Current considerations on the distribution of *Glossina* in West and Central Africa. *Acta Tropica* **47**, 185–187.
- Gouteux, J.P. and Lancien, J. (1986). Le piège pyramidal à tsétsé (Diptera: Glossinidae) pour la capture et la lutte: essais comparatifs et description de nouveaux systèmes de capture. *Tropical Medicine and Parasitology* **37**, 61–66.
- Gouteux, J.P. and Laveissière, C. (1982). Écologie des glossines en secteur pré-forestier de Côte d'Ivoire. 4. Dynamique de l'écodistribution en terroir villageois. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **20**, 199–229.
- Gouteux, J.P. and Sinda, D. (1990). Community participation in the control of tsetse flies. Large scale trials using the pyramidal trap in the Congo. *Tropical Medicine and Parasitology* **41**, 49–55.
- Gouteux, J.P., Boreham, P.F.L. and Laveissière, C. (1982). Écologie des glossines en secteur forestier de Côte d'Ivoire. 2. Les préférences trophiques de *Glossina palpalis s.l.* *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **20**, 3–18.
- Gouteux, J.P., Noireau, F., Malonga, J.R. and Frézil, J.L. (1989). 'Effet de case' et 'contamination familiale' dans la maladie du sommeil: essai d'interprétation du phénomène. Exemple de trois foyers congolais. *Annales de Parasitologie Humaine et Comparée* **63**, 315–333.
- Grubhoffer, L., Muska, M. and Volf, P. (1994). Midgut hemagglutinins in five species of tsetse flies (*Glossina* spp.): two different lectin systems in the midgut of *Glossina tachinoides*. *Folia Parasitologica* **41**, 229–232.
- Hargrove, J.W. (1975). Some changes in the flight and apparatus of tsetse flies during maturation. *Journal of Insect Physiology* **21**, 1485–1489.
- Harmsen, R. (1973). The nature of the establishment barrier for *Trypanosoma brucei* in the gut of *Glossina pallidipes*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 364–373.
- Henry, M.C. (1981). Importance de la contamination familiale dans la trypanosomiase à *Trypanosoma brucei gambiense*. *Bulletin de la Société de Pathologie Exotique* **74**, 65–70.
- Henry, M.C., Ruppolt, J.F. and Bruneel, H. (1982). Distribution de l'infection par *T. b. gambiense* dans une population du Bandundu en République du Zaïre. *Annales de la Société Belge de Médecine Tropicale* **62**, 301–313.

- Hervouet, J.P. and Laveissière, C. (1987). Facteurs humains de la transmission de la maladie du sommeil. *La Medicina Tropicale nella Cooperazione allo Sviluppo* **3**, 72–77.
- Hide, G. (1997). The molecular epidemiology of trypanosomatids. In: *Trypanosomiasis and Leishmaniasis. Biology and Control* (G. Hide, J.C. Mottram, G.H. Coombs and P.H. Holmes, eds), pp. 289–303. Wallingford: CAB International.
- Hide, G. (1999). History of sleeping sickness in East Africa. *Clinical Microbiology Reviews* **12**, 112–125.
- Hide, G., Tait, A., Maudlin, I. and Welburn, S.C. (1996). The origins, dynamics, and generation of *Trypanosoma brucei rhodesiense* epidemics in East Africa. *Parasitology Today* **12**, 50–55.
- Hoare, C.A. (1972). *The Trypanosomes of Mammals*. Oxford and Edinburgh: Blackwell Scientific Publications.
- Jannin, J., Penchenier, L., Eozenou, P., Ventrou, P., Mialebama, J., Louya, F., Bobenda, T., Samba, F. and Coddy Zitsamele, R. (1992). Recrudescence actuelle de la trypanosomiase humaine dans le foyer de la Sangha (Cuvette) au Congo. *Bulletin de la Société de Pathologie Exotique* **85**, 31–38.
- Janssens, P.G. and Burke, J. (1992). Les trypanosomiases africaines. In: *Médecine et Hygiène en Afrique Centrale de 1885 à Nos Jours* (P.G. Janssens, M. Kivits and J. Vuylsteke, eds), pp. 1399–1535. Brussels: Fondation Roi Baudouin.
- Jenni, L., Molyneux, D.H. and Livesey, J.L. (1980). Feeding behaviour of tsetse flies infected with salivarian trypanosomes. *Nature* **283**, 383–385.
- Kabeya, N.M., Pochet, A. and Mandiangu, M. (1988). Évolution et épidémiologie des trypanosomiases humaines africaines (THA) au Zaïre. *Médecine Tropicale* **48**, 277–283.
- Kageruka, P. (1989). Réervoir animal de *Trypanosoma (Trypanozoon) brucei gambiense* en Afrique Centrale. *Annales de la Société Belge de Médecine Tropicale* **69**, supplément 1, 155–163.
- Katondo, K.M. (1984). Revision of second edition of tsetse distribution maps. An interim report. *Insect Science and its Application* **5**, 381–388.
- Khonde, N., Pépin, J., Niyonsenga, T., Milord, F. and De Wals, P. (1995). Epidemiological evidence for immunity following *Trypanosoma brucei gambiense* sleeping sickness. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 607–611.
- Khonde, N., Pépin, J., Niyonsenga, T. and De Wals, P. (1997). Familial aggregation of *Trypanosoma brucei gambiense* trypanosomiasis in a very high incidence community in Zaïre. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 521–524.
- Koerner, T., de Raadt, P. and Maudlin, I. (1995). The 1901 Uganda sleeping sickness epidemic revisited: a case of mistaken identity? *Parasitology Today* **11**, 303–306.
- Küpper, W., Eibl, F., van Elsen, A.C. and Clair, M. (1982). The use of the biconical Challier-Laveissière trap impregnated with deltamethrin against *Glossina*. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* **35**, 157–163.
- Lancien, J. (1981). Description du piège monoconique utilisé pour l'élimination des glossines en République Populaire du Congo. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **19**, 235–238.
- Lancien, J. (1991). Lutte contre la maladie du sommeil dans le sud-est Ouganda par piégeage des glossines. *Annales de la Société Belge de Médecine Tropicale* **71**, 35–47.
- Langley, P.A. and Stafford, K. (1990). Feeding frequency in relation to reproduction in *Glossina morsitans morsitans* and *G. pallidipes*. *Physiological Entomology* **15**, 415–421.
- Lapeyssonnie, L. (1992). Géométrie et passion: la lutte contre la maladie du sommeil. *Annales de la Société Belge de Médecine Tropicale* **72**, supplément 1, 7–12.
- Laveissière, C. (1988). *Biology and control of Glossina species, vectors of human African trypanosomiasis*. Geneva: World Health Organization. Vector control series: tsetse flies. Training and information guide. WHO/VBC/88.958.

- Laveissière, C. and Boreham, P.F.L. (1976). Écologie de *Glossina tachinoides* Westwood, 1850, en savanne humide d'Afrique de l'Ouest. Préférences trophiques. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **14**, 7–20.
- Laveissière, C. and Couret, D. (1981). Essai de lutte contre les glossines riveraines à l'aide d'écrans imprégnés d'insecticide. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **19**, 271–283.
- Laveissière, C. and Grébaut, P. (1990). Recherches sur les pièges à glossines (Diptera: Glossinidae). Mise au point d'un modèle économique: le piège 'Vavoua'. *Tropical Medicine and Parasitology* **41**, 185–192.
- Laveissière, C. and Hervouët, J.P. (1981). Populations de glossines et occupation de l'espace. Enquête entomologique préliminaire dans la région de la Lobo (Côte d'Ivoire), février 1981. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **19**, 247–260.
- Laveissière, C. and Méda, H.A. (1992). La lutte par piégeage contre la maladie du sommeil: pas aussi simple qu'on le croit! *Annales de la Société Belge de Médecine Tropicale* **72**, supplément 1, 57–68.
- Laveissière, C. and Méda, H.A. (1993). Équipe mobile ou agents de santé: quelle stratégie contre la maladie du sommeil? *Annales de la Société Belge de Médecine Tropicale* **73**, 1–6.
- Laveissière, C. and Méda, H.A. (1999). Incidence de la maladie du sommeil et densité des campements de culture en forêt de Côte d'Ivoire: possibilité de prédiction des zones à risques pour la mise en place précoce d'un réseau de surveillance. *Tropical Medicine and International Health* **4**, 199–206.
- Laveissière, C., Couret, D. and Kienou, J.P. (1981). Lutte contre les glossines riveraines à l'aide de pièges biconiques imprégnés d'insecticide. 4. Expérimentation à grande échelle. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **19**, 41–48.
- Laveissière, C., Couret, D., Staak, C. and Hervouët, J.P. (1985). *Glossina palpalis* et ses hôtes en secteur forestier de Côte d'Ivoire. Relation avec l'épidémiologie de la trypanosomiase humaine. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **23**, 297–303.
- Laveissière, C., Couret, D. and Eouzan, J.P. (1986a). La campagne pilote contre la trypanosomiase humaine dans le foyer de Vavoua (Côte d'Ivoire). 3. Résultats des évaluations entomologiques. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **24**, 7–20.
- Laveissière, C., Hervouët, J.P. and Couret, D. (1986b). Localisation et fréquence du contact homme/glossines en secteur forestier de Côte d'Ivoire: 1. Recherche des points épidémiologiquement dangereux dans l'environnement végétal. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **24**, 21–35.
- Laveissière, C., Hervouët, J.P. and Couret, D. (1986c). Localisation et fréquence du contact homme/glossines en secteur forestier de Côte d'Ivoire: 2. Le facteur humain et la transmission. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **24**, 45–57.
- Laveissière, C., Couret, D. and Manno, A. (1987). Importance de la nature des tissus dans la lutte par piégeage contre les glossines. *Cahiers ORSTOM, Série Entomologie Médicale et Parasitologie* **25**, 133–143.
- Laveissière, C., Sané, B. and Méda, H. (1994a). Measurement of risk in endemic areas of human African trypanosomiasis in Côte d'Ivoire. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 645–648.
- Laveissière, C., Grébaut, P., Lemasson, J., Méda, H.A., Couret, D., Doua, F., Brou, N. and Cattand, P. (1994b). *Les Communautés Rurales et la Lutte contre la Maladie du Sommeil en Forêt de Côte d'Ivoire*. Geneva: World Health Organization.

- Laveissière, C., Médà, H.A., Doua, F. and Sané, B. (1998). Dépistage de la maladie du sommeil: comparaison de l'efficacité des équipes mobiles et des agents de santé communautaires. *Bulletin of the World Health Organization* **76**, 559–564.
- Leak, S.G.A. (1999). *Tsetse Biology and Ecology*. Wallingford: CABI Publishing.
- Legros, D., Evans, S., Maiso, F., Enyaru, J.C.K. and Mbulamperi, D. (1999). Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 439–442.
- Louis, J.P., Jannin, J., Hengy, C., Moulia-Pelat, J.P., Makuwa, M., Asonganyi, T., Noutoua, L., Fadat, G., Nguerenemo, P., Cattand, P. and Trébucq, A. (1991). Absence d'inter-relations épidémiologiques entre l'infection rétrovirale à VIH et la trypanosomiase humaine africaine (THA). Analyse de trois enquêtes cas-témoins réalisées en 1989 et 1990 en Afrique centrale. *Bulletin de la Société de Pathologie Exotique* **84**, 25–29.
- Magnus, E., Vervoort, T. and Van Meirvenne, N. (1978). A card-agglutination test with stained-trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. *Annales de la Société Belge de Médecine Tropicale* **58**, 169–176.
- Martin, G., Leboeuf, E. and Roubaud, E. (1909). *Rapport de la Mission d'Études de la Maladie du Sommeil au Congo français 1906–1908*. Paris: Masson.
- Maudlin, I. and Welburn, S.C. (1988). The role of lectins and trypanosome genotypes in the maturation of midgut infections in *Glossina morsitans*. *Tropical Medicine and Parasitology* **39**, 56–58.
- Maudlin, I. and Welburn, S.C. (1989). A single trypanosome is sufficient to infect a tsetse fly. *Annals of Tropical Medicine and Parasitology* **83**, 431–433.
- Maudlin, I., Welburn, S.C. and Milligan, P.J.M. (1998). Trypanosome infections and survival in tsetse. *Parasitology* **116**, supplement, S23–S28.
- Mbulamperi, D.B. (1989a). Possible causes leading to an epidemic outbreak of sleeping sickness: facts and hypotheses. *Annales de la Société Belge de Médecine Tropicale* **69**, supplément **1**, 173–179.
- Mbulamperi, D.B. (1989b). A review of human African trypanosomiasis (HAT) in Uganda. *East African Medical Journal* **66**, 743–747.
- Mbulamperi, D.B. (1990). Recent epidemic outbreaks of human trypanosomiasis in Uganda. *Insect Science and its Application* **11**, 289–292.
- McKelvey, J.J. (1973). *Man against Tsetse. Struggle for Africa*. Ithaca: Cornell University Press.
- Médà, H.A. (1997). *Épidémiologie et contrôle de la trypanosomiase humaine africaine dans le foyer de Zoukougbeu (Côte d'Ivoire). Un modèle de surveillance pour des foyers à faible endémicité*. Thesis: Vrije Universiteit Brussel, Faculteit Wetenschappen.
- Médà, H., Laveissière, C., de Muynck, A., Doua, F. and Diallo, P.B. (1993). Les facteurs de risque de la trypanosomiase humaine africaine dans les foyers endémiques de Côte d'Ivoire. *Médecine Tropicale* **53**, 83–92.
- Médà, H., Doua, F., Laveissière, C., Miézan, T.W., Gaens, E., Brattegard, K., de Muynck, A. and De Cock, K. (1995). HIV infection and human African trypanosomiasis: a case-control study in Côte d'Ivoire. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 639–643.
- Mehlitz, D., Brinkmann, U. and Haller, L. (1981). Epidemiological studies on the animal reservoir of *gambiense* sleeping sickness. Part 1. Review of literature and description of the study areas. *Tropical Medicine and Parasitology* **32**, 129–133.
- Mehlitz, D., Zillmann, U., Scott, C.M. and Godfrey, D.G. (1982). Epidemiological studies on the animal reservoir of *gambiense* sleeping sickness. 3. Characterization of *Trypanozoon* stocks by isoenzymes and sensitivity to human plasma. *Tropical Medicine and Parasitology* **33**, 113–118.

- Mentens, H., de Muynck, A., Van der Stuyft, P., Bruneel, H., Kabeya, N.M., Molisho, S. and Nkuadio, C. (1988). *Étude épidémiologique des déterminants de la maladie du sommeil à T. b. gambiense dans le foyer de Fankana-Kalaketini, Zaire*. Antwerpen: Prinz Leopold Instituut voor Tropische Geneeskunde, Epi Publicatie no. 3.
- Mérot, P., Galey, J.B., Politzar, H., Tamboura, I. and Cuisance, D. (1984). Résultats d'une campagne de lutte contre les glossines riveraines au Burkina par l'emploi d'écrans imprégnés de deltaméthrine. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux* **37**, 175–184.
- Miézan, T., Doua, F., Cattand, P. and de Raadt, P. (1991). Évaluation du Testryp® CATT appliqué au sang prélevé sur papier filtre et au sang dilué, dans le foyer de trypanosomiase à *Trypanosoma brucei gambiense* en Côte d'Ivoire. *Bulletin of the World Health Organization* **69**, 603–606.
- Miézan, T.W., Méda, A.H., Doua, F. and Cattand, P. (1994). Évaluation des techniques parasitologiques utilisées dans le diagnostic de la trypanosomose humaine à *Trypanosoma gambiense* en Côte d'Ivoire. *Bulletin de la Société de Pathologie Exotique* **87**, 101–104.
- Miézan, T.W., Méda, H.A., Doua, F., Yapo, F.B. and Baltz, T. (1998). Assessment of central nervous system involvement in *gambiense* trypanosomiasis: value of the cerebro-spinal white cell count. *Tropical Medicine and International Health* **3**, 571–575.
- Miézan, T.W., Méda, H.A., Doua, F., Djè, N.N., Lejon, V. and Büscher, P. (2000). Single centrifugation of cerebrospinal fluid in a sealed Pasteur pipette for simple, rapid and sensitive detection of trypanosomes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 293.
- Milord, F., Pépin, J., Loko, L., Ethier, L. and Mpia, B. (1992). Efficacy and toxicity of eflor-nithine for treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Lancet* **340**, 652–655.
- Moloo, S.K. (1993). The distribution of *Glossina* species in Africa and their natural hosts. *Insect Science and its Application* **14**, 511–527.
- Molyneux, D.H. (1980a). Host–trypanosomes interactions in *Glossina*. *Insect Science and its Application* **1**, 39–46.
- Molyneux, D.H. (1980b). Animal reservoirs and residual 'foci' of *Trypanosoma brucei gambiense* sleeping sickness in West Africa. *Insect Science and its Application* **1**, 59–63.
- Molyneux, D.H. and Ashford, R.W. (1983). *The Biology of Trypanosoma and Leishmania, Parasites of Man and Domestic Animals*. London: Taylor & Francis.
- Nantulya, V. (1997). TrypTect CIATT® – a card indirect agglutination trypanosomiasis test for diagnosis of *Trypanosoma brucei gambiense* and *T. b. rhodesiense* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 551–553.
- Nantulya, V., Musoke, A.J., Rurangirwa, F.R. and Moloo, S.K. (1984). Resistance of cattle to tsetse-transmitted challenge with *Trypanosoma brucei* or *Trypanosoma congolense* after spontaneous recovery from syringe-passaged infections. *Infection and Immunity* **43**, 735–738.
- Nekpeni, E.B., Dagnogo, M. and Eouzan, J.P. (1989). Détermination de la limite géographique entre deux sous-espèces de glossines en Côte d'Ivoire: *Glossina palpalis palpalis* (Robineau-Desvoidy, 1830) et *G. p. gambiensis* (Vanderplank, 1949). *Tropical Medicine and Parasitology*, **40**, 12–15.
- Nitcheman, S. (1990). Comparison of the susceptibility to deltamethrin of female *Glossina morsitans morsitans* Westwood, 1985 (Diptera: Glossinidae) uninfected and infected with *Trypanosoma (Nannomonas) congolense* Broden, 1904 (Kinetoplastida, Trypanosomatidae). *Annals of Tropical Medicine and Parasitology* **84**, 483–491.
- Noireau, F., Gouteux, J.P., Toudic, A., Samba, F. and Frézil, J.L. (1986). Importance épidémiologique du réservoir animal à *Trypanosoma brucei gambiense* au Congo. 1.

- Prévalence des trypanosomoses animales dans les foyers de maladie du sommeil. *Tropical Medicine and Parasitology* **37**, 393–398.
- Noireau, F., Lemesre, J.L., Nzoukoudi, M.Y., Louembet, M.T., Gouteux, J.P. and Frézil, J.L. (1988). Serodiagnosis of sleeping sickness in the Republic of Congo: comparison of indirect immunofluorescent antibody test and card agglutination test. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 237–240.
- Noireau, F., Paindavoine, P., Lemesre, J.L., Toudic, A., Pays, E., Gouteux, J.P., Steinert, M. and Frézil, J.L. (1989). The epidemiological importance of the animal reservoir of *Trypanosoma brucei gambiense* in the Congo. 2—Characterization of the *Trypanosoma brucei* complex. *Tropical Medicine and Parasitology* **40**, 9–11.
- Noireau, F., Lemesre, J.L. and Vervoort, T. (1991). Absence of serological markers of infection with *Trypanosoma brucei gambiense* in domestic animals in a sleeping sickness focus in South Congo. *Tropical Medicine and Parasitology* **42**, 195–196.
- Okia, M., Mbulamperi, D.B. and de Muynck, A. (1994). Risk factors assessment for *T. b. rhodesiense* sleeping sickness in south-east Uganda. *Annales de la Société Belge de Médecine Tropicale* **74**, 105–112.
- Okiria, R. (1985). The prevalence of human trypanosomiasis in Uganda, 1970 to 1983. *East African Medical Journal* **62**, 813–816.
- Okoth, J.O. (1999). Tsetse and trypanosomiasis control problems in south-east Uganda: past, present and alternative strategies. *Schweizerische Medizinische Wochenschrift* **129**, 1091–1098.
- Okoth, J.O., Kirumira, E.K. and Kapaata, R. (1991). A new approach to community participation in tsetse control in the Busoga sleeping sickness focus, Uganda. A preliminary report. *Annals of Tropical Medicine and Parasitology* **85**, 315–322.
- Otieno, L.H. and Darji, N. (1979). The abundance of pathogenic African trypanosomes in salivary secretions of wild *Glossina pallidipes*. *Annals of Tropical Medicine and Parasitology* **73**, 53–58.
- Paquet, C., Castilla, J., Mbulamperi, D., Beaulieu, M.F., Gastellu Etchegorry, M. and Moren, A. (1995). La trypanosomiase à *Trypanosoma brucei gambiense* dans le foyer du Nord-Ouest de l'Ouganda. Bilan de 5 années de lutte (1987–1991). *Bulletin de la Société de Pathologie Exotique* **88**, 38–41.
- Penchenier, L., Jannin, J., Moulia-Pelat, J.P., Elfassi de la Baume, F., Fadat, G., Chanfreau, B. and Ezenou, P. (1991). Le problème de l'interprétation du CATT dans le dépistage de la trypanosomiase humaine à *Trypanosoma brucei gambiense*. *Annales de la Société Belge de Médecine Tropicale* **71**, 221–228.
- Penchenier, L., Sarda, J. and Jannin, J. (1993). Où en est le foyer de trypanosomiase humaine de Mossaka (Congo)? *Bulletin de la Société de Pathologie Exotique* **86**, 347–350.
- Penchenier, L., Mathieu-Daudé, F., Brengues, C., Bañuls, A.L. and Tibayrenc M. (1997). Population structure of *Trypanosoma brucei* s.l. in Côte d'Ivoire assayed by multilocus enzyme electrophoresis: epidemiological and taxonomical considerations. *Journal of Parasitology* **83**, 19–22.
- Penchenier, L., Grébaut, P., Bodo, J.M., Ebo'o Eyenga, V., Njiokou, F., Simo, S.G., Nkinin, S., Ndong Asumu, P., Simarro, P., Herder, S. and Souala, G. (1998). Le foyer de trypanosomiase humaine de Campo (Cameroun) en 1998. Aspects épidémiologiques, état de l'endémie et comparaison des CATT 1.3 et CATT Latex dans le dépistage de masse. *Bulletin de Liaison Documentaire de l'OCEAC* **31**, 8–19.
- Pépin, J. and Khonde, N. (1996). Relapses following treatment of early-stage *Trypanosoma brucei gambiense* sleeping sickness with a combination of pentamidine and suramin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 183–186.

- Pépin, J. and Milord, F. (1994). The treatment of human African trypanosomiasis. *Advances in Parasitology* **33**, 1–47.
- Pépin, J., Guern, C., Milord, F. and Mpia, B. (1989). Intégration de la lutte contre la maladie du sommeil dans un réseau de centres de santé polyvalents. *Bulletin of the World Health Organization* **67**, 301–308.
- Pépin, J., Ethier, L., Kazadi, C., Milord, F. and Ryder, R. (1992). The impact of HIV infection on the epidemiology and treatment of *Trypanosoma brucei gambiense* sleeping sickness in Nioki, Zaire. *American Journal of Tropical Medicine and Hygiene* **47**, 133–140.
- Pépin, J., Milord, F., Khonde, A., Niyonsenga, T., Loko, L. and Mpia, B. (1994). Gambiense trypanosomiasis: frequency of, and risk factors for, failure of melarsoprol therapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 447–452.
- Pollock, J.N. (1982). *Training Manual for Tsetse Control Personnel. Tsetse Biology; Systematics and Distribution; Techniques*. Rome: Food and Agriculture Organization.
- Prothero, R.M. (1963). Population mobility and trypanosomiasis in Africa. *Bulletin of the World Health Organization* **28**, 615–626.
- Roberts, L.W. (1981). Probing by *Glossina morsitans morsitans* and transmission of *Trypanosoma (Nannomonas) congolense*. *American Journal of Tropical Medicine and Hygiene* **30**, 948–951.
- Robertson, D.H.H. and Baker, J.R. (1958). Human trypanosomiasis in south-east Uganda. 1. A study of the epidemiology and present virulence of the disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **52**, 337–348.
- Ross, R. and Thomson, D. (1910). A case of sleeping sickness studied by precise enumerative methods: regular periodical increase of the parasites disclosed. *Proceedings of the Royal Society of London, B* **82**, 411–415.
- Ross, R. and Thomson, D. (1911). A case of sleeping sickness studied by precise enumerative methods: further observations. *Proceedings of the Royal Society of London, B* **83**, 187–205.
- Ruppel, J.F. and Burke, J. (1977). Follow-up des traitements contre la trypanosomiase expérimentée à Kimpangu (République du Zaire). *Annales de la Société Belge de Médecine Tropicale* **57**, 481–491.
- Sané, B., Laveissière, C. and Médà, H.A. (2000). Diversité du régime alimentaire de *Glossina palpalis palpalis* en zone forestière de Côte d'Ivoire: relation avec la prévalence de la trypanosomiase humaine africaine. *Tropical Medicine and International Health* **5**, 73–78.
- Schares, G. and Mehltz, D. (1996). Sleeping sickness in Zaire: a nested polymerase chain reaction improves the identification of *Trypanosoma (Trypanozoon) brucei gambiense* by specific kinetoplast DNA probes. *Tropical Medicine and International Health* **1**, 59–70.
- Scott, D. (1970). The epidemiology of Gambian sleeping sickness. In: *The African Trypanosomiases* (H.W. Mulligan and W.H. Potts, eds), pp. 614–644. London: George Allen and Unwin.
- Scott, C.M., Frézil, J.L., Toudic, A. and Godfrey, D.G. (1983). The sheep as a potential reservoir of human trypanosomiasis in the Republic of Congo. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **77**, 397–401.
- Simarro, P.P., Sima, F.O., Mir, M., Mateo, M.J. and Roche, J. (1991). La lutte contre la trypanosomiase humaine africaine dans le foyer de Luba en Guinée équatoriale: bilan de trois méthodes. *Bulletin of the World Health Organization* **69**, 451–457.
- Smith, A.B., Esko, J.D. and Hajduk, S.L. (1995). Killing of trypanosomes by the human haptoglobin-related protein. *Science* **268**, 284–286.
- Smith, D.H., Pépin, J. and Stich, A.H.R. (1998). Human African trypanosomiasis: an emerging public health crisis. *British Medical Bulletin* **54**, 341–355.

- Southon, H.A.W. and Cunningham, M.P. (1966). Infectivity of trypanosomes derived from individual *Glossina morsitans* Westw. *Nature* **189**, 411–412.
- Stanghellini, A. and Duvallet, G. (1981). Épidémiologie de la trypanosomiase à *Trypanosoma gambiense* dans un foyer de Côte d'Ivoire. I. Distribution de la maladie dans la population. *Tropical Medicine and Parasitology* **32**, 141–144.
- Stanghellini, A., Josse, R., Cattand, P., Bopang, T., Tirandibaye, N., Emery, P., Milleliri, J.M. and Cordoliani, G. (1989). Aspects épidémiologiques de la trypanosomiase humaine africaine dans le sud du Tchad. *Médecine Tropicale* **49**, 395–400.
- Truc, P., Mathieu-Daudé, F. and Tibayrenc, M. (1991). Multilocus isozyme identification of *Trypanosoma brucei* stocks isolated in Central Africa: evidence for an animal reservoir of sleeping sickness in Congo. *Acta Tropica* **49**, 127–135.
- Truc, P., Bailey, J.W., Doua, F., Laveissière, C. and Godfrey, D.G. (1994). A comparison of parasitological methods for the diagnosis of gambian trypanosomiasis in an area of low endemicity in Côte d'Ivoire. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 419–421.
- Turner, D.A. (1980). A novel marking–release recapture method for possible use in determining aspects of tsetse fly behaviour. *Insect Science and its Application* **1**, 9–13.
- Van Hoof, L. (1947). Observations on the trypanosomiasis in the Belgian Congo. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **40**, 728–754.
- Van Nieuwenhove, S. (1983). Impact of serodiagnosis (capillary haemagglutination) test on the transmission of *gambiense* sleeping sickness. In: *Proceedings of the International Scientific Council on Trypanosomiasis Research and Control, 17th meeting*, pp. 203–205. Nairobi: ISCTR: Scientific Publication Division, publication no. 11.
- Van Nieuwenhove, S. (1992). Trypanosomiase: efficacité et efficience des dépistages répétés. In: *Rapport Final du Séminaire de Modellisation Appliquée pour l'Optimisation des Prises de Décisions et du Suivi des Programmes de Contrôle de la Maladie du Sommeil* (J.D.F. Habbema and A. de Muynck, eds), pp. 45–53. Rotterdam: Erasmus University.
- Vos, G., Moloo, S.K., Nelson, R.T. and Gardiner, P.R. (1988). Attempts to protect goats against challenge with *Trypanosoma vivax* by initiation of primary infections with large numbers of metacyclic trypanosomes. *Parasitology* **97**, 383–392.
- Welburn, S.C. and Maudlin, I. (1992). The nature of the teneral state in *Glossina* and its role in the acquisition of trypanosome infection in tsetse. *Annals of Tropical Medicine and Parasitology* **86**, 529–536.
- Welburn, S.C., Maudlin, I. and Ellis, D.S. (1989). Rate of trypanosome killing by lectins in midguts of different species and strains of *Glossina*. *Medical and Veterinary Entomology* **3**, 77–82.
- Welde, B.T., Chumo, D.A., Waema, D., Reardon, M.J. and Smith, D.H. (1989a). A history of sleeping sickness in Kenya. *Annals of Tropical Medicine and Parasitology* **83**, supplement 1, 1–11.
- Welde, B.T., Chumo, D.A., Reardon, M.J., Waema, D., Smith, D.H., Gibson, W.C., Wanayma, L. and Siongok, T.A. (1989b). Epidemiology of Rhodesian sleeping sickness in the Lambwe Valley, Kenya. *Annals of Tropical Medicine and Parasitology* **83**, supplement 1, 43–62.
- Willett, K.C. (1963). Some principles of the epidemiology of human trypanosomiasis in Africa. *Bulletin of the World Health Organization* **28**, 645–652.
- Williams, B.I. (1996). African trypanosomiasis. In: *The Wellcome Trust Illustrated History of Tropical Diseases* (F.E.G. Cox, ed.), pp. 178–191. London: The Wellcome Trust.
- Woodruff, A.W., Evans, D.A. and Owino, N.O. (1982). A healthy carrier of African trypanosomiasis. *Journal of Infection* **5**, 89–92.
- World Bank (1993). *World Development Report 1993: Investing in Health*, pp. 216–218. Oxford: Oxford University Press.

- World Health Organization (1986). *Epidemiology and Control of African Trypanosomiasis. Report of a WHO Expert Committee*. Geneva: World Health Organization, Technical Report Series, no. 739.
- World Health Organization (1997). *Division of Control of Tropical Diseases. Progress Report 1996*. Geneva: World Health Organization.
- World Health Organization (1998). *Control and Surveillance of African Trypanosomiasis. Report of a WHO Expert Committee*. Geneva: World Health Organization, Technical Report Series, no. 881.
- World Health Organization (2000). *The World Health Report 2000: Health Systems Improving Performance*. Geneva: World Health Organization.
- Wyatt, G.B., Boatin, B.A. and Wurapa, F.K. (1985). Risk factors associated with the acquisition of sleeping sickness in north-east Zambia; a case-control study. *Annals of Tropical Medicine and Parasitology* **79**, 385-392.
- Zillmann, U., Mehlitz, D. and Sachs, R. (1984). Identity of *Trypanozoon* stocks isolated from man and a domestic dog in Liberia. *Tropical Medicine and Parasitology* **35**, 105-108.

Apoptosis and Parasitism: from the Parasite to the Host Immune Response

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ABSTRACT

Apoptosis, a form of programmed cell death (PCD), plays a central role in normal tissue development as well as in the pathogenesis of different diseases. PCD is responsible for the non-inflammatory physiological elimination of potentially harmful or unnecessary cells during embryogenesis, and for the proper functioning of continuous cell renewal systems in adult organisms. Maturation of the immune system and the specific immune response are examples of situations where PCD plays important roles. This review discusses the importance of apoptosis in two fundamental elements of

a host–parasite interaction: the parasite (Section 1), and the host's immune response (Section 2). Section 1 discusses questions raised by the description of apoptosis in unicellular eukaryotes, such as the evolutionary origin of the molecular components of PCD, its role in the emergence and maintenance of parasitism, and the constraints of a multicellular organization for the proper operation of a cell death programme. The proposal is that PCD can occur in any situation where living cells display features of an organized network which operates through interactions within themselves and/or with elements of their environment. The possibility is also discussed that evolutionary relics of a complete cell death system may operate in unicellular parasites with functions other than inducing cell death. Section 2 reviews data on the mechanisms of host-cell PCD and the consequences of this phenomenon in host defence and pathogenesis. Infectious agents, from viruses to parasites, can either delay or induce apoptosis of different types of host cells. Apoptosis following lymphocyte polyclonal activation and stimulation of peripheral T lymphocytes, as a result of the engagement of specific counter-receptor systems, is of special interest for defining host immunocompetence and mechanisms of immunopathology.

1. THE PARASITE

1.1. Apoptosis in Unicellular Organisms

Multicellular organisms can clearly benefit from the death of infected, developmentally unnecessary or potentially harmful subpopulations of their own cells (Kinloch *et al.*, 1999; Vaux and Korsmeyer, 1999). Intuitively, this should not be true for a population of unicellular organisms, which can be regarded as constituted by homogeneous, self-sufficient, and non-gregarious individuals (Welburn *et al.*, 1997). A corollary to this concept is that programmed cell death evolved after the onset of multicellularity. However, progressively accumulating evidence indicates that populations of parasitic protists, from bacteria to eukaryotes, can establish interorganismal communication among members and with cells and molecules of their eventual hosts. It is therefore not surprising that the constraints and demands for the proper operation of a single multicellular organism, or of a multicellular organization of unicellular organisms, can be similar in some very basic aspects of their functionality, such as the control of cell multiplication, differentiation and survival (Christensen *et al.*, 1998). With this perspective, it is interesting to note that several examples have been described of productive interactions – e.g., induction of multiplication, protein phosphorylation, receptor binding, and protection from death – between protozoan parasites and mammalian cytokines (defined in the broadest sense) that

are otherwise regulators of programmed cell death (PCD) (Barcinski and Costa-Moreira, 1994). Some of these include epidermal growth factor (EGF) (Hide *et al.*, 1989) and interferon γ (IFN γ) (Bakhiet *et al.*, 1990) with *Trypanosoma brucei*; interleukin-2 (IL-2) (Mazingue *et al.*, 1989), granulocyte-macrophage colony stimulating factor (GM-CSF) (Charlab *et al.*, 1990; Barcinski *et al.*, 1992), and insulin-like growth factor (Goto *et al.*, 1998) with *Leishmania* spp., and insulin-like growth factor with *Giardia duodenalis* (= *G. lamblia*) (see Luján *et al.*, 1994). As for PCD, the progressive characterization of its mechanism, with the identification of the molecular domains involved in this complex process, and the increase in the power of database searching and sequence comparisons, have allowed the evolutionary origin of many such protein domains to be traced back to unicellular organisms (Aravind *et al.*, 1999).

Cytochrome *c*, in addition to its central role in cell survival, is, when translocated to the cytoplasm, a crucial participant of the apoptotic process (Kluck *et al.*, 1997; Yang, J. *et al.*, 1997). This apparent contradiction can be resolved by considering the mechanism of PCD as a metazoan evolutionary refinement of the conflict between levels of selection due to the symbiotic origin of mitochondria (Blackstone and Green, 1999). Apoptosis might have appeared in evolution in conjunction with the symbiotic process that gave rise to the eukaryotes rather than at the onset of multicellularity (Kroemer, 1997). This could explain the ancient origin of several molecules involved in the apoptotic cascade. As a matter of fact, descriptions of programmed death in unicellular organisms are restricted to several examples of apoptotic or apoptotic-like death or to situations where the introduction of molecules known to regulate PCD in metazoa enables them to exert their effect when expressed in yeast cells, showing that part of the 'death machinery' exists in these unicellular organisms (Greenhalf *et al.*, 1996; Ink *et al.*, 1997; Manon *et al.*, 1997). A clear-cut, developmentally regulated programme of cell death in a non-metazoan organism has been described only in *Dictyostelium discoideum* (see Cornillon *et al.*, 1994), an amoeboid fungus considered to be an example of an evolutionary step towards multicellularity (Kaiser, 1986). A programme of nuclear (but not cell) death has been described in ciliates such as *Tetrahymena* (see Mpoke and Wolfe, 1996) and *Stylonychia* (see Maercker *et al.*, 1999), eukaryotic organisms containing macronuclei and micronuclei. These facts may indicate that, in different unicellular organisms, evolutionary ancestors of the cell death machinery, which is complete and fully operative only in metazoa, might be used for other purposes than killing cells (see below).

1.2. Apoptosis in Unicellular Parasites

In this section, only apoptosis of unicellular parasites will be described and discussed.

Trypanosoma brucei, the causative agent of human African trypanosomiasis or sleeping sickness, alternates between proliferative and non-proliferative forms when switching hosts. Most infective forms fail to become established in the insect host (*Glossina*; tsetse fly), and the great majority of parasites die upon their encounter with the fly (Welburn and Maudlin, 1999). Although the exact mechanism underlying this refractoriness is still not fully understood, it is clear that tsetse midgut lectins play a role in parasite killing and in the maturation to forms infective to mammals (Maudlin and Welburn, 1987). The establishment of a model *in vitro* for lectin-induced killing of *Trypanosoma brucei rhodesiense* has allowed the characterization of this type of death as presenting several features of apoptotic death, such as oligonucleosomal fragmentation of deoxyribonucleic acid (DNA), nuclear condensation and blebbing of the surface membrane (Welburn *et al.*, 1996). This type of cell death has been shown to be associated with differentially transcribed messenger ribonucleic acids (mRNAs), with some late upregulated mRNA species indicating active participation of the parasite in the death process (Murphy and Welburn, 1997). Complementary DNAs (cDNAs), corresponding to two upregulated transcripts, have been cloned and sequence analysis revealed that they encode for prohibitin, a mitochondrial protein, and for TRACK, the trypanosome receptor for activated kinase (Welburn and Murphy, 1998). It has been postulated that, within the tsetse fly, the maintenance of a steady-state number of parasites is due to an equilibrium between proliferation and active death (Welburn and Maudlin, 1999).

Similarly to *Trypanosoma brucei*, *Trypanosoma cruzi* also alternates its life cycle between an insect vector and a mammalian vertebrate host. Epimastigotes in the late stationary phase of culture *in vitro* have been shown to be dying, with ultrastructural changes characteristic of apoptosis. In this situation, oligonucleosomal DNA cleavage could not be detected; however, *in situ* terminal deoxytransferase-mediated dUTP (deoxyuridine triphosphate) nick end labelling (TUNEL) staining was able to detect DNA fragments in dying epimastigotes. The frequency of apoptotic death of epimastigotes increased when cells were cultured either at low cell densities or in poor medium (with serum deprivation), suggesting that extracellular signals, as described in other systems, are necessary for inhibiting programmed parasite death. Furthermore, Ameisen *et al.* (1995) submitted epimastigotes to stressing conditions to which trypomastigotes are supposedly preadapted, like heat shock and human complement. Apoptotic death was observed in both situations, including typical DNA cleavage in the latter. The antibiotic G418 also induced epimastigote death with an apoptotic DNA cleavage pattern. Parasites were rescued from death by transfection with the *neo*^r gene, indicating that death was not due to non-specific toxicity of the antibiotic (Ameisen *et al.*, 1995).

Apoptotic *Trypanosoma cruzi* were also found, by the TUNEL assay, in dog

hearts suffering acute experimental myocarditis (Zhang, J. *et al.*, 1999). Interestingly, it was shown that the elongation factor 1- α of *Trypanosoma cruzi* (TcEF-1 α) migrates from the cytoplasm to the nucleus in epimastigotes undergoing apoptosis induced by prolonging the stationary phase of culture or by G418 treatment. This finding raises the possibility that TcEF-1 α could act as a transcription factor with a role in the expression of genes involved in PCD (Billaut-Mullot *et al.*, 1996). Similar events to both those described above were also reported to occur in heat-shocked promastigotes of *Leishmania amazonensis* (see Moreira *et al.*, 1996). When submitted to heat shock, about 20% of promastigotes displayed morphological features characteristic of apoptotic death, clearly different from necrotic parasites, which were also present in the same preparation. Fully formed apoptotic bodies or even membrane protuberances (blebs) – frequently observed in other cell types undergoing PCD and also described in apoptotic *Trypanosoma brucei* and *Trypanosoma cruzi* – were never found in promastigotes. DNA oligonucleosomal breakage was also detected, in spite of the small percentage of dying forms and the simultaneous occurrence of necrotic death following heat shock. Probably for this reason, its detection depended upon the oligonucleosomal pattern being made more conspicuous by radioactive end-labelling of the DNA fragments.

In the case of *Leishmania*, apoptotic death was clearly Ca²⁺-dependent, as shown to be the case in other PCD situations. An increase in the free intracytoplasmatic concentration of free Ca²⁺ was observed in promastigotes submitted to a non-lethal heat shock. Disruption of the mitochondrial Ca²⁺ homeostatic function by a sub-lethal concentration of the proton ionophore FCCP (carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone) sensitizes the parasite to heat and oxidative stresses, suggesting the involvement of mitochondria in the parasite's apoptotic death (unpublished results). Ca²⁺-dependent death, induced by reactive oxygen species (ROS) with nuclear features of apoptosis, has also been described in *Trypanosoma brucei brucei*. After ROS treatment of the parasite, most of the nuclei were labelled by the TUNEL assay; the frequency of positive nuclei decreased after loading the cell with the cell-permeant calcium chelator EGTA/AM (ethylene glycol-bis (β -aminoethyl ether)N, N, N, N'-tetraacetic acid/acetoxy-methyl ester). In this last situation, inhibitors of caspase-1, caspase-3, calpain, serine and cystein-protease or proteasomes were incapable of interfering with the parasite's death. Also, transformed procyclic forms expressing Bcl-2 were not rescued from this form of death (Ridgley *et al.*, 1999).

Treatment *in vitro* with a 50% inhibitory concentration (IC₅₀) of chloroquine induced apoptotic-type internucleosomal DNA breakage in a chloroquine sensitive strain of *Plasmodium falciparum* (IC₅₀ = 40 nM), but not in a chloroquine resistant strain (IC₅₀ = 360 nM). Based on the findings that *Plasmodium falciparum* is sensitive to topoisomerase inhibitors which are

inducers of apoptosis in mammalian cells, that genes for topoisomerase I and II have been identified in this parasite, and that there are similarities between multidrug resistance in tumour cells and chloroquine resistance, the interesting hypothesis has been raised that apoptosis induction in malarial parasites could be the end result of chloroquine action (Picot *et al.*, 1997).

1.3. Apoptosis and Parasitism

It is interesting to note that all the examples of apoptosis in unicellular parasites have been described in agents of vector-borne diseases. Heteroxenous life styles have appeared at different moments in the evolution of parasitism (Fernandes *et al.*, 1993). Whether apoptosis co-evolved with heteroxeny, or whether its apparent restriction to heteroxenous parasites is due merely to its not having been looked for in monoxenous parasites, is still an open question.

Regardless of this, apoptosis in unicellular parasites can be selectively advantageous if, as stated above, some features of this process can be operative without necessarily resulting in death or, alternatively, if individual death altruistically benefits clonal populations. It has been postulated that *Trypanosoma* and *Plasmodium* species are largely clonal in nature (Tibayrenc *et al.*, 1990) and potential clonal competition in the insect vector has been experimentally demonstrated (Deane *et al.*, 1984; Reifenberg *et al.*, 1997). With *Trypanosoma brucei*, as already mentioned, apoptosis could be an important event in the tsetse fly, where strict parasite population size control operates. Since parasites and flies compete for proline, the maintenance of such an equilibrium, in which parasite multiplication is compensated by parasite death, can be mutually advantageous (Welburn and Maudlin, 1999). Indeed, procyclic trypanosomes displaying morphological features of apoptosis have been found in the midgut of flies (Welburn *et al.*, 1989).

In the parasite-mammalian host interaction, apoptosis could be useful in situations where macrophage deactivation and avoidance of inflammatory reaction are conditions for parasite survival. *Leishmania*, an obligatory intracellular parasite of macrophages in its mammalian hosts, is an example. Indeed, the ability of this parasite to trigger the synthesis of IL-12 and of nitric oxide is far less efficient than that displayed by *Trypanosoma cruzi*, which can also infect macrophages but is not an obligatory resident of this cell type (Camargo *et al.*, 1997; Gazzinelli *et al.*, 1997). We have evidence that *Leishmania amazonensis* amastigotes express phosphatidylserine in their external surface membrane, which is one of the earliest signs of apoptosis in multicellular organisms, thus mimicking a mammalian apoptotic cell in its ability to deactivate host macrophages upon internalization (J.M.F. Balanco, M.D. Gomes, J.R. Chagas and M.A. Barcinski, manuscript in preparation). This is an example of a phenotypic change characteristic of apoptotic death

being used to aid parasite survival instead of causing death.

It is interesting to note that apoptosis, as well as caspases, have been described in *Hydra vulgaris*, an organism that stands at the base of metazoan evolution (Cikala *et al.*, 1999; Mihai *et al.*, 1999). Based on the fact that *Hydra* can survive by simply recycling apoptotic cells, the authors speculated that the selective advantage of PCD for very primitive multicellular organisms lies not in the death of certain subpopulations but in the silent process of phagocytosis of apoptotic cells. In the fungus *Dictyostelium discoideum*, caspases have been shown to play a role in development and morphogenesis, but not in cell death (Olie *et al.*, 1998).

A suggestion has been made that, in unicellular organisms, caspases may be involved mainly in reshaping cell morphology (Depraetere and Golstein, 1998), and that their involvement in cell death may be a step in eukaryote evolution acquired after the emergence of multicellularity. This could also be the case with *Leishmania*, a more primitive eukaryote than *Dictyostelium*, in which caspase-like activity has also been detected (J.M.F. Balanco *et al.*, manuscript in preparation). During the cell cycle, promastigotes suffer drastic morphological changes and the infective forms are clearly different in shape from non-infective ones (Charlab *et al.*, 1995). Death in culture could be due to the absence of survival factors on which the parasite is dependent and that are present in nature.

2. THE HOST'S IMMUNE RESPONSE

2.1. Host Cell Apoptosis in Parasitic Infections in General: Implications for Immunopathogenesis

Regulation of host cell apoptosis is a critical determinant factor in host-pathogen interactions. On one hand, intracellular microbial pathogens manipulate host cell apoptosis, either to increase their life span within infected cells or to spread infection to other cells. On the other hand, the host immune response induces apoptosis of infected target cells in order to damage intracellular pathogens. In addition, host and pathogen induce lymphocyte apoptosis in the course of infection, which could alter the quality of subsequent immune responses.

There are several well-documented examples of pathogen manipulation of host cell apoptosis in infections caused by viruses and bacteria. While certain pathogenic DNA viruses actively inhibit apoptosis of their host cells (Cuff and Ruby, 1996), other viruses such as measles virus (Fugier-Vivier *et al.*, 1997) and human immunodeficiency virus (HIV) (Groux *et al.*, 1992) exacerbate apoptosis of host dendritic cells (Fugier-Vivier *et al.*, 1997) and T lymphocytes

(Groux *et al.*, 1992; Fugier-Vivier *et al.*, 1997). An increasing number of pathogenic bacteria have been shown to rely on host macrophage apoptosis as a virulence mechanism (Zychlinsky and Sansonetti, 1997). A secreted protein named YopJ, encoded within a virulence-promoting plasmid carried by pathogenic *Yersinia*, has been identified as an inducer *in vivo* of macrophage apoptosis, being responsible for spread of the infection in mice (Monack *et al.*, 1998). Bacterial invasion of epithelial (Kim *et al.*, 1998; Rudi *et al.*, 1998) and endothelial (Menzies and Kourteva, 1998) cell layers leads to apoptosis in these cell types. Apoptosis of intestinal epithelial cells infected with *Salmonella* or *Escherichia coli* is protracted, compared with induction of macrophage apoptosis by the same agents (Kim *et al.*, 1998). It has been suggested that delayed apoptosis is important, both for attaining an adequate inflammatory response by the host and for allowing time for bacteria to adapt to the intracellular environment (Kim *et al.*, 1998).

Another important issue is that infectious agents such as viruses and parasites cause vigorous polyclonal immune responses in the host. These responses, in turn, are subjected to apoptotic mechanisms of containment, creating a complex balance for immunoregulation. Effector leucocytes are produced to cope with the infection and, at the same time, must be eliminated to avoid the immunopathology that results from their unwanted continual activity. When apoptosis-based immunoregulation shortens effector cell activity before the pathogen is eliminated, chronic infection may ensue.

The best understood mechanisms of cell death in the host immune system are those involving mature (peripheral) T lymphocytes. Two largely distinct mechanisms of PCD have been identified in T cells. One is passive cell death (Van Parijs and Abbas, 1996), where inappropriately stimulated T cells become committed to die. It has been demonstrated that T cells can be protected from passive cell death by exposure to IL-2, or by co-stimulatory signals delivered through B7/CD28 interactions, which upregulate expression of the anti-apoptotic protein Bcl-xL (Van Parijs and Abbas, 1996). The second mechanism is activation-induced cell death (AICD), in which chronically activated T cells initiate an active, single cell-dependent death response (suicide) or one dependent on cell interaction (fratricide). AICD requires appropriate expression and function of cell surface death receptors and ligands, such as the Fas/Fas ligand (FasL) counter-receptor system, which is the major molecular effector of AICD (Van Parijs and Abbas, 1996). Fas (APO-1 or CD95) is a member of the tumour necrosis factor (TNF) receptor superfamily expressed by a wide variety of tissues (Baker and Reddy, 1998). Fas ligand is a trimeric protein member of the TNF superfamily, which can be expressed in either cell-bound or secreted forms. Compared with Fas, FasL has more restricted tissue expression, and is under tight regulatory control in activated T lymphocytes (Baker and Reddy, 1998). Fas transduces PCD through activation of a biochemical cascade that leads to caspase activation (Enari *et al.*, 1995; Baker and Reddy,

1998). Upon engagement of the T cell receptor for antigen (TCR), FasL message and protein are induced in T cells (Yang, Y. *et al.*, 1995). Engagement of Fas on the target cell by a FasL-bearing activated CD4⁺ T cell triggers intracellular caspase activation and apoptosis. There is evidence that this mechanism is relatively independent of the levels of Bcl family proteins (Van Parijs *et al.*, 1996), and that it is, in fact, potentiated by IL-2 (Lenardo, 1991). AICD of CD4⁺ T cells can also be effected by non-lymphoid cells through FasL induction. It has been shown that AICD *in vivo* in response to superantigen results from T cell killing mediated by liver and intestinal non-lymphoid cells which have been instructed by T cells to express FasL (Bonfoco *et al.*, 1998). In addition, an alternative death pathway conferred by TNF receptors/TNF- α has been described for AICD in T cells (Sytwu *et al.*, 1996). More recently, it became clear that the Fas/FasL pathway is involved with PCD induced by a variety of other chemical and physical injuries, and that it can also be expressed by non-lymphoid cells, such as macrophages and epithelial cells, under conditions of stress or inflammation.

While T cell apoptosis can be induced by these two distinct mechanisms in the course of parasitic infections, the end product of apoptosis, namely production of apoptotic bodies, may not be without effect on the host's immune system. At sites of immune privilege, such as the anterior chamber of the eye, Fas-induced lymphocyte apoptosis not only prevents a local inflammatory response but also induces systemic immune tolerance to the antigen applied to the eye (Griffith *et al.*, 1996). Recognition and engulfment of apoptotic bodies are major functions of phagocytes, both in embryogenesis and during immune responses, and are mediated by a set of pattern-recognition receptors that partially overlap with receptors for pathogen-associated molecular patterns (Franc *et al.*, 1999). More importantly, phagocytosis of apoptotic bodies by macrophages triggers an autocrine, and possibly also a paracrine, anti-inflammatory response mediated by released factors such as prostaglandin E2 (PGE₂), transforming growth factor β (TGF β) and platelet activating factor (PAF) (Fadok *et al.*, 1998), which could have further consequences for the immune regulation of parasitic infections.

2.2. Host Cell Apoptosis in Specific Parasitic Infections

From an evolutionary point of view, there are three main ways in which the induction of host cell apoptosis could benefit parasites and thus confer a selective advantage. First, as a virulence mechanism; host cell apoptosis could assist in the spreading of the infection. Rupture of infected macrophages or other tissue cells, as well as direct killing of uninfected leucocytes by parasite molecules, could be included in this category. Second, parasitic infections hyperstimulate host lymphocytes and take advantage of the marked

immunoregulatory changes that follow polyclonal lymphocyte activation. A third reason is that host cell apoptosis could stimulate the production of essential growth factors for parasites, as will be discussed later. However, it should be stressed that, at the same time, intracellular parasites also need to inhibit apoptosis in their host cells, in order to complete their intracellular life cycle and be transported to distant sites. Therefore, it is not surprising that both anti- and pro-apoptotic molecules have been described in a single virulent intracellular pathogen (Rojas *et al.*, 1997), and it is likely that their expression is differentially regulated by the pathogen according to its needs.

Parasites induce vigorous and repeated stimulation of host T lymphocytes, namely, polyclonal lymphocyte activation. Several pathogenic parasites are potent inducers of host polyclonal lymphocyte activation, both *in vivo* and *in vitro*. For example, potent polyclonal T cell responses *in vitro* to live parasites or parasite antigens have been described for *Trypanosoma cruzi*, *Toxoplasma gondii*, *Plasmodium* and *Leishmania* infections in humans. Although parasite mitogens cannot be excluded, the primary response usually requires antigen processing, and major histocompatibility complex [MHC] presentation (Van Voorhis, 1992; Piuvezam *et al.*, 1993) is usually more intense than alloreactive responses, and engages an unbiased CD4⁺ T cell $\alpha\beta$ TCR repertoire in humans (Piuvezam *et al.*, 1993; Goodier and Targett, 1997a). A superimposed superantigen-type response has also been described in mice, favouring certain V β gene segments, in the cases of *Toxoplasma gondii* (see Denkers *et al.*, 1996) and *Plasmodium yoelii* (see Pied *et al.*, 1997), among other parasites. Both memory and naive T cell subsets are engaged in the different systems investigated. Cytokine secretion in these responses is of mixed Th1/Th2 type, or Th1-biased (Van Voorhis, 1992; Russo *et al.*, 1998). In the response of previously non-exposed humans to *Plasmodium falciparum*, activated CD4⁺ T cells largely overlap with memory T cells reactive with tetanus toxoid and influenza antigens (Goodier and Targett, 1997b). Therefore, protozoan parasites activate a large fraction of both naive and memory T cell repertoires directed against a large set of cross-reactive peptide–MHC complexes. Besides the biochemical complexity of unicellular organisms, protozoan parasites express unique surface proteins with tandem repeats of alternated amino acid motifs, which generate a vast number of distinct peptide sequences following proteolytic processing (Kahn and Wleklinski, 1997). Polyclonal lymphocyte activation is a surprising and aggressive strategy employed by intracellular parasites, in many ways resembling induction of peripheral T cell tolerance after a vigorous immune response. In the latter case, a rapid phase of clonal expansion is followed by host elimination of most of the activated T cells. Peripheral deletion of activated CD4⁺ T cells is effected mainly by Fas-mediated AICD (Van Parijs and Abbas, 1996), and leads to immunological tolerance, for example if initiated at immunoprivileged sites (Griffith *et al.*, 1996). Accordingly, several studies have demonstrated antigen-specific and

polyclonal T cell unresponsiveness following initial polyclonal activation by the parasitic infection and, as discussed below, there is evidence that, in many cases, unresponsiveness is due to T cell apoptosis. Immunosuppression appears to be a general consequence of excessive immunostimulation. In a model of chronic immunostimulation, mice were treated for 6 months with human serum (Galdiero *et al.*, 1997). There was a marked reduction in T cell number, mainly CD4⁺ T cells, an increased ratio of lymphocyte apoptosis, and increased susceptibility to bacterial infection (Galdiero *et al.*, 1997). Interestingly, patients with chronic helminth infections also express reduced CD4/CD8 T cell ratios, increased proportions of T cells with memory or activated phenotype, and a marked increase in lymphocyte apoptosis (Kalinkovich *et al.*, 1998). These alterations were reversed after eradication of the infection (Kalinkovich *et al.*, 1998). Such changes in T cell regulation could have long-lasting effects favouring establishment of chronic infection.

On the other hand, little attention has been given to the possibility that lymphocyte apoptosis might also be of survival value for hosts with genetic susceptibility to pathogenic parasites. Induction of T cell apoptosis would be beneficial, since Th1 T cells are preferentially targeted by AICD (Zhang, X. *et al.*, 1997), and immunopathology caused by type 1 cytokines can be lethal for the infected susceptible host (Hunter *et al.*, 1997). Chronic parasitic infection could be a lesser price to pay than death of the host as a result of acute immunopathology.

2.2.1. American trypanosomiasis (*Chagas disease*)

Chagas disease is a human cardiac illness caused by chronic infection with the protozoan parasite *Trypanosoma cruzi*. The finding by Lopes *et al.* (1995) of lymphocyte PCD induced by *Trypanosoma cruzi* was the first demonstration of this phenomenon in a parasitic disease, and employed a murine model of experimental Chagas disease. Infection of mice with *Trypanosoma cruzi* leads to apoptosis of CD4⁺ and CD8⁺ T cells *in vivo* in the spleen, and to spontaneous apoptosis in culture (Lopes *et al.*, 1995). Addition of exogenous IL-2 rescues CD4⁺ and, notably, CD8⁺ T cells from spontaneous death (Lopes *et al.*, 1995), indicating that host T cells are prone to passive cell death in acute *Trypanosoma cruzi* infection. In addition, helper CD4⁺, but not cytotoxic CD8⁺, T cells undergo AICD following stimulation with anti-TCR or anti-CD3 antibodies (Lopes *et al.*, 1995). Addition of IL-2 actually potentiates T cell AICD. It has been suggested that T cell apoptosis in Chagas disease generates immunosuppression and that polyclonal lymphocyte activation drives T cells to a death-susceptible state (DosReis *et al.*, 1995). This hypothesis is in line with the propriocide hypothesis of mature T cell apoptosis, following engagement of the TCR in

actively cycling cells and in the presence of IL-2 (Lenardo, 1991). In agreement with this possibility, treatment of mice with hydroxyurea, which eliminates cycling cells *in vivo*, ameliorates T cell suppression in acute *Trypanosoma cruzi* infection (Fuchs and Barcinski, 1987). It was later demonstrated that induction of lymphocyte PCD directly accounts for suppression of CD4⁺ T cell proliferative responses (Figure 1; Lopes and DosReis, 1996). Infection with *Trypanosoma cruzi* upregulates the Fas death pathway, and leads to Fas-mediated CD4⁺ T cell killing, according to the following evidence (as described by Lopes *et al.*, 1999). (i) Purified CD4⁺ T cells from mice infected with *Trypanosoma cruzi* upregulate Fas and FasL mRNA and their functional products. (ii) CD4⁺ T cell AICD can be blocked with anti-FasL antibody. This treatment also increases host T cell proliferation, indicating

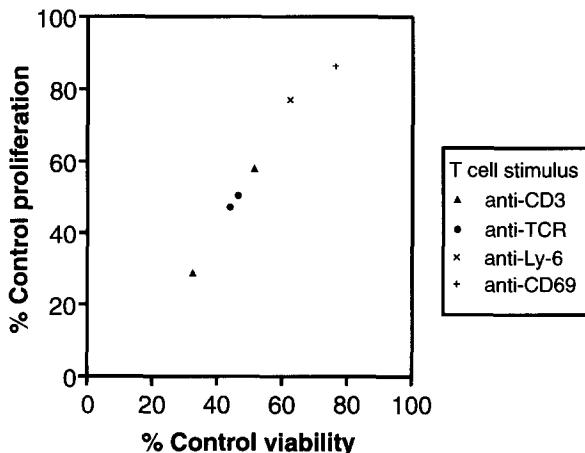


Figure 1 Linear relationship between CD4⁺ T cell unresponsiveness and programmed cell death in *Trypanosoma cruzi* infection. Splenic CD4⁺ T cells (containing accessory cells), from either *T. cruzi*-infected or control BALB/c mice, were stimulated *in vitro* with anti-CD3 (plastic bound and soluble forms), anti-TCR (T cell receptor for antigen), anti-Ly-6 and anti-CD69 monoclonal antibodies. Cell death was evaluated after 20 hours in culture, and expressed as a percentage of control T cell viability, as measured in cultures of control uninfected littermates. Proliferation was measured by tritiated thymidine uptake in sister cultures after 3 days, and expressed as a percentage of control (uninfected) T cell proliferation. Percentage of control cell viability and percentage of control proliferation were plotted for each T cell stimulus used. Unlike CD3 or TCR stimulation, Ly-6 or CD69 stimulation did not induce significant cell death in culture. [Data reproduced from Lopes, M.F., and DosReis, G.A. (1996), *Infection and Immunity* **64**, 1559–1564, with permission. Copyright © 1996 The American Society for Microbiology.]

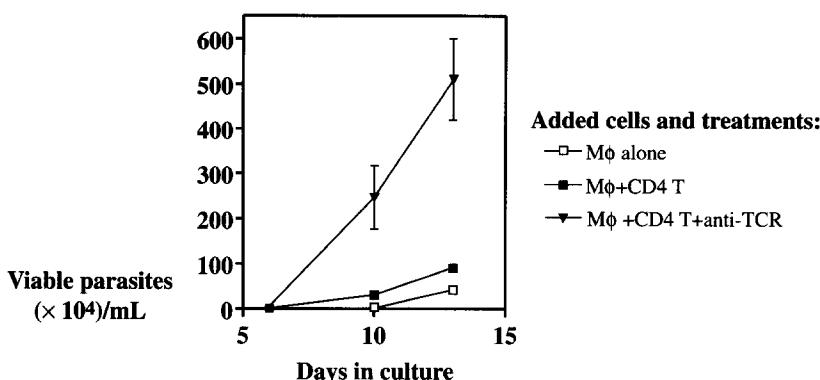


Figure 2 CD4⁺ T cell activation exacerbates replication of *Trypanosoma cruzi* in co-cultured macrophages. Splenic CD4⁺ T cells from BALB/c mice infected with *T. cruzi*-infected normal peritoneal macrophages (Mφ) infected with the same parasite and either stimulated with anti-TCR (T cell receptor for antigen) monoclonal antibody or left unstimulated. The number of motile trypomastigotes produced in the supernatants was measured after 6, 10 and 13 days in culture. The number of trypomastigotes produced by macrophages alone is also shown. [Data reproduced from Nunes, M.P., Andrade, R.M., Lopes, M.F. and DosReis, G.A. (1998), *Journal of Immunology* **160**, 1313–1319, with permission. Copyright © 1998 The American Association of Immunologists.]

that AICD targets fully functional, and not anergic, T cells. (iii) CD4⁺ T cell AICD is absent from mutant FasL-deficient *gld* mice infected with *Trypanosoma cruzi*.

The role of CD4⁺ T cell AICD in the control of intracellular replication of *Trypanosoma cruzi* has been investigated *in vitro* and *in vivo*. A model *in vitro* consisted of infection of normal peritoneal macrophages with *Trypanosoma cruzi*, followed by co-culture with CD4⁺ T cells from infected mice (Nunes *et al.*, 1998). If these CD4⁺ T cells were stimulated with anti-TCR, a large increase in parasite replication ensued (Figure 2; Nunes *et al.*, 1998). In this model, Fas ligation promoted lymphocyte apoptosis, markedly increased parasite growth, and ablated most of the T cell-derived IFN γ production (Nunes *et al.*, 1998). Accordingly, blockade of FasL prevented T cell apoptosis and reduced parasite growth (Nunes *et al.*, 1998). Treatment of macrophage monolayers alone with anti-FasL had no effect on macrophage viability or parasite replication, indicating that control by the Fas pathway was exerted at the level of the T cell. In agreement with these findings, CD4⁺ T cells from FasL-deficient *gld* mice infected with *Trypanosoma cruzi* were unable to exacerbate parasite growth in wild-type macrophages (Lopes *et al.*, 1999). These results

demonstrated that T cell activation in mice infected with *Trypanosoma cruzi* induces AICD and ablates IFN γ -producing T cells, favouring parasite replication in macrophages.

A model *in vivo* consisted of infection of FasL-deficient *gld* mice with *Trypanosoma cruzi*. Surprisingly, *gld* mice were more susceptible to infection, with higher and more sustained parasitaemia, than wild-type mice (Lopes *et al.*, 1999). Cytokine production by CD4 $^{+}$ T cells from infected *gld* mice showed a marked increase in Th2-type cytokine production (IL-4, IL-10), and treatment with anti-IL-4 *in vivo* abolished the increase in parasitaemia (Lopes *et al.*, 1999). These results suggested that increased susceptibility is due to an enhanced Th2-type immune response to infection in *gld* mice. The reason for this Th2 bias is not completely understood, since CD4 $^{+}$ T cell AICD preferentially targets Th1-type T cells (Varadachary *et al.*, 1997; Zhang, X. *et al.*, 1997), and an increased Th1 response would be expected in *gld* mice. However, the BALB background used in these experiments might have favoured Th2 responses, as BALB/c mice are prone to develop Th2 responses following infection with *Trypanosoma cruzi* (see Hoft *et al.*, 1993), and express a polymorphic trait for increased propensity of T cells to develop into Th2-type cells (Bix *et al.*, 1998). Phenotypic and functional alterations in T cell subsets associated with the *gld* mutation (Davidson *et al.*, 1986) could also be involved in increased Th2 responses. We have recently observed that the abnormal CD4 $^{-}/$ CD8 $^{-}$ B220 $^{+}$ T cell subset from infected *gld* mice increases parasite replication in macrophages following activation (A.C. Otero, M.P. Nunes, M.F. Lopes, and G.A. DosReis, unpublished observations). Another possible explanation of the increased susceptibility of *gld* mice is that a functional Fas/FasL pathway is required for clearance of parasite-infected target cells. Although normal peritoneal macrophages infected with *Trypanosoma cruzi* were not susceptible to the Fas/FasL pathway, the possibility that other tissue macrophages from infected hosts could be killed via the Fas pathway, especially after priming with inflammatory cytokines, cannot be excluded.

A third model to investigate the role of apoptosis in *Trypanosoma cruzi* infection was derived from the observation that physical contact between activated T cells and macrophages was required for increased parasite replication (Nunes *et al.*, 1998). This finding suggested a role for phagocytosis of apoptotic bodies. The model consisted of exposing macrophages infected with *Trypanosoma cruzi* to either apoptotic or necrotic T cells, produced either by heating or γ -irradiation (for apoptosis induction), or by freezing–thawing or chemical fixatives (for necrosis induction). Apoptotic, but not necrotic, T cells exacerbated *Trypanosoma cruzi* growth in infected macrophages (Freire-de-Lima *et al.*, 2000). The mechanism involved was related to the suppressive effects of apoptotic cells on the proinflammatory activity of phagocytes (Voll *et al.*, 1997; Fadok *et al.*, 1998). These effects are mediated by PGE₂ and TGF β secretion, and are triggered by ingestion of apoptotic cells (Fadok *et al.*,

1998). Macrophage uptake of apoptotic cells is effected by a set of pattern-recognition receptors that recognize a set of apoptotic cell-associated molecular patterns, and promote tissue remodelling and clearance of dying cells during development (Franc *et al.*, 1999). A major macrophage receptor for apoptotic cells is a complex formed by the scavenger receptor CD36 and the vitronectin receptor (VnR or $\alpha_v\beta_3$ integrin) (Savill, 1998). This receptor complex recognizes thrombospondin bound to the apoptotic cell surface (Savill, 1998). We identified a cascade of cell surface and biochemical events linking binding of apoptotic cells by macrophages to a burst of intracellular growth of *Trypanosoma cruzi* (Figure 3). We found that adhesion of apoptotic cells to macrophages engages VnR, and VnR cross-linkage triggers intense PGE₂ and TGF β production; TGF β secretion is required for parasite growth (Freire-de-Lima *et al.*, 2000). Through engagement of VnR and autocrine TGF β secretion, apoptotic cells induce marked *de novo* ornithine decarboxylase (ODC) activity and polyamine (mainly putrescine) synthesis by macrophages. Blockers of polyamine synthesis inhibit *Trypanosoma cruzi*

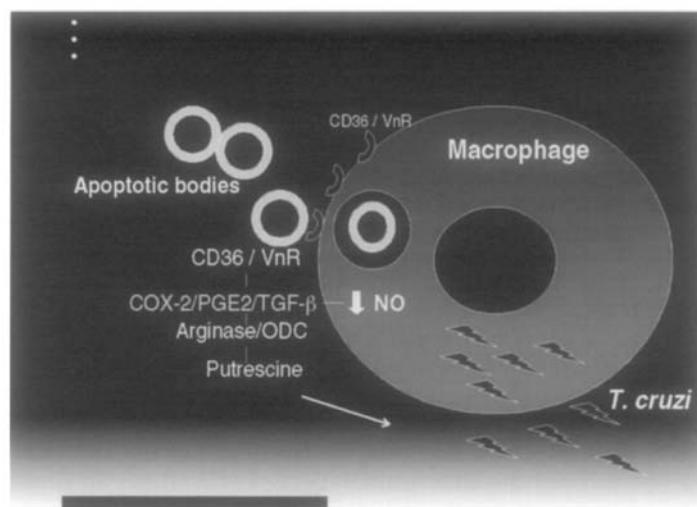


Figure 3 Phagocytosis of apoptotic cells drives parasite replication inside macrophages during *T. cruzi* infection. Acute infection of mice with *Trypanosoma cruzi* generates intense lymphocyte apoptosis and production of apoptotic bodies. These in turn are ingested by macrophages through a receptor complex that includes the vitronectin receptor (VnR). Engagement of the VnR generates prostaglandin and TGF β secretion, which in turn leads to induction of enzymes required for polyamine synthesis. These events ultimately block NO production and drive *Trypanosoma cruzi* growth (see text for details).

growth induced by apoptotic cells (Freire-de-Lima *et al.*, 2000). Moreover, parasitaemia can be exacerbated *in vivo* by injection of apoptotic cells, and pharmacological blockers of PGE₂ secretion prevent parasite replication *in vitro* and control parasitaemia *in vivo* (Freire-de-Lima *et al.*, 2000), suggesting that the phenomenon is immunopathogenic for *Trypanosoma cruzi* infection. Thus, the onset of host lymphocyte apoptosis is highly beneficial to the parasite, as it triggers intense TGF β production and, therefore, polyamine synthesis in macrophages, driving parasite growth (Figure 3). Additional mechanisms of parasite escape involving phagocyte receptors for apoptotic cells may exist. As noted previously (Franc *et al.*, 1999), there is an overlap between phagocyte receptors for apoptotic cells and for microorganisms, suggesting that certain pathogen-associated molecular patterns are similar to apoptotic cell molecular patterns.

A second mechanism of host T cell suppression in experimental Chagas disease is dependent on adherent cells, and involves nitric oxide (NO) production followed by suppressive effects on T cells (Abrahamsohn and Coffman, 1995). Secretion of proinflammatory cytokines such as IFN γ and TNF α is required for suppression (Abrahamsohn and Coffman, 1995). Moreover, NO production was involved in lymphocyte apoptosis in the course of infection with a virulent isolate of *Trypanosoma cruzi* (see Martins *et al.*, 1998). These results suggest that type 1 cytokines play a double role in the immune response to *Trypanosoma cruzi*, leading to both parasite control and host immunosuppression.

Recent studies have investigated the induction of host cell apoptosis by molecules purified or cloned from *Trypanosoma cruzi*. It has been reported that injection to mice of the enzyme *trans*-sialidase from *Trypanosoma cruzi* induces apoptosis of cells of the thymus, lymph nodes and spleen (Leguizamon *et al.*, 1999). Induction of apoptosis could contribute to alterations in the immune system and to the virulence-determinant effects previously ascribed to *trans*-sialidase (Chuenkova and Pereira, 1995). Glycoinositolphospholipids purified from *Trypanosoma cruzi*, or their isolated dihydroceramide moiety, are able to induce macrophage apoptosis in the presence of IFN γ , resulting in the release of viable intracellular parasites (Freire-de-Lima *et al.*, 1998). The differential role played by molecules that either block or promote apoptosis, and how these factors correlate with distinct degrees of virulence in *Trypanosoma cruzi* isolates, are issues that deserve investigation. Interestingly, induction of 65 kDa heat shock protein (hsp65) in macrophages from different murine strains correlates with their degree of resistance to *Trypanosoma cruzi* infection (Sakai *et al.*, 1999). Induction of hsp65 protected macrophages from apoptosis, and required natural killer (NK) cells, CD4 $^{+}$ T cells and IFN γ . It has been suggested that hsp65 plays a role in preventing macrophage apoptosis, and therefore hinders spread of *Trypanosoma cruzi* infection within the host (Sakai *et al.*, 1999). However, another report described massive destruction *in vivo* of

parasitized splenic macrophages in mice infected with *Trypanosoma cruzi*, coincident with a sudden drop in parasitaemia, onset of host mortality, and establishment of chronic infection (Cordeiro *et al.*, 1996). Further studies are necessary to clarify the role of macrophage apoptosis in *Trypanosoma cruzi* infection.

Finally, a possible role for apoptosis in myocardial damage following *Trypanosoma cruzi* infection has been proposed by DosReis *et al.* (1995). One study of human myocardial sections from patients with chronic Chagas disease found no evidence for myocardocyte apoptosis at this stage, although apoptosis was identified within mononuclear cell infiltrates (Rossi and Souza, 1999). However, this approach did not allow for the study of earlier stages of disease progression. Another study with a canine model of acute Chagas disease indicated that apoptosis is abundant in the inflammatory infiltrates of the heart, affecting lymphocytes, infected and uninfected myocytes, and also parasites (Zhang, J. *et al.*, 1999). This finding suggested that PCD can be important in the immunopathology of the earlier stages of Chagas disease, and showed that it applies to parasites, lymphoid cells and myocytes in the same inflammatory setting.

2.2.2. *Schistosomiasis*

In mice infected with *Schistosoma mansoni*, regional differences have been reported between T cells derived from hepatic granulomas and those from the spleen. While splenic T cells consisted of activated, actively cycling cells with high expression of intracellular IL-2, granuloma-derived T cells, although activated, were neither cycling nor expressing IL-2 to the same extent (Rumbley *et al.*, 1998). Increased apoptosis in granuloma-derived, but not splenic, T cells was observed (Rumbley *et al.*, 1998), suggesting a regional mechanism for modulation of *Schistosoma*-specific T lymphocytes. Another study found increased CD8⁺ and CD4⁺ T cell apoptosis in spleens of schistosome-infected mice following the onset of egg laying and of Th2-type responses (Fallon *et al.*, 1998). These results agree with previous findings of lymphocyte apoptosis *in vivo* in the spleen and in infiltrates surrounding egg deposits in the livers of mice infected with *Schistosoma mansoni* (see Estaquier *et al.*, 1997). Both spontaneous and activation-induced T cell apoptosis were found at the onset of a Th2-type response, and IL-10 appeared to be critically involved in premature T cell death (Estaquier *et al.*, 1997). Taken together, these results suggest an important role for T cell apoptosis in downregulation of a Th1 response, onset of a Th2 response, and establishment of chronic granulomatous reactions in experimental schistosomiasis.

2.2.3. Malaria

Tracking the fate of activated T cells in the course of experimental malaria suggests a role for T cell death in the immunopathogenesis of infection. Besides polyclonal T cell activation, murine infection by *Plasmodium yoelii* leads to peripheral deletion of CD4⁺ and CD8⁺ T cells expressing V β 9⁺ TCR (Pied *et al.*, 1997). The significance of peripheral deletion was tested in murine strains carrying endogenous retroviruses that eliminate V β 9⁺ T cells early in life. Mice lacking V β 9⁺ T cells were more susceptible to infection by *Plasmodium yoelii* than control mice (Pied *et al.*, 1997), suggesting that peripheral deletion of anti-parasite T cells favours the establishment of malarial infection. A second study employed cultured Th1-type CD4⁺ T cell lines specific for *Plasmodium berghei*. These cell lines control, but fail to eliminate completely, malarial parasites following adoptive transfer *in vivo* (Hirunpetcharat and Good, 1998). The transferred cell line was followed in the host after fluorescent labelling. Although the transferred cells could persist in the normal recipient host for more than 9 months, malarial infection of the recipient rapidly eliminated 99% of labelled T cells in the tissues, without any effect on T cells specific for ovalbumin (Hirunpetcharat and Good, 1998). The results suggest that infection-induced death of effector Th1 T cells limits the ability of the host to achieve complete elimination of the parasite.

2.2.4. Toxoplasmosis

Acute infection of mice with *Toxoplasma gondii* leads to expansion of T cells bearing the V β 5 TCR β chain, followed by partial clonal deletion and unresponsiveness in the chronic stage (Denkers *et al.*, 1996). Besides stimulation and deletion of V β 5⁺ T cells, acute infection of mice leads to polyclonal activation followed by apoptosis of host CD4⁺ T cells, which is related to transient T cell unresponsiveness (Khan *et al.*, 1996). In addition, oral infection of susceptible C57 BL6 mice with *Toxoplasma gondii* induces T cell deletion by apoptosis in the Peyer's patches, and apoptosis appears to result from a co-operative effect of IFN γ on Fas-mediated cell death (Liesenfeld *et al.*, 1997). Intraocular toxoplasmosis is more severe in Fas-deficient *lpr* C57 BL6 and FasL-deficient *gld* C57 BL6 mice, than in wild-type C57 BL6 mice, suggesting that intraocular Fas/FasL-mediated lymphocyte apoptosis helps to reduce intraocular inflammation in this model of toxoplasmosis (Hu *et al.*, 1999). Both virulence and protection against spread of *Toxoplasma* can be regulated by apoptosis of parasite-infected macrophages. Cells infected with *Toxoplasma* are resistant to multiple inducers of apoptosis (Nash *et al.*, 1998), and yet virulent *Toxoplasma* isolates induce macrophage apoptosis and parasite spread in the tissues (Hisaeda *et al.*, 1997). The host immune response

interferes with this pathogen-mediated regulation. Host-protective $\gamma\delta$ T cells induce heat shock protein hsp65 in infected macrophages, a protein that prevents both macrophage apoptosis and spread of virulent *Toxoplasma* infection (Hisaeda *et al.*, 1997). In some infections, apoptosis of the infected host cell may also damage the intracellular pathogen. However, lysis of *Toxoplasma*-infected cells by cytotoxic CD8⁺ T cells did not affect the viability of released *Toxoplasma gondii* (see Yamashita *et al.*, 1998). Resistance of *Toxoplasma* differs from that of mycobacteria. Perforin-mediated apoptosis of macrophages infected with mycobacteria reduced bacterial viability, while Fas-mediated apoptosis released fully viable bacteria (Stenger *et al.*, 1997). Following infected host cell apoptosis, the viability and fate of the released microorganisms could play an important role in protection or pathogenesis. Release of bacteria may be necessary to dislodge them from aged, unresponsive host cells, allowing uptake by recently activated, bactericidal macrophages (Fratazzi *et al.*, 1997). In the case of highly motile parasites, release of viable pathogens might help the spread of infection, as suggested previously (Barcinski and DosReis, 1999). Whether the outcome of microbial release is beneficial or deleterious for the host depends on biological properties of the pathogen (such as adhesion to, and ingestion by, passenger cells and haematogenous spread), on its location, and on the inflammatory setting associated with the infection.

2.2.5. *Leishmaniasis*

Bone marrow macrophages infected with *Leishmania donovani* are resistant to apoptosis by factor deprivation (Moore and Matlashewski, 1994). The effect was attributed to autocrine GM-CSF secretion by *Leishmania*-infected macrophages, and leishmanial lipophosphoglycan (LPG) was identified as responsible for this anti-apoptotic effect (Moore and Matlashewski, 1994). In murine visceral leishmaniasis induced by *Leishmania donovani*, host CD4⁺ T lymphocyte apoptosis and reduced Th1 cytokine production have been described at early phases of infection (Das *et al.*, 1999). In murine visceral leishmaniasis induced by *Leishmania chagasi*, no evidence for splenic CD4⁺ T cell AICD was found at the chronic stage, in spite of high parasite burdens in the spleens of infected animals. Reduced Th1 T cell activity was present and depended upon suppressive signals by the CTLA-4 (cytotoxic T lymphocyte associated antigen 4)/B7 negative co-stimulatory pathway (Gomes *et al.*, 1998). CTLA-4 engagement does not induce apoptosis (Blair *et al.*, 1998), but induces autocrine TGF- β secretion (Chen, W. *et al.*, 1998), and TGF- β inhibits T-cell AICD (Genestier *et al.*, 1999). Increased TGF- β secretion mediates the suppressive effects of CTLA-4 engagement in chronic murine visceral leishmaniasis (Gomes *et al.*, 2000). It is likely that, in the early stages of visceral

leishmaniasis, apoptosis could be induced in the T cells, as described (Das *et al.*, 1999), and could play a pathogenic role.

Recent studies suggest a novel role of host cell apoptosis in the course of *Leishmania major* infection (Conceição-Silva *et al.*, 1998; Huang *et al.*, 1998). Mice deficient in either Fas (Huang *et al.*, 1998) or FasL (Conceição-Silva *et al.*, 1998), and infected with *Leishmania major*, displayed a progressive increase in local parasite burden and non-healing lesions, suggesting a major protective role of the Fas/FasL pathway against parasite infection. Local injection of a soluble FasL construct restored the ability to control parasite number (Conceição-Silva *et al.*, 1998). Fas deficiency is sufficient to render the animals susceptible and non-healing regarding *Leishmania major* infection, in spite of exacerbated Th1-type responses and increased IL-12 and NO secretion (Huang *et al.*, 1998). It has been proposed that *Leishmania*-infected macrophages are killed by effector CD4⁺ T cells via the Fas/FasL pathway, and that this killing is required for control of *Leishmania major* infection (Conceição-Silva *et al.*, 1998). It remains to be investigated whether Fas-mediated killing of infected macrophages also kills released parasites, or whether released immature parasites are killed by other mechanisms.

2.2.6. Other parasitic infections

The protozoan parasite *Cryptosporidium parvum* causes persistent diarrhoea and malnutrition in immunocompromised patients with acquired immune deficiency syndrome. Infection of intestinal and biliary epithelial cell lines with *Cryptosporidium parvum* results in host cell apoptosis; caspase inhibitors block apoptosis and increase the number of infected cells (Chen, X.M. *et al.*, 1999; Ojcius *et al.*, 1999). Apoptosis of biliary epithelial cells is Fas/FasL-dependent, since infection increases both Fas and FasL expression by infected cells, induces FasL secretion, and promotes apoptosis of bystander uninfected cells across a cell-impermeable membrane (Chen, X.M. *et al.*, 1999). It was suggested that epithelial cell apoptosis plays a role in the pathogenesis of the infection. Acute infection of rats with the nematode parasite *Nippostrongylus brasiliensis* also induces apoptosis of villus epithelial cells in the small intestine, as assessed by nick end-labelling of apoptotic nuclei *in situ* (Hyoh *et al.*, 1999). Splenic accessory cells from mice infected with *Nippostrongylus brasiliensis* inhibit lymphocyte AICD and increase T cell proliferation through secretion of IL-6 (Liwska and Lee, 1999). It has been suggested that enhanced IL-6 secretion by accessory cells and blockade of AICD could be involved in the genesis of the Th2-biased immune responses seen after concomitant *Nippostrongylus brasiliensis* infection (Liwska and Lee, 1999).

3. CONCLUDING REMARKS

As discussed in the previous sections, the role of host cell apoptosis in parasitic infections is rather complex, and appears to depend in a unique way on each combination of host and parasite molecules. The relevant steps of infection in which apoptosis plays a role are briefly outlined in Figure 4. One important

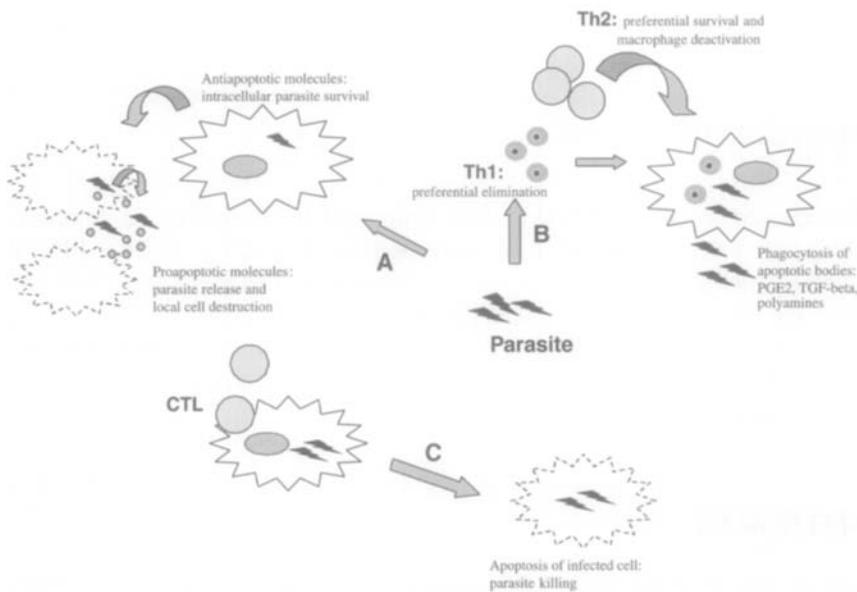


Figure 4 A summary of the roles of host cell apoptosis in parasitic infection. A. Parasite regulation of host cell apoptosis. Intracellular parasites express anti-apoptotic molecules that extend their survival by blocking apoptosis of the infected host cell, allowing spread of the passenger parasite to distant sites. When a critical level of intracellular parasite load is reached, pro-apoptotic molecules induce host cell apoptosis which spreads the infection. Bystander uninfected leucocytes can also be killed by pro-apoptotic parasite molecules. B. Immunoregulatory roles of lymphocyte apoptosis. Following parasite-induced polyclonal activation, Th1 T cells are preferentially eliminated by activation-induced cell death. Reduction of Th1 cytokines and effector cells aids parasite escape. Th2 T cells preferentially resist apoptosis, and help parasite persistence by deactivating macrophages. Phagocytosis of apoptotic bodies by macrophages induces autocrine and paracrine anti-inflammatory effects through generation of prostaglandin, TGF β and polyamines (see Figure 3), aiding parasite survival and growth. C. Immunoprotective role of apoptosis. Effector cytotoxic lymphocytes (CTL) can lyse cells infected with *Leishmania major* through the Fas death pathway, either damaging parasite DNA or promoting the release of immature parasites that are killed by additional mechanisms. However, other intracellular parasites, such as *Toxoplasma gondii*, are resistant to CTL-mediated killing. Some model interactions depicted in Figure 4 are also valid for extracellular, more complex parasites such as helminths (see text for discussion of a role for parasite apoptosis in regulation of the host immune response).

issue to be considered, as indicated in Figure 4, is whether either deleterious or protective immune mechanisms will be allowed to develop in the host, depending on innate genetic factors inherited as polymorphic traits. In the future, it is likely that effective manipulation of host cell apoptosis will become feasible, either through the use of soluble recombinant constructs and monoclonal antibodies that interfere with the Fas/FasL death pathway, or through pharmacological agents that interfere with the cellular apoptotic machinery. Such reagents would be helpful in the management of the host immune response to parasitic infection.

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REFERENCES

- Abrahamsohn, I.A. and Coffman, R.L. (1995). Cytokine and nitric oxide regulation of the immunosuppression in *Trypanosoma cruzi* infection. *Journal of Immunology* **155**, 3955–3963.
- Ameisen, J.C., Idziorek, T., Billot-Mulot, O., Loyens, M., Tissier, J.-P., Potentier, A. and Ouassi, A.(1995). Apoptosis in a unicellular eukaryote (*Trypanosoma cruzi*): implications for the evolutionary origin and role of programmed cell death in the control of cell proliferation, differentiation and survival. *Cell Death and Differentiation* **2**, 285–300
- Aravind, L., Dixit, V.M. and Koonin, E.V.(1999). The domains of death: evolution of the apoptosis machinery. *Trends in Biochemical Sciences* **24**, 47–53
- Baker, S. J. and Reddy, E. P. (1998). Modulation of life and death by the TNF receptor superfamily. *Oncogene* **17**, 3261–3270.
- Bakhiet, M., Olsson, T., van der Meide, P. and Kristensson, K. (1990). Depletion of CD8+ T cells suppresses growth of *Trypanosoma brucei brucei* and interferon-gamma production in infected rats. *Clinical and Experimental Immunology* **81**, 195–199.
- Barcinski, M.A. and Costa-Moreira, M.E. (1994). Cellular response of protozoan parasites to host-derived cytokines. *Parasitology Today* **10**, 352–355.
- Barcinski, M.A. and DosReis, G.A. (1999). Apoptosis in parasites and parasite-induced apoptosis in the host immune system: a new approach for the parasitic diseases. *Brazilian Journal of Medical and Biological Research* **32**, 395–401.
- Barcinski, M.A., Schehtman, D., Quintão, LG., Costa, D.A., Soares, L.R.B., Moreira, M.E.C.

- and Charlab, R. (1992). Granulocyte-macrophage colony stimulating factor increases the infectivity of *Leishmania amazonensis* by protecting promastigotes from heat-induced death. *Infection and Immunity* **60**, 3523–3527.
- Billaut-Mulot, Fernandez-Gomez, R., Loyens, M. and Ouassi, A. (1996). *Trypanosoma cruzi* elongation factor 1 α : nuclear localization in parasites undergoing apoptosis. *Gene* **174**, 19–26.
- Bix, M., Wang, Z.E., Thiel, B., Schork, N.J. and Locksley, R.M. (1998). Genetic regulation of commitment to interleukin 4 production by a CD4 $^{+}$ T cell-intrinsic mechanism. *Journal of Experimental Medicine* **188**, 2289–2299.
- Blackstone, N.W. and Green, D.G. (1999). The evolution of a mechanism of cell suicide. *BioEssays* **21**, 84–88.
- Blair, P.J., Riley, J.L., Levine, B.L., Lee, K.P., Craighead, N., Francomano, T., Perfetto, S.J., Gray, G.S., Carreno, B.M. and June, C.H. (1998). CTLA-4 ligation delivers a unique signal to resting human CD4 T cells that inhibits interleukin-2 secretion but allows Bcl-X(L) induction. *Journal of Immunology* **160**, 12–15.
- Bonfoco, E., Stuart, P.M., Brunner, T., Lin, T., Griffith, T.S., Gao, Y., Nakajima, H., Henkart, P., Ferguson, T.A. and Green, D.R. (1998). Inducible non-lymphoid expression of Fas ligand is responsible for superantigen-induced peripheral deletion of T cells. *Immunity* **9**, 711–720.
- Camargo, M.M., Andrade, A.C., Almeida, I.C., Travassos, L.R. and Gazzinelli, R.T. (1997). Glycoconjugates isolated from *Trypanosoma cruzi* but not from *Leishmania* species membranes trigger nitric oxide synthesis as well as microbicidal activity in IFN- γ -primed macrophages. *Journal of Immunology* **159**, 6131–6139.
- Charlab, R., Blaineau C., Schechtman, D. and Barcinski, M.A. (1990). Granulocyte-macrophage colony-stimulating factor is a growth factor for promastigotes of *Leishmania mexicana amazonensis*. *Journal of Protozoology* **37**, 352–357.
- Charlab, R., Tesh, R.B., Rowton, E.D. and Ribeiro, J.M.C. (1995). *Leishmania amazonensis*: sensitivity of different promastigote morphotypes to salivary gland homogenates of the sandfly *Lutzomyia longipalpis*. *Experimental Parasitology* **80**, 167–175.
- Chen, W., Jin, W. and Wahl, S. (1998). Engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) induces transforming growth factor β (TGF- β) production by murine CD4 $^{+}$ T cells. *Journal of Experimental Medicine* **188**, 1849–1857.
- Chen, X.M., Gores, G.J., Paya, C.V. and La Russo, N.F. (1999). *Cryptosporidium parvum* induces apoptosis in biliary epithelia by a Fas/Fas ligand-dependent mechanism. *American Journal of Physiology* **277**, G599–G608.
- Christensen, S.T., Leick, V., Rasmussen, L. and Wheatley, D.N. (1998). Signaling in unicellular eukaryotes. *International Review of Cytology* **177**, 181–253.
- Chuenkova, M. and Pereira, M.E.A. (1995). *Trypanosoma cruzi* trans-sialidase: enhancement of virulence in a murine model of Chagas' disease. *Journal of Experimental Medicine* **181**, 1693–1703.
- Cikala, M., Wilm, B., Hobmayer, E., Bottger, A. and David, C.N. (1999). Identification of caspases and apoptosis in the simple metazoan *Hydra*. *Current Biology* **9**, 959–962.
- Conceição-Silva, F., Hahne, M., Schroter, M., Louis, J. and Tschoopp, J. (1998). The resolution of lesions induced by *Leishmania major* in mice requires a functional Fas (APO-1, CD95) pathway of cytotoxicity. *European Journal of Immunology* **28**, 237–245.
- Cordeiro, Z.M., Dahia, A.C. and Andrade, Z.A. (1996). Kinetics of *Trypanosoma cruzi* destruction in the mouse spleen. *Revista da Sociedade Brasileira de Medicina Tropical* **30**, 3–9.
- Cornillon, S., Foa, C., Davoust, J., Buonavista, N., Gross, J.D. and Golstein, P. (1994). Programmed cell death in *Dictyostelium*. *Journal of Cell Science* **107**, 2691–2704.

- Cuff, S. and Ruby, J. (1996). Evasion of apoptosis by DNA viruses. *Immunology and Cell Biology* **74**, 527–537.
- Das, G., Vohra, H., Rao, K., Saha, B. and Mishra, G. C. (1999). *Leishmania donovani* infection of a susceptible host results in CD4⁺ T-cell apoptosis and decreased Th1 cytokine production. *Scandinavian Journal of Immunology* **49**, 307–310.
- Davidson, W.F., Dumont, F.J., Bedigian, H.G., Fowlkes, B.J. and Morse, H.C., III (1986). Phenotypic, functional, and molecular genetic comparisons of the abnormal lymphoid cells of C3H-lpr/lpr and C3H-gld/gld mice. *Journal of Immunology* **136**, 4075–4084.
- Deane, M.P., Souza, M.A., Pereira, N.M., Gonçalves, A.M., Momen, H. and Morel, C.M. (1984). *Trypanosoma cruzi*: inoculation schedules and re-isolation methods select individual strains from doubly infected mice, as demonstrated by schizodeme and zymodeme analysis. *Journal of Protozoology* **31**, 276–280.
- Denkers, E.Y., Caspar, P., Hiieny, S. and Sher, A. (1996). *Toxoplasma gondii* infection induces specific nonresponsiveness in lymphocytes bearing the V-beta 5 chain of the mouse T cell receptor. *Journal of Immunology* **156**, 1089–1094.
- Depraetere, V. and Golstein, P. (1998). Dismantling in cell death: molecular mechanisms and relationship to caspase activation. *Scandinavian Journal of Immunology* **47**, 523–531.
- DosReis, G.A., Fonseca, M.E.F. and Lopes, M.F. (1995). Programmed T-cell death in experimental Chagas disease. *Parasitology Today* **11**, 390–394.
- Enari, M., Hug, H. and Nagata, S. (1995). Involvement of an ICE-like protease in Fas-mediated apoptosis. *Nature* **375**, 78–81.
- Estquier, J., Marguerite, M., Sahuc, F., Bessis, N., Auriault, C. and Ameisen, J. C. (1997). Interleukin-10-mediated T cell apoptosis during the T helper type 2 cytokine response in murine *Schistosoma mansoni* parasite infection. *European Cytokine Network* **8**, 153–160.
- Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y. and Henson, P. M. (1998). Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE-2 and PAF. *Journal of Clinical Investigation* **101**, 890–898.
- Fallon, P.G., Smith, P. and Dunne, D.W. (1998). Type 1 and type 2 cytokine-producing mouse CD4⁺ and CD8⁺ T cells in acute *Schistosoma mansoni* infection. *European Journal of Immunology* **28**, 1408–1416.
- Fernandes, A.P., Nelson, K. and Beverley, S.M. (1993). Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspectives on the age and origins of parasitism. *Proceedings of the National Academy of Sciences of the USA* **90**, 11608–11612.
- Franc, N.C., White, K. and Ezekowitz, R.A.B. (1999). Phagocytosis and development: back to the future. *Current Opinion in Immunology* **11**, 47–52.
- Fratazzi, C., Arbeit, R.D., Carini, C. and Remold, H.G. (1997). Programmed cell death of *Mycobacterium avium* serovar 4-infected human macrophages prevents the mycobacteria from spreading and induces mycobacterial growth inhibition by freshly added, uninfected macrophages. *Journal of Immunology* **158**, 4320–4327.
- Freire-de-Lima, C.G., Nunes, M.P., Corte-Real, S., Soares, M.P., Previato, J.O., Mendonça-Previato, L. and DosReis, G. A. (1998). Proapoptotic activity of a *Trypanosoma cruzi* ceramide-containing glycolipid turned on in host macrophages by IFN-γ. *Journal of Immunology* **161**, 4909–4916.
- Freire-de-Lima, C.G., Nascimento, D.O., Soares, M.B.P., de Mello, F.G., Bozza, P.T., Castro-Faria-Neto, H.C., DosReis, G.A. and Lopes, M.F. (2000). Uptake of apoptotic cells drives the growth of a pathogenic trypanosome in macrophages. *Nature* **403**, 199–203.
- Fuchs, R. and Barcinski, M.A. (1987). Effect of hydroxyurea on the concanavalin A proliferative response of *Trypanosoma cruzi*-infected mice. *Immunology Letters* **15**, 317–321.

- Fugier-Vivier, I., Server-Delprat, C., Rivailler, P., Rissoan, M.C., Liu, Y.J. and Rabourdin-Combe, C. (1997). Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. *Journal of Experimental Medicine* **186**, 813–823.
- Galdiero, F., Galdiero, M., Nuzzo, I., Vitiello, M., Bentivoglio, C. and Romano-Caratelli, C. (1997). Polyclonal T cell elimination by prolonged immunostimulation in an experimental model. *Clinical and Experimental Immunology* **110**, 182–188.
- Gazzinelli, R.T., Camargo, M.M., Almeida, I.C., Morita, Y.S., Giraldo, M., Acosta-Serrano, A., Hieny, S., Englund, P.T., Ferguson, M.A.J., Travassos, L.R. and Sher, A. (1997). Identification and characterization of protozoan products that trigger the synthesis of IL-12 by inflammatory macrophages. *Chemical Immunology* **68**, 136–152.
- Genestier, L., Kasibhatla, S., Brunner, T. and Green, D.R. (1999). Transforming growth factor β 1 inhibits Fas ligand expression and subsequent activation-induced cell death in T cells via downregulation of c-Myc. *Journal of Experimental Medicine* **189**, 231–239.
- Gomes, N.A., Barreto-de-Souza, V., Wilson, M.E. and DosReis, G.A. (1998). Unresponsive CD4 $^{+}$ T lymphocytes from *Leishmania chagasi*-infected mice increase cytokine production and mediate parasite killing after blockade of B7-1/CTLA-4 molecular pathway. *Journal of Infectious Diseases* **178**, 1847–1851.
- Gomes, N.A., Gattass, C.R., Barreto-de-Souza, V., Wilson, M.E. and DosReis, G.A. (2000). Transforming growth factor-beta mediates CTLA-4 suppression of cellular immunity in murine kala-azar. *Journal of Immunology* **164**, 2001–2008.
- Goodier, M.R. and Targett, G.A. (1997a). Polyclonal T-cell responses to *Plasmodium falciparum* gametocytes in malaria non-exposed donors. *Parasite Immunology* **19**, 419–425.
- Goodier, M.R. and Targett, G.A. (1997b). Evidence for CD4 $^{+}$ T cell responses common to *Plasmodium falciparum* and recall antigens. *International Immunology* **9**, 1857–1865.
- Goto, H., Gomes, C.M.C., Corbett, C.E.P., Monteiro, H.P. and Gidlund, M. (1998). Insulin-like growth factor I is a growth-promoting factor for *Leishmania* promastigotes and amastigotes. *Proceedings of the National Academy of Sciences of the USA* **95**, 13211–13216.
- Greenhalf, W., Stephan, C. and Chaudhuri, B. (1996). Role of mitochondria and C-terminal membrane anchor of Bcl-2 in Bax induced growth arrest and mortality in *Saccharomyces cerevisiae*. *FEBS Letters* **380**, 169–175.
- Griffith, T.S., Yu, X., Herndon, J.M., Green, D.R. and Ferguson, T.A. (1996). CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. *Immunity* **5**, 7–16.
- Groux, H., Torpier, G., Monté, D., Capron, A. and Ameisen, J.C. (1992). Activation-induced death by apoptosis in CD4 $^{+}$ T cells from human immunodeficiency virus-infected asymptomatic individuals. *Journal of Experimental Medicine* **175**, 331–340.
- Hide, G., Gray, A., Harrison, C.M. and Tait, A. (1989). Identification of an epidermal growth factor receptor homologue in trypanosomes. *Molecular and Biochemical Parasitology* **36**, 51–60.
- Hirunpetcharat, C. and Good, M.F. (1998). Deletion of *Plasmodium berghei*-specific CD4 $^{+}$ T cells adoptively transferred into recipient mice after challenge with homologous parasite. *Proceedings of the National Academy of Sciences of the USA* **95**, 1715–1720.
- Hisaeda, H., Sakai, T., Ishikawa, H., Maekawa, Y., Yasutomo, K., Good, R.A. and Himeno, K. (1997). Heat shock protein 65 induced by gamma-delta T cells prevents apoptosis of macrophages and contributes to host defense in mice infected with *Toxoplasma gondii*. *Journal of Immunology* **159**, 2375–2381.
- Hoft, D.F., Lynch, R.G. and Kirchhoff, L.V. (1993). Kinetic analysis of antigen-specific

- immune responses in resistant and susceptible mice during infection with *Trypanosoma cruzi*. *Journal of Immunology* **151**, 7038–7047.
- Hu, M.S., Schwartzmen, J.D., Yeaman, G.R., Collins, J., Seguin, R., Khan, I.A. and Kasper, L.H. (1999). Fas–FasL interaction involved in pathogenesis of ocular toxoplasmosis. *Infection and Immunity* **67**, 928–935.
- Huang, F.P., Xu, D., Esfandiari, E.O., Sands, W., Wei, X.Q. and Liew, F.Y. (1998). Mice defective in Fas are highly susceptible to *Leishmania major* infection despite elevated IL-12 synthesis, strong Th1 responses, and enhanced nitric oxide production. *Journal of Immunology* **160**, 4143–4147.
- Hunter, C.A., Ellis-Neyes, L.A., Slifer, T., Kanaly, S., Grunig, G., Fort, M., Rennick, D. and Araújo, F.G. (1997). IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *Journal of Immunology* **158**, 3311–3316.
- Hyoh, Y., Nishida, M., Tegoshi, T., Yamada, M., Uchikawa, R., Matsuda, S. and Arizono, N. (1999). Enhancement of apoptosis with loss of cellular adherence in the villus epithelium of the small intestine after infection with the nematode *Nippostrongylus brasiliensis* in rats. *Parasitology* **119**, 199–207.
- Ink, B., Zornig, M., Baum, B., Hajibagheri, N., James, C., Chittenden, T. and Evan, G. (1997). Human Bak induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells. *Molecular and Cellular Biology* **17**, 2468–2474.
- Kahn, S.J. and Wlekliński, M. (1997). The surface glycoproteins of *Trypanosoma cruzi* encode a superfamily of variant T cell epitopes. *Journal of Immunology* **159**, 4444–4451.
- Kaiser, D. (1986). Control of multicellular development: *Dictyostelium* and *Myxococcus*. *Annual Review of Genetics* **20**, 539–566.
- Kalinkovich, A., Weisman, Z., Greenberg, Z., Nahmias, J., Eitan, S., Stein, M. and Bentwich, R.Z. (1998). Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. *Clinical and Experimental Immunology* **114**, 414–421.
- Khan, I.A., Matsuura, T. and Kasper, L.H. (1996). Activation-mediated CD4⁺ T cell unresponsiveness during acute *Toxoplasma gondii* infection in mice. *International Immunology* **8**, 887–896.
- Kim, J.M., Eckmann, L., Savidge, T.C., Lowe, D.C., Witthoft, T. and Kagnoff, M.F. (1998). Apoptosis of human intestinal epithelial cells after bacterial invasion. *Journal of Clinical Investigation* **102**, 1815–1823.
- Kinloch, R.A., Treherne, J.M., Furness, L.M. and Hajimohammadreza, I. (1999). The pharmacology of apoptosis. *Trends in Pharmacological Sciences* **20**, 35–42.
- Kluck, R.M., Bossy-Wetzel, E., Green, D.R. and Newmeyer, D.D. (1997). The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* **275**, 1132–1136.
- Kroemer, G. (1997). Mitochondrial implication in apoptosis: towards an endosymbiont hypothesis of apoptosis evolution. *Cell Death and Differentiation* **4**, 443–456.
- Leguizamón, M.S., Mocetti, E., García-Rivello, H., Argibay, P. and Campetella, O. (1999). Trans-sialidase from *Trypanosoma cruzi* induces apoptosis in cells from the immune system *in vivo*. *Journal of Infectious Diseases* **180**, 1398–1402.
- Lenardo, M.J. (1991). Interleukin-2 programs mouse $\alpha\beta$ T lymphocytes for apoptosis. *Nature* **353**, 858–861.
- Liesenfeld, O., Kosek, J.C. and Suzuki, Y. (1997). Gamma interferon induces Fas-dependent apoptosis of Peyer's patch T cells in mice following peroral infection with *Toxoplasma gondii*. *Infection and Immunity* **65**, 4682–4689.
- Liwicki, R. S. and Lee, T. D. (1999). Nematode infection enhances survival of activated T cells by modulating accessory cell function. *Journal of Immunology* **163**, 5005–5012.

- Lopes, M.F. and DosReis, G.A. (1996). *Trypanosoma cruzi*-induced immunosuppression: selective triggering of CD4⁺ T cell death by the T-cell receptor-CD3 pathway and not by the CD69 or Ly-6 activation pathway. *Infection and Immunity* **64**, 1559–1564.
- Lopes, M.F., Veiga, V.F., Santos, A.R., Fonseca, M.E.F. and DosReis, G.A. (1995). Activation-induced CD4⁺ T cell death by apoptosis in experimental Chagas' disease. *Journal of Immunology* **154**, 744–752.
- Lopes, M.F., Nunes, M.P., Henriques-Pons, A., Giese, N., Morse, H.C., III, Davidson, W.F., Araújo-Jorge, T.C. and DosReis, G.A. (1999). Increased susceptibility of Fas ligand-deficient *gld* mice to *Trypanosoma cruzi* infection due to a Th2-biased host immune response. *European Journal of Immunology* **29**, 81–89.
- Luján, H.D., Mowatt, M.R., Helman, L.J. and Nash, T.E. (1994). Insulin-like growth factors stimulate growth and L-cysteine uptake by the intestinal parasite *Giardia lamblia*. *Journal of Biological Chemistry* **269**, 13069–13072.
- Maercker, C., Kortwig, H., Nikiforov, M.A., Allis, C.D. and Lipps, H.J. (1999). A nuclear protein involved in apoptotic-like DNA degradation in *Styloynchia*: implications for similar mechanisms in differentiating and starved cells. *Molecular Biology of the Cell* **10**, 3003–3014.
- Manon, S., Chaudhuri, B. and Guerin, M. (1997). Release of cytochrome c oxidase in Bax-expressing yeast cells, and prevention of these effects by coexpression of Bcl-x_L. *FEBS Letters* **415**, 29–32.
- Martins, G.A., Cardoso, M.A., Aliberti, J.C. and Silva, J.S. (1998). Nitric oxide-induced apoptotic cell death in the acute phase of *Trypanosoma cruzi* infection in mice. *Immunology Letters* **63**, 113–120.
- Maudlin, I. and Welburn, S.C. (1987). Lectin mediated establishment of midgut infections of *Trypanosoma congolense* and *Trypanosoma brucei* in *Glossina morsitans*. *Tropical Medicine and Parasitology* **38**, 167–170.
- Mazingue, C., Cottrez-Detoeuf, F., Louis, J., Kweider, M., Auriault, C. and Capron, A. (1989). *In vitro* and *in vivo* effects of interleukin 2 on the protozoan parasite *Leishmania*. *European Journal of Immunology* **19**, 487–491.
- Menzies, B.E. and Kourteva, I. (1998). Internalization of *Staphylococcus aureus* by endothelial cells induces apoptosis. *Infection and Immunity* **66**, 5994–5998.
- Mihai, C., Wilm, B., Hobmayer, E., Bottger, A. and David, C.N. (1999). Identification of caspases and apoptosis in the simple metazoan *Hydra*. *Current Biology* **9**, 959–962.
- Monack, D.M., Meissas, J., Bouley, D. and Falkow, S. (1998). *Yersinia*-induced apoptosis *in vivo* aids the establishment of a systemic infection in mice. *Journal of Experimental Medicine* **188**, 2127–2137.
- Moore, K.J. and Matlashewski, G. (1994). Intracellular infection by *Leishmania donovani* inhibits macrophage apoptosis. *Journal of Immunology* **152**, 2930–2937.
- Moreira, M.E.C., Del Portillo, H., Milder, R.V., Balanco, J.M.F. and Barcinski, M.A. (1996). Heat shock induction of apoptosis in promastigotes of the unicellular organism *Leishmania (Leishmania) amazonensis*. *Journal of Cellular Physiology* **167**, 305–313.
- Mpope, S. and Wolfe, J. (1996). DNA digestion and chromatin condensation during nuclear death in *Tetrahymena*. *Experimental Cell Research* **225**, 357–365.
- Murphy, N.B. and Welburn, S.C. (1997). Programmed cell death in procyclic *Trypanosoma brucei rhodesiense* is associated with differential expression of mRNAs. *Cell Death and Differentiation* **4**, 365–370.
- Nash, P.B., Purner, M.B., Leon, R.P., Clarke, P., Duke, R.C. and Curiel, T. J. (1998). *Toxoplasma gondii*-infected cells are resistant to multiple inducers of apoptosis. *Journal of Immunology* **160**, 1824–1830.
- Nunes, M.P., Andrade, R.M., Lopes, M.F. and DosReis, G.A. (1998). Activation-induced T

- cell death exacerbates *Trypanosoma cruzi* replication in macrophages cocultured with CD4⁺ T lymphocytes from infected hosts. *Journal of Immunology* **160**, 1313–1319.
- Ojcius, D.M., Perfettini, J.L., Bonnin, A. and Laurent, F. (1999). Caspase-dependent apoptosis during infection with *Cryptosporidium parvum*. *Microbes and Infection* **1**, 1163–1168.
- Olie, R.A., Durrieu, F., Cornillon, S., Loughran, G., Gross, J., Earnshaw, W.C. and Golstein, P. (1998). Apparent caspase independence of programmed cell death in *Dictyostelium*. *Current Biology* **8**, 955–958.
- Picot, S., Burnod, J., Bracchi, V., Chumpitazi, B.F.F. and Ambroise-Thomas, P. (1997). Apoptosis related to chloroquine sensitivity of the human malaria parasite *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 590–591.
- Pied, S., Voegtle, D., Marussig, M., Renia, L., Miltgen, F., Mazier, D. and Cazenave, P.A. (1997). Evidence for superantigenic activity during murine malaria infection. *International Immunology* **9**, 17–25.
- Piuvezam, M.R., Russo, D.M., Burns, J.M., Jr, Sheiky, Y.A., Grabstein, K.H. and Reed, S.G. (1993). Characterization of responses of normal human T cells to *Trypanosoma cruzi* antigens. *Journal of Immunology* **150**, 916–924.
- Reifenberg, J.M., Cuisance, D., Frézil, J.L., Cuny, G. and Duvallet, G. (1997) Comparison of the susceptibility of different *Glossina* species to simple and mixed infections with *Trypanosoma (Nannomonas) congolense* savannah and riverine forest types. *Medical and Veterinary Entomology* **3**, 246–252.
- Ridgley, E.L., Xiong, Z-H. and Ruben, L. (1999). Reactive oxygen species activate a Ca²⁺-dependent cell death pathway in the unicellular organism *Trypanosoma brucei brucei*. *Biochemical Journal* **340**, 33–40.
- Rojas, M., Barrera, L.F., Puzo, G. and Garcia, L.F. (1997). Differential induction of apoptosis by virulent *Mycobacterium tuberculosis* in resistant and susceptible murine macrophages: role of nitric oxide and mycobacterial products. *Journal of Immunology* **159**, 1352–1361.
- Rossi, M.A. and Souza, A.C. (1999). Is apoptosis a mechanism of cell death in chronic chagasic myocarditis? *International Journal of Cardiology* **68**, 325–331.
- Rudi, J., Kuck, D., Strand, S., von Herbay, A., Mariani, S.M., Krammer, P.H., Galle, P.R. and Stremmel, W. (1998). Involvement of the CD95 (APO-1/Fas) receptor and ligand system in *Helicobacter pylori*-induced gastric epithelial apoptosis. *Journal of Clinical Investigation* **102**, 1506–1514.
- Rumbley, C.A., Zekavat, S.A., Sugaya, H., Perrin, P.J., Ramadan, M.A. and Phillips, S.M. (1998). The schistosome granuloma: characterization of lymphocyte migration, activation, and cytokine production. *Journal of Immunology* **161**, 4129–4137.
- Russo, D.M., Chakrabarti, P. and Burns, J.M., Jr (1998). Naive human T cells develop into Th1 or Th0 effectors and exhibit cytotoxicity early after stimulation with *Leishmania*-infected macrophages. *Journal of Infectious Diseases* **177**, 1345–1351.
- Sakai, T., Hisaeda, H., Ishikawa, H., Maekawa, Y., Zhang, M., Nakao, Y., Takeuchi, T., Matsumoto, K., Good, R.A. and Himeno, K. (1999). Expression and role of heat-shock protein 65 (HSP65) in macrophages during *Trypanosoma cruzi* infection: involvement of HSP65 in prevention of apoptosis of macrophages. *Microbes and Infection* **1**, 419–427.
- Savill, J. (1998). Phagocytic docking without shocking. *Nature* **392**, 442–443.
- Stenger, S., Mazzaccaro, R.J., Uyemura, K., Cho, S., Barnes, P.F., Rosat, J.P., Settezz, A., Brenner, M.B., Porcelli, S.A., Bloom, B.R. and Modlin, R.L. (1997). Differential effects of cytolytic T cell subsets on intracellular infection. *Science* **276**, 1684–1687.
- Sytwu, H.K., Liblau, R.S. and McDevitt, H.O. (1996). The roles of Fas/APO-1 (CD95) and

- TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. *Immunity* **5**, 17–30.
- Tibayrenc, M., Kjellberg, F. and Ayala, F. (1990). A clonal theory of parasitic protozoa: the population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas* and *Trypanosoma* and their medical and taxonomical consequences. *Proceedings of the National Academy of Sciences of the USA* **87**, 2414–2418.
- Van Parijs, L. and Abbas, A.K. (1996). Role of Fas-mediated cell death in the regulation of immune responses. *Current Opinion in Immunology* **8**, 355–361.
- Van Parijs, L., Ibraghimov, A. and Abbas, A.K. (1996). The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity* **4**, 321–328.
- Van Voorhis, W.C. (1992). Coculture of human peripheral blood mononuclear cells with *Trypanosoma cruzi* leads to proliferation of lymphocytes and cytokine production. *Journal of Immunology* **148**, 239–248.
- Varadachary, A.S., Perdow, S.N., Hu, C., Ramanarayanan, M. and Salgame, P. (1997). Differential ability of T cell subsets to undergo activation-induced cell death. *Proceedings of the National Academy of Sciences of the USA* **94**, 5778–5783.
- Vaux, D.L. and Korsmeyer, S.J. (1999). Cell death in development. *Cell* **96**, 245–254.
- Voll, R.E., Herrmann, M., Roth, E.A., Stach, C. and Kalden, J.R. (1997). Immunosuppressive effects of apoptotic cells. *Nature* **390**, 350–351.
- Welburn S.C. and Maudlin, I. (1999). Tsetse–trypanosome interactions: rites of passage. *Parasitology Today* **15**, 399–403.
- Welburn, S.C. and Murphy, N.B. (1998). Prohibitin and RACK homologues are up-regulated in trypanosomes induced to undergo apoptosis and in naturally occurring terminally differentiated forms. *Cell Death and Differentiation* **5**, 615–622.
- Welburn, S.C., Maudlin, I. and Ellis, D.S. (1989). Rate of trypanosome killing by lectins in midguts of different species and strains of *Glossina*. *Medical and Veterinary Entomology* **3**, 77–82.
- Welburn, S.C., Dale, C., Ellis, D., Beecroft, R., Pearson, T.W. and Maudlin, I. (1996). Apoptosis in procyclic *Trypanosoma brucei rhodesiense* in vitro. *Cell Death and Differentiation* **3**, 229–236.
- Welburn, S.C., Barcinski, M.A. and Williams, G.T. (1997). Programmed cell death in trypanosomatids. *Parasitology Today* **13**, 22–26.
- Yamashita, K., Yui, K., Ueda, M. and Yano, A. (1998). Cytotoxic T-lymphocyte-mediated lysis of *Toxoplasma gondii*-infected target cells does not lead to death of intracellular parasites. *Infection and Immunity* **66**, 4651–4655.
- Yang, J., Liu, X., Bhalla, K., Kim, C.N., Ibrado, A.M., Cai, J., Peng, T.-I., Jones, D.P. and Wang, X. (1997). Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* **275**, 1129–1132.
- Yang, Y., Mércep, M., Ware, C.F. and Ashwell, J.D. (1995). Fas and activation-induced Fas ligand mediate apoptosis of T cell hybridomas: inhibition of Fas ligand expression by retinoic acid and glucocorticoids. *Journal of Experimental Medicine* **181**, 1673–1682.
- Zhang, J., Andrade, Z.A., Yu, Z.X., Andrade, S.G., Takeda, K., Sadigursky, M. and Ferrans, V.J. (1999). Apoptosis in a canine model of acute Chagasic myocarditis. *Journal of Molecular and Cellular Cardiology* **31**, 581–596.
- Zhang, X., Brunner, T., Carter, L., Dutton, R.W., Rogers, P., Sato, T., Reed, J.C., Green, D. and Swain, S.L. (1997). Unequal death in T helper (Th) 1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *Journal of Experimental Medicine* **185**, 1837–1849.
- Zychlinsky, A. and Sansonetti, P. (1997). Apoptosis in bacterial pathogenesis. *Journal of Clinical Investigation* **100**, 493–495.

Biology of Echinostomes Except *Echinostoma*

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ABSTRACT

This review examines the significant literature on the biology of echinostomes, except *Echinostoma*, for the following species rich genera: *Echinoparyphium*, *Echinochasmus*, *Himasthla*, and *Hypoderaeum*. Coverage of these genera includes descriptive studies, life cycle studies, experimental and manipulative studies, and biochemical and molecular studies. Coverage of other echinostome genera, i.e., *Petasiger*, *Euparyphium*, *Stephanoprora*, *Isthmiophora*, *Acanthoparyphium*, and *Patagifer* was mainly concerned with descriptive and life cycle studies. Some groups that are considered echinostome-like were covered because species in these genera have medical, economic, or biological significance (particularly *Parorchis*, *Philophthalmus*, and *Ribeiroia*). A dominant theme of the review is the availability of key species of echinostomes for use in biological research; coverage also includes methods for obtaining and maintaining these organisms in the laboratory. Some species with significant medical, veterinary, and biological importance that have been covered extensively include the *Echinoparyphium recurvatum* species complex, *Echinochasmus liliputanus*, *Himasthla quissetensis*, *Himasthla rhigadana*, and *Hypoderaeum conoideum*.

1. INTRODUCTION

The purpose of this review is to examine the significant literature on various genera of Echinostomatidae except *Echinostoma*. Emphasis is placed on descriptive and life cycle studies; experimental and manipulative studies; and physiological, biochemical, and molecular studies. Genera other than *Echinostoma*, for which there is considerable literature with coverage in some or most of the aforementioned topics, include *Echinoparyphium*, *Echinochasmus*, *Himasthla*, and *Hypoderaeum*. Less information is available on species in the genera *Petasiger*, *Euparyphium*, *Stephanoprora*, *Isthmiophora*, *Acanthoparyphium*, and *Patagifer*. Table 1 summarizes literature citations for the above genera using various computer searches for different time periods. As expected, the most extensive citations to these genera were found in *Helminthological Abstracts* (CAB).

Taxonomic studies are mentioned only as they relate to descriptive, life cycle, and experimental studies covered in the review. The systematics of the Echinostomatidae is unsettled and has been reviewed by Kostadinova and Gibson (2000). Table 2 summarizes information from Kostadinova and Gibson (2000) on echinostome genera containing numerous species. The table provides morphological data for distinguishing the genera *Echinostoma*,

Table 1 Echinostome genera^a with ten or more citations in CAB from 1972–2000

Rank	Genus	CAB ^b	BIOSIS ^c	MEDLINE ^d
1	<i>Echinostoma</i>	867	749	441
2	<i>Echinoparyphium</i>	238	151	27
3	<i>Echinochasmus</i>	166	89	25
4	<i>Himasthla</i>	92	86	18
5	<i>Hypoderaeum</i>	87	52	11
6	<i>Petasiger</i>	40	27	1
7	<i>Euparyphium</i>	37	22	8
8	<i>Stephanoprora</i>	30	20	4
9	<i>Isthmiophora</i>	29	32	9
10	<i>Acanthoparyphium</i>	19	19	3
11	<i>Patagifer</i>	16	11	1

^a Genera chosen based on at least 10 citations in CAB between 1972–2000.

^b Citations in *Helminthological Abstracts* from 1972 to June 2000.

^c Citations in Biosis Previews from 1969 to June 2000.

^d Citations in MEDLINE from 1966 to June 2000.

Note: Rank is based on CAB citations and is slightly different in BIOSIS and MEDLINE based on differences in citation rates to some genera.

Echinoparyphium, *Echinochasmus*, *Stephanoprora* and *Himasthla*, and should be consulted for morphological features that distinguish these genera.

Although several reviews on *Echinostoma* have appeared since 1990 (Huffman and Fried, 1990; Fried and Huffman, 1996) there is no comprehensive review that considers the other echinostome genera. Fried and Graczyk (2000) edited a book on the use of echinostomes as experimental models for biological research. At least 80% of the coverage is on species of *Echinostoma*. Salient information from the book on echinostomes except for *Echinostoma* is summarized below.

Kanev *et al.* (2000) provided an overview of the biology of echinostomes including information on the literature and history of these digenleans, life cycle studies and consideration of form and function of the larval and adult stages. Although most of their information is on *Echinostoma*, there is coverage of the following genera: *Euparyphium*, *Echinochasmus*, *Echinoparyphium*, *Hypoderaeum*, *Moliniella*, *Petasiger*, *Acanthoparyphium*. Information on echinostome-related genera such as *Parorchis*, *Philopthalmus*, and *Ribeiroia* is also included. Kostadinova and Gibson (2000) are mainly concerned with the systematics of the confusing 37-collar spined ‘revolutum’ group in the genus *Echinostoma*. However, they do discuss systematic concerns in the more speciose (species rich) genera of echinostomes such as *Echinoparyphium*, *Echinochasmus*, *Stephanoprora*, and *Himasthla*.

Table 2 Characteristic features of the species-rich genera of the Echinostomatidae.

Feature	<i>Echminostoma</i>	<i>Echinopygium</i>	<i>Echimochasmus</i>	<i>Stephanopora</i>	<i>Himasthila</i>
Body size	Medium to large ^a	Small to medium	Small	Small to large	Medium to large
Body shape	Elongate (NBW = 10–20%)	Elongate to elongate-oval (BW = 13–33%)	Elongate-oval (BW = 17–50%)	Elongate (BW = 7–20%)	Notably elongate and slender (BW = 2.5–14%)
Forebody	Very short (FO = 10–20%)	Long (FO = 20–40%)	Long (FO = 25–50%)	Short (FO = 15–26%)	Extremely short (FO = 3–10%)
Head collar					
Ventral ridge	Present	Present	Absent	Absent	Present
Dorsal interruption	Absent	Absent	Present	Absent	29, 31 or 34–40 (type only)
Spines	31–55	29–45	20–24 or 30–34	22 (26 type only)	Single row
Lateral spines	Single row	Double row	Single row	Single row	Single row
Dorsal spines	Double row	Double row	2 × 2	2 × 2	2 × 2
Angle spines	2 × 5	2 × 4			
Testes	In 3rd quarter of body	In mid-hindbody	In mid-hindbody	Equatorial or pre-equatorial	Fairly close to posterior extremity
Cirrus sac	Elongate-oval	Elongate-oval	Elongate-oval	Elongate-oval	Very long and slender
Internal seminal vesicle	With saccular posterior and tubular anterior portion	Simple or bipartite-saccular, or twisted tubular	Bipartite-saccular	Bipartite-saccular	Tubular, with dilated posterior part
Cirrus	Strong, tubular, unspined	Long, tubular, unspined	Short, unspined	Small, unspined	Long, muscular, spined
Uterus	Long to very long (U = 20–45%)	Very short to short (U = 3–20%)	Very short (U = 0–10%)	Moderately long	Very long (U = 40–80%)
Viteline fields	Between VS and posterior extremity	Not reaching VS anteriorly	Anterior limits between VS and pharynx	Anterior limits at anterior testis	Anterior limits variable, up to mid-level of cirrus-sac

Table 2 cont.

Feature	<i>Echinostoma</i>	<i>Echinopyrygium</i>	<i>Echinochasmus</i>	<i>Stephanopora</i>	<i>Himasthla</i>
First intermediate hosts	Freshwater pulmonate and prosobranch gastropods	Freshwater pulmonate and prosobranch gastropods	Freshwater prosobranch gastropods	Prosobranch (occasionally pulmone) gastropods	Marine prosobranch gastropods
Second intermediate hosts	Freshwater gastropods, bivalves, planarians and tadpoles	Freshwater gastropods, bivalves and tadpoles	Freshwater teleosts, occasionally snails and tadpoles	Freshwater, brackish-water and marine teleosts	Marine bivalves, prosobranch gastropods, annelids
Final hosts	Wide range of birds and mammals	Wide range of birds and mammals	Wide range of fish-eating birds and mammals	Wide range of fish-eating birds, mammals and reptiles	Birds ^a ; incidentally in fish mammals
Distribution	Cosmopolitan	Cosmopolitan	Cosmopolitan	Cosmopolitan	Cosmopolitan

^a Small (< 5 mm); medium (5–10 mm); large (> 10 mm).^b Charadriiformes, Anseriformes and Ciconiiformes.

BW, maximum body width as a proportion of body length; FO, length of the forebody as a proportion of body length; U, length of the uterine field as a proportion of body length; VS, ventral sucker.

Table 2 was reproduced from Table 2 on page 38 of Fried, B. and Graczyk, T.K. (2000), *Echinostomes as Experimental Models for Biological Research*, with the permission of the publisher, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Huffman (2000) in her coverage on echinostomes in veterinary and wildlife parasitology considered mainly species in the genus *Echinostoma*. However, information on echinostomiasis and epidemiology, pathological effects of echinostomes in wildlife, domestic animals and experimental hosts will be transferable to studies on echinostomes other than *Echinostoma*. Haseeb and Eveland (2000) discussed various genera, in addition to *Echinostoma*, that have been implicated as human parasites. They provided information on species that are human parasites in the genera *Echinochasmus*, *Echinoparyphium*, *Himasthla*, and *Hypoderaeum*. Fried (2000) covered information on maintenance, cultivation, and excystation of echinostomes. Although most of the information on these topics is on *Echinostoma*, some coverage on *Himasthla*, *Echinoparyphium*, *Echinochasmus*, and *Acanthoparyphium* is given. Although not considered as echinostomes by many workers, information on parorchids (*Parorchis*) and philophthalmids (*Philophthalmus*) was included in the Fried (2000) chapter. Fujino and Ichikawa (2000) reviewed the salient transmission and scanning electron microscopical studies on echinostomes and noted that, although most of the work is on the genera *Echinostoma* and *Echinoparyphium*, there are ultrastructural studies on other genera such as *Echinochasmus*.

Nollen (2000) reviewed studies on the reproductive physiology and behavior of echinostomes. Such studies appear to be restricted to the genus *Echinostoma*. He mentioned some reports in the literature on abnormalities in the male reproductive system of some species of *Echinoparyphium*, including missing anterior and posterior testes and odd testicular structures. Adema *et al.* (2000) reviewed the salient features on the immunobiological relationships of echinostomes and their snail intermediate hosts. They also covered some of the general literature on echinostome-snail host parasite relationships and noted that most of these studies were on the genus *Echinostoma*. Haas (2000) reviewed the behavioral biology of echinostomes including studies on the free-living larval stages, the intramolluscan parasitic stages, and the adult parasitic stages. Cercarial behavioral studies have been done with members of the genera *Echinoparyphium*, *Himasthla*, *Pseudoechinoparyphium*, *Hypoderaeum*, and *Isthmiophora*, in addition to the *Echinostoma*. However, studies on the behavior of the parasitic intramolluscan stages, and on juvenile and adult worm stages from vertebrate hosts, are restricted to species of *Echinostoma*. Barrett (2000) reviewed studies on the physiology and biochemistry of echinostomes; specific studies other than on *Echinostoma* are not mentioned in his chapter. Humphries *et al.* (2000) reviewed structural and functional correlates of echinostome neuromusculature; information on genera other than the *Echinostoma* was not mentioned. Graczyk (2000) reviewed studies on the immunobiology and immunodiagnosis of echinostomes, with major emphasis on species of *Echinostoma*; no mention of studies on genera other than *Echinostoma* was made. Morgan and Blair (2000) reviewed studies on the

molecular biology of echinostomes with emphasis on *Echinostoma*. Information, mainly on karyotyping, was given for some genera other than *Echinostoma*, including *Echinoparyphium*, *Hypoderaeum*, and *Neoacanthoparyphium*.

2. THE GENUS *ECHINOPARYPHIUM*

2.1. Descriptive Studies

Members of the genus *Echinoparyphium* are small to average in size with a narrow and elongated body in most specimens; the body is expanded posteriorly in some specimens. The oral collar is kidney-shaped and contains a double continuous row of collar spines. The spines in the aboral and adoral rows may be equal or unequal in size. The tegument is usually spinose but may be smooth. A large ventral sucker is located about midway between the first and second quarter of the body or slightly posterior to that. The intestinal cecal bifurcation is at the anterior edge of the ventral sucker. The cirrus sac is small and does not extend beyond the posterior edge of the ventral sucker. The testes are rounded, median, and in tandem in the posterior half of the body. The ovary is round or transversely oval, median or somewhat lateral, and situated near the anterior aspect of the anterior testis. The vitellaria consist of large or average-sized lateral follicles situated in the posterior part of the body. Except for a few follicles, the vitellaria do not reach the posterior edge of the ventral sucker. The uterus is short with relatively few large eggs about 52–108 µm in length and 52–68 µm in width. Further distinguishing characteristics of this genus can be found in the third column of Table 2. A line drawing of an adult *E. recurvatum* is shown in Figure 1. By contrast, Figure 2 shows a line drawing of perhaps the best-studied echinostome, *Echinostoma revolutum*. Major contrasting features of the species relate to the number of collar spines (43 to 45 in *E. recurvatum* compared with 37 in *E. revolutum*) and egg size and number (relatively few and large eggs in *E. recurvatum* versus many, but small, eggs in *E. revolutum*). The following descriptive coverage is chronological and represents only a fraction of such studies reported in the literature on numerous valid and dubious species in this genus.

Wang (1976) found that *Echinoparyphium gallinarum* from the intestine of *Gallus gallus* in Fuzhou, China differed from *Echinoparyphium cinetorchis* in that it has a larger body, larger collar spines, larger suckers and eggs, and testes with six to eight deep lobes and tegumentary spines extending to the margin of the acetabulum. Bykhovskaya-Pavlovskaya (1978) examined species of Echinostomatidae from the Zoological Institute of the Academy of Sciences at Leningrad, Russia and found that *Echinoparyphium paracinctum*

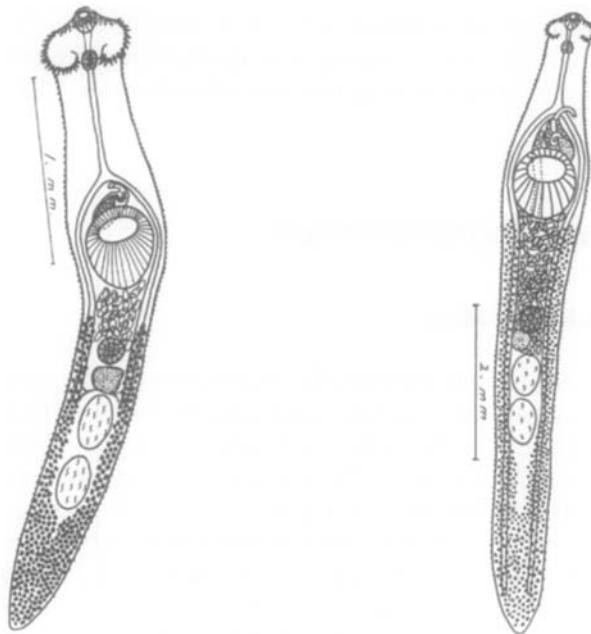


Figure 1 *Echinoparyphium recurvatum*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

Figure 2 *Echinostoma revolutum*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

was mistakenly described with 43 collar spines. She found that this species had 45 collar spines and therefore belonged to *Echinoparyphium mordwilkoi*. The collection also contained *Echinoparyphium petrowi*, which has 49 collar spines. Gupta and Jahan (1979) showed that *Echinoparyphium magniovum* differed from all known species of *Echinoparyphium* in having 27 collar spines and vitellaria that extended from the anterior part of the ventral sucker to the hind part of the body. Gorchilova and Kanev (1986) used electron microscopy to study the cyst walls of *Echinoparyphium echinatum*, *E. revolutum*, and *Echinoparyphium aconiatum*. All three species had an external plasmalemma with adhering collagenous fibers; the first two species, *E. echinatum* and *E. revolutum*, had three cyst layers while *E. aconiatum* had only two layers.

Grabda-Kazubska and Kiseliene (1989) studied cercariae released by *Radix pereger* and compared the cercariae with those of *E. recurvatum* on the basis of chaetotaxy and the structure of the excretory system; they noted that most of the important structural features were similar. Kiseliene and Grabda-Kazubska (1990) reported that *Echinoparyphium pseudorecurvatum* differed

from *E. recurvatum* in all life cycle stages, particularly in the cercarial and adult stages. The species situation is very unsettled in this genus and many authors speak of an *Echinoparyphium recurvatum* complex. Grabda-Kazubska and Kiseliene (1991) also noted that the echinostome cercaria released by *Valvata piscinalis* is the larval stage of *E. mordwilkoii*; it has 45 collar spines and is similar to *E. recurvatum* and *E. pseudorecurvatum*. However, it differs from the latter species in the length of its redial gut, cercarial chaetotaxy, and tegumentary spines in the adult. Kiseliene and Grabda-Kazubska (1993) suggested that the *E. recurvatum* complex consists of at least three different species: *E. recurvatum*, *E. pseudorecurvatum* and *E. mordwilkoii*.

Semenas *et al.* (1999) obtained 37- and 43-collar-spined encysted metacercariae from tissues of the unionid bivalve *Diplodon chilensis* in Patagonia, Argentina. Chicken and mice were used as experimental hosts to obtain echinostome adults. Chickens were refractory to infection and mature adults with 43 collar spines were obtained only from mice. Their echinostome was described as *Echinoparyphium megacirrus* sp.n. The susceptibility of laboratory hosts to 43-collar-spined *Echinoparyphium* species is variable; some infect only mice, others infect chicks, and yet others develop in both avian and mammalian hosts. Susceptibility of vertebrate hosts to both 43- and 45-collar-spined *Echinoparyphium* species needs further work.

Fried *et al.* (1998a) made comparative observations on cercariae of *Echinoparyphium* sp. from *Physa gyrina* in Charlie's Pond, Stoke's County, North Carolina, USA and cercariae of *Echinostoma trivolvis* from *Helisoma trivolvis* in Northampton County, Pennsylvania, USA. The cercaria of *Echinoparyphium* sp. has 43 collar spines, lacks penetration and paraesophageal glands, and has a conical tail without fin folds. The cercaria of *E. trivolvis* has 37 collar spines, penetration and paraesophageal glands and a finger-like process at the tip of the tail; the tail contains fin folds. The length of the cercarial body and tail of *E. trivolvis* was significantly greater than that of *Echinoparyphium* sp. Cercariae of both species encysted in *Biomphalaria glabrata* snails in single and concurrent infections. In concurrent infections with a single cercaria of each species, two encysted metacercaria were adjacent to each other in the saccular kidney of the snail at 24 h postinfection. The diameter of the encysted metacercaria of *E. trivolvis* was significantly greater than that of *Echinoparyphium* sp. The metacercariae of *Echinoparyphium* sp. excysted at 39°C in an alkaline trypsin–bile salts medium used previously to excyst *E. trivolvis*. The length of excysted metacercariae of *E. trivolvis* was significantly greater than that of *Echinoparyphium* sp. This is an important paper to examine for comparative differences in the cercarial and metacercarial stages of representative species of *Echinostoma* versus *Echinoparyphium*.

2.2. Life Cycle Studies

Najarian (1953, 1954) described the life cycle of *Echinoparyphium flexum* from North America. Miracidia developed and hatched in freshwater at room temperature in about 14 days and then penetrated pulmonate snails *Lymnaea (Stagnicola) palustris*. The intramolluscan stages included a typical sporocyst stage and two generations of rediae. Cercariae released from second generation rediae emerged from *L. palustris* and encysted in the same or other snails. Cercarial encystment was not host specific and occurred in a variety of snails, i.e., *Helisoma*, *Physa*, or other species of *Lymnaea*, and in various genera of tadpoles, i.e., *Rana*, *Hyla*, and *Pseudacris*. Unidentified echinostome metacercarial cysts with 45 collar spines from pulmonate snails and tadpoles in the USA could be referable to *E. flexum*. Blue-winged teal, *Anas discors*, and the American black scoter, *Oidemia americana*, are often naturally infected with this echinostome, which they acquire by eating tainted snails and tadpoles. Domestic chicks serve as experimental hosts for this echinostome. The species situation is unsettled in the USA with both the 43- and 45-collar-spined *Echinoparyphium* species, and some authors consider these forms together with the *Echinoparyphium recurvatum* complex. See Kanev *et al.* (1998) for further information on this topic.

Lie and Umathevy (1965) described the life history of *Echinoparyphium dunnii* from the first intermediate host *Lymnaea rubiginosa* in Kuala Lumpur, Malaysia. Howell (1968a) described the life cycle of a 37-collar-spined echinostome, *Echinoparyphium serratum*, beginning with the cercarial stage released from the freshwater snail *Isidorella brazieri*, in Australia. Mouahid and Mone (1988) described the life cycle of *Echinoparyphium elegans*; the miracidia infected *Bulinus truncatus* snails and mother sporocysts developed in the head-foot and mantle regions. Redial generations developed in the digestive gland-gonad complex of the snail. Cercariae released from the first intermediate host encysted in the pericardial sac and kidney of *B. truncatus*, *B. globosus*, *Physa acuta* and *Biomphalaria glabrata*. Experimental definitive hosts were domestic chicks, pigeons, and canaries.

Kiseliene and Grabda-Kazubska (1990) described the life cycle of *Echinoparyphium pseudorecurvatum* with *Planorbis planorbis* as first intermediate host and tadpoles and frogs as second intermediate hosts; chickens, ducks, and pigeons were experimental definitive hosts. Grabda-Kazubska and Kiseliene (1991) described the life cycle of *Echinoparyphium mordwilkoii* in which the prosobranch snails *Valvata piscinalis* served as first and second intermediate hosts and charadriid birds (wading and shore birds such as plovers and killdeers) as the final hosts. They also showed that cercariae released from *V. piscinalis* in Lithuania have morphological characteristics representative of the *E. recurvatum* complex; these cercariae appeared identical to those of *E. mordwilkoii*.

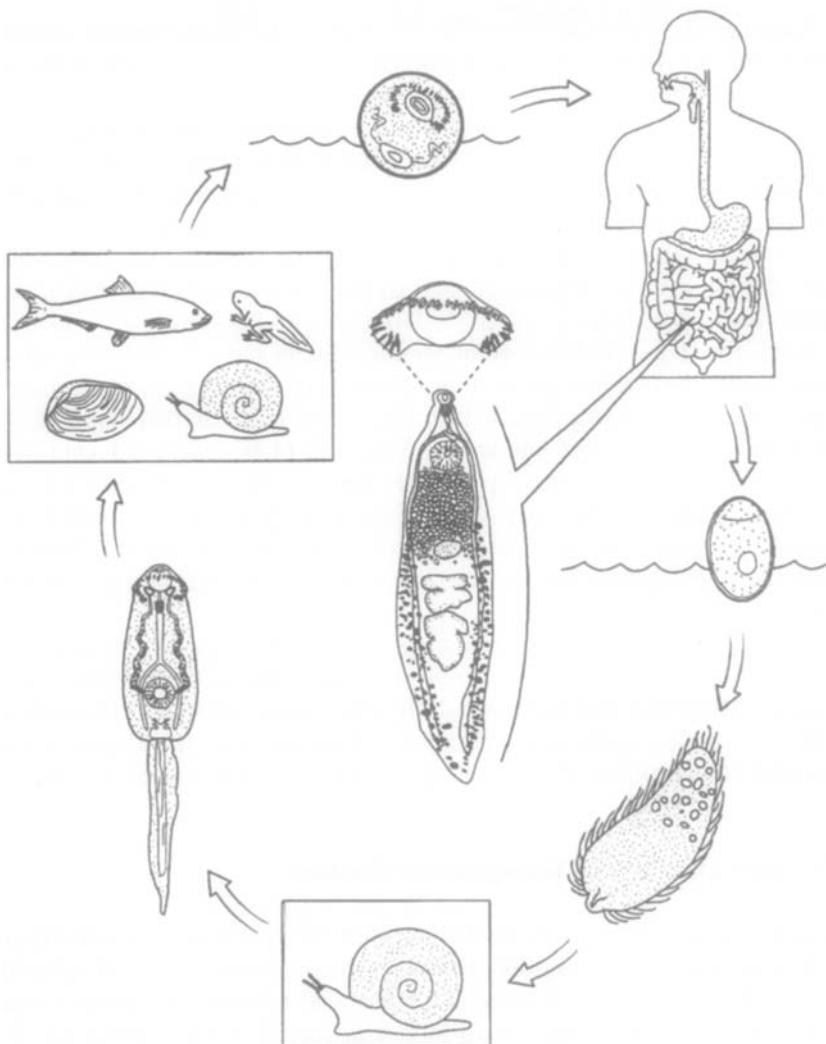


Figure 3 Life cycle of a representative echinostome. Reproduced from Figure 2 on page 89 of Fried, B. and Graczyk, T.K. (2000), *Echinostomes as Experimental Models for Biological Research*, with the permission of the publisher, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Kanev *et al.* (1994) described the life cycle of *Echinoparyphium cinctum* with lymnaeid snails as first intermediate hosts; snails, frogs and turtles as second intermediate hosts; and various birds as definitive hosts. King and Van As (1996) used light and scanning electron microscopy to describe the life cycle of *Echinoparyphium elegans* from South Africa. *Bulinus tropicus* served as first and second intermediate hosts and adult echinostomes were obtained by feeding infected snails to rats. Kanev *et al.* (1998) described the life cycle of *E. rubrum* from naturally infected snails, *Physa gyrina*, and *Physa occidentalis*, from Douglas Lake, Michigan and Golden Gate Park, San Francisco, California, respectively.

Sohn (1998) observed characteristics of the life history of a 45-collar-spined species of *E. recurvatum* under both natural and experimental conditions in Korea. The snail intermediate host in Pusan was *Radix auricularia*. Of 106 snails necropsied in the summer of 1992, 52 (49%) were infected with intramolluscan stages including encysted metacercariae. Cercariae released from *R. auricularia* encysted in snails of the same species and in loach fishes. Chicks and ducks, but not rats and mice, served as experimental definitive hosts. Detailed morphological descriptions of this 45-collar-spined species were provided.

Figure 3 shows a representative diagram of a life cycle of a hypothetical species of *Echinoparyphium* or *Echinostoma* in humans. The encysted metacercariae are eaten along with tainted shellfish, fish or tadpoles as shown here. If this were a life cycle of a species of *Echinocasmus*, the diagram would show direct ingestion of the free cercarial stage from contaminated water.

2.3. Experimental and Manipulative Studies

Howell (1968b) devised a chemical procedure for excysting metacercariae of *Echinoparyphium serratum* obtained from the pericardial tissue of naturally infected freshwater snails *Isidorella brazieri*. He achieved postmetacercarial development of this echinostome in various media with a procedure that involved an acid pepsin pretreatment, followed by treatment in a 0.02 M sodium dithionite reductant solution. The final treatment consisted of an excystation medium of 0.3% trypsin plus 0.5% sodium cholate in Hanks basal salt solution (BSS) at pH 7.3 ±1°C. There was about 75% excystation within 10 min. Howell (1970) later described factors involved in the excystation of this species and found that acid pepsin pretreatment was not obligatory for excystation. Howell's (1968b, 1970) studies are still state of the art in regard to chemical excystation of any species of *Echinoparyphium*. Howell (1968b) cultivated *E. serratum* metacercariae *in vitro*. The best development, although suboptimal when compared with development of worms in experimental duckling hosts, was in a yolk-albumen-saline medium supplemented with yeast

extract (for details, see Fried, 1978). Worms cultivated in this medium at 39°C with a gas phase of air developed to the vitellogenesis stage, and some worms developed eggs. Worms grown *in vitro* were stunted and development was delayed when compared with worms grown in ducklings. Vitellogenesis was achieved in about 140 h *in vitro* compared with 60 h in worms grown in ducklings. Nevertheless, this study is still state of the art for the cultivation *in vitro* of *Echinoparyphium* species and should be consulted by anyone who attempts studies *in vitro* on these echinostomes.

Kubliciene and Ciuniene (1973) infected ducks with *Echinoparyphium recurvatum*. Between 3 and 14 days postinfection, intestinal changes such as hyperaemia, haemorrhagia, desquamation, and necrosis occurred. Between 14 and 38 days postinfection, necrotic lesions were found in the duodenum along with desquamation of the mucosa of the ileum and necrotic changes in the rectal mucosa. Fried and Grigo (1975) obtained *Echinoparyphium flexum* metacercarial cysts from the kidney and pericardial sac of naturally infected *Physa* sp. snails in Pennsylvania, USA. They found that excystation requirements for *E. flexum* were less fastidious than those of *E. serratum* as described by Howell (1968b, 1970). Fried and Grigo (1975) were able to excyst their species of *Echinoparyphium* in an alkaline medium of trypsin–bile salts in Earle's balanced salt solution at 39°C. There was no need for an acid pretreatment, a reductant, or special gases. Excystation requirements vary from one *Echinoparyphium* species to another.

Fried and Grigo (1975) studied the infectivity of *E. flexum* metacercariae in domestic chicks. Attempts to increase infectivity by pretreating cysts in 7.5% NaHCO₃ or in 0.5% bile salts plus 0.5% trypsin in Earle's BSS at pH 8.1 were unsuccessful. Evans (1983) reported that metacercarial cysts of *E. recurvatum* from *Lymnaea peregra* became established in the small intestines of experimentally infected domestic ducks within 12 h postinfection. The maximal survival time of adults in this host was 28 days.

McCarthy (1990) provided evidence for the existence of two first intermediate host-specific sibling species of the 45-collar-spined echinostome *E. recurvatum*. One sibling utilized *L. peregra* as the first intermediate host while the other used *Valvata piscinalis*. Both siblings used ducks (*Anas platyrhynchos*) as experimental definitive hosts. Conn and Conn (1995) experimentally infected zebra mussels, *Dreissena polymorpha*, from the St Lawrence River in New York with metacercariae of *Echinoparyphium* sp. This was the first report of an echinostomatid infection in *D. polymorpha* and also the first time echinostome metacercariae were found in the gonads of zebra mussels. The potential of using species of *Echinoparyphium* as biological control agents of zebra mussels needs to be explored further. King and Van As (1997) used scanning electron microscopy to examine echinostome cercariae released from *Bulinus tropicus* snails in South Africa. The cercariae had 43 collar spines and were identified as *Echinoparyphium elegans*. Toledo *et al.* (1998a) studied

larval trematode infections in freshwater gastropods from the Albufera Naturae Park in Spain and reported the presence of cercariae of *E. recurvatum* species for the first time in Spain.

McCarthy (1999a) studied the photoperiodic cercarial emergence of *E. recurvatum* from *L. peregra* in the United Kingdom. Cercariae emerged during the light phase of the experiment from 08:00–20:00 hours; McCarthy (1999b) also studied the phototactic responses of the cercariae of *E. recurvatum* from infected *L. peregra* snails and found that cercariae have an initially positively phototactic, low infectivity, dispersal phase followed by a negatively phototactic, maximally infective host location and infection phase. McCarthy (1999c) studied the influence of temperature on the survival and infectivity of *E. recurvatum* cercariae at 10, 15, 20, 25, and 30°C. Cercarial survival was temperature-dependent with the maximum survival time being reduced from 68 h at 10°C to 12 h at 30°C. The LD₅₀ declined from 48 h at 10°C to 8 h at 30°C. Cercarial infectivity to a gastropod host was also temperature dependent; infectivity was zero at 10°C, maximum at 25°C, and declining at 30°C. Transmission efficiency of this cercaria was maximum at 20°C and relatively high between 10 and 25°C; it was zero at 10°C and very low at 30°C. This *E. recurvatum* species shows transmission optima at water temperatures likely to be encountered in natural habitats in the United Kingdom.

McCarthy (1999d) examined the potential influence of the second intermediate host species on the infectivity of metacercarial cysts of *E. recurvatum* to experimental duckling hosts *A. platyrhynchos*. The metacercarial cysts were obtained 14 days post-cercarial infections from the following second intermediate hosts: *L. peregra*, *Lymnaea stagnalis*, *Physa fontinalis*, *Planorbus planorbis*, *Biomphalaria glabrata*, and *Rana temporaria* tadpoles. Encysted metacercariae from each of these hosts were fed 50 cysts per duckling, and ducklings were necropsied 15 days post-exposure. All worms recovered were gravid and about the same size. It was concluded that the species of second intermediate hosts did not influence the infectivity of the metacercarial cyst of *E. recurvatum*, nor the subsequent reproductive status, growth or development of this echinostome in experimentally infected *A. platyrhynchos*. This is the first detailed study in which the effects of metacercarial encystment in various second intermediate hosts was studied in terms of subsequent infectivity of the echinostome in the experimental definitive host.

2.4. Biochemical and Molecular Studies

Grabda-Kazubska and Moczon (1988) localized the nervous system of *Echinoparyphium recurvatum* on the basis of cholinesterase activity; they used acetylthiocholine as the substrate in their assay. Grabda-Kazubska *et al.* (1998) sequenced the internal transcribed spacer region of ribosomal DNA of

Echinoparyphium elegans, *E. recurvatum*, *Echinoparyphium pseudorecurvatum*, *Pseudoechinoparyphium echinatum*, *Neoacanthoparyphium echinatoides*, and *Hypoderæum conoideum*. The most closely related species were those of *Echinoparyphium*, while *P. echinatum* and *N. echinatoides* were more distant. *H. conoideum* was found to be closely related to species of *Echinoparyphium*.

Muller *et al.* (1999) provided quantitative data on the neutral lipid content of a 45-collar-spined *Echinoparyphium* sp. cercaria from *Physa* sp. snails in Pennsylvania, USA. High performance thin-layer chromatographic analysis (HPTLC) was used to determine the major neutral lipids in cercariae obtained following snail isolation. The cercarial lipids were identified as free sterols, free fatty acids, and triacylglycerols. The most abundant fraction (free sterols) was quantified by densitometric HPTLC and the amount of free sterol (mainly cholesterol) was $0.022 \pm 0.0021 \mu\text{g}$ per cercaria. This was the first time that quantitative HPTLC was used to quantify neutral fats in an echinostome cercaria. The function of neutral lipids in cercariae is speculative. Cholesterol is probably an important structural constituent of the cells and tissues of the cercaria. Echinostome cercariae have abundant excretory concretions (calcareous corpuscles) of dubious function and cholesterol may be associated with these concretions.

3. THE GENUS *ECHINOCHASMUS*

3.1. Descriptive Studies

Distinguishing morphological characteristics of the genus *Echinochasmus* are shown in the fourth column of Table 2. Numerous species have been described in the genus; some of the better studied ones are *Echinochasmus liliputanus*, *Echinochasmus schwartzi*, *Echinochasmus perfoliatus*, *Echinochasmus bagulai*, *Echinochasmus fujianensis*, and *Echinochasmus japonias*. A species found in the USA is *E. donaldsoni* and a line drawing of an adult of that species is shown in Figure 4. Some representative descriptive studies of members of this genus are presented below.

The adult stage of *E. liliputanus* was described by Xiao *et al.* (1992) as leaf-shaped with an oral collar containing 24 spines; the spines are arranged symmetrically on the ventral and dorsal side of the collar. The adult has an oral sucker, a prepharynx and a pharynx. The intestinal ceca extend to the posterior end of the body and the acetabulum is located in the anterior region of the body. The cirrus pouch is kidney shaped, located between the bifurcation of the intestine and the acetabulum; it contains the seminal vesicle and cirrus. The vitellaria are distributed on either side of the acetabulum and contain many follicles. The male and female reproductive organs are located in the middle and

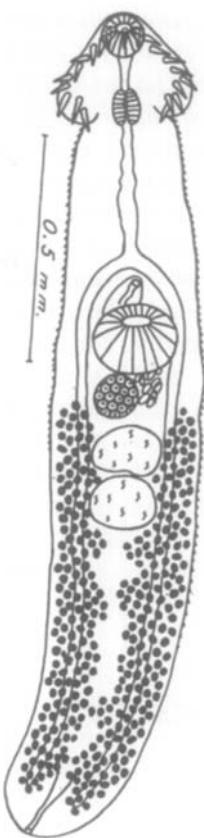


Figure 4 *Echinochasmus donaldsoni*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

posterior parts of the body; the two testes are oval and located in the posterior third. The ovary is also oval and located in the mid-body region. The uterus is convoluted, located between the anterior testis and acetabulum, and usually contains from one to six eggs.

Barse (1998) found encysted metacercariae of *E. schwartzi* in the gills of the mummichog, *Fundulus heteroclitus*, in Chesapeake Bay, Maryland. *E. schwartzi* was identified by feeding the infected gills to mice (*Mus musculus*) and ducks (*Anas platyrhynchos*); adult worms referable to *E. schwartzi* were recovered in the small intestines of both experimental hosts. *E. schwartzi* metacercariae were found in both male and female mummichogs and there was no difference in metacercarial infection rate as a function of the size of the *F.*

heteroclitis. Therefore, host size and sex were not dependent factors of infection.

E. perfoliatus adults are intestinal echinostomes with 24 collar spines (Dimitrov *et al.*, 1998). The miracidia were prepared for argentophilic studies by treatment in 0.5% aqueous AgNO₃. The location of the argentophilic structures was determined using the nomenclature of Dimitrov *et al.* (1995). There are 52 argentophilic papillae-like structures in each miracidium. Twenty-one epidermal plates were observed with a 6 + 9 + 4 + 2 pattern. On the terebratorium, 23 papilla-like structures were observed; they were asymmetrical and arranged in three axes and the papillae were distributed in three groups per axis. Both small (1 µm) and large (2.5 µm) papillae occurred in these groups. The eyespots were formed by three pairs of crystalline lenses. The excretory pores opened laterally between the third and fourth epidermal plates.

3.2. Life Cycle Studies

Dhanumkumari *et al.* (1991) described the life history of *Echinochasmus bagulai*, a species that infects avian hosts. The cercariae are released from the snail, *Thiara tuberculata*, and metacercarial cysts are formed in the gills of various fishes, i.e., *Aplocheilus panchax*, *Oryzias melastigma*, *Gambusia affinis*, and *Channa punctata*. Adult worms are found mainly in the small intestine of the bird *Ardeola grayi*. The eggs of *E. bagulai* are golden yellow, 66–78 µm in length and 39–45 µm in width. The free swimming miracidia are oval, ciliated, and contain an anterior terebratorium. There are two pigmented eyespots and two pairs of prominent anterior sensory papillae. There are two flame cells, and the posterior region of the miracidium contains the germinal cells. The redia is motile with a saccular gut that extends about half the radial length. The redia measures 300–564 µm in length and 42–45 µm in width. The cercarial body is oval and measures 132–147 µm in length by 72–75 µm in width. The collar spines are not visible in the cercaria. The tail is muscular and the excretory system is of the stenostomate type. The cercarial body has nine pairs of flame cells. The encysted metacercaria is oval with bluntly rounded ends. The oral collar region and excretory system are not clearly visible in the metacercaria.

Ditrich *et al.* (1996) described the life cycle of *Echinochasmus macrocaudatus* n. sp. from the mother sporocyst to the adult stage under both natural and experimental conditions. Prosobranch snails *Pyrgophorus coronatus*, belonging to the family Hydrobidae, from sinkholes (cenotes) in the Yucatan Peninsula, Mexico, were the first intermediate hosts of this echinostome. Cercariae released from *P. coronatus* have very large tails (zygocerous cercaria) and these cercariae encyst on the gills of characid fish *Astyanax fasciatus* (natural hosts) and experimentally on the gills of poeciliid fishes, *Xiphophorus*

variatus and *Poecilia velifera*. Encysted metacercariae of this echinostome developed into adults in the intestine of domestic chicks and ducks. The adult collar possesses 22 spines with two angle spines on each side of the collar. *E. macrocaudatus* differs from the closely related species *Echinocasmus schwartzii* by its larger oral sucker and by the location of the acetabulum situated at two-fifths of the body length. Dimitrov *et al.* (1998) studied *Echinocasmus perfoliatus*, a European and Asian species that infects both mammals and birds. Numerous fresh water snails serve as first intermediate hosts and cercariae released from the snails have a broad specificity towards numerous second intermediate host fish species.

3.3. Experimental and Manipulative Studies

Various species of *Echinocasmus* infect humans. For instance, Seo *et al.* (1985) reported *Echinocasmus japonicus* infections from humans in Korea; they noted that infection with this species caused abdominal pain, diarrhea, and lethargy; diagnosis was based on recovering eggs in the feces. Chai and Lee (1991) noted that, in humans, in mild infections consisting of less than 100 worms, eggs were absent from the feces. Chai *et al.* (1989) used an enzyme-linked immunosorbent assay (ELISA) to detect infection of *E. japonicus* in the absence of fecal eggs. Xiao *et al.* (1995) did experimental and epidemiological studies in Chenquiaozhou Village, China on the mode of infection of *Echinocasmus liliputanus*. They found that this echinostome infects humans in two ways, i.e., by humans consuming raw fish containing *E. liliputanus* metacercariae or by humans drinking unboiled water containing cercariae. This is the first echinostome for which human infection can occur by the ingestion of the cercarial stage. Luty and Mizgajska (1999) identified eggs of *Echinocasmus perfoliatus* in the feces of dogs and cats during a 1997–1998 survey of intestinal parasites in 445 dogs and 205 cats in Poznan, Poland. The behavior of *E. japonicus* cercarial emergence was altered by changes in water temperature. Infected *Parafossarulus striatulus* snails maintained in pond water at 27–32°C showed the highest rate of cercarial emergence (Chen *et al.*, 1990). Cercarial emergence decreased when the water temperature was below 24°C and ceased at 14°C.

Humans infected with *Echinocasmus fujianensis* have been treated with praziquantel and mebendazole-medicated salt (Chen *et al.*, 1997). Both treatments relieved the symptoms of infection such as abdominal pain, diarrhea, distension and anorexia. There were, however, mild side effects associated with treatment. Praziquantel, in comparison with mebendazole-medicated salt, induced a greater reduction rate of infection; egg reduction rate after treatment was 95.4%. The suggested praziquantel dosage was 100 mg for children under 12 years old, and 200 mg for older patients. Mebendazole-medicated salt was

useful when treating patients who had multiple infections including *E. fujianensis* and nematode parasites. Wang *et al.* (1998) conducted a survey in a primary school in Anhaii Province, China to investigate the seasonal distribution of *E. liliputanus* in children. They found that infection with this species peaked in the fall, particularly in September and October. The main reason for the seasonal distribution was that children drank untreated water from local ponds containing *E. liliputanus* cercariae.

Xiao *et al.* (2001) studied encystment *in vivo* and *in vitro* of *E. liliputanus* cercariae from *Bellamya aeruginosa* snails and examined the biological activity of the metacercariae. Encystment *in vivo* occurred in the gills of goldfish second intermediate hosts. However, cercariae were induced to encyst in a variety of saline solutions including Locke's solution. Cysts formed *in vivo* and *in vitro* were capable of excystation in 0.1% sodium deoxycholate at 37°C. Cysts formed *in vivo* and *in vitro* were equally infective to experimental rabbit definitive hosts.

3.4. Biochemical and molecular studies

Cheng *et al.* (1999) used random amplified polymorphic DNA analysis (RAPD) to examine the relationship between *Echinochasmus fujianensis* and two related species (*Echinochasmus japonicus* and *Echinochasmus liliputanus*) in Eastern China. Four hundred and sixty-nine polymorphic DNA fragments were obtained by 28 primers from the three species of *Echinochasmus*; 20.8% of the fragments in *E. japonicus* and 97.6% in *E. fujianensis* were the same as *E. liliputanus*. It was concluded that *E. fujianensis* and *E. liliputanus* are conspecific, but distinct from *E. japonicus*. Multiple infections with *E. fujianensis* and *E. japonicus* occur in humans in Eastern China, with *E. fujianensis* being the dominant species.

4. THE GENUS *HIMASTHLA*

4.1. Descriptive Studies

The characteristic features of the genus *Himasthla* are described in the last column of Table 2. Adults of *Himasthla* are characterized by a long body, and an anterior collar usually with 31 collar spines. Figure 5 is a line drawing of *Himasthla rhigedana*. Members of this genus use various intermediate and definitive hosts including snails, bivalves, gulls, herons, fishes, and occasionally humans. The species implicated as a human pathogen is *Himasthla muehlensi*, and considerable biological work has been done with three well-described

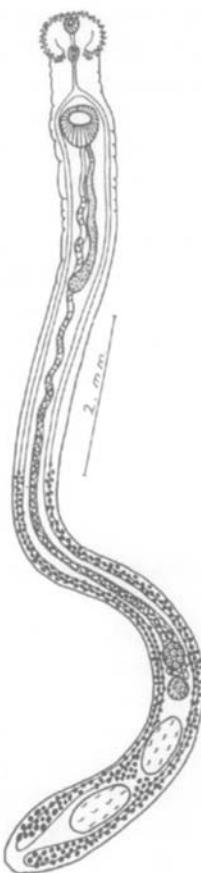


Figure 5 *Himasthla rhigedana*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

species, i.e., *Himasthla leptosoma*, *Himasthla quissetensis* and *Himasthla secunda*.

Stunkard (1938) described the life cycle of *H. quissetensis*. Echinostome cercariae develop in daughter rediae in the prosobranch marine snail *Ilyanassa obsoletus*, and encyst in a number of marine bivalves including *Mya*, *Mytilus*, and *Pectin*, and in some gastropods including *Crepidula*. Because of the lack of specificity of the second intermediate host of this echinostome, infection of humans by eating raw or insufficiently cooked shellfish infected with encysted metacercariae is a possibility. A natural definitive host is the herring gull. Experimental infections can be obtained in various avian hosts and on the

chick chorioallantois, making this a good echinostome for use in the laboratory.

The metacercarial stage of *H. leptosoma* has an oral collar with 29 cephalic spines; the collar spines may not be readily noticeable because they can be introverted into the head region by muscles in the collar (Irwin *et al.*, 1984). Sensory papillae surround the oral sucker but do not occur on the surface of the acetabulum; the tegumentary spines in this species are peg-like (Irwin *et al.*, 1984). Using the *H. leptosoma* model of introverted collar spines, Fujino *et al.* (1994) made extensive studies on the retraction and extension of collar spines in *Echinostoma trivolvis*.

The cercarial stage of *H. quissetensis* is released from the first intermediate snail host, *Ilyanassa*=(*Nassarius*) *obsoletus*. The cercariae of *H. quissetensis* lack eyespots (Hellmann, 1975). *I. obsoletus* snails infected with *H. quissetensis* rarely are doubly-infected; of 89 *I. obsoletus* snails infected with *H. quissetensis*, only three were doubly-infected with other larval trematodes (Vernberg *et al.*, 1969). The cercaria of *H. secunda* has been used for behavioral studies by Chapman and Wilson (1972). The cercarial behavioral pattern of this species includes bending waves of the tail. The tail has been observed to propagate waves associated with complex swimming motions; these motions occur even when the body is removed.

4.2. Life Cycle Studies

Adams and Martin (1963) described the life cycle of *Hismathla rhigedana*. Miracidia develop within the egg in about 18 days at 22–24°C. After the miracidia hatch they penetrate the marine snail *Cerithidea californica*. Development continues through sporocyst and redial stages and cercariae, released from the second generation rediae, encyst on vegetation, mainly marine plants in the intertidal zone. Cysts removed from vegetation or from the sides and bottom of glassware in the laboratory are infective to domestic chicks. In the wild, the definitive host is the curlew. Because of the availability of this echinostome from naturally infected *C. californica*, the tendency of cercariae to encyst on glassware or substrates, and the ease of infecting domestic chicks, this echinostome can provide a good model for experimental work on *Himasthla*.

Vanoverschelde and Vaes (1980) described the life cycle of *Himasthla militaris* and also studied the effects of light and dark on cercarial release from *Hydrobia stagnorum* snails. The cercariae emerge in the dark but remain in the snail host during the light phase. The study used a 9 h light and 15 h dark schedule; when the schedule was reversed so was the cercarial emergence pattern.

4.3. Experimental and Manipulative Studies

Irwin *et al.* (1984) studied excystment of *Himasthla leptosoma* metacercariae from bivalves in the genera *Cardium* (cockles) and *Mytilus* (mussels) in the United Kingdom. They treated the cysts in a medium consisting of 0.8% sodium taurocholate, 0.3% trypsin, and 0.8% L-cysteine at 41°C. Such treatment resulted in intense metacercarial activity and within 20 min newly excysted metacercariae emerged through the cyst wall. Scanning and transmission electron microscopy showed that the excysted metacercariae emerged through a small area of the cyst that did not have a layer of lamellae, present elsewhere towards the innermost surface. The appearance of ruptured cyst walls suggested to Irwin *et al.* (1984) that the walls had been softened by the excystation medium. However, the intense activity of the larva within the cyst during excystation suggested that metacercarial enzymic factors were probably released from the organism within the cyst. Results of their study suggested that excystation involved both intrinsic and extrinsic factors, a conclusion that has been reached for many other species of digeneans in regard to excystation.

Kirschner and Bacha (1980) used encysted metacercariae of *Himasthla quissetensis* to study excystation *in vitro*. Encysted metacercariae were obtained using Laurie's (1974) method of artificially inducing encystment of cercariae following their release from *Ilyanassa obsoletus* snails. In the field, cysts of this species occur in the tissues of numerous species of marine bivalves and some marine gastropods. Excystment of this species involves both extrinsic and intrinsic factors. First the cyst wall is weakened and then the organism shows vigorous muscular movements within the cyst wall. Maximum excystment occurred following low pH treatment in Earle's BSS at pH 2.5 followed by the reductant, sodium dithionite (0.015 M) and a final treatment in 0.3% trypsin plus 0.05% bile salts in Earle's BSS adjusted to pH 7–8 with NaHCO₃ at 41°C. Since *H. quissetensis* cysts are found in numerous marine mollusks, they are available for chemical excystation studies. The medium described above is recommended for this purpose.

LeFlore and Bass (1982) studied excystation *in vitro* of *Himasthla rhigedana* metacercariae. Cysts of this species are obtained following cercarial release of this echinostome from naturally infected marine snails *Cerithidea californica*. Cysts are obtained in the laboratory on the bottom and sides of glassware containing salt water and the infected snails. Maximum excystation was obtained following pretreatment of cysts in 0.015 M sodium dithionite for 7 min and then treatment in a medium containing 0.2% sodium taurocholate plus 0.5% trypsin in alkaline Hank's BSS at pH 7.8 for 1 h at 42°C. Some differences in excystation patterns were found between this species and that of *H. quissetensis* studied by Kirschner and Bacha (1980). Differences in patterns were explained in part based on the fact that *H.*

rhigedana encysts in the open on a substrate whereas *H. quissetensis* encysts in or on molluscan tissues.

H. quissetensis was the first echinostome to be cultivated on the chick chorioallantois from the cercarial stage (Fried and Groman, 1985). This echinostome, which typically uses bivalves as its second intermediate host, has been grown to the adult stage in chick embryos in the absence of the metacercarial stage. Infected *I. obsoletus* snails from Barneget Bay, New Jersey, USA were used as the source of cercariae. The *H. quissetensis* cercariae from the snails were also used to infect 6-day-old white leghorn fertile eggs prepared by Zwilling's (1959) method. Of 28 eggs examined at necropsy, 23 were infected and most of the exposed eggs contained adult worms (a total of 224 worms were recovered). In some cases, encysted metacercariae were also found. The adult worms showed considerable size variation; one 9-day-old chorioallantoic worm measured 0.24 mm, whereas another measured 0.47 mm in length. The worms contained hematin and evidence of other blood ingesta in their intestinal ceca. The adult worms also had well-developed reproductive structures and the 31 collar spines typical of *H. quissetensis* were easily seen. The cercariae contained cystogenous material, excretory concretions, and genital anlage typical of the species.

The life cycle of *Himasthla militaris* is correlated with external environmental factors (Vanoverschelde, 1982), including both temperature and salinity. The longevity of the miracidium was tested as well as the rate of miracidial infection in the first intermediate host. The miracidial half-life was greatest at 14°C and least at 30°C. The miracidium infected snail hosts at 25 and 30°C, but not at 14°C, and miracidia showed optimal survival at a salinity of 17 parts per 1000. The encysted metacercariae of *H. leptosoma* excysted in an alkaline trypsin–bile salts solution at 41°C and larvae were released after 20 min in the medium (Irwin *et al.*, 1984). Electron microscopy was used to observe the excystation process and larvae emerged through a small opening in the metacercarial cyst. Development of *H. quissetensis* occurred on the cloacal lips of experimentally infected domestic chicks (Herman and Bacha, 1978). Cercariae placed on the cloacal lips of 1-, 10-, and 20-day-old chicks developed rapidly into sexually mature adults in that site. Each chick was infected with 50–60 cercariae obtained from *I. obsoletus*. As worms aged they showed a preference for the ileum and worms that localized in the bursa of Fabricius did not do as well there as those in the ileum.

Laurie (1974) studied the structure of the *H. quissetensis* cyst using an *in vitro* encystment technique on the cercariae. This *in vitro* technique induced encystment and allowed for studies on the ultrastructure of ectopically formed cysts. Electron microscopy revealed that the cyst was composed of two layers, an inner and outer layer. The outer layer was homogeneous and composed of granules released from the tegument, whereas the inner layer was formed by scrolled rods originating from subtegumental bodies.

4.4. Biochemical and Molecular Studies

Hoskin and Cheng (1974) used autoradiographic and respirometric techniques to examine the uptake of exogenous glucose from the rediae of *Himasthla quissetensis*. Starved rediae of this species utilized oxygen aerobically and took up glucose. Daughter rediae of *H. quissetensis* were dissected from the digestive-gonad gland of *Ilyanassa obsoletus* and placed in sterile seawater. Glucose was added at different time intervals and the uptake of glucose was measured. Starved rediae showed elevated oxygen uptake. The rediae of *H. quissetensis* contain a yellow-orange pigment. Thin layer chromatography (TLC) was used to determine the chemical nature of this pigment. Hoskin and Cheng (1975) extracted the pigment in chloroform-methanol from both the rediae and tissue of the first intermediate host *I. obsoletus*. The pigment was analyzed on silica gel sheets; spectrophotometric techniques were also used to confirm the analyses. The retention factor (R_f) values of the pigments (i.e., the distance moved by the solute over the distance moved by the mobile phase front) as determined by TLC and the wavelengths determined by spectrometry provided evidence that the main compound was β -carotene. The function of β -carotene in the redia and snail is not clear.

5. THE GENUS *HYPODERAEUM*

5.1. Descriptive Studies

The collar spines in members of the genus *Hypoderæum* are weakly developed; the excretory ducts are without lateral branches and the uterus contains many eggs. The ventral sucker is at least five times larger than the oral sucker. Although a number of species have been described in this genus, most of the experimental work is focused on *Hypoderæum conoideum*. Figure 6 shows a line drawing of this species.

Lie *et al.* (1973) described the biology of larval and adult stages of *Hypoderæum dingeri* from *Lymnaea rubiginosa* snails in Thailand; adults were obtained by feeding encysted metacercariae to ducklings. Williams (1978) described *Hypoderæum* sp. adults based on experimental infections in Khaki Campbell ducklings. This species is closely related to *H. dingeri*, but differs from other members of the genus in the number and arrangement of the circumoral spines, distribution of tegumentary spines, shape of testes, size of cirrus sac, structure of cirrus, and extent of the vitellaria. Mekhraliev (1988) described cercarial morphometrics of *H. conoideum* from *Lymnaea auricularia* in Lake Glyulalan, Azerbaijan. Skovronskii (1985) described *Lymnaea truncatula* as the first and second intermediate hosts of *H. conoideum*.

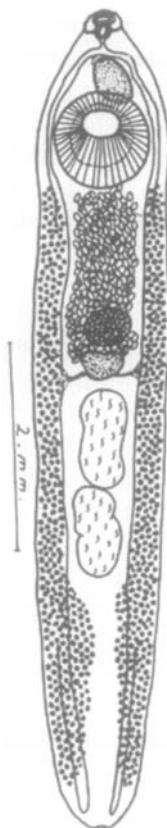


Figure 6 *Hypoderæum conoideum*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

in Russia. The identity of *H. conoideum* was confirmed by infecting domestic chicks with encysted metacercariae.

5.2. Life Cycle Studies

Mathias (1925) studied the life cycle of *Hypoderæum conoideum*. The miracidium required about 3 weeks to develop prior to penetration in pulmonate snails *Lymnaea stagnalis* and *Lymnaea limosa* in which sporocysts and first and second generation rediae developed. Echinostome cercariae developed in the second generation and then after leaving the first intermediate host encysted in the same snail or another snail of the same or a different species.

Adult flukes developed in the intestine of swans, geese, and ducks. Lie (1964) studied the life cycle of *Hypoderæum dingeri* in Holland. The miracidia penetrated and developed into sporocysts in *Lymnaea rubiginosa* snails. The pattern of mother and daughter redial development is as described for *H. conoideum*. Likewise, cercarial release and re-entry into the same or other snails to form metacercarial cysts is as described for *H. conoideum*. Geese and ducks serve as natural and experimental vertebrate definitive hosts. *H. dingeri* may be conspecific with *H. conoideum*.

Yurlova (1987) described the life cycles of *Hypoderæum conoideum* and *H. cubanicum* (the latter was described as a new species) from the Lake Chany area of West Siberia, Russia. Of ten species of gastropods used as experimental first intermediate hosts, only *Lymnaea tumida* served as a host for both *H. conoideum* and *H. cubanicum*. Cercariae were first released from *L. tumidae* at 43 days postmiracidial infection for *H. conoideum* and at 79 days postmiracidial infection for *H. cubanicum*. Infective metacercariae of both species were obtained in *L. tumidae* and *Planorbis planorbis*. Mature *H. conoideum* adults were obtained 11 days after cysts were fed to domestic ducks; *H. cubanicum* adults were obtained in ducks 9 days after the cysts were fed. The developmental stages of both species were described.

5.3. Experimental and Manipulative Studies

Stadnichenko (1971) described histopathological effects and metabolic changes in *Lymnaea auricularia* snails infected with the intramolluscan stages of *Hypoderæum conoideum*. Kublickiene and Ciuniene (1973) infected 8-week-old domestic ducks with encysted metacercariae of *H. conoideum*; the ducks were necropsied at various postinfection intervals to assess the intestinal damage associated with experimental echinostomiasis. Intestinal changes included hyperemia, hemorrhagia, desquamation and necrosis between 3 and 14 days postinfection. Necrotic lesions were found in the duodenum, along with desquamation of the ileum, and proliferative necrotic changes in the rectum at 14 to 38 days postinfection. By 60 days postinfection, most of the lesions had healed. Kublickiene and Ciuniene (1973) described the pathological changes in the intestinal mucosa of ducks experimentally infected with *H. conoideum* at 3 to 60 days postinfection.

KeQiang *et al.* (1995) used transmission electron microscopy (TEM) and scanning electron microscopy (SEM) to study the ultrastructure of the tegument of *H. conoideum*. As seen by SEM, the head collar was semicircular, and the collar spines were located in tegumentary folds; the body spines were scale-like and distributed from the collar to the acetabulum. The tegument was thick, contained bar- and disc-shaped secretory bodies, and numerous

mitochondria. KeQiang *et al.* (1996a) used TEM to examine mature sperms in *H. conoideum*. The sperm structure was typical of that of digenleans, i.e., the anterior region originated from the zone of differentiation and there was a complete circle of peripheral microtubules and two axonemes of the 9 + 1 arrangement in the sperm body. KeQiang *et al.* (1996b) used TEM to study vitellogenesis in *H. conoideum*. Vitelline follicles were surrounded by a basal lamina, and the immature follicles had relatively little cytoplasm, but numerous ribosomes. The mature vitelline cells had considerable cytoplasm, and remnants of rough endoplasmic reticulum around the nucleus; glycogen was abundant in the cells. Lipid droplets were detected in both the developing and mature vitelline cells.

Toledo *et al.* (1999a) studied the infectivity of *H. conoideum* miracidia in an array of laboratory raised freshwater snails, i.e., *Lymnaea peregra*, *Lymnaea corvus*, *Physella acuta* and *Gyraulus chinensis*. In the wild, these snails live in the same habitat as the echinostome. *L. corvus* and *L. peregra* were equally susceptible to the echinostome when specimens of each snail species were singly exposed to miracidia. When miracidia could choose either lymnaeid species they showed a high degree of specificity toward *L. peregra*. The results suggested that *H. conoideum* miracidia can distinguish between these two species of lymnaeids in their host orientation, therefore miracidia could achieve specificity before contacting the snail host. During the snail-host orientation process, miracidia might respond to signals different from those generated upon snail contact and invasion. For further discussion of this topic, see Haas (2000). Toledo *et al.* (1999a) studied survival characteristics of *H. conoideum* from lymnaeid snails in Valencia, Spain. The survival patterns were almost identical to those found in the cercariae of *Echinoparyphium albuferensis*. For details of this study see Section 6.2.

5.4. Biochemical and Molecular Studies

Shaymardanov (1995) used a variety of histochemical staining procedures to document egg-shell formation in *Hypoderæum conoideum*. Although not strictly a biochemical study, the tests showed the presence of basic proteins, phenols, and phenolases. Since all three precursors of sclerotin were demonstrated, it is apparent that quinone-tanning is the mode of egg-shell formation in this echinostome. More detailed biochemical studies and molecular studies on this genus appear not to be available.

6. OTHER ECHINOSTOME GENERA

6.1. Genus *Petasiger*

Members of the genus *Petasiger* are avian parasites. They have a short, plump body, widest at the mid region. The length of adults is about 1 to 2 mm and the testes are oblique. In *Petasiger*, the number of collar spines varies between 19 and 27. The acetabulum is in the mid-body region or posterior to the middle; the uterus is short and contains a few eggs. Beaver (1939) described *Petasiger nitidus* with the cercaria of the magnacauda type (large tail) that develops in rediae in planorbid snails in the genus *Helisoma*. Figure 7 shows a line drawing of the adult *P. nitidus*. After cercariae of *P. nitidus* emerge from the snails they are eaten by several species of freshwater fishes in which they encyst and develop

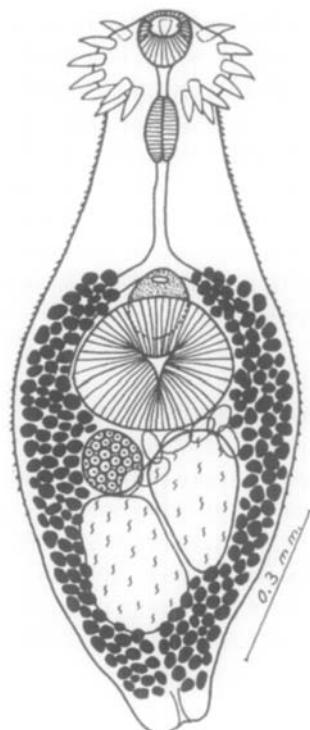


Figure 7 *Petasiger nitidus*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

into the metacercarial stage. Experimental infections were established in canaries by feeding them metacercariae. The natural definitive host is the horned grebe, *Colymbus auritus*. Descriptive, life cycle and experimental studies were done on species of *Petasiger* in the 1990s and representative studies are listed below.

Kostadinova and Chipev (1992) found larval stages of a species of *Petasiger* in *Planorbis planorbis* snails from brackish water on the Black Sea coast of Bulgaria. The cercaria was of the magnacauda type and contained 19 collar spines. The life cycle was completed using numerous laboratory-raised aquarium fishes mainly in the genus *Puntius* as experimental second intermediate hosts and canaries as the definitive host. The authors provided detailed descriptions of the parasitic and free-living stages. The species was described as *Petasiger grandivesicularis* and the authors also provided a key to the magnacauda cercariae from the Palaearctic region. Kostadinova (1997) used argentophilic procedures to provide chaetotaxy data (sensory papillae and other silver-staining structures on the tegument) on the cercariae of two species of *Petasiger*. Kostadinova (1999) reported a new geographical record of *P. grandivesicularis* from the little grebe, *Tachybaptus ruficollis*, in Germany. Additional information on intraspecific variation of adults of this species of *Petasiger* was given.

King and Van As (2000) described the biology and life cycle of a new species, *Petasiger variospinosus*. This species has an oral collar with 27 spines. Cercariae were obtained by isolating freshwater snails *Bulinus tropicus*, in orange Free State, South Africa. Tadpoles of the African clawed toad, *Xenopus laevis*, from the same locale as the snails, contained encysted metacercariae with 29 collar spines. Laboratory reared cormorants, *Phalacrocorax africanus*, were used as experimental definitive hosts. Adult worms were obtained after feeding the infected tadpoles to the cormorants and were referable to *P. variospinosus*. In addition to completing the life cycle experimentally, all stages were described by light and electron microscopy.

Shostak (1992) examined the survival of *P. nitidus* cercariae released from *Helisoma trivolvis* in relation to temperature, pH, and salinity. Cercariae of this species were maintained at various temperatures (4 to 35°C), pH levels (5–9), and salinities (5 mg to 29 g NaCl/L). The optimal temperature for survival was 8°C. Some variation in survival was noted as a factor of pH at 8 and 23°C. Best survival in terms of salinity at 23°C was in 2.9 g NaCl/L. All cercariae died within 1 h at a salinity of 17.5 g/L or higher. Cercariae tolerated a range of environmental conditions similar to that tolerated by the molluscan host *H. trivolvis*.

6.2. Genus *Euparyphium*

The genus *Euparyphium* is characterized by a short cirrus sac located anterodorsal to the acetabulum; there are 26 or more collar spines and the

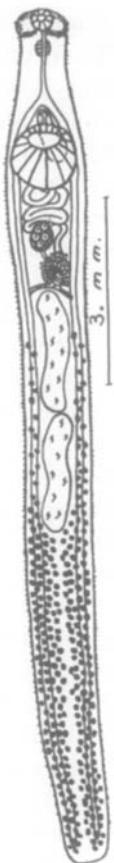


Figure 8 *Euparyphium inerme*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

testes are elongate and twisted spirally. A line drawing of a representative species, *Euparyphium inerme*, is shown in Figure 8. Hoberg *et al.* (1997) reported the presence of *E. inerme* from the intestines of the river otter *Lutra canadensis* in the Pacific Northwest. This was the first report of this echinostome in river otters. Beaver (1941) described a species of *Euparyphium* subsequently named in his honor as *Euparyphium beaveri*. Cercariae of this echinostome develop in second generation rediae in the pulmonate snail *Stagnicola (Lymnaea) angulata*, and following emergence from the snail encyst in the cloacal wall of tadpoles. Acquisition of adults is obtained following ingestion of infected tadpoles by mink, otter, and snowshoe hares. It appears that metamorphosed frogs retain the metacercarial infection and also

provide a source of infection to warm-blooded vertebrate definitive hosts. The parasitology group in Valencia, Spain, under the direction of Drs J.G. Esteban and R. Toledo, have been doing considerable work with a species of *Euparyphium* from Valencia. Their work is featured below.

Esteban *et al.* (1997) described the life cycle and illustrated the various life-history stages of a new species of echinostome, *Euparyphium albuferensis*, from a nature park near Valencia, Spain. *Rattus norvegicus* and *Rattus ratto* served as natural definitive hosts for this species. Experimental hosts consisted of albino rats, mice, and golden hamsters. Adult worms with 45 collar spines were obtained from both natural and experimental hosts. Avian hosts tested, i.e., chickens, ducks, and pigeons, were not suitable definitive hosts for this species of echinostome. The freshwater planorbid snail *Gyraulus chinensis* served as both the natural and experimental first intermediate host. *G. chinensis*, along with a variety of other snails, i.e., *Lymnaea truncatula*, *Lymnaea peregra*, *Lymnaea palustris*, and *Physa acuta* served as second intermediate hosts. Toledo *et al.* (1998b) described the argentophilic pattern (cercarial chaetotaxy) of cercariae of *E. albuferensis* released from naturally infected *G. chinensis* snails from Spain. Some sensillary arrangements, particularly in the dorsal cephalic region of the body and in the tail of the cercaria of *Euparyphium*, differed from that of other closely related genera, i.e., *Echinoparyphium*, *Echinostoma*, *Hypoderæum*, and others. The authors point out that this is very important because when dealing with adult worms there are sometimes few distinct characteristics for dealing with closely related genera. The authors have presented a useful table on the cercarial sensilla of *Euparyphium* spp. and echinostome cercariae in closely related genera. Toledo *et al.* (1999b) studied survival characteristics of cercariae of *E. albuferensis* at 20 and 30°C. Cercarial survival was age dependent and was higher at 20°C. The maximum life span for this cercaria was 26 h at 20°C and 16 h at 30°C. Its time to 50% mortality was the same at each temperature. This cercaria seems well adapted for transmission in its natural habitat. The age dependency factor of cercarial survival may be related to diminishing endogenous energy levels, particularly glycogen. For a similar study on depletion of glycogen reserves in an echinostome cercaria as a function of cercarial aging see Fried *et al.* (1998b).

Toledo *et al.* (1999c) studied the production and chronobiology of emergence of the cercariae of *E. albuferensis* from *G. chinensis* experimentally infected with single miracidia (monomiracidia infections). Studies were made for 28 consecutive days after the first emergence of cercariae. The effect of a sudden change in the light-dark cycle was also investigated. The authors noted that although the daily cercarial shedding rates were very variable, a progressive increase in cercarial production was observed in the first weeks of cercarial shedding. Under a 12 h light-dark cycle, the cercariae emerged in the light and the rhythm was circadian. A sudden change in the light-dark cycle resulted in corresponding changes in the emergence patterns, showing that

cercarial emergence in this species is correlated to light-dark changes. The implications of the work to the biology of this life cycle in its natural habitat in Spain is discussed in Toledo *et al.* (1999c).

6.3. Genus *Stephanoprora*

Adult members of the genus *Stephanoprora* have characteristics that are typically echinostome-like, as seen in the line drawing of *Stephanoprora pseudoechinata* in Figure 9. A distinguishing characteristic of members of

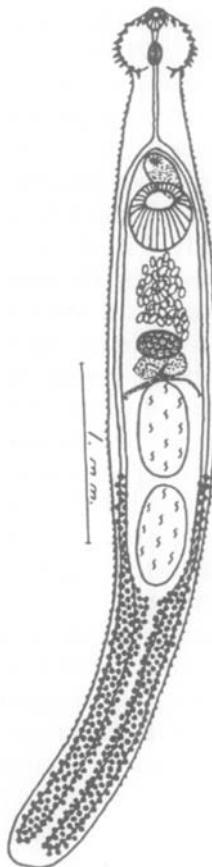


Figure 9 *Stephanoprora pseudoechinata*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

this genus is that the vitelline follicles extend forward as far as the posterior margin of the anterior testis (see Figure 9). Kostadinova and Gibson (2000) included *Stephanoprora* with the genera that are species rich and the characteristic features of the genus are shown in the fifth column of Table 2 (see Section 1). The genus may not be a natural group since some species have cercariae of the magnacauda type, whereas others have cercariae of the gymnocephalus type but lacking collar spines. Schell (1985) reviewed the earlier descriptive literature on this genus and discussed the life cycle of *Stephanoprora denticulata*, which has cercariae of the magnacauda type that develop in rediae in *Biomphalaria glabrata* snails. Cercariae are released from the snails, enter the pharynx of fishes, and encyst in the gills. Natural infections have been found in the gills of killifish, *Fundulus heteroclitus*. Experimental infections were established in various vertebrate hosts, i.e., hamsters, chicks, gulls; sexually mature worms were obtained 7 days after feeding the metacerarial cysts. Natural definitive avian hosts include the skimmer, crow, heron, and sandpiper. Schell (1985) noted that in contrast to *S. denticulata*, the life cycle of *Stephanoprora paradenticulata* is one that does not have a cercaria of the magnacauda type, but has a typical gymnocephalous cercaria that lacks collar spines. However, adult worms from the intestines of the sandpiper have an oral collar with spines.

Other recent studies on species of the genus are reported below. Holeman-Spector and Olaque (1989) reported two new species of *Stephanoprora* from seagulls, *Larus dominicanus*, in Uruguay. The first species, *Stephanoprora dogieli*, was characterized by a small peristomic disk armed with hook-shaped spines and a long esophagus; these features distinguished it from the related species, *Stephanoprora manei*, *Stephanoprora uruguayense*, and *Stephanoprora conciliata*. *S. uruguayense* was characterized by 11 small spines on the anterior margin of the oral sucker, absence of a prepharynx, which along with other features differentiates it from related species, i.e., *S. dogieli*, *S. manei*, and *S. conciliata*. Etchegoin and Martorelli (1997) described *Stephanoprora podicepsi* as a new species from the small intestine of the great grebe, *Podiceps major*, from Buenos Aires, Argentina. The new species has features in common with *S. conciliata*, *S. manei*, *S. dogieli*, and *S. uruguayense* but can be distinguished from these by the smaller size of the body, the peristomic disk, testes, and ovary and a shorter esophagus and uterus. Only descriptive and life cycle studies appear to be available for members of the genus *Stephanoprora*.

6.4. Genus *Isthmiophora*

Adults in the genus *Isthmiophora* are characterized by 27 collar spines, and testes that are irregular in shape or lobed and located in the middle of the posterior half of the body. A line drawing of *Isthmiophora melis* is shown in

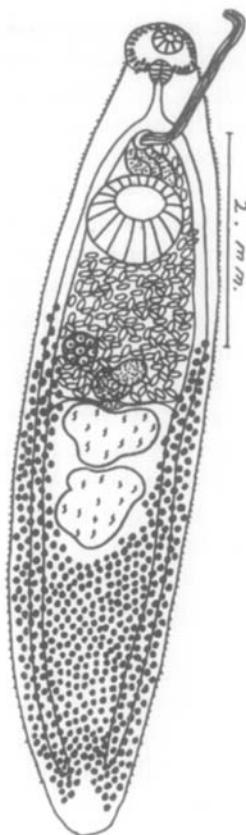


Figure 10. *Isthmiophora melis*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

Figure 10. Kanev *et al.* (2000) considered *Echinoparyphium* as possibly cogenetic with *Isthmiophora*. Kostadinova and Gibson (2000) in their recent classification of the Echinostomatidae retained *Isthmiophora* as a genus separate from *Euparyphium*.

Dönges (1963) infected snails with individual rediae of *Isthmiophora spiculator* to determine the exact sequence of larval stage development. An interesting finding of echinostome redial transplant studies is the occurrence of unlimited multiplication of the redial stages of *I. spiculator* (see Dönges and Gotzelman, 1988).

Smales and Blanksespoor (1984) did SEM studies on adults of *I. melis*. The tegument of this species had a cobblestone-like appearance with interspersed pits. The taxonomic position of *I. melis* and the significance of cirrus morphology as a useful character were discussed. The distribution and possible

function of the four types of sensory papillae seen in *I. melis* (i.e., domed, button, ciliated, and bilobed) was also discussed.

6.5. Genus *Acanthoparyphium*

Members of the genus *Acanthoparyphium* have a long cirrus sac, extending some distance posterior to the acetabulum. They are characterized by 23 collar spines and testes that are round or oval. The best known species in this genus is *Acanthoparyphium spinulosum* and a line drawing of this species is shown in Figure 11. Bearup (1960) examined the life cycle of *A. spinulosum* in Australia where the estuarine mudflat snail *Pyzarus australis* is the first intermediae host. He did not find the sporocyst stage, but noted the usual two generations of rediae and the release of cercariae from daughter rediae. Cercariae encysted in gastropods in the genera *Salinator* and *Pyrizus* and in

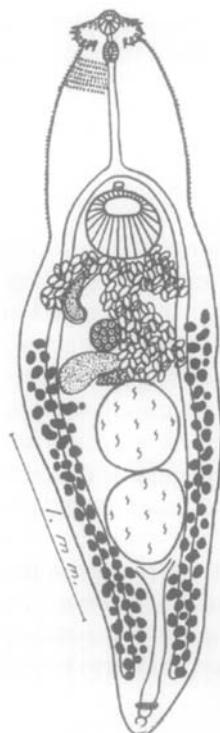


Figure 11 *Acanthoparyphium spinulosum*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

polychaete worms. Infected *Salinator* snails fed to silver gulls, *Larus vovae-hollandiae*, produced adult worms. Plovers and ducks serve as natural definitive hosts of the species in the wild. Martin and Adams (1961) studied *A. spinulosum* in California where the marine snail *Cerithidea californica* serves as both first and second intermediate host. Domestic chicks can be infected with encysted metacercariae of this echinostome and, in the wild, avocets and plovers are natural definitive hosts as a result of feeding on infected *C. californica* snails. Because of the availability of *C. californica* snails naturally infected with *A. spinulosum*, this echinostome should provide a good model for experimental work.

Bass and LeFlore (1984) examined excystation *in vitro* of *A. spinulosum* metacercariae from the radula of naturally infected *C. californica* snails. Optimal excystation of encysted metacercariae of this species involved pre-treatment in 0.5% pepsin in Hank's BSS at pH 2.0 (acidified with HCl) for 1 h; then treatment in a reductant of 0.015 M sodium dithionite for 10 min followed by incubation for 1 h at 42°C in a medium containing 0.2% sodium taurocholate plus 0.5% trypsin in Hank's BSS at pH 7.8. The ability to obtain excysted metacercariae of this species allows for a variety of experimental studies beginning with the newly excysted metacercariae. See Fried (1994) for further details.

6.6. Genus *Patagifer*

Adult members of the genus *Patagifer* have an oral collar with deep dorsal and ventral indentations; the collar spines are in a single row, the testes are elongate and the acetabulum is unusually large. Figure 12 is a line drawing of *Patagifer vioscai*. A useful paper on this genus is that of Jain and Srivastava (1970), who provided a key to the species of *Patagifer*, and discussed the validity of characters used to distinguish species in the genus. These characters include the number of collar spines; the presence of a notch at the posterior end of the acetabulum; the extent of overlapping of the cirrus sac by the acetabulum; the distance between the ovary and testes; the relative size of the oral sucker and prepharynx; and the width of the collar in relation to that of the body. The authors synonymized some of the species in the genus as follows: *Patagifer chadrapuri*, *Patagifer srivastavai*, and *Patagifer sarai* were synonymized with *Patagifer wesleyi*; *Patagifer simarai* was synonymized with *Patagifer bilolris*. Studies other than descriptive appear not to be available for any member of this genus.

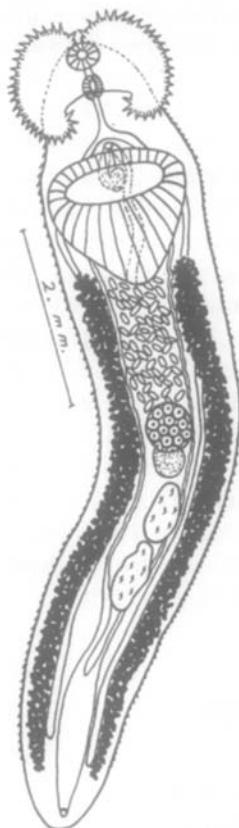


Figure 12 *Patagifer vioscai*. Reproduced from Schell, S.C., 1985, *Trematodes of North America, North of Mexico*, University Press of Idaho, with the permission of the publisher.

7. OTHER ECHINOSTOME-LIKE GENERA

A variety of genera are often considered as echinostome-like based on either the cercaria, the adult or the life cycle. One such echinostome-like genus is *Ribeiroia*. Species of *Ribeiroia* have echinostome-like affinities in terms of their biology, structure, and life history. Johnson *et al.* (1999) implicated a species of *Ribeiroia* (species not designated at the time of writing) as causing deformities in amphibians. Cercariae of *Ribeiroia*, released from *Helisoma* snails, encysted in the nervous system of tadpoles and led to abnormal and multiple limb development. This work was reported in *Science* and certainly increased interest in and coverage of echinostome-like digenaeans in the lay press.

Two genera often included with echinostomes are *Parorchis* and *Philophthalmus*. Species of *Parorchis* are associated with marine birds where they live in the cloaca, rectum, and bursa of Fabricius. Perhaps the best studied species both in the USA and in the United Kingdom is *Parorchis acanthus* whose life cycle was described by Stunkard and Cable (1932). Adults of *P. acanthus* have served as an interesting model in studies on germinal cells and gametogenesis (Rees, 1939). I have used encysted metacercariae of *P. acanthus* to develop an excystation medium for digeneans (see Fried and Roth, 1974). This alkaline medium, consisting of trypsin and bile salts in Earle's BSS, is now used widely to obtain chemically excysted metacercariae of echinostome and non-echinostome digeneans. Eyeflukes in the genus *Philophthalmus* often attract attention as occasional parasites of man. Several cases of human ocular philophtalmiasis have been reported in Israel based on infection with *Philophthalmus lucipetus* (see Gold and Nollen, 1999). The eyefluke *Philophthalmus hegeneni* was the first digenean grown on the chick chorioallantois from excysted metacercariae to the ovigerous adults (Fried, 1962). Nollen (1997) has done extensive studies on the reproductive biology, physiology, and behavior of eyeflukes in the genus *Philophthalmus*; the subject of *Philophthalmus* and philophtalmiasis was reviewed by Nollen and Kanev (1995).

8. OBTAINING MATERIAL

This section is concerned with obtaining material from genera other than *Echinostoma* for use in research and teaching. Carolina Biological (Elon, North Carolina, USA) supplies *Physa* sp. snails infected with cercarial and metacercarial stages of unidentified species of *Echinoparyphium*. Frogs and tadpoles obtained commercially from numerous suppliers or collected in the field often contain mixed infections of metacercarial cysts of species of *Echinostoma* and *Echinoparyphium*. To attempt to identify these cysts requires infecting experimental avian and mammalian hosts, typically domestic chicks, ducklings, mice, rats, or hamsters, *per os* with the cysts.

Several suppliers can provide marine gastropods infected with larval echinostomes. A reliable source of such material is Jones Biological (Long Beach, California, USA), which can supply workers with the prosobranch snail *Cerithidea californica*. This snail is infected with the intramolluscan stages of echinostomes in the genera *Echinostoma*, *Echinoparyphium*, *Himasthla*, and *Acanthoparyphium*. Martin (1972) has provided a useful key for identifying these larval stages from *C. californica*. The Marine Biological Laboratory (MBL) of Woods Hole, Massachusetts, USA can provide the mudflat snail *Ilyanassa obsoletus* infected with the intramolluscan stages of *Himasthla quis-* *setensis*.

The Biomedical Research Institute (Rockville, Maryland, USA) is a good source for obtaining uninfected *Biomphalaria glabrata* snails, which can serve as an experimental second intermediate host for a number of genera of echinostome cercariae including species of *Echinoparyphium* (see Fried *et al.*, 1998a).

Echinostome adults from various genera are available in the wild from numerous avian and mammalian hosts, mainly in the small and large intestines and other sites such as the stomach, bile duct, liver, cloaca and bursa of Fabricius. Note that obtaining vertebrate hosts in the wild may require governmental permits and even licenses in some countries. Likewise, the necropsy procedures may be governed by institutional and other guidelines. For an interesting discussion of this topic, see Irwin (1997). Vertebrate hosts infected with adult echinostomes may be available from commercial suppliers, zoological parks, hunters, and trappers. Cable (1940) and Schmidt (1988) have provided a list of species of echinostomes that may be found in vertebrate hosts at necropsy. Fried (2000) has provided information on maintenance of larval and adult echinostomes in intermediate and definitive hosts; *in vivo*, *in ovo*, and *in vitro* cultivation of larval and adult echinostomes; and transplantation of larval and adult echinostomes to new sites. The work described by Fried (2000) is on species of *Echinostoma*, except for: *in vitro* cultivation studies of the excysted metacercariae of *Echinoparyphium serratum* by Howell (1968b); cultivation *in ovo* of *Himasthla quissetensis* cercariae by Fried and Groman (1985); and transplantation of larval stages of *Isthmiophora spiculator* to new snails (Dönges and Gotzelmann, 1988). However, the information is certainly transferable to similar studies that attempt to use species of the echinostome genera that comprise the bulk of this review.

9. CONCLUDING REMARKS

This review has focused mainly on echinostomes except *Echinostoma*, for which a significant body of literature is available. Thus, most of the coverage is on *Echinoparyphium*, *Echinochasmus*, *Himasthla*, and *Hypoderaeum*. For these genera coverage includes salient and representative descriptive, life cycle, experimental, biochemical, and molecular studies. For other genera such as *Petasiger*, *Euparyphium*, *Stephanoprora*, *Isthmiophora*, *Acanthoparyphium*, and *Patagifer* information is mainly on descriptive and life cycle studies. Various echinostome and echinostome-like genera mentioned in Kanev *et al.* (2000) and Kostadinova and Gibson (2000), such as *Protechinostoma*, *Aporchis*, *Pelmatostomum*, *Longicollia*, *Baschkirovitrema*, *Ignasia*, *Prionosoma*, *Drepanocephalus*, *Moliniella*, *Pegosumum*, and others, were not

covered because of either the sparsity of literature on these groups or their unsettled taxonomic status.

Information on some echinostome-like genera was included because the organisms selected are important in human and veterinary medicine, in wildlife disease, or serve as models for biological research. The genera *Parorchis*, *Philophthalmus*, and *Ribeiroia* were therefore considered in the review.

For studies on the biology of *Echinostoma*, some key species have been used extensively in research, including *Echinostoma revolutum*, *Echinostoma trivolvis*, *Echinostoma caproni*, and *Echinostoma paraensei*. Stock material of these species is maintained either in the form of encysted metacercariae, eggs or infected snails and can be made available by exchange to other workers. Thus, collaborative efforts are made to examine various areas of biological research with these better known species of *Echinostoma* for which taxonomic information is reasonably reliable (however, see Kostadinova and Gibson (2000) for some reservations). The situation in regard to known species of echinostomes in the other genera is often less clear. To my knowledge there are no available stocks of encysted metacercariae to exchange for any species in genera other than *Echinostoma*. To facilitate cooperative research on genera other than *Echinostoma*, the availability of known stocks referable to well-described species would be useful.

The number of model systems available for continuous research in genera other than *Echinostoma* is scant. One can mention recent studies on *Hypoderaeum* and *Euparyphium* by R. Toledo and J.G. Esteban in Spain or work on the *Echinoparyphium recurvatum* complex by A. McCarthy in the United Kingdom, or the work on *Echinochasmus liliputanus* by X. Xiao and colleagues in China. These studies provide a reasonable body of literature on various topics related to model organisms. Such studies may serve as useful models for biological research with the non-*Echinostoma* genera discussed in this review.

Lastly, some mention should be made of taxonomic studies in the non-*Echinostoma* groups. Some recent models that provide excellent descriptive work, and further our knowledge of the systematics of these groups, include taxonomic and descriptive studies by W. M. Sohn on *Echinoparyphium recurvatum* in Korea and P. H. King and J. G. Van As in South Africa on *Petasiger variospinosus*. These model studies should be examined by workers interested in systematic studies on echinostome groups discussed in this review.

Echinostomes that are of medical significance continue to attract attention for obvious reasons. The fact that echinostomes can infect man orally via the cercarial stage in contaminated water is of considerable interest to the medical community. Numerous cases of human infection with *E. liliputanus* in China have been recently reported by X. Xiao and collaborators. As some of these echinostomes from genera other than *Echinostoma* gain more attention in the medical community, further research will be initiated.

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REFERENCES

- Adams, J.E. and Martin, W.E. (1963). Life cycle of *Himasthla rhigedana* Dietz, 1909 (Trematoda: Echinostomatidae). *Transactions of the American Microscopical Society* **82**, 1–6.
- Adema, C.M., Sapp, K.K., Hertel, L.A. and Loker, E.S. (2000). Immunobiology of the relationship of echinostomes with snail intermediate hosts. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 149–173, Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Barrett, J. (2000). Physiology and biochemistry of echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 199–212, Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Barse, A.M. (1998). Gill parasites of mummichogs, *Fundulus heteroclitus* (Teleostei: Cyprinodontidae): effects of season, locality, and host sex and size. *Journal of Parasitology* **84**, 236–242.
- Bass, H.S. and LeFlore, W.B. (1984). In vitro excystation of the metacercariae of *Acanthoparyphium spinulosum* (Trematoda: Echinostomatidae). *Proceedings of the Helminthological Society of Washington* **51**, 149–153.
- Bearup, A.J. (1960). Life history of *Acanthoparyphium spinulosum*. Johnston, 1917 (Trematoda: Echinostomatidae). *Australian Journal of Zoology* **8**, 217–225.
- Beaver, P. (1939). The morphology and life history of *Petasiger nitidus* Linton (Trematoda: Echinostomidae). *Journal of Parasitology* **25**, 269–276.
- Beaver, P.C. (1941). Studies on the life history of *Euparyphium melis* (Trematoda: Echinostomatidae). *Journal of Parasitology* **27**, 35–44.
- Bykhovskaya-Pavlovskaya, I.E. (1978). On the taxonomy of the family Echinostomatidae Dietz, 1909 (Trematoda). *Parazitologicheskii Sbornik* **28**, 16–28.
- Cable, R. (1940). *An Illustrated Laboratory Manual of Parasitology*. Minneapolis: Burgess Publishing Co.
- Chai, J. and Lee, S. (1991). Intestinal trematodes infecting humans in Korea. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**, 163–170.
- Chai, J.Y., Yu, J.R., Lee, S.H., Jung, H.C., Song, I.S. and Cho, S.Y. (1989). An egg negative patient of acute metagonimiasis diagnosed serologically by ELISA. *Seoul Journal of Medicine* **30**, 139–142.
- Chapman, H.D. and Wilson, R.A. (1972). The propulsion of the cercariae of *Himasthla leptostoma* (Trematoda: Echinostomatidae) and newly emerged metacercariae. *International Journal for Parasitology* **14**, 415–421.
- Chen, Y., Lin, J. and Fang, Y. (1990). Ecological studies on the cercariae of *Echinocasmus japonicus*. *Southeast Asian Journal of Tropical Medicine and Public Health* **8**, 26–28.
- Chen, Y., Xu, G., Feng, Z., Guo, Z., Lin, J. and Fang, Y. (1997). Studies on efficiency of praziquantel and mebendazole-medicated salt in treatment of *Echinocasmus fujianensis* infection. *Southeast Asian Journal of Tropical Medicine and Public Health* **28**, 344–346.
- Cheng, Y.Z., Zhang, Y.J., Lin, C.X., Zhan, Z.H., Yu, X.Z., Lin, J.X., Cai, M.J. and Che, W.H.

- (1999). Taxonomic studies on *Echinochasmus fujianensis* and the related species by random amplified polymorphic DNA analysis and experimental infection. *Chinese Journal of Parasitology and Parasitic Diseases* **17**, 135–139.
- Conn, D.B. and Conn, D.A. (1995). Experimental infection of Zebra mussels *Dreissena polymorpha* (Mollusca: Bivalvia) by metacercariae of *Echinoparyphium* sp. (Platyhelminthes:Trematoda). *Journal of Parasitology* **81**, 304–305.
- Dhanumkumari, K., Hanumantha, R. and Shyamasundari, K. (1991). The life cycle of *Echinochasmus bagulai* (Trematoda: Echinostomatidae). *International Journal for Parasitology* **21**, 259–263.
- Dimitrov, V., Kanev, I., Fried, B. and Radev, V. (1995). Argentophilic structures of the miracidium of *Echinostoma trivolvis* (Cort, 1914) (Trematoda, Echinostomatidae). *Journal of Parasitology* **81**, 306–307.
- Dimitrov, V., Kanev, I., Bezproz Vanich, V. and Radev, V. (1998). Argentophilic structures of the miracidium of *Echinochasmus perfoliatus* (Trematoda: Echinostomatidae). *Parasite* **5**, 185–188.
- Ditrich, O., Scholz, T. and Vargas Vazquez, J. (1996). Life cycle of *Echinochasmus macrocaudatus* n. sp. (Trematoda: Echinostomatidae). *Systematic Parasitology* **33**, 225–235.
- Dönges, J. (1963). Die experimental Bestimmung der Anzahl der Rediengeneration bei Trematoden. *Naturwissenschaften* **50**, 103–104.
- Dönges, J. and Gotzelmann, M. (1988). Digenetic trematodes: multiplication of the intramolluscan stages in some species is potentially unlimited. *Journal of Parasitology* **74**, 884–885.
- Esteban, J.G., Toledo, R., Sanchez, L. and Munoz-Antoli, C. (1997). Life-cycle of *Euparyphium albuferensis* n. sp. (Trematoda: Echinostomatidae) from rats in Spain. *Systematic Parasitology* **38**, 211–219.
- Etchegoin, J.A. and Martorelli, S.R. (1997). A new species of the genus *Stephanoprora* Odhner, 1902 (Digenea, Echinostomatidae) from Argentina, *Acta Parasitologica* **42**, 74–76.
- Evans, N.A. (1983). The establishment and survival of *Echinoparyphium recurvatum* in the definitive host. *Zeitschrift für Parasitenkunde* **69**, 401–402.
- Fried, B. (1962). Growth of *Philophthalmus* sp. (Trematoda) on the chorioallantois of the chick. *Journal of Parasitology* **48**, 545–550.
- Fried, B. (1978). Trematoda. In: *Methods of Cultivating Parasites In Vitro*. (C.A. Taylor and J.R. Baker, eds), pp. 151–192. London: Academic Press.
- Fried, B. (1994). Metacercarial excystment of trematodes. *Advances in Parasitology* **33**, 91–144.
- Fried, B. (2000). Maintenance, cultivation, and excystation of echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 99–118. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Fried, B. and Graczyk, T.K. (eds) (2000). *Echinostomes as Experimental Models for Biological Research*, p. 273. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Fried, B. and Grigo, K.L. (1975). Infectivity and excystation of the metacercaria of *Echinoparyphium flexum*. *Proceedings of the Pennsylvania Academy of Science* **49**, 79–81.
- Fried, B. and Groman, G.M. (1985). Cultivation of the cercariae of *Himasthla quissetensis* (Trematoda) on the chick chorioallantois. *International Journal for Parasitology* **15**, 219–223.
- Fried, B. and Huffman, J.E. (1996). The biology of the intestinal trematode *Echinostoma caproni*. *Advances in Parasitology* **38**, 311–368.
- Fried, B. and Roth, M. (1974). In vitro excystment of the metacercariae of *Parorchis acanthus*. *Journal of Parasitology* **60**, 465.

- Fried, B., Frazer, B.A. and Kanev, I. (1998a). Comparative observations on cercariae and metacercariae of *Echinostoma trivolvis* and *Echinoparyphium* sp. *Journal of Parasitology* **84**, 623–626.
- Fried, B., Eyster, L.S. and Pečhenik, J.A. (1998b). Histochemical glycogen and neutral lipids in *Echinostoma trivolvis* cercariae and effects of exogenous glucose on cercarial longevity. *Journal of Helminthology* **72**, 83–85.
- Fujino, T. and Ichikawa, H. (2000). Ultrastructural studies on echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 119–136. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Fujino, T., Fried, B. and Hosier, D.W. (1994). The expulsion of *Echinostoma trivolvis* from ICR mice: extension/retraction mechanisms and ultrastructure of the collar spines. *Parasitology Research* **80**, 581–587.
- Gold, D. and Nollen, P. (1999). Life history and identification of *Philophthalmus lucipitus* in Israel. *Journal of Parasitology* **85**, 608.
- Gorchilova, L. and Kanev, I. (1986) Electron microscope studies of the metacercariae of *Echinostoma revolutum* (Frolich, 1802), *E. echinatum* (Zeder, 1803) and *Echinoparyphium aconiatum* Dietz, 1909 (Trematoda: Echinostomatidae). *Khelminlogiya* **22**, 3–8.
- Grabda-Kazubska, B. and Kiseliene, V. (1989). Chaetotaxy and excretory system of the cercariae of *Echinoparyphium recurvatum* (Linstow 1873) (Digenea, Echinostomatidae). *Acta Parasitologica Polonica* **34**, 325–335.
- Grabda-Kazubska, B. and Kiseliene, V. (1991). The life cycle of *Echinoparyphium mordwilkoi* Skrjabin, 1915 (Trematoda, Echinostomatidae). *Acta Parasitologica Polonica* **36**, 167–173.
- Grabda-Kazubska, B. and Moczon, T. (1988). The nervous system of *Echinoparyphium recurvatum* (Linstow, 1873) cercaria and adult (Digenea, Echinostomatidae). *Acta Parasitologica Polonica* **33**, 177–184.
- Grabda-Kazubska, B., Borsuk, P., Laskowski, Z. and Mone, H. (1998). A phylogenetic analysis of trematodes of the genus *Echinoparyphium* and related genera based on sequencing of internal transcribed spacer region of rDNA. *Acta Parasitologica* **43**, 116–121.
- Graczyk, T.K. (2000). Immunobiology and immunodiagnosis of echinostomiasis. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 229–244. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Gupta, V. and Jahan, A. (1979). On four new trematodes of Echinostomatidae Poche, 1926 from birds. *Indian Journal of Helminthology* **29**, 57–72.
- Haas, W. (2000). The behavioral biology of echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 175–197. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Haseeb, M.A. and Eveland, L.K. (2000). Human echinostomiasis: mechanisms of pathogenesis and host resistance. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 83–98. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Hellman, L. (1975). *Himasthla quissetensis* and *Lepocreadium setiferoides*: emergence patterns from their molluscan host, *Nassarius obsoletus*. *Experimental Parasitology* **38**, 56–63.
- Herman, S. and Bacha, W.J. Jr. (1978). Site location of the trematode *Himasthla quissetensis* in the cloacal drop infections with cercariae. *Journal of Parasitology* **64**, 827–830.
- Hoberg, E.P., Henny, C.J., Hedstrom, O.R. and Grove, R.A. (1997). Intestinal helminths of river otters (*Lutra canadensis*) from the Pacific Northwest. *Journal of Parasitology* **83**, 105–110.

- Holeman-Spector, B. and Olaque, G. (1989). Digenetic trematodes of the genus *Stephanophrora*. Odhner, 1902 of birds of Uruguay with the description of two new species. *Acta Parasitologica Polonica* **34**, 311–317.
- Hoskin, G.P. and Cheng, T.C. (1974). *Himasthla quissetensis*: uptake and utilization of glucose by rediae as determined by autoradiography and respirometry. *Experimental Parasitology* **35**, 61–67.
- Hoskin, G.P. and Cheng, T.C. (1975). Occurrence of carotenoids in *Himasthla quissetensis* rediae and the host, *Nassarius obsoletus*. *Journal of Parasitology* **61**, 381–382.
- Howell, M.J. (1968a). The life-cycle of *Echinoparyphium serratum* sp. nov. (Digenea: Echinostomatidae). *Parasitology* **58**, 573–582.
- Howell, M.J. (1968b). Excystment and in vitro cultivation of *Echinoparyphium serratum*. *Parasitology* **58**, 583–597.
- Howell, M.J. (1970). Excystment of the metacercariae of *Echinoparyphium serratum*. *Journal of Helminthology* **44**, 35–36.
- Huffman, J.E. (2000). Echinostomes in veterinary and wildlife parasitology. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 59–82. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Huffman, J.E. and Fried, B. (1990). *Echinostoma* and echinostomiasis. *Advances in Parasitology* **29**, 215–269.
- Humphries, J.E., Mousley, A., Maule, A.G. and Halton, D.W. (2000). Neuromusculature – structure and functional correlates. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 213–227. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Irwin, S.W.B. (1997). Excystation and cultivation of trematodes. In: *Advances in Trematode Biology* (B. Fried and T.K. Graczyk, eds), pp. 57–86. Boca Raton, Florida: CRC Press.
- Irwin, S., McKerr, B. and Moran, I. (1984). Studies on metacercarial excystment in *Himasthla leptosoma* (Trematoda: Echinostomatidae) and on newly emerged metacercariae. *International Journal for Parasitology* **14**, 415–421.
- Jain, S.P. and Shrivastava, O.N. (1970). On the validity of some species of the genus *Patagifer* (Trematoda: Echinostomatidae). *Proceedings of the Indian Academy of Sciences* **72**, 156–161.
- Johnson, P.T.J., Lunde, K.B., Ritchie, E.G. and Launer, A.T. (1999). The effect of trematode infection on amphibian limb development and survivorship. *Science* **284**, 802–804.
- Kanev, I., Radev, V., Vassilev, I., Dimitrov, V. and Minchella, D. (1994). The life cycle of *Echinoparyphium cinctum* (Rudolphi 1803) (Trematoda: Echinostomatidae) with re-examination and identification of its allied species from Europe and Asia. *Helminthologia* **31**, 73–82.
- Kanev, I., Sorensen, R., Sterner, M., Cole, R. and Fried, B. (1998). The identification and characteristics of *Echinoparyphium rubrum* (Cort, 1914) new comb. (Trematoda, Echinostomatidae) based on experimental evidence of the life cycle. *Acta Parasitologica* **43**, 181–188.
- Kanev, I., Radev, V., Sterner, M. and Fried, B. (2000). An overview of the biology of echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 1–29. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- KeQiang, C., Chen, L.Y., Mao, Q.R., Xu, J.M. and Ji, L.M. (1995). Tegumentary ultrastructure of *Hypoderæum conoideum* and *Echinostoma miyagawai* (Digenea: Echinostomatidae). *Journal of Shanghai Agricultural College* **13**, 170–175.
- KeQiang, C., Huang, H., Lu, W. and Wan Bao, D. (1996a). Ultrastructure of sperm and spermatogenesis of *Hypoderæum conoideum*, Bloch 1872 (Digenea: Echinostomatidae). *Journal of Shanghai Agricultural College* **14**, 186–195.

- KeQiang, C., Chen, L.Y., Liu, Y.J., Xu, J.M. and Ji, L.M. (1996b). Vitellogenesis of *Echinostoma miyagawai* and *Hypoderæum conoideum* (Digenea: Echinostomatidae). *Journal of Shanghai Agricultural College* **14**, 79–85.
- King, P.H. and Van As, J.G. (1996). A description of the life stages of *Echinoparyphium elegans* (Trematoda: Echinostomatidae). *South African Journal of Zoology* **31**, 145–153.
- King, P.H. and Van As, J.G. (1997). Morphology and scanning electron microscopy of cercariae shed by *Bulinus tropicus* (Krauss, 1848) in the Free State, South Africa. *Journal of African Zoology* **111**, 301–312.
- King, P.H. and Van As, J.G. (2000). Morphology and life history of *Petasiger variospinosus* (Trematoda: Echinostomatidae) in the Free State, South Africa. *Journal of Parasitology* **86**, 312–318.
- Kirschner, K. and Bacha, W.J. Jr (1980). Excystment of *Himasthla quissetensis* (Trematoda: Echinostomatidae) metacercariae in vitro. *Journal of Parasitology* **66**, 263–267.
- Kiseliene, V. and Grabda-Kazubska, B. (1990). *Echinoparyphium pseudorecurvatum* sp. n. (Trematoda: Echinostomatidae) and its life cycle. *Acta Parasitologica Polonica* **35**, 285–295.
- Kiseliene, V. and Grabda-Kazubska, B. (1993). The structure and life cycle of sibling species of trematodes of the genus *Echinoparyphium* Dietz. *Biologija* **1**, 40–41.
- Kostadinova, A. (1997). A comparative study of cercarial chaetotaxy in two species of *Petasiger* Dietz, 1909. (Digenea, Echinostomatidae). *Systematic Parasitology* **37**, 105–110.
- Kostadinova, A. (1999). First record of *Petasiger grandivesicularis* Ishii, 1935 (Digenea: Echinostomatidae) in Germany. *Helminthologia* **36**, 101–103.
- Kostadinova, A. and Chipev, N. (1992). Experimental data on the life cycle of *Petasiger grandivesicularis* Ishii, 1935 (Trematoda, Echinostomatidae). *Systematic Parasitology* **23**, 55–65.
- Kostadinova, A. and Gibson, D.I. (2000). The systematics of the echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 31–57. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Kublickiene, O. and Ciuniene, E. (1973). Histological changes in the mucous membrane of duck intestine during experimental echinostomatidosis. *Acta Parasitologica Lituanica* **11**, 73–80.
- Laurie, J.S. (1974). *Himasthla quissetensis*: induced in vitro encystment of cercariae and ultrastructure of the cyst. *Experimental Parasitology* **35**, 350–362.
- LeFlore, W.B. and Bass, H.S. (1982). In vitro excystment of the metacercaria of *Himasthla rhigedana* (Trematoda: Echinostomatidae). *Journal of Parasitology* **68**, 1177–1178.
- Lie, K.J. (1964). Studies on Echinostomatidae (trematoda) in Malaya. VI. The life history of *Hypoderæum dingeri* n. sp. *Tropical and Geographical Medicine* **16**, 61–71.
- Lie, K.J. and Umathevy, T. (1965). Studies on Echinostomatidae (Trematoda) in Malaya. X. The life history of *Echinoparyphium dunni* sp. n. *Journal of Parasitology* **51**, 793–799.
- Lie, K.J., Nasemary, S. and Impand, P. (1973). Five echinostome species from Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **4**, 96–101.
- Luty, T. and Mizgajska, H. (1999). Prevalence of *Toxocara* spp. and other intestinal parasites in dogs and cats. *Medycyna Weterynaryjna* **55**, 759–761.
- Martin, W.E. (1972). An annotated key to the cercariae that develop in the snail *Cerithidea californica*. *Bulletin of the Southern California Academy of Science* **71**, 39–43.
- Martin, W.E. and Adams, J.E. (1961). Life cycle of *Acanthoparyphium spinulosum* Johnston, 1917 (Echinostomatidae: Trematode). *Journal of Parasitology* **47**, 777–782.
- Mathias, P. (1925). Recherches experimentales sur le cycle evolutif de quelques trématodes. *Bulletin Biologic France et Belgique* **59**, 1–123.
- McCarthy, A.M. (1990). Speciation of echinostomes: evidence for the existence of two

- sympatric sibling species in the complex *Echinoparyphium recurvatum* (von Linstow 1873) (Digenea: Echinostomatidae). *Parasitology* **101**, 35–42.
- McCarthy, A.M. (1999a). Photoperiodic cercarial emergence patterns of the digeneans *Echinoparyphium recurvatum* and *Plagiorchis* sp. from a mixed infection in *Lymnaea peregra*. *Journal of Helminthology* **73**, 59–62.
- McCarthy, A.M. (1999b). Phototactic responses of the cercaria of *Echinoparyphium recurvatum* during phases of sub-maximal and maximal infectivity. *Journal of Helminthology* **73**, 63–65.
- McCarthy, A.M. (1999c). The influence of temperature on the survival and infectivity of the cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology* **118**, 383–388.
- McCarthy, A.M. (1999d). The influence of second intermediate host species on the infectivity of metacercarial cysts of *Echinoparyphium recurvatum*. *Journal of Helminthology* **73**, 143–145.
- Mekhraliev, A.A. (1988). Trematode larvae from molluscs in Lake Glyulalan in Azerbaijan. Part II. Echinostomatid cercariae (Echinostomata). *Izvestiya Akademii Nauk Azerbaidzhanskoi SSR, Biologicheskii Nauki* **5**, 56–59.
- Morgan, J.A.T. and Blair, D. (2000). Molecular biology of echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 245–266. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Mouahid, A. and Mone, H. (1988). *Echinoparyphium elegans* (Looss, 1899) (Digenea: Echinostomatidae): the life cycle and redescription of the adult with a revision of the 43-spined members of the genus *Echinoparyphium*. *Systematic Parasitology* **12**, 149–157.
- Muller, E.E., Fried, B. and Sherma, J. (1999). HPTLC determination of neutral lipids in the cercariae of *Echinostoma trivolvis* and *Echinoparyphium* sp. (Trematoda). *Journal of Planar Chromatography – Modern TLC* **12**, 306–308.
- Najarian, H.H. (1953). The life history of *Echinoparyphium flexum* (Linton 1892) (Dietz 1910) (Trematoda: Echinostomatidae). *Science* **117**, 564–565.
- Najarian, H.H. (1954). Developmental stages in the life cycle of *Echinoparyphium flexum* (Linton, 1982) Dietz, 1910. (Trematoda: Echinostomatidae). *Journal of Morphology* **84**, 165–197.
- Nollen, P.M. (1997). Reproductive physiology and behavior of digenetic trematodes. In: *Advances in Trematode Biology*. (B. Fried and T.K. Graczyk, eds), pp. 117–147. Boca Raton, Florida: CRC Press.
- Nollen, P.M. (2000). Reproductive physiology and behavior of echinostomes. In: *Echinostomes as Experimental Models for Biological Research* (B. Fried and T.K. Graczyk, eds), pp. 137–148. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Nollen, P.M. and Kanev, I. (1995). The taxonomy and biology of philophthalmid eyeflukes. *Advances in Parasitology* **36**, 205–269.
- Rees, F.G. (1939). Studies on the germ-cell of the digenetic trematode, *Parorchis acanthus* Nicoll. Part I. Anatomy of the genitalia and gametogenesis in the adult. *Parasitology* **31**, 417–433.
- Schell, S.C. (1985). *Trematodes of North America, North of Mexico*. Moscow, Idaho: University Press of Idaho, 263 pp.
- Schmidt, G. (1988). *Essentials of Parasitology*, 4th edn. Dubuque, Iowa: WMC Brown.
- Semenas, L., Brugni, N. and de Nunez, M.O. (1999). Metacercariae of Echinostomatidae in *Diplodon chilensis* (Unionacea, Pelecypoda) and description of *Echinoparyphium megacirrus* sp.n. in Patagonia (Argentina). *Acta Parasitologica* **44**, 63–67.
- Seo, B.S., Lee, S.H., Chai, J.Y. and Hong, S.J. (1985). Studies on intestinal trematodes in Korea. XX. Four cases of natural human infection by *Echinocasmus japonicus*. *Korean Journal of Parasitology* **23**, 214–220.

- Shaymardanov, Z.K. (1995). Observations on the histochemistry of egg shell formation in *Hypoderaeum conoideum* and *Liorchis sotiae*. *Turkiye Parazitoloji Dergisi* **19**, 587–591.
- Shostak, A.W. (1992). Survival of *Petasiger nitidus* (Digenea: Echinostomatidae) cercariae in relation to temperature, pH, and salinity. *Canadian Journal of Zoology* **71**, 431–434.
- Skovronskii, R.V. (1985). *Lymnaea truncatula*, first and second intermediate host of *Echinostoma revolutum* and *Hypoderaeum conoideum*. *Parazitologiya* **19**, 323–324.
- Smales, L.R. and Blakespoor, H.D. (1984). *Echinostoma revolutum* (Froelich, 1802) Loos, 1899 and *Isthniophora melis* (Schrank, 1788) Luhe, 1909 (Echinostomatidae, Digenea): scanning electron microscopy of the tegumental surfaces. *Journal of Helminthology* **58**, 187–195.
- Sohn, W.M. (1998). Life history of *Echinopyryphium recurvatum* (Trematoda: Echinostomatidae) in Korea. *Korean Journal of Parasitology* **36**, 91–98.
- Stadnichenko, A.P. (1971). Pathogenic effect of larval trematodes on *Lymnaea auricularia* (Gastropoda, Pulmonata). *Materialy Nauchnykh Konferentsii Vsesoyuznogo Obshchestva Gel'mentologov* **23**, 260–267.
- Stunkard, H.W. (1938). The morphology and life cycle of the trematode *Himasthla quis-setensis* (Miller and Northrup, 1926). *Biological Bulletin* **75**, 145–164.
- Stunkard, H.W. and Cable, R.M. (1932). The life history of *Parorchis avitus*, a trematode from the cloaca of the gull. *Biological Bulletin* **62**, 328–338.
- Toledo, R., Munoz-Antoli, C., Perez, M. and Esteban, J.G. (1998a). Larval trematode infections in freshwater gastropods from the Albufera Natural Park in Spain. *Journal of Helminthology* **72**, 79–82.
- Toledo, R., Munoz-Antoli, C., Sanchez, L., Bayssade-Dufour, C. and Esteban, J.G. (1998b). Cercarial chaetotaxy of *Euparyphium albuferensis* Esteban *et al.*, 1997 (Trematoda: Echinostomatidae), with a review of some genera of the Echinostomatinae. *Systematic Parasitology* **39**, 35–44.
- Toledo, R., Munoz-Antoli, C., Perez, M. and Esteban, J.G. (1999a). Miracidial infectivity of *Hypoderaeum conoideum* (Trematoda: Echinostomatidae): differential susceptibility of two lymnaeid species. *Parasitology Research* **85**, 212–215.
- Toledo, R., Munoz-Antoli, C., Perez, M. and Esteban, J.G. (1999b). Survival and infectivity of *Hypoderaeum conoideum* and *Euparyphium albuferensis* cercariae under laboratory conditions. *Journal of Helminthology* **73**, 177–182.
- Toledo, R., Munoz-Antoli, C. and Esteban, J.G. (1999c). Production and chronobiology of emergence of the cercariae of *Euparyphium albuferensis* (Trematoda: Echinostomatidae). *Journal of Parasitology* **85**, 263–267.
- Vanoverschelde, R. (1982). Studies on life cycle of *Himasthla militaris* (Trematoda: Echinostomatidae): influence of temperature and salinity on the life span of the miracidium and infection of the first intermediate host, *Hydrobia ventrosa*. *Parasitology* **84**, 131–135.
- Vanoverschelde, R. and Vaes, F. (1980). Studies on the life cycle of *Himasthla militaris* (Trematoda: Echinostomatidae). *Parasitology* **81**, 609–617.
- Vernberg, W., Vernberg, J. and Beckerlite, F. (1969). Larval trematodes: double infections in the common mud-flat snails. *Science* **164**, 1287–1288.
- Wang, P. (1976). Studies on the trematode family Echinostomatidae Dietz, 1909 from Fujian. *Acta Zoologica Sinica* **22**, 288–293.
- Wang, T.P., Zhu, C.G., Fang, G.R., Xiao, X., Lu, D.B., Wu, D.G., Zhang, B.Z. and Li, H.B. (1998). Studies on seasonal distribution and influence factors of *Echinocasmus* infection. *Chinese Journal of Parasitic Disease Control* **11**, 295–297.
- Williams, E.A. (1978). The morphology of *Hypoderaeum* sp. nov. (Trematoda: Echinostomatidae). *Parasitology* **77**(3), XIV.
- Xiao, X., Wang, T.P. and Lu, D.B. (1992). The first record of human natural infection of

- Echinochasmus liliputanus*. *Chinese Journal of Parasitology and Parasitic Diseases* **10**, 132–136.
- Xiao, X., Lu, D., Wang, T., Gao, J., Zhu, C., Zhang, B., An, J., Pong, H., Xu, M. and Wu, W. (1995). Studies on the mode of human infection with *Echinochasmus liliputanus*. *Chinese Journal of Parasitology and Parasitic Diseases* **13**, 197–199.
- Xiao, X., Wang, T., Wang, Y., Sheng, G. and Wang, Q. (2001). In vivo and in vitro encystment of *Echinochasmus liliputanus* cercariae and biological activity of the metacercariae. *Journal of Parasitology* **87**, in press.
- Yurlova, N.I. (1987). Morphology and biology of the trematodes *Hypoderæum cubanicum* n. comb. and *H. conoideum* (Echinostomatidae). *Parazitologiya* **21**, 35–42.
- Zwilling, E. (1959). A modified chorioallantoic grafting procedure. *Transplantation Bulletin* **6**, 115–116.

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