ANIMAL MODEL STUDIES ON THE ANTELOPE SCHISTOSOMES, *SCHISTOSOMA MARGREBOWIEI* AND *S. LEIPERI*, WITH PARTICULAR REFERENCE TO THEIR PROPOSED ROLE IN LIMITING THE DISTRIBUTION OF HUMAN INTESTINAL SCHISTOSOMIASIS

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PREFACE

The experimental work described in this thesis was carried out in the laboratories of the former Research Institute for Diseases in a Tropical Environment (now the Natal branch of the South African Medical Research Council), under the supervision of Dr. C H J Schutte and co-supervision of Dr. B I Huckett.

These studies represent original work conducted by the author and have not been submitted in alternative form to any other University. Where use was made of the work of others it has been duly acknowledged in the text.

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During the period when the majority of this work was carried out I shared a close collaborative partnership with Dr Sue Higgins-Opitz and benefited greatly from the many hours which we spent together discussing diverse theoretical and practical aspects of schistosomiasis research. Her perceptiveness, particularly with regard to possible pitfalls in experimental design, and her familiarity with the extensive literature pertaining to this field are things which I much admire. I am also extremely grateful to her for the assistance which she so willingly provided in the laboratory, especially with worm counts and with granuloma and portal pressure measurements, as well as for coordinating certain of the activities of technical staff and for helping to organise and collate data.

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ABSTRACT

It has been postulated that the absence of human and cattle schistosomiasis in parts of southern Africa where lechwe antelope (*Kobus leche*) occur is a consequence of an immunologically-mediated protection induced by repeated exposure to the cercariae of *Schistosoma margrebowiei* and *S. leiperi*, which are common parasites of these animals. The aim of the studies described was the development of animal models in which to investigate this hypothesis.

The infection characteristics of the antelope schistosomes in BALB/c mice and *Mastomys coucha* were assessed. Both schistosome species reached full patency in these hosts, although *S. margrebowiei* infections deteriorated rapidly in *M. coucha*. While they differed markedly in terms of egg production rates and preferred sites of tissue egg deposition, both species caused severe hepatosplenomegaly and portal hypertension in the mouse model. Modulation of the granulomatous responses to ova in the tissues was demonstrated.

Mice harbouring mature antelope schistosome infections displayed strong partial resistance to challenge infections with both homologous parasites and the human schistosome, *S. mansoni*. However, the failure of challenge parasites to become established was considered to be due largely to changes in the portal-hepatic vasculature resulting from egg-induced immunopathology. Resistance to *S. mansoni* challenge did not develop in mice infected with radiation-attenuated cercariae of the antelope schistosomes.

The suitability of rats and guinea pigs as alternative models was assessed. Worm recoveries from rats were low and there was no

evidence of egg-deposition. Worm yields from the guinea pig were relatively high, but sexual development was poor and short-lived. Since excretion of *S. margrebowiei* eggs has occasionally been reported from humans, and since the guinea pig supports full sexual maturation of *S. mansoni*, this animal appeared to provide a particularly appropriate model for the present investigation. However, repeated exposure of guinea pigs to cercariae of the antelope schistosomes, over a period of 24 weeks, failed to induce significant resistance to *S. mansoni* challenge infection.

The need for further experimental and field studies is discussed. An area in the Okavango Delta (Ngamiland, Botswana) has been identified as a possible site for field work.

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CHAPTER ONE

GENERAL INTRODUCTION

Schistosomiasis is a water-borne disease of tropical and sub-tropical regions caused by blood-dwelling digenetic trematode parasites belonging to the family Schistosomatidae. All mammalian schistosomiasis is caused by genera belonging to the sub-family Schistosomatinae (Figure 1.1), although this sub-family also includes some genera which are parasitic in avian hosts.

1.1 GLOBAL DISTRIBUTION AND SIGNIFICANCE OF SCHISTOSOMIASIS

Schistosome infections occur in a wide variety of mammalian hosts, including rodents, ruminants, carnivores, non-human primates and man (Pitchford, 1977a; Rollinson and Southgate, 1987). For the most part infections in wild animals and birds attract little attention. In contrast, however, schistosomiasis in humans and domestic livestock is a problem of enormous proportions.

1.1.1 Human Schistosomiasis

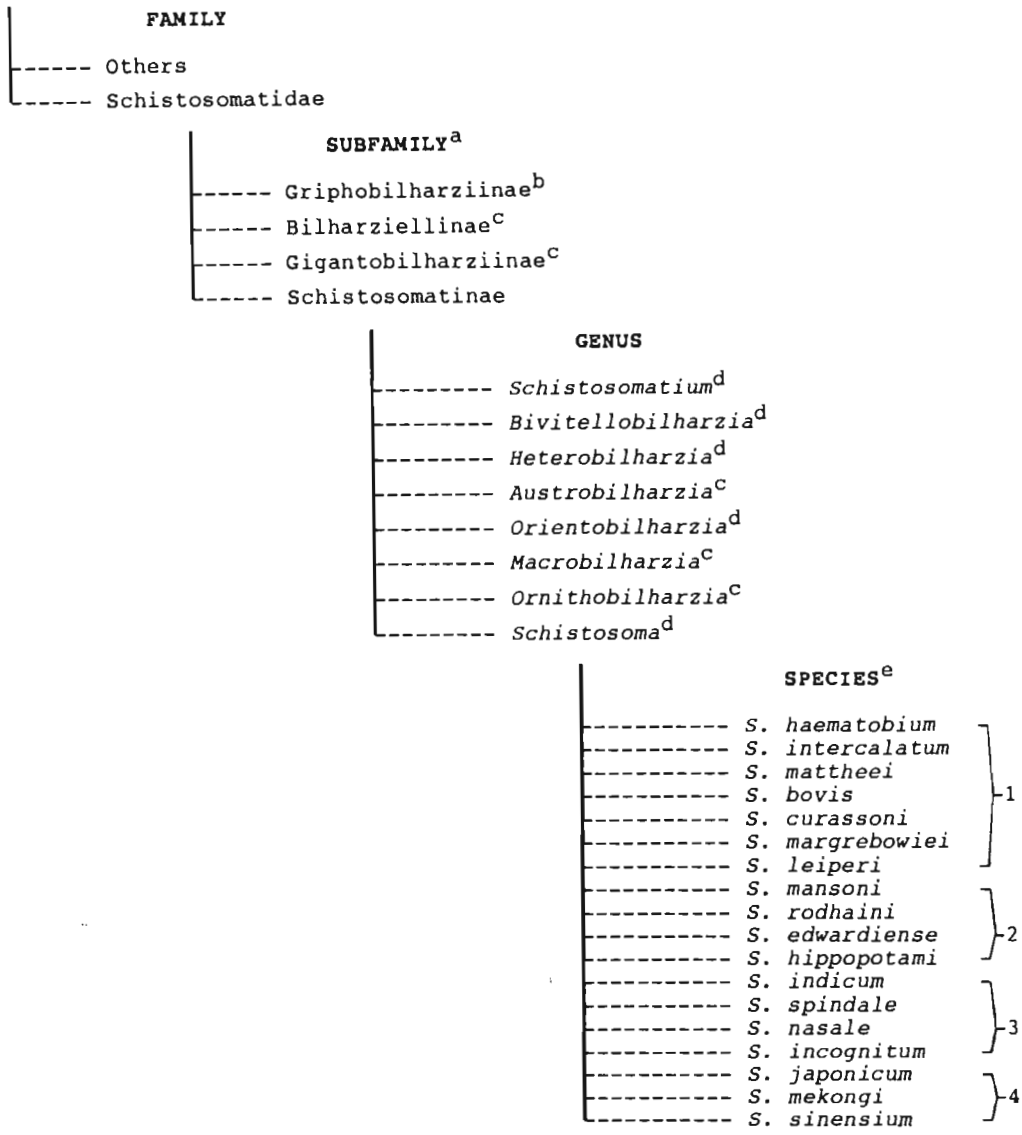
Human schistosomiasis is endemic in no less than 76 countries (Yoon and Mott, 1991), largely within the under-developed regions, with between 500 and 600 million people estimated to be at risk of infection (Doumenge *et al*, 1987). At least 200 million are thought to actually harbour the parasites, resulting in a wide spectrum of acute and chronic clinical manifestations (von Lichtenberg, 1987) and annual mortality rates which are estimated at between 250 000 and 1 million (Walsh, 1989; Warren, 1989).

It is generally agreed that measureable morbidity is seen only in a small proportion of infected individuals. Butterworth (1988), for example, suggests that even in areas of particularly high prevalence, less than 10% of infected persons are likely to develop severe complications. However, the impact of schistosomiasis cannot simply be measured in terms of overt morbidity and mortality. Of considerable significance also are the more subtle debilitating

FIGURE 1.1 TAXONOMIC POSITION OF THE MAMMALIAN SCHISTOSOMES

Summary of successive relevant taxa, from Phylum down to Family levels (Beaver *et al*, 1984):

Phylum Platyhelminthes, Class Trematoda, Subclass Digenea, Order Prosostomata, Suborder Strigeata, Superfamily Schistosomatoidea.



a This sub-division follows the approach of Farley (1971), rather than that of Mehra (1940), who erected an additional subfamily, namely Dendritobilharziinae, to accomodate the genus *Dendritobilharzia*; Farley favours the inclusion of the latter genus, together with the genus *Gigantobilharzia* into the sub-family Gigantogbilharziinae. Malek (1980) favours the sub-division proposed by Mehra.

b This subfamily is included on the basis of a recent publication (Platt *et al*, 1991) dsecribing *Griphobilharzia amoena*, a blood fluke recovered from freshwater crocodiles, which represents the first member of the family Schistosomatidae known to be parasitic in a poikilothermic hosts.

c Parasitic in birds

d Parasitic in mammals

e For convenience, the various *Schistosoma* species are assigned informally to one of four groups, as specified below, on the basis of egg morphology, common relationships with snail hosts, and geographical distribution (Rollinson & Southgate, 1987):
1, '*S. haematobium*' group; 2, '*S. mansoni*' group; 3, '*S. indicum*' group; 4, '*S. japonicum*' group.

effects associated with chronic infection, which, although extremely difficult to quantify, are believed to exert a substantial influence on human productivity (Pant, 1987). It has been estimated, for example, that the total number of days of disability per case of schistosomiasis is in the region of 600-1000 (Walsh, 1984); when considered in terms of the global population of infected individuals, this represents a massive loss in productive manpower.

The majority of human schistosomiasis is caused by three species of schistosomes, namely *S. haematobium*, which affects the urogenital organs, and *S. mansoni* and *S. japonicum*, both of which cause intestinal forms of the disease. *S. haematobium* is widely distributed in Africa, as well as in parts of the Middle East. The distribution of *S. mansoni* is essentially similar, but also includes parts of Latin America and the Caribbean, whilst that of *S. japonicum* is limited to the Far East, specifically China, Indonesia, Thailand and the Phillipines (Doumenge *et al*, 1987). Two other schistosome species, with far more restricted distributions, are also recognised as agents of human intestinal schistosomiasis: *S. intercalatum* occurs over a limited range in west and central Africa, while *S. mekongi* has thus far only been reported from Democratic Kampuchea and Lao People's Democratic Republic (Doumenge *et al*, 1987). Finally, it is of interest to note that a number of cases of human schistosomiasis have been reported from indigenous inhabitants of Malaysia (Orang Asli aborigines), current evidence suggesting that this is due to a previously unrecognised species (Shekhar and Pathmanathan, 1987).

1.1.2 Veterinary Schistosomiasis

In Africa the most important agents of schistosomiasis in livestock are *S. mattheei*, which occurs in the southern sub-continent, and *S. bovis*, which is distributed widely in the central and northern regions, as well as in parts of the Middle East and the Mediterranean. The former species is parasitic in sheep and cattle, while the latter occurs in these hosts as well as in goats. *S. curassoni* also occurs in domestic ruminants in West Africa, but is considered to be of minor veterinary significance (Vercruysse *et al*, 1985).

Pitchford (1959) observed the presence of *S. mattheei*-like ova in urine samples recovered from humans in parts of the Eastern Transvaal, South Africa. However, since they were found in an area where this parasite occurs sympatrically with *S. haematobium*, he suspected them to be the product of hybrids between the two species. Subsequent work (e.g. Wright and Ross, 1980; Kruger and Evans, 1990) has confirmed this. There have also been occasional reports of apparent *S. bovis* infections in humans (Chunge *et al*, 1986), but these do not appear to have been investigated in any detail.

In Asia the schistosomes of greatest veterinary significance include *S. japonicum*, *S. indicum*, *S. spindale*, *S. nasale* and *S. incognitum*. *S. japonicum* is distinctive in that it occurs naturally in a wide range of wild and domestic animals, some of which, notably cattle, pigs and dogs are considered to play an important role in the epidemiology of human infections (Rollinson and Southgate, 1987). *S. indicum*, *S. spindale*, *S. nasale* and *S. incognitum* occur predominantly on the Indian subcontinent. Between them, the former three species infect a variety of domestic animals, including horses, cattle, buffalo, sheep and goats. Although *S. incognitum* also occurs in sheep, goats and cattle, the pig is considered to be its main definitive host (Agrawal and Shah, 1989).

There have been numerous reports describing the pathological consequences of natural and experimental infection of various hosts with the above-mentioned non-human schistosomes (reviewed by Taylor, 1987). The bulk of studies in this regard have focused on *S. mattheei* and *S. bovis*, presumably because they are known to be capable of causing severe disease outbreaks (Hurter and Potgieter, 1967; van Wyk *et al*, 1974; Hussein *et al*, 1975). However, all of the other species are known to cause significant and sometimes severe morbidity (Rollinson and Southgate, 1987; De Bont *et al*, 1989 & 1991; Fransen *et al*, 1990). Considering the number of different schistosome species involved, the range over which they are prevalent, and the variety of domestic animals affected, there can thus be no doubt that the impact of schistosome infections on livestock productivity is very substantial (Massoud and Nelson, 1972), although as yet, no attempts appear to have been any made to estimate this in global terms (Dargie, 1987).

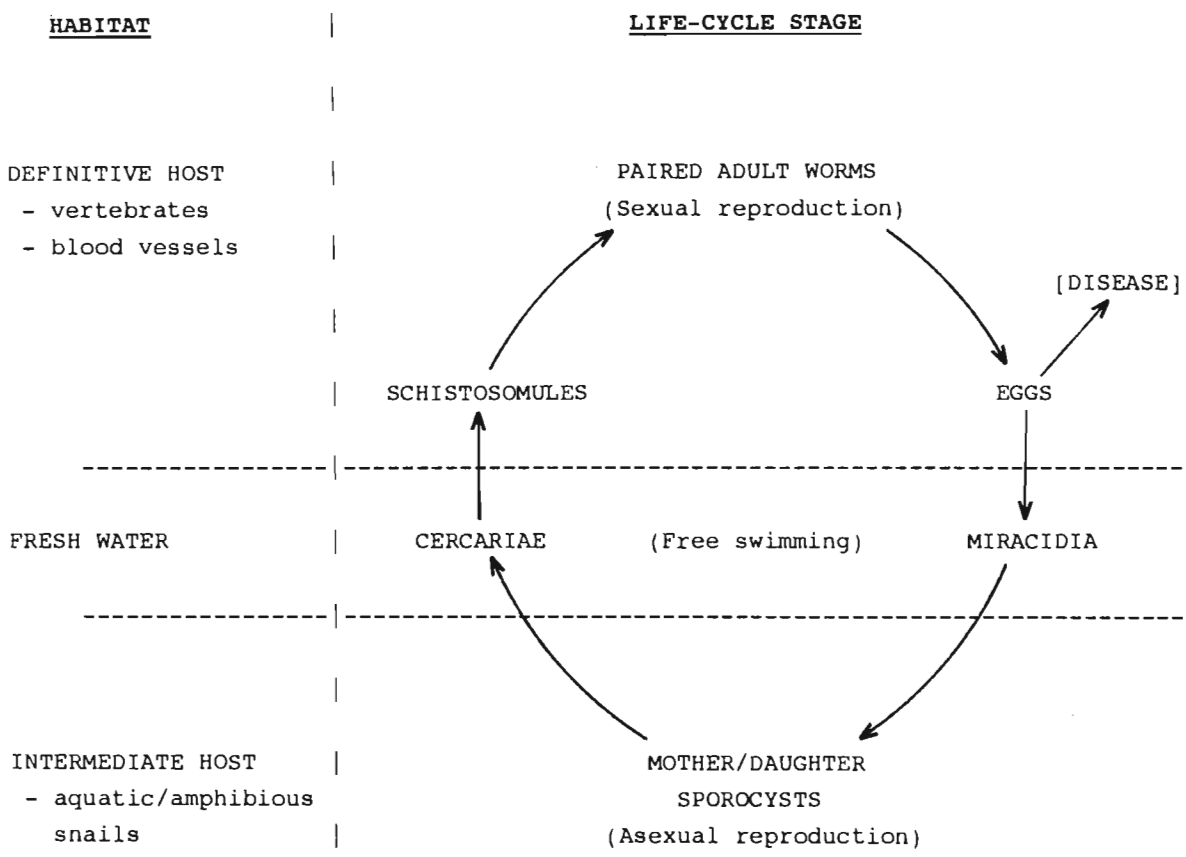
1.2 LIFE-CYCLE AND BIOLOGY OF MAMMALIAN SCHISTOSOMES

The schistosome life-cycle (Figure 1.2) involves alternating passage through a vertebrate ('definitive') host and an aquatic or amphibious snail ('intermediate') host; between each of these hosts are short-lived, free-swimming aquatic larval forms. The adult worms, which are dioecious, reside *in copula* in the veins of the definitive host, apparently in a state of monogamous association (Popiel, 1986). The female worms produce ova at daily rates which show pronounced inter- and intra-specific variation (Loker, 1983; Higgins-Opitz and Dettman, 1990). A substantial proportion of these ova become trapped within the host tissues where they evoke immunologically-mediated reactions which represent the fundamental process in the development of chronic host morbidity, as discussed below. However, the balance of ova are released in the excreta of the infected host, thereby contributing to the perpetuation of the life-cycle. Egg excretion is achieved most commonly via the faecal route since by far the majority of species within the genus *Schistosoma* inhabit the mesenteric veins. Notable exceptions to this rule are *S. haematobium*, which occurs predominantly in the veins of the urogenital system in man, and *S. nasale*, which inhabits the veins of the nasal mucosa in bovines; in these instances egg excretion is via the urine and nasal secretions, respectively. Schistosome ova are deposited into host venules in an unembryonated state and mature during the course of migration through the tissues, which takes about a week (Jourdane and Theron, 1987).

Contact of the schistosome egg with fresh water stimulates the emergence of a ciliated larval form known as the miracidium, which remains infective for a relatively short period of time (6-12 hours), during which time it swims about in search of a compatible host snail. Snails belonging to the family Planorbidae serve as hosts for all schistosomes falling into the *S. haematobium* and *S. mansoni* groups (Figure 1.1), as well as for some of those in the *S. indicum* group. The balance of the latter group utilize hosts of the family Lymnaeidae, while schistosomes of the *S. japonicum* group develop in snails of the family Pomatiopsidae (Rollinson and Southgate, 1987). For most schistosomes compatibility is limited to single genera and, in some instances, single species of snail hosts.

Having located and penetrated a suitable host, the miracidium undergoes a process of asexual reproduction, developing initially

FIGURE 1.2 LIFE CYCLE OF THE SCHISTOSOMES



into a mother sporocyst, which subsequently gives rise to a number of daughter sporocysts (Jourdane and Theron, 1987). On maturation the daughter sporocysts release large numbers of the second free-swimming larval stage, infective to the mammalian host, and known as cercariae. The duration of the intra-molluscan phase of development, while generally in excess of 3 weeks, tends to vary considerably, depending on the snail/schistosome combination and on environmental factors, in particular temperature (Rollinson & Southgate, 1987).

Emission of cercariae from snails follows a fairly well-defined daily pattern characteristic of each schistosome species, with peak shedding generally corresponding with periods of optimum water contact by the definitive host (Jourdane and Theron, 1987). Once released from the snail, cercariae may survive for more than 24 hours. However, studies with *S. mansoni* (Wilson & Coulson, 1983) have demonstrated that cercarial infectivity remains high for only about 5-8 hours; thereafter mortalities increase steadily, and although survivors apparently retain the ability to penetrate mammalian skin, their ability to complete the post-penetration migration and maturation process is markedly impaired.

Penetration of cercariae into the host skin is accompanied by tail loss and is rapidly followed by extensive structural, physiological and biochemical changes which adapt the larvae to their new environment and prepare them for the process of migration to their final habitat (reviewed by Wilson, 1987). The transformed larvae, referred to as schistosomula, escape from the site of penetration by entry, in most instances, into the venous drainage, or less commonly, the lymph drainage of the skin. Thereafter they are transported passively with the blood flow, via the right heart to the lungs. Once in the lungs they undergo morphological changes resulting most notably in enhanced extensibility, which greatly facilitates their further passage through the vasculature.

After traversing the lungs the schistosomula pass into the left heart via the pulmonary vein and are subsequently distributed around the body, predominantly by the arterial blood. Those which arrive in the hepatic portal system transform from a migratory to a non-migratory form, whilst those which are distributed to other organs make their way through the appropriate capillary beds and are routed back to the heart-lung circuit prior to redistribution through the systemic

circulation. In small experimental hosts, such as mice, a substantial number of schistosomula die during the course of negotiating the lung capillary bed, apparently accounting for the relatively low adult worm recoveries (often well below 50% of invading cercariae) from these hosts (Wilson, 1987). However, in larger hosts, such as the baboon, the lungs are traversed with less difficulty (Wilson *et al*, 1990). Relatively few schistosomula are thought to be lost due to delivery to unfavourable systemic sites (Wilson, 1987).

On arrival in the liver schistosomula develop into adult worms, following which pairing takes place. Within the genus *Schistosoma*, the male worm plays a vital role at this point of the process, firstly by serving to carry the female to the sites of egg deposition, and secondly by facilitating her full growth and sexual maturation; in regard to the latter, it appears that the role of the male is primarily to assist the female to ingest sufficient nutrients to meet the energy demands associated with intensive egg production (Popiel, 1986; Basch, 1990).

It should be noted that the preceding description of the migration and maturation of schistosomes within the definitive host is derived primarily from studies on the species responsible for disease in humans, in particular, *S. mansoni*. Relatively little is known about the behaviour of other species, although it seems likely that similar processes would apply to most, since all, with the exception of *S. nasale*, are adapted to a similar locality within the circulatory system.

The interval between the penetration of cercariae into the skin and the onset of egg-production by female worms shows considerable inter-specific variation, ranging from approximately 4 weeks to well over 8 weeks (Loker, 1983).

1.3 CLINICAL MANIFESTATIONS OF SCHISTOSOME INFECTION

The different stages of schistosome infection, namely cercarial penetration, mating and sexual maturation, and long-term parasitization are associated with a variety of clinically-distinct disease manifestations in humans.

1.3.1 Cercarial Dermatitis

Cercarial dermatitis, or "swimmer's itch" (Baird and Wear, 1987) is a pruritic, papular rash resulting from an immediate-type hypersensitivity response to cercariae in the skin. It is most widely recognised as a condition occurring within minutes to hours after exposure to the cercariae of non-human, and in particular avian schistosomes, many of which are unable to migrate much beyond the skin stage in humans. Repeated exposure to these organisms is associated with accelerated and intensified reactions, indicative of host sensitization. However, dermatitis is also known to occur as a result of exposure to *S. haematobium* and *S. mansoni*, although it would appear to be most pronounced in individuals who have had no previous exposure to schistosome infection (Chapman *et al*, 1988; Evans *et al*, 1990; Ong and Ellis, 1989; King, 1991); Baird and Wear (1987) suggest that in such cases the condition should be referred to as 'schistosomal' dermatitis, to differentiate it from that caused by non-human schistosomes.

1.3.2 Acute Schistosomiasis

At a stage corresponding approximately with the mating and sexual maturation of the worms, and the onset of egg deposition in the tissues (3 weeks to 3 months after exposure), individuals exposed to primary infections with human schistosomes may develop a variety of symptoms, including severe fevers, malaise, abdominal cramping and diarrhoea, hepatomegaly and/or splenomegaly, arthralgia and urticaria. This phase of the disease, which is referred to as acute (toxaemic) schistosomiasis, or Katayama fever, is usually transient (Boros, 1989), with symptoms recurring at intervals over a period of some weeks. However, the condition may become so severe as to be life-threatening (Evans *et al*, 1991). It is believed to be a form of immune complex disease resulting from the relatively sudden increase in the amount of schistosome-derived antigenic material released into the circulation at the time of parasite maturation. Maturation and mating of worms is associated with a marked increase in their metabolic activity (Wilson, 1987), presumably resulting in a corresponding increase in the output of excretory and secretory products; in addition, schistosome eggs, which are deposited in large numbers once the female worms become gravid, secrete soluble

metabolites which are highly antigenic (von Lichtenberg, 1987). Acute schistosomiasis is traditionally associated with exposure to heavy infections (Warren, 1973) but has also been reported from patients with relatively light parasite loads (Farid *et al*, 1986).

The sequential occurrence of schistosomal dermatitis and acute schistosomiasis appears to be quite common in persons from non-endemic areas experiencing infection for the first time (Chapman *et al*, 1988; Ong and Ellis, 1989; Anon, 1990; Evans *et al*, 1990; King, 1991). In contrast, these manifestations apparently occur relatively infrequently in the inhabitants of areas endemic for *S. haematobium* and *S. mansoni* and are generally considered to be of minor significance compared with the sequelae of long term egg-accumulation in the tissues. The situation in areas endemic for *S. japonicum* differs somewhat in that acute symptoms are both common and severe (Olveda and Domingo, 1987).

1.3.3 Chronic Schistosomiasis

Schistosome worms have evolved sophisticated mechanisms of evading the mammalian host immune system (Pearce and Sher, 1987) and, once they have established themselves in the final sites of habitation, are capable of surviving for many years. The mean life-span of schistosome worms is generally thought to be in the range of 3-10 years, although there is some uncertainty in this regard (Wilkins, 1987). While studies with both *S. haematobium* and *S. mansoni* have indicated a period of about three and a half years (Wilkins *et al*, 1984; Goddard and Jordan, 1980), there is adequate evidence that *S. mansoni* worms are capable of surviving for more than 35 years (Hornstein *et al*, 1990). Even assuming a life-span of only a few years and a reduction in fecundity with worm senescence (Hornstein *et al*, 1990), there is evidence of continuous worm replacement due to repeated infection, particularly during childhood and adolescence (Wilkins *et al*, 1984). It is thus clear that the deposition of ova in the host tissues by female schistosomes may continue uninterruptedly for many years. Estimates indicate that the daily rates of egg production by *S. haematobium*, *S. mansoni* and *S. japonicum*, may exceed 200, 300 and 3000, respectively (Loker, 1983).

In contrast to the worms, which, as mentioned, rely for survival on a strategy of immune evasion, the eggs of schistosomes are highly antigenic and evoke an intense, immunologically-mediated granulomatous response. However, this also appears to represent a 'deliberate' tactic on the part of the parasite, since there is evidence that the granulomatous reaction facilitates the extrusion of the egg into the lumen of the excretory organ, thereby promoting parasite survival (Doenhoff *et al*, 1986; Damian, 1987). From the host's perspective the response is 'aimed' primarily at the destruction of the egg, and also appears to play a role in sequestering toxic products released by the egg (Byram and von Lichtenberg, 1977).

The mechanisms of granuloma formation, which have been studied in great detail (see reviews by von Lichtenberg, 1987, and Warren, 1987), involve dynamic interactions between a complex array of soluble factors and cell types, the former including interleukins, lymphokines and monokines, and the latter, eosinophils, macrophages, fibroblasts, and T-lymphocytes. Recent evidence suggests that eosinophils (Lenzi *et al*, 1987) and platelets (Ngaiza and Doenhoff, 1990) may play important roles in facilitating egg excretion. One of the features of the process is a localised increase in collagen synthesis, with the eventual result that as eggs are destroyed and the granulomas resolve, fibrotic lesions remain.

The two fundamental consequences of schistosome egg deposition underlying subsequent morbidity are thus intense inflammation and, in particular, progressive fibrosis, which develops as a result of the continued deposition of large numbers of eggs into the tissues over prolonged periods.In schistosomiasis mansoni and japonica, the primary outcomes are damage to the intestinal mucosa, and more importantly, portal hypertension due to occlusion of hepatoportal venous blood flow, which in turn leads to the proliferation of portosystemic collateral vessels. The clinical manifestations of intestinal pathology include abdominal pain, mucosal ulceration and diarrhoea, and tend to be associated with the earlier stages of chronic disease, whilst those of hepatic damage include variceal bleeding and pronounced hepatosplenomegaly and are generally regarded as features of the more advanced stages of disease (Abdel-Wahab and Mahmoud, 1987). A significant association between hepatosplenic schistosomiasis and progressive nephropathy has also been recognised

(von Lichtenberg, 1987). While the severity of hepatosplenic disease appears to be related primarily to the intensity of infection, as discussed in Section 1.5, there is evidence that genetic characteristics, independent of intensity of infection, may influence the clinical outcome (Prata, 1988).

S. haematobium primarily affects the pelvic organs, in particular the bladder, ureters and kidneys. Clinical features include bladder ulceration and calcification, obstructive uropathy and hydronephrosis. Between them these may result in various manifestations, including haematuria, pelvic pain, dysuria, a predisposition to serious urinary tract infection, and kidney failure; there also appears to be considerable evidence indicating a link between *S. haemtobium* infection and the development of bladder cancer (von Lichtenberg, 1987). In addition, *S. haematobium* worms quite commonly occur in the mesenteric veins of the lower intestine, resulting in the accumulation of eggs in the liver and intestines, notably the rectum and sigmoid colon: however, they are considered to evoke somewhat less pathology than those of *S. mansoni* (Smith and Christie, 1986).

1.3.4 Ectopic Schistosomiasis

While the majority of lesions resulting from schistosome infections occur in the tissues closest to the sites of egg excretion, both worms and eggs may be deposited in numerous other sites within the body, largely by passage through portosystemic anastomoses. A wide variety of less common clinical manifestations are thus recognised: in particular, affectations of the central nervous system (Pittella, 1991), often including severe paraplegia, and the lungs (von Lichtenberg, 1987), with associated cor pulmonale, are most noteworthy. Other organs affected may include the appendix (Satti et al, 1987), skin (Uhltman *et al*, 1990; Dickinson *et al*, 1990) and eyes (Dickinson *et al*, 1990).

1.4 THE MAJOR DETERMINANTS OF SCHISTOSOMIASIS TRANSMISSION

Human schistosomiasis is essentially a disease of rural and/or poor communities, in which contact with natural water bodies and their

pollution by human excreta is an everyday occurrence. The most fundamental determinants of this disease should therefore perhaps be considered to be human behaviour and socio-economic status (Ree, 1982). Changes in the economic and social characteristics of communities may thus drastically alter transmission patterns (Kloetzel, 1989).

In terms of the transmission process itself, fresh water is the vital link, in that it is the essential ingredient in ensuring the viability of human communities, the intermediate snail hosts of schistosomes, and the free-living stages of the parasite life-cycle. Changes in the characteristics of water bodies thus exert a powerful influence on the transmission, and hence the epidemiological patterns, of schistosomiasis. The development of improved water resources, often comprising large dams and irrigation schemes, is a major priority in the under-developed regions of the world, many of which are experiencing rapid population growth. Not only do these resources provide new habitats for snails, but they also attract massive influxes of people, and they are thus considered to play a major role in the exacerbation of transmission, particularly in existing endemic areas (Iarotski and Davis, 1981). A rare example of the reverse scenario is to be seen in Japan, where the disappearance of schistosomiasis japonica from previously known endemic areas has been linked to the drainage of large areas of rice paddies, due to urban development (Kitani and Iuchi, 1990).

A variety of other climatic and physical factors play an important role in schistosomiasis transmission, including for example, temperature, rainfall, water quality, and the availability of suitable nutrient materials for snails. While interactions between multiple influences must be assumed, the first two factors are recognised as playing a particularly dominant part (Southgate and Rollinson, 1987), especially in areas characterised by seasonal transmission patterns (Chandiwana *et al*, 1987; Ozumba *et al*, 1989). Temperature exerts a strong regulatory influence on snail breeding and distribution, the survival and infectivity of cercariae and miracidia, and the intra-molluscan development of schistosomes. Rainfall may either promote or reduce transmission, for example by creating temporary transmission foci, or by washing snails away from existing foci. Extended periods of drought may also result in changes in transmission, for obvious reasons (Zein, 1989).

1.5 PATTERNS OF INFECTION AND MORBIDITY IN ENDEMIC COMMUNITIES

1.5.1 Individual Differences

Within individual communities infection intensities are typically highly variable, and are characteristically over-dispersed, with the majority of infected subjects harbouring light to moderate parasite loads and a small proportion carrying very heavy loads. The latter group, sometimes referred to as the 'wormy' group (Schutte, 1983), comprises individuals with an apparent predisposition to the development of heavy parasite burdens (Anderson, 1987); this characteristic appears, at least in part, to be genetically determined (Abel *et al*, 1991). These individuals are of particular importance in terms of transmission, since they contribute an inordinately large proportion of the total number of eggs released by a community into the aquatic environment (Wilkins, 1987). They are also the group most likely to present with the severest forms of disease.

1.5.2 Age-related Patterns

As a rule, prevalence and intensity of schistosome infections in endemic communities (assessed on the basis of presence and quantity of eggs in the excreta, respectively) rise rapidly during the first decade of life, peak between the ages of 10 and 15 years, and decline during the subsequent decades. This age-related pattern appears to be most pronounced in areas affected by *S. haematobium*, while in areas endemic for *S. mansoni* and *S. japonicum* the patterns tend to be somewhat more variable, in particular with respect to prevalence, which sometimes takes longer to peak, remains high over a more extended age range, and declines at a slower rate. In all instances the rates of decline in intensity of infection appear to be greater in communities which display high rates of transmission (i.e. those with higher peak infection intensities) (Anderson, 1987; Wilkins, 1987).

1.5.3 Regional Differences

In general, there are positive correlations between prevalence and intensity of infection (Wilkins, 1987), and between both of these

criteria and the frequency of urinary tract or hepato-intestinal abnormalities (Doumenge *et al*, 1987). Thus communities with the highest prevalences also tend to have the highest mean infection intensities, and are also likely to present the highest levels of morbidity.

Prevalence and intensity patterns vary considerably from one community to the next, with pronounced differences sometimes being observed even between communites separated by short distances (Wilkins, 1987). These variations to some extent reflect differences in the physical and social characteristics of the specific transmission sites. On the one hand, they may differ in terms of their suitability as habitats for the intermediate snail hosts (Brown, 1980). On the other hand communities may differ in a variety of ways which influence water contact patterns and the extent to which water bodies are contaminated by excreta; these include differences in socio-economic status, cultural behaviour and means of subsistence (Ree, 1982; Lima e Costa *et al*, 1987; Mott *et al*, 1990). Differences between communities in terms of morbidity are also frequently observed, which to some degree may directly relate to differences in infection levels; however, the influence of various other compounding factors, such as genetic differences, the presence of other infectious diseases, nutritional status, etc. cannot be discounted (Butterworth, 1990).

1.6 CONTROL OF SCHISTOSOMIASIS

The concept of schistosomiasis control implies the disruption of the transmission cycle at one or more levels. Viewed in relation to the schistosome life-cycle (Figure 1.2) this could theoretically be achieved at virtually any point, as indicated by the following list:

i) preventing the penetration of cercariae into the skin

ii) preventing or attenuating the development and maturation of the parasite in the human host (see section 1.7)

iii) interfering with the fecundity of worm pairs, thereby reducing the quantity and or viability of ova produced (see section 1.7)

iv) killing the adult worms

v) preventing or reducing the introduction of infected excreta (i.e. ova) into snail habitats

vi) reducing or eliminating populations of susceptible vector snails

vii) interfering with the ability of miracidia to locate, penetrate or develop in the intermediate host

viii) preventing or reducing contact between cercariae and the human hosts

1.6.1 Strategies With Limited Current Application Potential

All of these options have been subjected to a greater or lesser degree of laboratory and/or field investigation. For practical reasons some are considered to hold little promise in terms of their potential to make a significant impression on transmission. A case in point is the first of the above-mentioned options, i.e. interfering with cercarial penetration, for example by using soaps or lotions with cercaricidal properties (e.g. Hunter *et al*, 1956; van Rensburg, 1972), which has received little serious consideration as a tool in large-scale control. Extensive studies have been carried out in relation to option (vii) involving a number of different approaches (Jordan *et al*, 1980), including the use of non-susceptible decoy snails to divert miracidia from susceptible ones, the use of predators (e.g. fish, filter-feeding and carnivorous invertebrates, and carnivorous plants) to consume miracidia, the use of toxins to kill them, and the use of antagonistic trematode species (based on the exposure of snails to trematodes which produce redial stages that attack schistosome sporocysts) (Combes, 1982). However, none of these appears as yet to have reached a stage where they might be of significant practical benefit. Studies on the intramolluscan development of schistosomes have spawned an entire field dealing with the immune responses of snails to parasitic infections, the main contribution of which appears to have been in the area of comparative and evolutionary immunology (Loker and Bayne, 1986).

In other instances, where a number of different approaches have been adopted with a view to the same end, the application potential differs according to the approach taken and the current 'state of the art'; this is particularly so in the case of option (vi). On the one hand highly effective synthetic molluscicides (McCullough and Mott, 1983) have been available for many years and are an integral component of most current control schemes (see below). On the other hand, extensive research continues along a variety of other avenues. These include the development of 'natural' (e.g. plant-derived) mollusicides (Hostettmann, 1984), which potentially will be more target-specific and more environmentally 'friendly', the genetic manipulation of snail susceptibility to infection by the introduction of large numbers of non-susceptible snails into natural populations (Minchella and LoVerde, 1983), and the use of various biological control agents (Madsen, 1990), such as snail pathogens, non-schistosome trematodes which cause sterilization of snails, and competitive snail species. In the case of natural molluscicides, at least one product, extracted from the berries of *Phytolacca dodencandra* and known as 'endod', appears to have considerable application potential (Lemma *et al*, 1984). However, the remaining alternatives remain essentially at an experimental stage.

1.6.2 Morbidity Control Versus Transmission Control or Eradication

It is important to note that the current global approach to the control of schistosomiasis is not aimed at eradication of the disease, or even necessarily at drastically curtailing transmission, but rather at reducing to insignificant levels the degree of schistosome-induced morbidity (Mott, 1987). Current control programmes thus employ a multi-dimensional approach centred around the fourth, fifth, sixth and eighth of the above-listed options, namely elimination of the adult worms, decreasing the degree of faeco-urinary contamination of water bodies, reducing snail populations in and around transmission foci, and reducing human water contact at potentially infective sites (Butterworth, 1988; Mott, 1987). One of the advantages of such an approach is that of synergistic interaction between the different interventions.

1.6.3 Chemotherapy

The benefits of a reduction in worm loads in infected communities are two-fold: firstly it results in a decreased amount of egg-excretion, thereby reducing parasite transmission potential, and secondly, it reduces the extent of tissue egg-accumulation, thereby reducing the probability of significant morbidity. A number of highly effective anti-schistosomal drugs are currently available (Shekhar, 1991), including oxamniquine, metrifonate and praziquantel, of which the latter is presently the drug of choice (King and Mahmoud, 1989). Chemotherapy is almost invariably used as one of the main weapons during the initial stages of control programmes, usually resulting in pronounced decreases in both prevalence and intensity of infection. This may be accompanied by a rapid improvement in general health status, as measured, for example, in terms of physical fitness and appetite (Latham *et al*, 1990). Drug treatment also plays an important role in the follow-up and maintenance phases of control programmes, in terms of retreatment of partially-cured or reinfected individuals.

Unfortunately schistosomicidal drugs, especially praziquantel, are expensive, and this represents one of the major constraints on their use. Application strategies vary from mass treatment (treatment of all members of the target community), through selective treatment (treatment of all infected members of the community), to selected group treatment (treatment of selected groups of infected individuals only) (Mott, 1987; Butterworth, 1990), according to epidemiological, logistical and economic considerations. The need to monitor chemotherapy campaigns with a view to the early detection of possible drug resistant schistosome strains has been recognised; at this stage, while there are no reports of resistance to praziquantel, there is some evidence of resistance of *S. mansoni* to oxamniquine (Marshall, 1987).

1.6.4 Molluscicides

Molluscicides represent the second of the rapid-effect 'attack' weapons available for schistosomiasis control. However, the most effective and widely used of the available compounds, niclosamide, suffers from two disadvantages: (a) it is expensive, and (b) it

exhibits a significant amount of toxicity to non-target organisms, notably fish. For this reason, current control programmes focus on a policy of highly localised (focal) application (Teesdale, 1986).

1.6.5 Environmental and Behavioural Modification

The major disadvantage of both chemotherapy and molluscicide is that if their application is not sustained, transmission is likely to revert to pre-intervention levels within a relatively short space of time. As regards long-term control, the limitations of chemotherapy, in particular, are becoming increasingly evident, resulting in a reassessment of its role (Wilkins, 1989; Gryseels and Polderman, 1991). For this reason, considerable emphasis is being given to strategies which are likely to affect transmission on a more sustained basis.

On the one hand, these focus on the modification and management of the physical environment, essentially using the principles of civil engineering, many of which can be appropriately adapted for use in underdeveloped regions (Pike, 1987). Examples of such measures include, among others, improved sanitation, provision of pathogen-free water supplies, planning of village siting in relation to dams or irrigation canals (e.g. resettlement villages), and rendering of water bodies unsuitable for snail habitation. On the other hand, these measures are unlikely to be of much benefit without the communities for whom they are intended fully appreciating their merits and the principles upon which they are based, and being motivated to make them work. Thus, an increasing accent is being placed on the need to develop appropriate health education strategies (Teesdale, 1986; World Health Organization, 1990), and to deploy socio-anthropological methodologies in order to understand how different communities perceive and deal with the disease, with a view to 'custom-designing' intervention strategies according to the characteristics of individual communities or ethnic groups (Robert *et al*, 1989). In regard to the latter, it is often beneficial to identify the reasons for poor versus satisfactory outcomes of past control efforts.

With respect to the control of schistosomiasis *per se*, the value of approaches based on environmental and behavioural modifications lies

in the fact that they serve to reduce the likelihood of contact between the free-living stages of the parasite and their snail or human hosts. However, they offer an additional benefit in that they involve measures which, although in some instances are by necessity schistosomiasis-specific, in many other instances are able to exert a substantial influence on water-borne diseases in general. As such they can be included as part of more broad-based health programmes aimed at reduction of morbidity due to multiple diseases. An additional benefit is that they often do not require sophisticated technology or equipment, or highly-trained 'imported' personnel; this is of particular importance in view of the realization that in order for health interventions to have any chance of long-term success, they must be built on the foundation of full community participation during all phases, including initiation, design, implementation and maintenance (Ndamkou and Ratard, 1990; Ali *et al*, 1989).

1.6.6 Economic and Operational Requirements: Management, Monitoring, Diagnosis and Training

Apart from the wide range of measures discussed above, which are aimed essentially at the disruption of the transmission process itself, it must be mentioned at this point that the success of all schistosomiasis control programmes is dependent, first and foremost, on the availability of adequate financial resources, and secondly on the establishment of sound operational structures. The latter include (a) effective local management, (b) the availability of teams capable of implementing interventions and of monitoring their efficacy, using the appropriate diagnostic methods, and (c) opportunities for training of personnel (Ali *et al*, 1989).

With reference to diagnostic techniques, it is worth noting that in terms of establishing prevalences and intensities of infection the preferred methods are still those based on the demonstration of schistosome ova in the excreta or in tissue biopsies (Peters and Kazura, 1987). Most available serodiagnostic techniques suffer from the disadvantages that they lack species specificity, do not differentiate between past and present infection, do not indicate intensity of infection, are relatively expensive, and usually require specialised equipment or facilities; their usefulness is thus limited to preliminary screening of samples for evidence of infection

(Weiland, 1989). Tests based on the detection in serum or urine of a circulating antigen excreted by schistosome worms show considerable promise, but are not as yet available for wide-scale use (De Jonge *et al*, 1988). In view of the shift in emphasis from transmission control to morbidity control, as mentioned above, considerable attention has recently been given to the application of ultrasonography as a tool for the assessment of pathological lesions (Hatz *et al*, 1990; World Health Organization, 1991a).

1.7 IMMUNITY AND IMMUNOPROPHYLAXIS

Of the various strategies which are considered to have an important part to play in the long term control of human schistosomiasis, immunoprophylaxis warrants special mention, by virtue of the fact that it has elicited a prodigious amount of research effort, but has not as yet reached a stage of practical application. Intensive experimental studies aimed primarily at the development of a schistosomiasis vaccine capable of limiting either the development and maturation of worms or their fecundity (options ii and iii) have been in progress for more than three decades. Although early work, most notably that in the rhesus monkey, provided evidence that strong protective immunity could be elicited in some animal hosts, it was soon realised that the schistosome presents an extremely complex immunological target and that the development of a vaccine would be no simple task (Sadun, 1963; Smithers and Terry, 1969a).

Subsequent years thus saw a proliferation of *in vivo* and *in vitro* investigations which yielded extensive insights into the nature of the interactions between the vertebrate immune system and the various developmental stages of the schistosome worm. As a result a great deal is now known about the nature, variety and regulation of immunological mechanisms evoked by schistosomes in different hosts and how they contribute to host protection and immunopathogenesis, as well as about how the worms evade and even exploit potentially damaging immune responses. Studies on the immunology of schistosomiasis are in fact widely recognised to have made a significant contribution to the development of the broader field of immunology *per se*, one example being the discovery that eosinophils are a necessary component of the immune armoury, with a particular role in the defence against multicellular parasites (Ellner and

Mahmoud, 1982). However, since it is not intended here to attempt to review in detail the entire field of experimental schistosomiasis immunology (which in any case appears to be subject almost annually to multiple reviews), the reader is directed to the following articles which, between them, cover many of the major developments and current concepts applicable to this field: Damian (1984, 1987), Pearce and Sher (1987), Boros (1989) and Sher and Colley (1989). Where appropriate, some of the key concepts that have emerged over the years will be dealt with in detail in the context of subsequent chapters. The intention at this point is rather to briefly focus on current knowledge regarding immunity to schistosomes in man, and on the state of the art as regards the development of candidate vaccines.

1.7.1 Evidence for Acquired Immunity to Schistosomes in Man

As discussed by Davis (1987), it has long been assumed that at least some measure of protective immunity to schistosomes must develop in man, perhaps the most compelling evidence being that of an age-related decline in morbidity and mortality, which in some endemic areas has been observed to occur in spite of continuing high levels of exposure to infection. However, opportunites for investigating those aspects of this phenomenon which might facilitate the development of an effective vaccine (such as the rate at which resistance develops, the antigens responsible for eliciting protective responses, and the immunological mechanisms which mediate these responses) were for many years limited, due to the problems inherent in conducting adequately controlled studies in human subjects. Hence the extensive investment in animal model studies, as mentioned above and in section 1.8.

In more recent years the advent of highly effective and safe drugs, in particular praziquantel, coupled with improved methods of quantifying exposure to infection, have facilitated the design and implementation of a number of ground-breaking investigations, based largely on the assessment of reinfection rates and intensities in various drug-cured target groups. Studies in a *S. mansoni*-endemic region in Kenya (Butterworth, 1990) have, in the first place, confirmed that a targeted approach to chemotherapy, in particular one focused on schoolchildren, results in a pronounced and persistent

reduction in intensity of infection, morbidity, and transmission. More importantly, however, they demonstrated that the rate of reinfection in young children, especially those in the 9 to 12 year age-range, was far higher than that in older children and adults. Although it was demonstrated that exposure to infection decreases with age, the magnitude of this decrease was shown to be insufficient to account for the drop-off in reinfection levels.

Similar studies in a *S. haematobium*-endemic area of the Gambia (Wilkins *et al*, 1987) have also shown a strong inverse relationship between age and the intensity of reinfection, again after allowing for age-related differences in levels of exposure to infection.

The results of the above-mentioned investigations thus provide compelling evidence of a progressive, age-related development of acquired resistance to schistosomes in man, and have served to greatly reinforce the belief that the production of an effective anti-schistosomal vaccine is feasible. Corresponding studies aimed at dissecting the immunological mechanisms underlying this phenomenon have been carried out by both groups. In summary, these indicate a dynamic relationship between the production of protective and non-protective antibody isotypes. Levels of protective antibodies apparently rise considerably more slowly than those of non-protective forms. Consequently, in younger children the latter predominate, and it is suggested that by occupying the majority of available schistosome antigenic sites, they block the effect of the former. In the case of *S. mansoni* the blocking antibodies are thought to belong to the IgM and IgG2 classes and are elicited by egg polysaccharide antigens (Butterworth, 1990), while in the case of *S. haematobium* they appear to belong to the IgG4 subclass and are reactive to both worm and egg antigens (Hagan *et al*, 1991). It should be noted, however, that the blocking antibody theory is based essentially on *in vitro* studies of the abilities of sera or serum fractions to inhibit schistosomulum killing, and the *in vivo* significance of this phenomenon remains to be determined. It has been shown in passive transfer experiments with monoclonal antibodies that the demonstration of blocking *in vitro* does not necessarily imply *in vivo* efficacy (Bickle and Andrews, 1988).

Recent evidence indicates that IgE antibodies play a major role in the development of protective immunity (Hagan *et al*, 1991; Rihet *et*

al, 1991). The role of cellular immune effector mechanisms in the development of acquired resistance has not as yet been elucidated, although there is some evidence of a role for eosinophils (Butterworth and Hagan, 1987).

1.7.2 Current Approaches to the Development of Vaccines

In the light of the above discoveries, the primary aim of an anti-schistosome vaccine would be to promote the development of strong protective immune responses at an early stage of exposure to schistosomes, thereby forestalling the production of blocking antibodies. However, in view of the shift in emphasis towards the reduction of schistosomiasis-related morbidity rather than eradication of the disease, it is now recognised that a vaccine which provides only partial immunity might well be acceptable. It may even be preferable, since according to the concept of concomitant immunity (Smithers and Terry, 1969b) (see Chapter 4) the establishment of a low worm burden, insufficient to result in significant morbidity, may be beneficial in terms of sustaining long-term resistance. Furthermore, since eggs are the major cause of morbidity, even a vaccine that interfered with oogenesis or egg-productivity *per se* might be sufficient to achieve an adequate degree of morbidity reduction. Similarly, an 'anti-pathology' vaccine (Warren, 1972) which resulted in a modified granulomatous response, such that the fibrotic processes were reduced while the benefits of toxin sequestration were retained, may also be effective.

Attempts to develop vaccines have involved a vast number of experiments with both living and non-living schistosome preparations. Of the former, studies with radiation-attenuated cercariae and schistosomules have been particularly rewarding. However, while attenuated vaccines show considerable promise for the control of bovine schistosomiasis, they are unlikely to be acceptable for use in humans (Taylor and Bickle, 1986). Current approaches thus focus on the use of defined antigens. Recent advances in molecular biology have greatly facilitated this process and have led to the identification of a considerable number of candidate vaccine molecules (Simpson and Cioli, 1987; Berquist, 1990). While some of these are surface membrane epitopes with apparently little functional significance, there has also been considerable emphasis on the

identification of antigens which have important functional roles. The latter include enzymes, such as glutathione-S-transferase (GST), and structural proteins, such as paramyosin. In this context the idea of a vaccine which, as suggested above, disrupts the process of sexual maturation or oogenesis without necessarily destroying the worms seems quite conceivable, since it may be possible to design a vaccine targeted at the underlying molecular processes; these are likely to be elucidated with continued studies on schistosome reproductive biology (Erasmus, 1987). Experimental support for the concept of such an 'anti-fecundity' vaccine comes from a recent report by Xu *et al* (1991), who observed markedly reduced *S. mansoni* egg production and egg viability in mice treated with a monoclonal antibody raised against a recombinant *S. mansoni*-specific 28-kDa GST.

The rapid progress that has been made in the past few years, not only with respect to the identification of potential candidate vaccines, as dicussed above, but also as regards the development of a variety of promising immunological adjuvants (reviewed by Bomford, 1989), has led to considerable enthusiasm about the prospects for the development of an effective vaccine in the not-too-distant future. This is reflected in the strong support expressed by the World Health Organization, which recently sponsored a meeting of international experts to discuss strategies for the development and testing of candidate vaccines (World Health Organization, 1991b). However, while the consensus among many leading schistosomiasis researchers is that vaccines are an essential weapon in the war against schistosomes (Butterworth *et al*, 1987), strong reservations have been expressed, in particular by Kloetzel (1989), largely on the basis of cost-benefit considerations. Vaccine development efforts have and will continue to absorb a tremendous amount of manpower and expense, and it is argued that if these resources were invested into existing options for control, especially those which contribute to a general improvement in living conditions, the overall benefits would be far greater. This concern deserves careful consideration, especially in the light of the observation by Nelson (1986) that, as has been demonstrated for a variety of other diseases, the development of effective vaccines does not necessarily imply their effective utilization.

1.8 THE ROLE OF ANIMAL MODELS IN SCHISTOSOMIASIS RESEARCH

Direct, in-depth investigations on schistosome infections in humans are obviously subject to severe technical and ethical constraints. Much of the extensive body of existing knowledge on the biology of the mammalian schistosomes and the spectrum of responses which they evoke in their vertebrate hosts has thus been acquired through studies in animal models. This has been possible due to the fortunate fact that the life-cycles of many schistosome species can be maintained in the laboratory using widely available hosts such as the mouse and the hamster. In contrast, methods for the *in vitro* cultivation of the intra-mammalian stages of the schistosome life-cycle have proven difficult to develop and are far from perfected (Clegg and Smith, 1987). Thus, while they have been applied extensively and to great benefit, for example in studies on the effects of host-derived humoral and/or cellular factors on schistosomula (Clegg and Smith, 1987), adult worms (Bosshardt and Damian, 1986) and eggs (de Brito *et al*, 1984), *in vitro* methods are clearly in no way able to replace *in vivo* models. Considering the complexities of schistosome development, it seems unlikely that an *in vitro* system which adequately simulates the *in vivo* environment will be realized in the foreseeable future.

The majority of studies involving animal models have, for obvious reasons, focused on the most important agents of schistosomiasis in man, namely *S. mansoni*, *S. japonicum* and *S. haematobium*. Of the three, *S. haematobium* has proven most fastidious in terms of its definitive host requirements and for the most part has not been successfully maintained in long-term culture. One of the few exceptions in this regard concerns the establishment in 1960 of a life-cycle in a southern African indigenous rodent, *Saccostomus campestris* (the 'pouch mouse') (Pitchford and Visser, 1965), which continues to thrive in the present author's laboratory.

S. mansoni and *S. japonicum* are far less discriminating in their host requirements, being capable of sexual maturation in a remarkably wide range of animals (Rollinson and Southgate, 1987). As a result the definitive host stages of these two species are quite easily maintained in the laboratory. However, while a number of highly prolific laboratory cultures of *S. mansoni* have been available to researchers for at least four decades, *S. japonicum* life-cycles have

proven more difficult to maintain on a long-term basis. This difference reflects the fact that the aquatic intermediate host snails of *S. mansoni* (*Biomphalaria* spp.) have proven far easier to cultivate in the laboratory (Lewis *et al*, 1986) than the amphibious hosts of *S. japonicum* (*Oncomelania* spp.). Thus, in spite of recently improved opportunities for laboratory studies on *S. japonicum*, resulting from advances in snail maintenance methods (Moloney *et al*, 1987a), even a cursory glance at the schistosomiasis literature reveals that the vast majority of experimental studies to date have involved the use of *S. mansoni*.

While the range of animal hosts used for schistosome life-cycle maintenance is generally limited to a few species, most commonly mice and hamsters, essentially on the basis of availability and susceptibility to infection, a wide variety of host types have been tested as possible models for research purposes. In the case of *S. mansoni*, for example, this includes a number of somewhat unusual hosts, such as woodchucks and chipmunks which are not indigenous to schistosomiasis endemic areas (Knopf, 1982). However, the great majority of studies have involved the use of well-characterised laboratory hosts, in particular mice, rats and non-human primates, and, to a lesser extent, hamsters, guinea pigs and rabbits.

Hosts are traditionally classified as either 'permissive' or 'non-permissive'. These terms were first used by Cioli *et al* (1977) to differentiate between animals that support stable, well-developed infections, with long-term oviposition, and those in which 'development of worms is incomplete and/or oviposition is interrupted after a short period'. Knopf (1982) applied a more rigorous definition, differentiating between permissive or non-permissive hosts purely on the basis of whether or not they are capable of passing viable schistosome ova in their excreta. According to this definition, the term 'non-permissive' accommodates a particularly wide range of possibilities. At the one extreme it includes hosts which do not permit schistosomulum development much beyond the skin stage, and at the other it includes those which may support full, and possibly prolonged, sexual maturation and oviposition, but which are unable to expel the eggs into the lumina of the excretory organs.

No attempt will be made here to discuss in depth the various applications of animal models in schistosomiasis research. Their most

prominent use has undoubtedly been in studies on the mechanisms of schistosome-related immunity and immunopathogenesis and in this regard the reader is directed to the reviews by McLaren and Smithers (1987) and Damian (1989) which discuss the relative merits of the various models and cover most of the major developments in this field. It is important to note, as is stressed in both of the above articles, that no single model system can be considered as fully representative of the human/schistosome host-parasite relationship, extensive insights having been gained from studies in both permissive and non-permissive hosts. Instead, each of the most commonly used models has specific characteristics which have been shown to represent aspects of human infections, or have provided clues as to some of the possible mechanisms operative in man. Thus, as Damian (1989) observes, 'mice are much better models for the study of egg granuloma-induced fibrosis than either rhesus monkeys or baboons, despite their much more distant relationship to humans'. Similarly, in spite of the fact that laboratory rats (*Rattus norvegicus*) are non-permissive hosts, they have played a central role in studies on so-called antibody-dependent cellular cytotoxicity (ADCC) mechanisms, which are believed to be a component of the human immune response to schistosomes (Capron and Capron, 1986).

Apart from their use in studies on immunity and immunopathology, animal models have also been extensively utilised for many other purposes, including comparative investigations on the infection characteristics of different schistosome species (Loker, 1983), studies on definitive host factors influencing growth and survival of schistosomes (Knopf, 1982), studies on the processes of development and migration of the intra-mammalian life-cycle stages (Wilson, 1987), and experimental chemotherapy (Marshall, 1987). Furthermore, infections in laboratory animals represent an important source of worms and eggs used, for example, for the preparation of schistosome antigens.

An additional aspect of animal-based investigations, discussed at length by Taylor (1987) but often overlooked, concerns the use of ruminants, in particular cattle, sheep and goats. Firstly, some of the characteristic lesions associated with chronic schistosomiasis in man, such as Symmer's (clay pipestem) fibrosis, are also seen in these animals, and since they are amenable to long-term studies, they may be useful for investigations on the etiology of chronic disease.

Secondly, cattle and sheep have been used with considerable success in the development and field testing of irradiated vaccines against *S. mattheei*, *S. bovis* and zoonotic schistosomiasis japonica (see Section 1.9). It is suggested that the extension of this work could yield valuable insights into the types of factors which might influence the success or failure of human vaccines, once they reach the stage of field trials.

1.9 STUDIES ON NON-HUMAN SCHISTOSOMES

'Non-human' schistosomes are those species for which man is not the primary definitive host. Some of these species have been mentioned in previous sections; those not previously mentioned include *S. margrebowiei*, *S. leiperi*, *S. rodhaini*, *S. edwardiense*, *S. hippopotami* and *S. sinensium*.

Studies on non-human schistosomes comprise only a small proportion of the total body of research on the genus *Schistosoma*. This no doubt reflects the fact that they pose minimal health risk to man, coupled with the ready availability of animal models for studies on human schistosomiasis, as discussed above. As a result there appear to be relatively few areas in which there have been prolonged, systematic investigations. Nevertheless, it is possible to summarize much of the work on non-human schistosomes in terms of four major areas, namely, (i) taxonomic relationships between schistosome species, (ii) pathology and pathogenesis in naturally and experimentally infected hosts, (iii) development of live attenuated vaccines for use in domestic ruminants, and (iv) zoonoses and heterologous immunity.

1.9.1 Taxonomic Relationships Between Schistosome Species

Studies on the the taxonomic relationships between schistosomes obviously include both human and non-human species. These relationships have been explored using a variety of approaches, including comparative analyses of morphological, life-cycle and behavioural characteristics, karyotypes, intermediate and definitive host specificities, enzyme patterns and DNA probes (reviewed by Rollinson and Southgate, 1987). The information accumulated during the course of such studies provides, as it were, a series of

biological 'character sketches' by which the various human and non-human schistosome species may be identified. In practical terms this has proven particularly useful in studies on natural hybridization between schistosomes (see Southgate and Rollinson, 1987), helping, for example, to demonstrate that apparent infections of *S. mattheei* in man in South Africa were, in fact, caused by hybrids resulting from exposure to cercariae of both *S. mattheei* and *S. haematobium* (Kruger and Evans, 1990).

1.9.2 Pathology and Pathogenesis

Many of the studies on the pathology and pathogenesis of infections with non-human schistosomes were initiated directly as a result of observed morbidity in domestic livestock, as mentioned in Section 1.1. Much of this work is covered in detail in the review by Taylor (1987) and will not be discussed at length here. In short, the pathological sequelae of natural and experimental infections with *S. bovis* and *S. mattheei* in cattle, sheep and goats appear to have been more intensively described than for any of the other animal schistosomes. However, there have also been a variety of reports describing pathology in some of the natural defintive hosts of *S. spindale* (goats), *S. indicum* (horses, sheep and goats), *S. nasale* (cattle and buffaloes) and *S. incognitum* (dogs and pigs). Apart from the studies reviewed by Taylor, there have in recent years been various additional publications dealing with, for example, *S. mattheei* in cattle (Obwolo and Rogers, 1988), *S. bovis* in sheep and goats (Kassuku *et al*, 1986), and *S. curassoni* in sheep and goats (Vercruysse *et al*, 1985 and 1988). Although *S. japonicum* is regarded as a pathogen of considerable veterinary importance in the Orient (Mao and Shao, 1982), only one strain, namely that from Taiwan (better known as the 'Formosan' strain), is recognised as being strictly zoophilic (Rollinson and Southgate, 1987) and can therefore be categorised as a non-human schistosome. However, since there appear to be few, if any, detailed English language reports on the pathology resulting from infections with either the Formosan or any other strains of *S. japonicum* in livestock, no attempt will be made to address this subject here.

Studies on the pathogenesis of non-human schistosomiasis in laboratory animals appear to have been very limited, with only a few

references being encountered in the literature consulted. Experiments describing the effects of *S. mattheei* and *S. bovis* in mice (based on two publications) and of *S. incognitum* in mice (also based on two publications) are dealt with in the reviews by Taylor (1987) and Agrawal and Shah (1989), respectively. The effects of *S. curassoni* in mice and hamsters have been described by Vercruysse *et al* (1986). With respect to *S. japonicum*, there have been numerous studies on the pathology of experimental infection (reviewed by Cheever, 1985); however, work on the Formosan strain has been very limited and has yielded equivocal results. In general it would appear that the effects of non-human schistosomes in small laboratory animals do not necessarily reflect those in the natural definitive hosts. The value of such models probably lies in their potential as alternatives to, or for comparison with, the well-characterised *S. mansoni*/mouse model.

1.9.3 Development of Live, Radiation-attenuated Vaccines

The use of live, radiation-attenuated cercariae or schistosomulae of *S. bovis*, *S. mattheei* and *S. japonicum*, as a means of vaccinating livestock against homologous natural infections has been intensively investigated (Taylor, 1987). The results of these studies have been extremely promising, demonstrating that substantial levels of homologous protection can be achieved with all three species. Effective methods of cryopreserving schistosomulae have been developed and it therefore appears that live vaccines for domestic animals may become a feasible proposition, assuming that the necessary quantities of parasite material can be made available on a reliable basis. The *S. bovis* vaccine has been most intensively assessed and is presently undergoing field trials aimed at defining the optimum immunization procedures (Taylor *et al*, 1991).

1.9.4 Zoonoses and "Zooprophylaxis"

Zoonoses are defined as "diseases naturally transmitted between animals and man" (World Health Organization, 1959). For the most part, the emphasis appears to have been on organisms for which animals are the reservoir hosts, but which sometimes infect man, resulting in some measure of detectable disease. However, the

apparent simplicity of the above definition belies an extraordinary degree of complexity, since a great variety of permutations have been recorded, including uni- or bi-directional transmission and the possible involvement of either or both domestic and wild animals (Figure 1.3) (Nelson, 1988; Schwabe, 1991).

With reference to schistosomiasis, the anthropophilic (non-Formosan) strains of *S. japonicum* represents the most advanced state of complexity; while transmission can be maintained independently either in man or a variety of animal hosts, extensive bi-directional transmission between man and animals also occurs. In contrast, the Formosan strain is apparently capable only of uni-directional transmission to man, since it is restricted to animal hosts; in man this parasite apparently undergoes partial maturation (Hsu and Hsu, 1956), resulting in what Malek (1980) has referred to as 'nonpatent visceral schistosomiasis'.

As regards the non-human schistosomes in general, evidence suggests that they are mostly incapable of full sexual maturation in man, except perhaps on rare occasions. Although there have been various reports of the recovery of non-human schistosome ova from human excreta (Malek, 1980), these have characteristically dealt with isolated cases, and have often been inadequately documented. While Pitchford (1959) reported a high prevalence of *S. mattheei* infections in man in South Africa, it has now been established that this was more than likely due to the production of ova by *S. mattheei* female worms *in copula* with *S. haematobium* (see Section 1.9.1). Only the zoonotic potential of *S. incognitum* remains in dispute (Agrawal and Shah, 1989). However, in spite of their apparent innocuousness to man, the possible influence of non-human schistosomes on the epidemiology of human schistosomiasis (and, to a lesser extent, *vice versa*) has been a subject of intense study, largely due to the endeavours of Professor G S Nelson (Kinoti, 1991).

Nelson's interest in this subject was apparently aroused by observations made by Le Roux (1961) pertaining to the absence of *S. haematobium* in parts of Sardinia, Corsica and Sicily. Since the appropriate snail hosts of *S. haematobium* were present, Le Roux suggested that humans were experiencing a form of 'natural' immunization as a result of exposure to the cercariae of *S. bovis*, which was prevalent in cattle in these regions. A similar situation

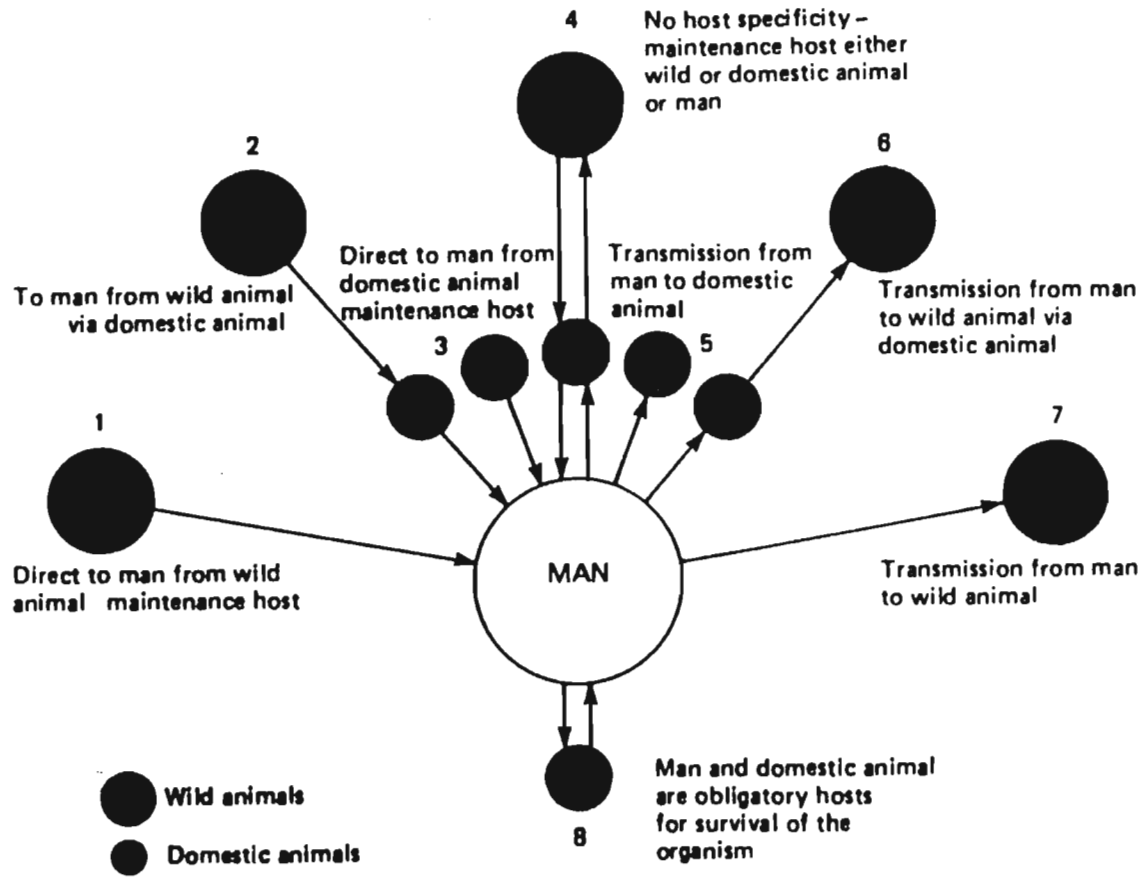


FIGURE 1.3 Zoonoses: epidemiological categories (from Nelson, 1988; with permission).

was encountered by Nelson *et al* (1962) who observed marked differences in the severity of complications (especially bladder cancer) associated with urinary schistosomiasis in the Lake Victoria and coastal areas of Kenya. It was suggested that the less severe complications in the former focus were in some way related to probable frequent human exposure to the cercariae of *S. bovis*, which was not present in the coastal area.

Nelson *et al* (1962) recognised that, if true, such a phenomenon might have broad implications for the epidemiology of schistosomiasis in Africa as a whole, in view of the diversity and widespread prevalence of non-human schistosomes in many parts of the continent. They suggested that repeated human exposure to the cercariae of such non-pathogenic species was probably common in some areas and might elicit some measure of 'natural heterologous immunity' (Nelson *et al*, 1967) against *S. mansoni* and *S. haematobium*. It was proposed that the phenomenon be referred to as 'zooprophylaxis' (a term adopted from the field of medical entomology - see Footnote), initially defined as "protection against a pathogenic strain of a particular parasite by natural infections by non-pathogenic strains of the same or related parasites". This definition was subsequently refined to "the prevention or amelioration of disease in man as a result of previous exposure to heterologous infections of animal origin" (Nelson, 1974).

In an effort to obtain sound empirical evidence for the hypothesis of zooprophylaxis Nelson initiated an extensive series of experimental investigations (thoroughly reviewed by Taylor, 1991) aimed at assessing the ability of non-human schistosomes to induce protection against susequent infection with human schistosomes and *vice versa*. Studies involving *S. mansoni*, *S. haematobium*, *S. mattheei*, *S. bovis* and *S. rodhaini*, as well as the related cattle parasite *Ornithobilharzia turkestanicum* (now known as *Orientobilharzia turkestanicum*; Farley, 1971) were carried out in a variety of different hosts, including mice, rhesus monkeys, baboons,

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Footnote: Entomological Definition of Zooprophylaxis: 'the use of wild or domestic animals, which are not the reservoir hosts of a given disease, to divert the blood-seeking mosquito vectors from the human hosts of that disease' (World Health Organization, 1982).

sheep and cattle. The results of these investigations, together with those of similar studies carried out by various other workers, involving *S. japonicum* (human and zoophilic strains), some of the Indian schistosomes (*S. indicum*, *S. spindale* and *S. incognitum*), and species belonging to genera other than *Schistosoma* (specifically *Heterobilharzia americana* and *Trichobilharzia szidati*, parasites of mammalian and avian hosts, respectively), provided substantial evidence that heterologous immunity in schistosomiasis was indeed possible. Between them, the majority of heterologous immunity experiments to date are covered in the reviews by Taylor (1987) and Christensen *et al* (1987). However, it was observed that the degree of resistance, and the circumstances under which it was induced differed substantially from one host to the next. It was thus clear that extrapolation of these observations to man was inappropriate.

Subsequently the attention of those involved in this area of research shifted increasingly towards the development of homologous radiation-attenuated vaccines for use in livestock (*S. bovis* and *S. japonicum*), and the exploitation of the homologous *S. mansoni* attenuated vaccine model for the identification of important immune effector mechanisms and candidate defined antigen vaccines (Taylor and Bickle, 1986). As a result of this change in emphasis the issue of heterologous immunity as it pertains to man has remained largely neglected.

1.10 REVIEW OF THE LITERATURE PERTAINING TO *SCHISTOSOMA MARGREBOWIEI* AND *S. LEIPERI*

1.10.1 Initial Description and Distinctive Characteristics

S. margrebowiei was first described by Le Roux (1933), on the basis of specimens recovered from cattle and a variety of wild ungulates. Its distinguishing features included the similar appearance of its eggs to those of *S. japonicum* (Figure 1.4) and the large size of the adult worms. Le Roux (1955) was also responsible for first recognizing *S. leiperi* as a distinct species. It was differentiated largely on the basis of the shape and size of its eggs, which are characteristically long and thin (Figure 1.4), and had in fact been reported by Le Roux from cattle, sheep and a particularly wide range of wild herbivores from as early as 1931 (Pitchford, 1977a). However, as he subsequently acknowledged (Le Roux, 1961), he had regarded it

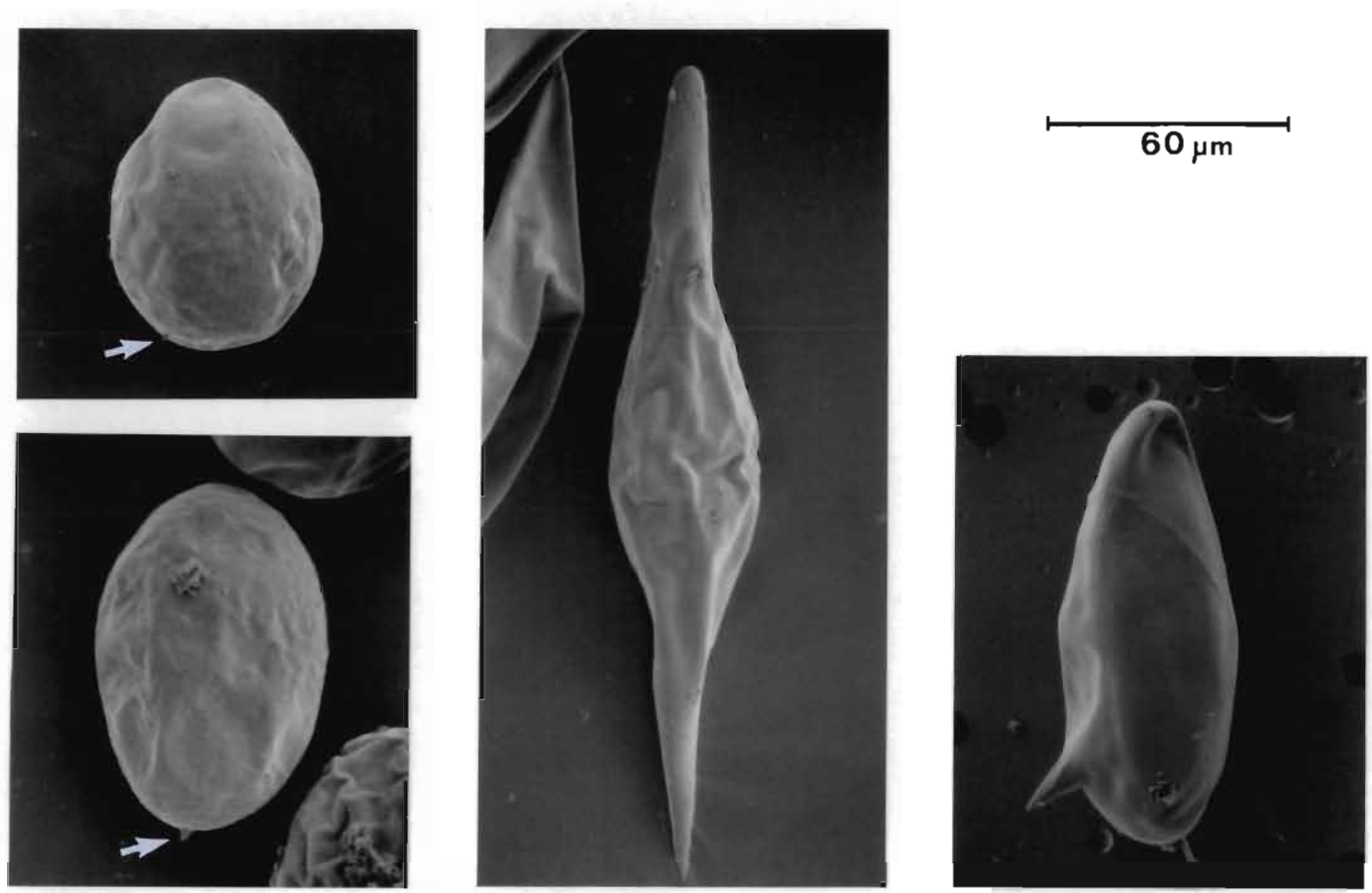


FIGURE 1.4 Morphology of schistosome ova, recovered from the livers of infected rodents, as shown by scanning electron microscopy. (A) *Schistosoma margrebowiei*: two examples are presented, representing the upper and lower extremes of size, and showing variations in the position of the spine (arrows). (B) *S. leiperi*: while the shape of this egg is typical, its length (212µm) is less than that reported by other workers (see Table 1.1). (C) *S. mansoni*: shown for comparative purposes.

prior to 1955 as *S. spindalis* (*S. spindale*) in Africa, due to the resemblance between the eggs of the two species. According to Le Roux (1933), dual infections with *S. margrebowiei* and *S. leiperi* (referred to at this time as *Bilharzia margrebowiei* and *B. spindalis*, respectively) were common, at least in what was then Northern Rhodesia (now Zambia). In most hosts the adult worms of both species were located in the porto-mesenteric veins.

The dimensions of the eggs and adult worms of *S. margrebowiei* and *S. leiperi*, as determined by various authors, are shown in Table 1.1, highlighting the contrasts between the two species. The adult worms of *S. margrebowiei* are the largest in the *S. haematobium* group, while the ova, which are oval and possess a small terminal spine (sometimes not visible), are the smallest; on the other hand, the worms of *S. leiperi* are the smallest in this group, while its eggs, which are particularly elongate, are longer than those of any other African schistosome (Loker, 1983). Furthermore, whereas the number of intra-uterine eggs of mature *S. margrebowiei* females is usually between one and two hundred (Southgate and Knowles, 1977), that of *S. leiperi* is usually between ten and twenty (Southgate *et al*, 1981).

1.10.2 Definitive Hosts and Distribution

A detailed list of all the hosts from which *S. margrebowiei* and *S. leiperi* eggs and/or worms were reported prior to 1976 was compiled by Pitchford (1977a). Before the early 1970s, information about these two species was limited largely to observations of their presence in various wild and domestic herbivores recorded by Le Roux (see Pitchford, 1977a), plus a smattering of incidental observations noted by other authors. However, commencing in 1973, Pitchford carried out a series of field studies in parts of northern Namibia, northern Botswana and western Zimbabwe, aimed specifically at obtaining additional information pertaining to the distribution and definitive host preferences of these two schistosomes.

In an attempt to determine which animal species were likely to serve as the primary definitive hosts for *S. margrebowiei* and *S. leiperi*, Pitchford considered each of the 18 game species reported by Le Roux to harbour either or both schistosomes (see Pitchford, 1977). Working from the premise that "only those non-migratory, water-loving,

TABLE 1.1 Dimensions of worms and eggs of *S. margrebowiei* and *S. leiperi* recovered from naturally- and experimentally-infected hosts as reported by various authors.

	LENGTH OF WORMS (mm)		EGG SIZES (µm)		REFERENCE AND NOTES
	Males	Females	Length x Width	HOSTS	
S. margrebowiei	12 - 18	14 - 20	60-70 x 42-45	Various natural hosts	1
	16 - 17	20 - 23	78-84 x 48-54	Antelope	2
	16.6 [23.3]	25.0 [33.8]	87(10) x 62(8)	Hamster	3
	13.5 [17.5]	25.0 [28.2]	-	Hamster	4
	13.4 [?]	20.3 [22.8]	-	Mouse	4
	13.0 [15.6]	19.0 [20.1]	-	Gerbil	4
S. leiperi	5.5 - 7.5	6.5 - 8.5	195-245 x 35-40 240-300 x 45-60	Various natural hosts	5
	7.5	9.5	258-273 x 46-59	Bushbuck	6
	11.6	14.8	-	Hamster	7

NOTES:

1 Le Roux (1933): A range of worm sizes is specified, without comment as to whether they were mature and or paired; apparently only uterine eggs were measured.

2 Graber (1978): Again only a range of worm sizes is specified, without further comment. Ova dimensions also appear to refer to uterine eggs.

3 Southgate and Knowles (1977): Mean size of fully-grown paired worms is shown, together with the maximum individual length observed (in square parentheses); egg dimensions represent means and standard deviations (in parentheses) and are based on measurement of eggs recovered from the tissues of infected hosts.

4 Ogbe MG (1985): Mean size of mature paired worms is shown, together with maximum individual length (in square parentheses); the size of the longest male worm recovered from the mouse is stated as 12.6mm, which is clearly an error. Ova dimensions are not supplied.

5 Le Roux (1955). A range of worm sizes is specified without any comment as to whether they were sexually mature and or paired. Both intra-uterine (upper line) and faecal eggs were measured.

6 Malek and Ongom (1984): It is unclear whether only single male and female worms were measured or whether the values given represent means of a number of measurements. Only intra-uterine eggs were measured.

7 Southgate *et al* (1981). Only mean worm lengths are given; maximum individual lengths were not indicated, nor were egg dimensions.

As stressed by Le Roux (1933), worm length is heavily influenced by method of specimen preparation; direct comparison of results given by different authors therefore requires caution. However, a standardised method was used in the cases of Southgate & Knowles (1977), Ogbe (1985) and Southgate *et al* (1981).

gregarious species with suitable faecal characteristics would, if susceptible, maintain a specific schistosome population indefinitely in nature if isolated from other definitive hosts harbouring the same species", he concluded that only lechwe (*Kobus leche*), puku (*K. vardoni*), waterbuck (*K. ellipsiprymnus* and *K. defassa*) and buffalo (*Syncerus caffer*) fully satisfied all the requirements (Pitchford, 1976). An additional species with highly aquatic habits, namely the sitatunga (*Tragelaphus spekei*), was excluded on the grounds that it is largely solitary and apparently quite rare. Other species, such as impala (*Aepyceros melampus*), kudu (*T. strepsiceros*) and bushbuck (*T. scriptus*) were considered to partially fulfil the requirements.

Pitchford's field studies (Pitchford, 1974 and 1976; Pitchford and Wolstenholme, 1977) indicated that prevalences of *S. margrebowiei* and *S.leiperi* infections in lechwe and puku were indeed high. In contrast, examination of a substantial number of buffalo droppings from lechwe habitats in Chobe and Eastern Caprivi revealed no evidence of schistosome infection, leading to the conclusion that they are unsuitable hosts. Prevalences in waterbuck appeared to be variable. On the basis of these observations, together with the existing information on the geographical distribution and host range of *S. margrebowiei* and *S. leiperi*, Pitchford argued that the distribution of these schistosomes is essentially linked to that of lechwe and puku. Although he considered waterbuck to be capable of maintaining these parasites independently of other hosts, he did not regard them as primary hosts, at least on the eastern side of Africa, contending that their distribution was too discontinuous and localised. His opinion presumably also reflected the fact that infections with *S. margrebowiei* and *S. leiperi* had been observed considerably less frequently in these hosts than in lechwe and puku (see Pitchford, 1977a).

Pitchford thus proposed that *S. margrebowiei* and *S.leiperi* were, for the most part, confined to a fairly restricted geographical range, including the Okavango Delta and Chobe Game Reserve in northern Botswana, Eastern Caprivi (Namibia), Zambia, southern Zaire and central southern Tanzania. However, in his first paper dealing with this subject, Pitchford (1976) made allowances for a possible role for waterbuck in areas where lechwe and puku were not present, suggesting that *K. defassa* and *K. kob* might be found to sustain transmission in parts of west and west central Africa. In support of

this he quoted the observation of an apparent human infection with *S. margrebowiei* in Mali (Lapierre and Hien, 1973). Stronger evidence, of which he only subsequently became aware (Pitchford and Wolstenholme, 1977), was in fact already in existence, indicating a transmission focus of *S. margrebowiei* in Chad involving both antelope species (Graber, 1969). No evidence of *S. leiperi* transmission in this region was found. However, more recent observations by Howard *et al* (1982) indicate that waterbuck are indeed capable of maintaining both schistosomes independently of lechwe. These authors observed transmission of both *S. margrebowiei* and *S. leiperi* by lechwe and waterbuck independently, in separate game conservation areas in Zambia. It is stated that the two populations of antelopes shared the same territory in 'former times', although the duration of their separation is not specified.

As regards all the other hosts reported to be susceptible to infection with these schistosomes, including cattle, sheep and goats, Pitchford concluded that they would probably be unable to sustain transmission on a long-term basis in the absence of lechwe or puku (Pitchford, 1976; Pitchford and Wolstenholme, 1977).

More recently, Pitchford's views on the restricted distribution of *S. leiperi* have been challenged. Firstly, Malek and Ongom (1984) have reported finding this schistosome in a bushbuck in Uganda, and secondly, *S. leiperi*-like eggs have been observed from Nile lechwe (*K. megaceros*) from Sudan (Rollinson and Southgate, 1987). While these observations certainly suggest that the distribution of *S. leiperi* is considerably wider than suggested by Pitchford, neither of them can be considered to refute his opinion that its primary definitive host range is likely to include only a small range of species, with fairly well-defined characteristics. If anything, the observation of a possible *S. leiperi* focus in an entirely separate lechwe population far to the north of the previously recognised range attests to the accuracy of the criteria which he used to decide which animals were most likely to play the dominant role in maintaining transmission. Whether or not the bushbuck is capable of maintaining *S. leiperi* independently of other hosts remains to be established.

The recent report of an apparent *S. margrebowiei* infection in a girl in Mozambique (see Section 1.10.4) suggests that the distribution of

this species may extend considerably further to the south-east than previously suspected.

1.10.3 Intermediate Hosts

Very little was known about the intermediate hosts of *S. margrebowiei* and *S. leiperi* prior to Pitchford's studies. A role for bulinid snails (Brown, 1980), at least in the transmission of *S. leiperi*, was demonstrated by Le Roux (1955), who recovered worms of this species from mice exposed to cercariae shed by wild caught *Bulinus africanus* snails. During a visit to Chobe in 1974, Pitchford demonstrated that groups of laboratory-bred rodents (*Saccostomus campestris*) subjected to partial immersion in side pools of the Chobe River contaminated with snails of the *B. africanus* group became infected with *S. leiperi*, whilst those exposed to pools contaminated with snails of the *B. tropicus/truncatus* complex (specifically *B. tropicus* and *B. depressus*) acquired *S. margrebowiei* infections (Pitchford, 1975a). While it cannot be assumed that the pools were exclusively inhabited by one or the other species only, this observation provided some evidence of a difference in the intermediate host requirement of the two schistosomes. This was substantiated by the finding that after experimental exposure to miracidia hatched from filtered lechwe droppings, laboratory-bred *B. africanus* group snails subsequently shed only *S. leiperi* cercariae, while *B. tropicus* shed only *S. margrebowiei* (Pitchford, 1975a).

Natural infections with *S. margrebowiei* were subsequently discovered in Lochinvar National Park in Zambia, firstly in *B. forskali* and *B. scalaris* (both *B. forskali* group snails) by Wright *et al* (1979), and secondly in *B. tropicus* by Southgate *et al*,1985). Southgate and Knowles (1977) demonstrated experimentally that *S. margrebowiei* is compatible with a wide range of snails belonging to the *B. tropicus/truncatus* complex, including diploid, tetraploid and octoploid varieties originating from both within and without the known distribution of this schistosome. With respect to the latter, snail populations from as far afield as Sardinia, Israel, Libya, Sudan, Cameroon and Ethiopia were shown to be susceptible. They also found it to be compatible with *B. wrighti* (*B. reticulatus* group) and weakly compatible with two species belonging to the *B. forskali* group,

namely *B. bavayi* and *B. beccarii*. However, attempts to infect various snails belonging to the *B. africanus* group were unsuccessful.

Except for the above-mentioned finding by Le Roux (1955), there have been no reports of *S. leiperi* infections in wild-caught snails. However, it appears to have a wide-ranging compatibility with snails of the *B. africanus* group, since successful infections of laboratory-bred *B. africanus* from South Africa, *B. globosus* from South Africa, Zimbabwe, Zambia, Malawi and Ghana, *B. nasutus* from Kenya, and *B. hightoni*, also from Kenya, were achieved by Southgate *et al* (1981). These authors also showed that whereas it is compatible with *B. wrighti* (*B. reticulatus* group), *S. leiperi* is apparently incompatible with snails of the *B. tropicus/truncatus* complex (9 species tested) and the *B. forskali* group (2 species tested). Circumstantial evidence for natural transmission of *S. leiperi* by *B. globosus* was presented by Howard *et al* (1982), who recovered these snails from the waterbodies of *S. leiperi*-endemic areas in Zambia.

S. margrebowiei has also been shown to be incompatible with *Biomphalaria glabrata* and *Bi. sudanica* (hosts for *S. mansoni*) as well as *Helisoma* sp., and neither *S. margrebowiei* nor *S. leiperi* are compatible with *Planorbarius metidjensis* (host for *S. bovis* in Spain) or *Indoplanorbis exustus* (host for *S. spindale* in India) (Southgate and Knowles, 1977; Southgate *et al*, 1981).

1.10.4 Infectivity of *S. margrebowiei* and *S.leiperi* in Man

Interestingly, the first ever records of *S. margrebowiei*, published even before Le Roux's first reports of infections in animals, appear to have been the discovery of *S. japonicum*-like ova in human stools. Firstly, Walkiers (1928), found viable ova in the stools of five fishermen suffering from diarrhoea in the Belgian Congo (now Zaire). On the basis of this finding he proposed the erection of a new species, designated *S. faradjei*; however, this was never recognised. Secondly, Cawston (1930) recovered a single ovum from the faeces of a man with dysentery in Durban, who had previously been resident in Zambia. It seems highly likely that the ova observed in these cases were those of *S. margrebowiei*, since there is nothing to suggest that any of the individuals involved had been exposed to *S. japonicum*, and

S. margrebowiei is the only mammalian schistosomes with *S. japonicum*-like ova in Africa.

Since these early observations there have been a number of other reports which suggest that *S. margrebowiei* is capable of occasionally producing patent infections in man. Le Roux (1961) mentions finding ova of this species in human stools in Tanzania, suggesting that this may have been the result of the ingestion of infected antelope tissues. Another case (mentioned in Section 1.10.2; Lapierre and Hien, 1973) involved the finding of what were apparently *S. margrebowiei* eggs, together with those of *S. mansoni* and *S. haematobium*, in a rectal biopsy. In 1975 Pitchford found *S. margrebowiei* eggs in 2 out of a total of 471 stool samples collected from schoolboys in East Caprivi (Pitchford, 1976). On a subsequent visit to the area he found ova in 4 out of 39 stools from schoolchildren in a village situated in an area where lechwe, with a high prevalence of *S. margrebowiei* infection, were common (Pitchford and Wolstenholme, 1977). It is possible that these ova may have been ingested, since faecal egg excretion following consumption of schistosome-infected tissues (e.g. liver; Kinoti and Mumo, 1988) is known to be possible. However, as pointed out by Pitchford and Wolstenholme (1977), if the ova had been acquired by consumption of infected lechwe organs, one would expect to have encountered *S. leiperi* eggs in at least some of the stools, due to the high prevalence of this parasite in lechwe. They thus regarded the finding of *S. margrebowiei* eggs in stools in East Caprivi as indicative of genuine infections, suggesting that man is capable of supporting either short-lived, low-grade bisexual infections, or predominantly unisexual (male) infections, as noted in cattle (see section 1.10.4).

More recently, Giboda *et al* (1988) have reported the finding of *S. margrebowiei* eggs in the stool of a Zambian student living in Czechoslovakia. Although these authors did not assess the viability of the ova (Giboda, personal communication), they were first detected some nine months after the student arrived in Czechoslovakia, clearly demonstrating the presence of a patent infection. In this article reference was also made to a report of an apparent human infection with *S. margrebowiei* in Mozambique. This was followed up by the present writer. It was established by correspondence that the case involved a 15 year old girl from Maputo, who presented in April 1985 with pain in the hepatic region and mild diarrhoea (Jurasek, personal

communication). Ova containing active miracidia and identified as those of *S. margrebowiei* were subsequently found in a stool sample. Treatment of the patient with praziquantel (Droncit) resulted in an alleviation of the symptoms and the cessation of egg excretion. It was suggested that this infection may have been acquired in an area in southern Mozambique, some thirty kilometres north of Maputo, where human and animal schistosomiasis is apparently common.

There is no evidence of patent *S. leiperi* infections in man. Buckley (1946) observed degenerate eggs of this species in a human stool in Zambia, but regarded this as a 'pseudo-infection', presumably resulting from the ingestion of infected animal tissues. There have been no further reports of this nature. A possible explanation for the absence of *S. leiperi* infections in man has been suggested by Pitchford and du Toit (1975), based on their observation that the cercariae of this species are released from infected snails at a time when the likelihood of human exposure is low, i.e. during the late afternoon and early evening. In contrast, while *S. margrebowiei* shedding also exhibits a circadian pattern, with peaks in both early morning and early evening, cercariae are also released in smaller numbers throughout the day, thereby increasing the opportunity for human exposure (see section 1.10.6).

1.10.5 Interactions Between *S. margrebowiei*, *S.leiperi* and Other Schistosome Species

During a preliminary survey of schistosome infections in Kafue lechwe (*K. leche kafuensis*) in Lochinvar National Park, Zambia, Wright *et al* (1979) were able to make species identifications on the worms recovered from three young animals (not more than one year old) and three mature adults (five or more years old). The young animals proved to be exclusively infected with *S. margrebowiei*, whereas the older group harboured only *S. leiperi* worms. In a subsequent survey, involving a larger sample of animals, this trend was convincingly confirmed (Howard *et al*, 1981). Kafue lechwe of up to one year old (8 animals) were again found to carry only *S. margrebowiei* infections. Thereafter a distinct age-related inversion in the proportions of the two species was noted, with *S. leiperi* worms comprising 5% of the total worms recovered from 1-2 year old animals (4 animals), 50% of those from 2-3 year olds (2 animals), 73% of

those from 3-4 year olds (6 animals), and 100% of those from animals older than four years (7 animals).

Wright *et al* (1979) suggested that the occurrence of pure *S. margrebowiei* infections in young lechwe might be related to the migration of the lechwe population in response to the annual flood cycle, i.e. during the months immediately after calving they are restricted to an area in which only the snail hosts of *S. margrebowiei* are present. However, as these authors acknowledge, migration back into areas favourable for *S. leiperi* transmission must presumably occur before the juveniles are a year old, and there is still a need to explain the relatively slow establishment of infections with this species. Furthermore, migration-linked changes in exposure patterns are insufficient to explain the complete disappearance of *S. margrebowiei* from older animals. It was thus speculated that the age related changes in the prevalences of the two schistosomes in lechwe might be due, at least in part, to some form of immunologically-mediated competition between them.

The survey by Howard *et al* (1982) also included a study of schistosome infections in waterbuck from a separate area of Zambia, where lechwe were not present. While the sample comprised only eight mature animals, there was no evidence of any obvious trends, with some animals harbouring pure infections of either *S. margrebowiei* or *S. leiperi* and others harbouring mixed infections comprising both of these species together with *S. mattheei* and/or an as yet unidentified schistosome (possibly a hybrid).

In the course of his aforementioned investigations on the antelope schistosomes (section 1.10.2), Pitchford (1976) failed to find any evidence of *S. mattheei* infection in a total of 281 cattle dropppings sampled from numerous localities in Eastern Caprivi. There was also no evidence of this parasite in areas directly west of Eastern Caprivi (Kavango and Ovamboland), either in cattle or wildebeest (the latter animals were included because of their recognised ability to maintain *S. mattheei*). However, he did find schistosome worms, which he considered to be those of *S. margrebowiei* and *S. leiperi*, in the mesenteric veins of slaughtered cattle coming from the south of Eastern Caprivi. Virtually all of these worms were observed to be males, supporting his view that cattle are poor hosts of these schistosomes. The absence of *S. mattheei* in the above areas could not

be explained by the absence of suitable host snails. It was thus postulated that this parasite, which is common in cattle and game in the region to the east and south-east of the above area, was prevented from spreading in a westerly direction by the presence of the antelope schistosomes, possibly through the mechanism of heterologous immune protection (Pitchford, 1976).

Pitchford (1976) also examined human urines and stools from various parts of Eastern Caprivi and found that the prevalences of *S. mansoni* or *S. haematobium* infections were high in those areas where no lechwe were present, low in areas where they occurred in small numbers, and negligible in areas where they were plentiful. In a subsequent study in this area (Pitchford and Wolstenholme, 1977) excreta were obtained from children in four schools, selected according to their schistosomiasis status and their proximity to lechwe populations. Two of these schools were situated in an area devoid of lechwe and were shown to be highly endemic for human schistosomiasis, one for *S. mansoni* the other for *S. haematobium*. In contrast, the other two schools, which were in areas frequented by substantial numbers of lechwe, were found to be almost free of human schistosomiasis, with only 3% of children excreting *S. haematobium* ova and 6% excreteing *S. mansoni* ova. Of the remainder (4 of whom, all from the same village, were excreting *S. margrebowiei* eggs, as noted in section 1.10.3) 68% were found to have positive complement fixation (CFT) and/or intradermal skin (ID) tests. After considering various possible explanations, it was concluded that this phenomenon was most likely due to immunological stimulation resulting from exposure to the cercariae of *S. margrebowiei* and/or *S. leiperi*.

Pitchford and Wolstenholme (1977) also presented data showing an increase in the prevalence of *S. mansoni* infections in schoolchildren in Maun, a town situated at the south western periphery of the Okavango Delta, from 0% in 1956 to 60-70% in 1976. It was noted that during this twenty year period the human and domestic stock population had increased greatly and there had been a corresponding withdrawal of lechwe and other *Kobus* sp. into the deeper reaches of the swamp.

On the basis of all of the above evidence it was suggested (Pitchford, 1975b and 1976; Pitchford and Wolstenholme, 1977) that the absence of *S. mansoni* and *S. haematobium* in man in those areas

where lechwe are plentiful was a consequence of an immunologically-mediated protection induced by repeated contact with the cercariae of *S. margrebowiei* and *S. leiperi*. It is this postulate which provides the *raison d'etre* for the studies described in the subsequent chapters of the present dissertation.

1.10.6 Summary of Laboratory-based Studies on the Antelope Schistosomes

Laboratory life-cycles of *S. margrebowiei* and *S. leiperi*, both originating from the Chobe Game Reserve were first established in 1974 by Pitchford (1975a). The former species was passaged through *Saccostomus campestris* rodents and *Bulinus tropicus* snails (an albino strain), and the latter through *Mastomys coucha* (the multimammate mouse) and snails of the *B. africanus* group. Shortly thereafter, snails infected with each species were sent to the British Museum (Natural History) (BMNH) in London, where the hamster was subsequently used as the definitive host for both species, and *B. natalensis* (a snail of the *B. tropicus/truncatus* complex) as the intermediate host for *S. margrebowiei* (Southgate and Knowles, 1977; Southgate *et al*, 1981). Isolates of both species originating from the Lochinvar National Park in Zambia were also introduced into the laboratories of the BMNH in 1978 (Southgate *et al*, 1981; Southgate, personal comunication).

A variety of experimental or laboratory-based studies involving *S. margrebowiei* and *S. leiperi* have been carried out. For convenience these are summarised below under a number of sub-headings. It is notable that studies involving *S. margrebowiei* comprise by far the major portion of the investigations described to date.

Egg and Miracidium: In addition to measuring, photographing and describing the ova of *S. margrebowiei*, Southgate and Knowles (1977) carried out a detailed descriptive study of the miracidium, demonstrating similarities between this species and *S. haematobium* in terms of the epidermal cell plate formula and the distribution of sensory receptors.

Survival and Infectivity of Miracidia: Evans (1985) found that the survival of *S. margrebowiei* miracidia remained high for about six

hours after hatching, whereafter it declined rapidly, being reduced to 50% by nine and a half hours and zero after thirteen hours. Infectivity in *Bulinus natalensis* snails began to decline at some stage between four and six hours after hatching; eleven hour old miracidia produced an infection rate approximately one third that of fresh miracidia, whilst twelve hour old miracidia failed to produce viable infections.

Compatibility with Intermediate Hosts: Researchers at the BMNH tested the compatibility of *S. margrebowiei* and *S. leiperi* with a wide range of snail species, mostly those belonging to the genus *Bulinus* (Southgate and Knowles, 1977; Southgate *et al*, 1981); the results of these investigations are summarised in section 1.10.3. In general, the prepatent period of *S. margrebowiei* in the intermediate host appears to be considerably shorter than that of *S. leiperi*, although the minimum prepatent periods of the two species in the various hosts tested were observed to range between 19 and 39 days, and 29 and 40 days, respectively.

Cercarial Shedding and Behaviour: Pitchford and du Toit (1976) reported that the Chobe isolate of *S. margrebowiei* exhibited two well-defined peaks of cercarial shedding from *B. tropicus* snails (albino strain) under outdoor conditions, *viz* during the early morning and late afternoon. It was noted, furthermore, that cercariae were released in low numbers virtually continuously between the periods of peak emergence. More recently, Raymond and Probert (1991), have shown that the shedding characteristics of the Zambian isolate of this parasite, passaged through *B. natalensis* snails, conform to this pattern. They showed also that the ultradian rhythm persists during a period of 24 hour illumination, and that deviations of the light:dark cycle from a standard 12:12 hour schedule exert no significant influence on the timing of the emission peaks. Whether or not a prolonged period of constant illumination would result in the abolition of the rhythm remains to be seen.

With respect to *S. leiperi*, it was reported by Pitchford and du Toit (1976) that shedding from *B. africanus* occurred predominantly during the late afternoon and early evening. However, there is apparently a need for further investigations on this species, since Visser (personal communication), who was responsible for the routine maintenance of schistosome life-cycles in Pitchford's laboratory over

a period of many years, consistently found that large numbers of cercariae were released during the early morning. This has also been observed by the present author. Neither Visser nor the present author have assessed the evening shedding capacity of *S. leiperi*.

Notwithstanding the uncertainties regarding whether *S. leiperi* expresses one or two daily emission peaks, the merits of dawn and dusk shedding patterns are easily appreciated in the case of the antelope schistosomes, since these are the times when the definitive hosts typically frequent watering places.

Southgate and Knowles (1977) observed that the cercariae of *S. margrebowiei* respond to small elevations in temperature by discharging the contents of their post-acetabular glands. This causes them to adhere to one another, resulting in the formation of aggregates. Since the discharge of the post-acetabular glands vitiates the ability of cercariae to penetrate the definitive host, it was proposed that the geographic distribution of *S. margrebowiei* might be restricted by the need for specific climatic conditions, favourable to the retention of cercarial penetrative ability.

Infection Characteristics in Definitive Hosts: The growth and maturation in hamsters of *S. margrebowiei* (Chobe isolate), over the period 30-60 days of infection, and of *S. leiperi* (Chobe isolate), over the period 40-100 days of infection, was studied by Southgate and Knowles (1977) and by Southgate *et al* (1981), respectively. *S. margrebowiei* worm returns (i.e. the percentage of cercariae reaching the liver) and worm maturation rates were, respectively, considerably higher and more rapid than those of *S. leiperi*. The two species displayed converse tissue egg deposition patterns, with the majority of ova being deposited in the intestines in the case of *S. margrebowiei*, and in the liver in the case of *S. leiperi*. A high mortality rate was encountered in the hamsters exposed to *S. margrebowiei*. In this regard, it is worth noting that Pitchford (1975a) reported high mortality rates in *S. margrebowiei*-infected *S. campestris* and *M. coucha*.

Ogbe (1983 and 1985) studied the post-cercarial development of *S.margrebowiei* (Chobe isolate; Southgate, personal communication) over the period 40-100 days in three different laboratory rodents, namely hamsters, mice and gerbils. Similar rates of schistosomulum

migration were observed in all three hosts, with maximum lung schistosomulum recoveries being obtained on the sixth day of infection in each case. Worms were found to mature at a similar rate to that reported by Southgate and Knowles (1977). However, it was concluded (Ogbe, 1985) that gerbils are unsuitable as hosts for passage of *S. margrebowiei*, firstly because they suffer high mortalities, and secondly because percentage worm maturation and egg viability in these animals is poor in comparison to that in hamsters and mice. In contrast, this author considered the mouse to be highly compatible with this schistosome and particularly suitable for relatively long-term studies on the host-parasite relationship.

In view of its comparatively rapid rate of *in vivo* development, it was suggested that *S. margrebowiei* might prove more amenable to *in vitro* cultivation than species with a longer maturation phase (Southgate and Knowles, 1977). An attempt to this end by Ogbe (1983) met with little success, with schistosomula developing far more slowly than in the *in vivo* situation and not progressing beyond the so-called 'closed gut' stage.

Where appropriate, aspects of the above-mentioned studies will be discussed in more detail in Chapter 2.

Biochemistry: Enzyme analysis of worms recovered from lechwe and waterbuck in Zambia, by means of isoelectric focusing, revealed that *S. margrebowiei* and *S. leiperi* can be differentiated by alleles at the phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PDH) loci (Rollinson and Ross, 1983). Southgate *et al* (1981) compared the migration patterns of six different enzymes of the Chobe and Zambian isolates of *S. leiperi* and found only minor differences. However, comparison of individual *S. leiperi* worms recovered from two Zambian lechwe revealed polymorphisms in two out of seven enzymes tested (i.e. G6PDH and malate dehydrogenase); on the basis of this analysis it was established that the animals had been exposed to cercariae from at least eleven genetically-distinct sporocysts (Rollinson and Southgate, 1985).

Hybridisation of *S. margrebowiei* DNA extracted from adult worms with ribosomal RNA (rRNA) gene probes prepared from *S. mansoni* revealed that *S. margrebowiei* is characterised by the presence of variable numbers of a 0.4 kb insert in the non-transcribed spacer region of

the gene unit and by a 0.1 kb insert in the transcribed spacer (Walker *et al*, 1986). Comparison of different life-cycle stages of *S. margrebowiei* by the same method showed no stage-related differences (Rollinson *et al*, 1990). Hamburger *et al* (1991) used *S. margrebowiei* DNA to assist in the selection of a S. mansoni-specific probe.

Both *S. margrebowiei* and *S. leiperi* have been shown to possess chromosome complements of 2n=16 (Southgate and Knowles, 1977; Southgate *et al*, 1981).

Electron Microscopy: A number of descriptive scanning electron microscopy studies have been carried out on both of the antelope schistosomes, although, as with most other types of laboratory investigation, *S. margrebowiei* has received by far the majority of attention. Interest has focused in particular on the role of tubercular spines on the teguments of male worms and whether or not they are useful for species identification purposes. However, as the following discussion reveals, the role of spines remains enigmatic, and there is, at this stage little to support the notion that they are likely to aid in the differentiation of the antelope schistosomes from other species.

With respect to *S. margrebowiei*, Ogbe (1982) found the teguments of adult male worms to be devoid of tubercular spines, Evers *et al* (1983) observed vestigial spines on a small proportion of tubercules, and Probert and Awad (1987) noted a range, even on individual worms, from heavily spined to aspinous tubercles. The last mentioned authors suggested that the degree of spination is related to the age of the worms and whether or not they are *in copula* and, as a corollary, that spines may play a role in enabling female-bearing males to retain their positions in blood vessels. However, this suggestion appears less tenable in the light of observations made by Kruger *et al* (1988), who found that whereas sexually-mature worms recovered from lechwe in Eastern Caprivi were completely aspinous, those of the Chobe isolate maintained in *Saccostomus campestris* displayed numerous spines. These authors proposed that the presence or absence of spines is instead subject to phenotypic plasticity, being dependent on factors such as the diameter of the blood vessels in which they are resident, the parasite density level, and the nature of the immunological responses to which they are subjected. In regard to the

latter, Kruger and Wolmarans (1990) found there to be few leucocytes on the surfaces of *S. margrebowiei* worms recovered from lechwe.

As regards *S. leiperi*, only two studies have been reported. Southgate *et al* (1981) observed considerable individual variation in the degree of tubercular spination of male worms, while Evers *et al* (1983) noted a predominance of aspinous tubercles, except on the posterior regions of the tegument.

In view of its rapid development in laboratory rodents, *S. margrebowiei* has attracted some interest as a model for research on schistosomes belonging to the *S. haematobium* group. Detailed scanning and transmission electron microscopy studies have been carried out on the tegumental structures (Probert and Awad, 1987) and the reproductive systems of adult male and female worms (Awad and Probert, 1989 and 1990), as a prelude to studies on the effects of drug treatment. More recently these authors have also described the severe damage caused by praziquantel to the tegumental and sub-tegumental tissues of male and female worms; in this study evidence was also obtained of rapidly occurring damage to the female reproductive system (Awad and Probert, 1991).

1.11 AIM OF THE PRESENT STUDY

As noted by Christensen *et al* (1987), there is a need for experimental investigations of the hypothesis put forward by Pitchford that prior exposure of non- or poorly-susceptible definitive hosts to infection by *S. margrebowiei* and/or *S. leiperi* may inhibit the subsequent development of *S. mansoni*. The aim of the studies described in the following chapters of this thesis was thus to develop animal models in which to carry out such investigations.

CHAPTER TWO

THE INFECTION CHARACTERISTICS OF *SCHISTOSOMA MARGREBOWIEI* AND *S. LEIPERI* IN INBRED BALB/C MICE AND IN *MASTOMYS COUCHA*.

2.1 INTRODUCTION

The experimental studies reported in this and the following chapters were preceded by a series of experiments in *Mastomys coucha*, aimed at assessing the degree of heterologous resistance induced by prior infection with the antelope schistosomes against subsequent challenge with *S. mattheei*. The choice of this host seemed warranted, since Jackson (1980), working in the same laboratory and with the same colony of animals, had previously demonstrated the development of resistance in homologous immunity experiments with *S. mattheei*. Furthermore, Pitchford (1976) claimed to have obtained preliminary evidence indicating that "developed *S. margrebowiei* greatly reduces the development of *S. mansoni* in *Mastomys*". However, attempts by the present author to induce resistance against *S. mattheei* in *M. coucha* by prior infection with either *S. leiperi* or *S. margrebowiei* were consistently unsuccessful, leading to the conclusion that the available colony of animals was, in fact, unsuitable for studies of this nature (Dettman and Higgins-Opitz, 1989), and that an alternative host should be sought.

As an alternative small rodent model the inbred BALB/c mouse was selected. This selection was based on a combination of factors: firstly, these animals are genetically consistent, well-characterised and extensively used in biomedical research (Festing, 1979); secondly, they have been used by other workers for studies on immunity to schistosomes (Dean, 1983; Garcia *et al*, 1984); and thirdly, at the time when these studies were being initiated very few inbred mouse strains were available in South Africa, and, of these, BALB/c were by far the most readily available and proved the easiest to breed in large numbers. Of the other inbred mouse strains commonly used in studies on schistosomiasis (Dean, 1983), only C57Bl/6 mice were available. A small colony of these animals was obtained for breeding purposes but failed to thrive, and it was thus decided to focus efforts on the BALB/c model.

A series of experiments was subsequently carried out in BALB/c mice in which it was shown that primary infections with *S. margrebowiei* or *S. leiperi* resulted in substantial reductions in the establishment of *S. mattheei* or *S. mansoni* challenge infections (unpublished data). However, these studies were somewhat unsatisfactory, largely due to the fact that the mortality rates in the groups of animals subjected to the initial infections were unacceptably high. As a result it was decided to adopt a more systematic approach, starting with a detailed assessment of the infection characteristics of the antelope schistosomes in BALB/c mice.

As mentioned in Section 1.10.6 the only previous reports on the development of *S. margrebowiei* in laboratory rodents are those of Southgate and Knowles (1977), who used hamsters, and Ogbe (1983 and 1985), who used hamsters, mice of the TF1 strain, and gerbils. With respect to *S. leiperi*, only one report exists, namely that of Southgate *et al* (1981), involving hamsters. While these studies give some indications as to the expected worm returns and rates of maturation of these parasites in rodents, and of their preferred sites of tissue egg deposition, they were not considered to provide sufficient information to facilitate the design of the studies envisaged by the present author. More importantly, as the study by Ogbe (1985) demonstrated, the infectivity, fecundity and effects of an individual schistosome isolate may differ from host to host. It was thus considered essential to obtain data pertaining specifically to the behaviour of *S. margrebowiei* and *S. leiperi* in BALB/c mice. The results of studies aimed at obtaining such data are presented in this chapter.

It was subsequently decided to carry out additional studies of infection characteristics in *M. coucha*, primarily for comparative purposes. The results of these studies are also presented in this chapter. Preliminary observations (Dettman and Higgins-Opitz, 1989, and unpublished results) had indicated that while *S. leiperi* appeared to produce successful infections in both *M. coucha* and BALB/c mice, *S. margrebowiei* infections in *M. coucha* were poor in comparison to those in BALB/c mice. These observations contrasted with the results of studies on two strains of *S. mansoni*, originating from South Africa and Puerto Rico, respectively (Higgins-Opitz and Dettman, 1991). In this instance, it was found that although the strains differed markedly from each other, their individual infection

characteristics remained remarkably consistent in BALB/c mice and *M. coucha*, in spite of the considerable differences in the responses of these two hosts to schistosome infections. It was thus considered worthwhile to assess the degree to which the infection characteristics of *S. margrebowiei* and *S. leiperi* were influenced by the type of definitive host used.

2.2 MATERIALS AND METHODS

2.2.1 Source and Routine Passage of Schistosomes

The Chobe isolates of *S. margrebowiei* and *S. leiperi*, initially introduced into the laboratory by Pitchford (1975) were used throughout the studies described in this dissertation. *Mastomys coucha* infected with *S. leiperi* and *Saccostomus campestris* infected with *S. margrebowiei* were obtained from the former Bilharzia Field Research Unit in Nelspruit (BFRU; now the Eastern Transvaal Branch of the South African Medical Research Council) and used as a source of miracidia for the establishment of life-cycles. *S. leiperi* was subsequently passaged through *M. coucha* and *Bulinus africanus* or *B. globosus* snails, and *S. margrebowiei* through BALB/c mice and an albino strain of *B. tropicus*.

In general, the methods of handling the schistosomes and their snail hosts were based on the principles established by earlier workers (see reviews by Webbe and James, 1971, and Lewis *et al*, 1986). Details of the specific methods used in the present study are presented in Appendix A.

2.2.2 Sources and Maintenance of Rodents

Rodents were bred in the animal facility of the Research Institute for Diseases in a Tropical Environment (RIDTE; now the Natal Branch of the South African Medical Research Council). The inbred BALB/c mouse colony was established from stocks obtained in 1982 from the Natal Institute of Immunology in Pinetown. The origins and physical characteristics of the *M. coucha* have been described elsewhere (Higgins-Opitz *et al*, 1987; Dettman *et al*, 1987).

Only young adult male animals were used for experimental purposes. For individual experiments, groups of animals were age-matched to within one week of each other; however, the average ages of the animals (at the time of infection) in the different experiments ranged between 8 and 14 weeks.

The rodents were housed under conventional conditions in polypropylene cages (dimensions 445 x 280 x 125mm; Labotec, Johannesburg) on woodshavings. Tap water and commercially-produced rat pellets (Epol, Pietermaritzburg) were provided *ad libitum*. The animal rooms (dimensions 5.7 x 3.0 x 2.6M), which were fitted with wall-mounted shelves, were maintained under positive pressure at a temperature of approximately 23 C and a relative humidity of 50-60%, and were illuminated by a single 65 watt fluoresecent tube. They received 10-13 air changes per hour and were subjected to a 12/12 hour light/dark cycle.

2.2.3 Infection of Rodents

Animals were infected percutaneously using the paddling method of Dettman *et al* (1989). Briefly, this entailed subjecting them to a 4-step pre-soaking procedure, then placing them for 40-45 minutes into individual 600ml plastic beakers containing conditioned water (80ml and 100ml/beaker for mice and *M. coucha*, respectively) and the required number of cercariae (Figure 2.1). Details pertaining to the methods of harvesting cercariae and of estimating cercarial loads are provided in Appendix A. All infections were carried out in a controlled temperature laboratory with an ambient temperature of 26 C. In most instances cercariae were less than 4.5 hours old at the time of animal exposure.

Following infection the health status of the animals was monitored by a qualified laboratory animal technologist who ensured that animals showing signs of infection-related distress were promptly euthanased.

2.2.4 Measurement of Faecal Egg Excretion

Faecal pellets passed by groups of animals over a 24-hour period immediately prior to worm recovery were pooled, fixed for 24 hours

FIGURE 2.1 Rodents in infection vessels.

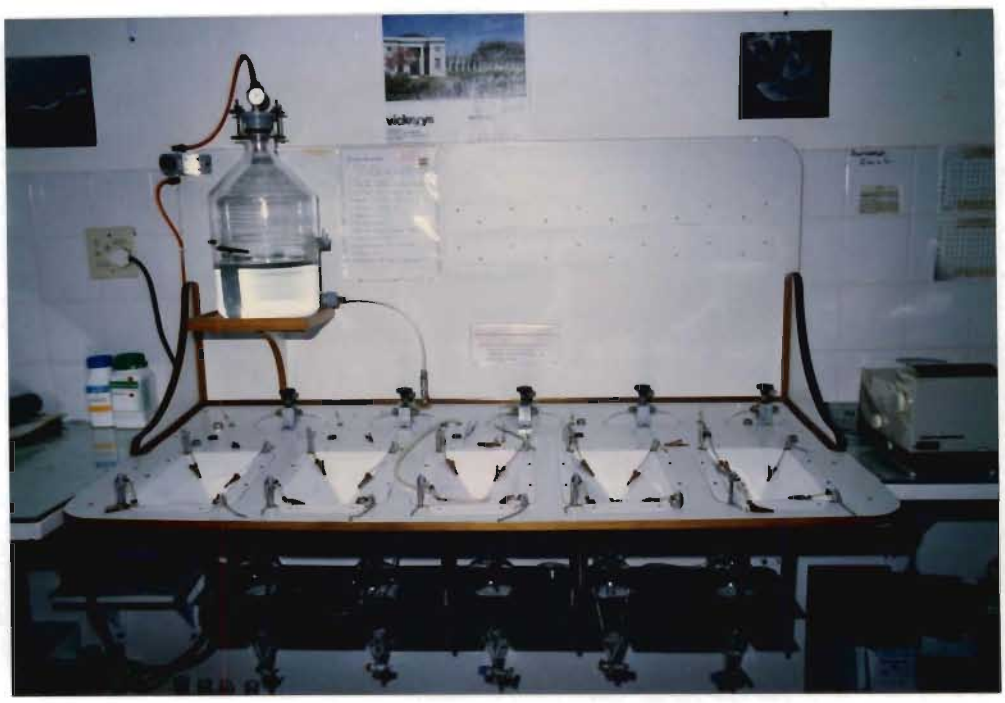


FIGURE 2.2 Perfusion apparatus used for the recovery of adult schistosome worms from mice and *Mastomys coucha*. This apparatus differs slightly from that described by Jackson *et al* (1982) in that worms are harvested under gravity rather than by suction. Tray dimensions (length x width x vertical depth) are 270 x 160 x 75mm.

in 25ml of 10% formalin (v/v), then homogenised by blending for about 1 minute in a Waring blender. The number of ova in a measured volume of the faecal suspension was determined by means of the formol-ether concentration technique of Ritchie (1948).

2.2.5 Anaesthesia of Animals

Rapid anaesthesia was induced by intra-peritoneal injection of pentobarbitone sodium at a dose of 0.20-0.25mg/g body mass. Specifically, BALB/c mice received 1ml and *M.coucha* 2ml of a 1-in-10 solution (v/v) in distilled water of Sagatal (Maybaker (S.A.) (Pty) Ltd, South Africa; containing 60mg/ml of pentobarbitone sodium). Tri-sodium citrate was added to the anaesthetic solution, at a concentration of 0.75% (mass/vol), to prevent the formation of blood clots in the occasional event of internal bleeding caused by the injection needle. The plane of anaesthesia in *M.coucha* was evaluated by placing them on a metal surface and assessing their responses to the sound and vibration produced by sharply tapping the surface with a metal object. This was found to be a more sensitive indicator than toe-pinching, which was satisfactory for mice.

2.2.6 Recovery and Counting of Schistosome Worms

Care was taken to ensure that worm recovery procedures were initiated either before the death of the animal due to the anaesthetic, or shortly thereafter, i.e. before intravascular clotting could occur. Worms were recovered by means of the perfusion method of Jackson *et al* (1982), using the apparatus shown in Figure 2.2. Briefly, this entailed dissection of the animal to expose the heart and portal vein and expulsion of the worms by the introduction of perfusion fluid (comprising tap water containing 0.85% sodium chloride (mass/vol), 0.75% tri-sodium citrate (mass/vol) and 1g/L of pentobarbitone sodium) into the left ventricle of the heart. The worm-bearing perfusate was treated with saponin, at an approximate concentration of 0.05-0.1mg/ml, in order to lyse the red blood cells, and a few drops of an anti-foaming agent (Antifoam A Emulsion; Sigma Chemical Co.,St Louis, USA).

Worms were examined and counted with the aid of a dissection microscope. They were subjectively classified as adult or juvenile on the basis of their stage of development and/or their size. Worms which were obviously smaller than their adult counterparts were regarded as juveniles, taking into account the fact that unpaired females (and, to a lesser extent, unpaired males) tend to be noticeably smaller than paired ones (Malek, 1980). Adult female worms were classified as gravid or non-gravid on the basis of obvious evidence of vitelline gland development and/or the presence of eggs *in utero*. For each individual animal, the number of potential worm pairs was defined as the maximum number of monogamous pairs that could be formed from the available adult male and female worms. The number of gravid worm pairs was taken as the number of sexually mature female worms, whether *in copula* or not. On rare occasions the number of gravid females was observed to be one greater than the number of males; in such instances it was assumed that a male worm had remained lodged within the tissues, or that it had been lost during the recovery process. Total worm burdens were expressed both in real terms and as percentages of the estimated numbers of cercariae to which the animals were exposed (i.e. percentage worm recovery).

2.2.7 Determination of Tissue Egg Burdens

On completion of the perfusion process, livers, spleens and intestines were dissected out and weighed, using a fine balance, following which they were digested in 4% potassium hydroxide solution (mass/vol) (2.5ml/g tissue) at 37 C for 16 hours (Cheever, 1968). Intestines were digested without first removing their contents and, in some instances, they were subdivided into three portions, namely small intestine, caecum and large intestine, for independent egg load assessment. In the cases of livers and intestines, digests were diluted with water prior to removal of samples for counting. Diluted digests were mixed by means of a magnetic stirrer and eggs were counted in duplicate 0.5 or 1ml aliquots, with the aid of counting chambers (Appendix B) and a stereo microscope. Sample volumes and the degree of digest dilution was varied in accordance with the density of eggs in suspension, with an attempt being made to ensure that individual egg counts were, on average, not more than 200. If the variation between individual counts exceeded 10%, a further two

samples were taken. Whenever possible, all the eggs in the spleen digests were counted, with dilution and sampling being carried out only when necessitated by high egg densities. In all cases where dilution and sampling was carried out, the total volume of the diluted digest was measured so that the total number of eggs in the digest could be calculated. Tissue egg burdens were expressed in terms of eggs per gravid worm pair.

2.2.8 Statistical Methods

In view of the need to correct for time, results of the various experiments were compared by means of the one-way analysis of covariance (Snedecor and Cochran, 1989). Relationship analysis on the tissue egg accumulation data was done using simple linear regression (Montgomery, 1984), with Pearson's correlation coefficient (Cangelosi *et al*, 1983) being used as a measure of the strength of relationships. For each host-parasite combination the time of onset of tissue egg deposition was estimated from the regression equations pertaining to liver eggs, intestinal eggs and total tissue eggs (liver + intestines), respectively, by solving for y = 0.

2.3 RESULTS

Data pertaining to the infection characteristics of *S. margrebowiei* and *S. leiperi* in BALB/c mice were obtained, in each instance, from two separate experiments, referred to as the 'Initial' and 'Repeat' studies. Data pertaining to *M. coucha* were obtained from studies carried out in parallel with the repeat mouse studies, animals being infected simultaneously, with cercariae from the same pool.

Details, for each study, of the estimated cercarial infection loads and of the intervals after exposure at which worms and tissues were recovered are summarised as follows:

S. margrebowiei

BALB/c Initial Study: 36 cercariae/animal; perfusions at 4, 5, 6, 7, 8, 9, 10, 12 & 20 weeks.

BALB/c Repeat Study: 32 cercariae/animal; perfusions at 6, 8, 12 & 20 weeks.

M. coucha Study: 61 cercariae/animal; perfusions at 6, 8, 12 & 20 weeks.

S. leiperi

BALB/c Initial Study: 45 cercariae/animal; perfusions at 5, 6, 7, 8, 9, 10, 12 & 22 weeks.

BALB/c Repeat Study: 40 cercariae/animal; perfusions at 8 & 22 weeks only.

M. coucha Study: 55 cercariae/animal; perfusions at 8, 10, 14 & 22 weeks.

2.3.1 Infectivity of Cercariae and Maturation of Worms

In the initial studies with BALB/c mice (Table 2.1) the percentage worm recoveries of *S. margrebowiei* were found to be slightly, but significantly ($P < 0.05$) higher than those of *S. leiperi*, and there was a substantial drop-off in worm recovery of the latter species between 12 and 22 weeks. A distinct difference between the two schistosomes was also noted in terms of the rates at which the worms grew, with the majority of *S. margrebowiei* worms having reached adulthood some two weeks earlier than those of *S. leiperi* (i.e. 5-6 weeks versus 7-8 weeks, respectively). The ratio of male:female worms was seen to be biased in favour of males in both cases, but particularly in the case of *S. leiperi*, where females constituted only about one-third of the total number of adult worms.

In the subsequent study with *S. margrebowiei* (Table 2.2) the percentage worm recoveries from BALB/c mice were found to be significantly lower ($P < 0.001$) than those of the first study, and there was evidence of a drop-off in worm numbers between 12 and 20 weeks of infection. Sex ratios again appeared to be biased in favour of male worms, although to a lesser extent than in the initial study. The development of *S. margrebowiei* in *M. coucha* was initially very

TABLE 2.1 Worm recoveries of *Schistosoma margrebowiei* (MB) and *S. leiperi* (LP) from BALB/c mice (initial studies).

		Weeks of Infection[a]									
		4	5	6	7	8	9	10	12	20	22
		$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)
% Worm recovery[b]	MB	35.7 (9.6)	34.1 (9.2)	42.1 (14.0)	39.3 (12.3)	37.3 (3.6)	33.7 (10.6)	30.5 (8.2)	33.3 (9.5)	28.1 (5.1)	ND
	LP	ND	21.9 (9.6)	31.1 (7.0)	32.7 (8.2)	32.4 (11.7)	31.7 (10.5)	29.9 (7.9)	32.4 (8.1)	ND	13.0 (7.1)
Adult male worms	MB	5.6 (2.8)	6.1 (2.1)	8.4 (5.8)	7.0 (3.7)	6.0 (1.4)	6.9 (3.0)	6.0 (3.7)	7.5 (2.5)	5.9 (1.8)	ND
	LP	ND	0.4 (0.8)	7.1 (3.0)	9.3 (2.9)	9.4 (3.3)	9.9 (2.1)	8.9 (3.8)	9.6 (2.5)	ND	3.0 (1.4)
Adult female worms	MB	4.7 (1.7)	4.0 (1.6)	5.1 (2.0)	5.7 (2.5)	5.6 (0.5)	4.7 (2.1)	3.4 (1.1)	4.5 (2.2)	4.2 (1.6)	ND
	LP	ND	0	2.1 (1.8)	4.3 (2.4)	3.4 (2.3)	4.3 (3.4)	4.3 (1.4)	5.0 (3.2)	ND	2.8 (1.8)
Juvenile worms[c]	MB	3.1 (2.3)	2.1 (2.6)	1.6 (2.2)	1.4 (2.6)	1.9 (1.1)	0.4 (1.1)	1.6 (2.3)	0	0	ND
	LP	ND	9.4 (3.9)	4.7 (1.6)	1.1 (0.9)	1.7 (2.1)	0.1 (0.4)	0.3 (0.5)	0	ND	0
Total worms	MB	12.9 (3.4)	12.3 (3.3)	15.1 (5.0)	14.1 (4.4)	13.4 (1.3)	12.1 (3.8)	11.0 (2.9)	12.0 (3.4)	10.1 (1.8)	ND
	LP	ND	9.9 (4.3)	14.0 (3.2)	14.7 (3.7)	14.6 (5.3)	14.3 (4.7)	13.4 (3.6)	14.6 (3.7)	ND	5.8 (3.2)
Juvenile/total (%)	MB	24%	17%	11%	10%	14%	3%	15%	0	0	ND
	LP	ND	95%	34%	7%	12%	1%	2%	0	ND	0

Means ($\bar{x}$) and standard deviations (s.d.) are shown; ND represents 'not done'.

a 7 animals from each group were studied at the various time intervals, except in the cases of the 12 and 20 week MB groups and the 22 week LP group, which consisted of 8, 9 and 6 animals, respectively.

b Worm recoveries of the MB group are significantly different from those of the LP group ($P < 0.05$; analysis of covariance, using data from the 5 to 12 week study intervals only).

c See text (Section 2.2.6) for definition of juvenile worms.

TABLE 2.2 Worm recoveries of *Schistosoma margrebowiei* from BALB/c mice (initial and repeat studies) and *M. coucha*[a].

		Weeks of Infection[b]			
		6	8	12	20
		$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)
% Worm recovery[c]	B1	42.1 (14.0)	37.3 (3.6)	33.3 (9.5)	28.1 (5.1)
	B2	24.1 (9.8)	29.9 (6.5)	27.2 (8.6)	18.1 (7.9)
	Mc	42.2 (12.5)	21.3 (8.9)	16.4 (16.8)	12.0 (8.9)
Adult male worms	B1	8.4 (5.8)	6.0 (1.4)	7.5 (2.5)	5.9 (1.8)
	B2	4.0 (2.6)	5.7 (1.5)	4.1 (1.7)	3.4 (2.4)
	Mc	13.7 (5.1)	5.6 (5.1)	6.1 (5.2)	2.2 (3.1)
Adult female worms	B1	5.1 (2.0)	5.6 (0.5)	4.5 (2.2)	4.2 (1.6)
	B2	3.0 (1.3)	3.9 (2.0)	4.6 (1.4)	2.3 (1.5)
	Mc	11.4 (4.6)	7.3 (3.2)	3.9 (5.3)	4.2 (4.6)
Juvenile worms[d]	B1	1.6 (2.2)	1.9 (1.1)	0	0
	B2	0.7 (1.0)	0	0	0.1 (0.3)
	Mc	0.6 (1.1)	0.1 (0.4)	0	0.9 (2.9)
Total worms	B1	15.1 (5.0)	13.4 (1.3)	12.0 (3.4)	10.1 (1.8)
	B2	7.7 (3.2)	9.6 (2.1)	8.7 (2.8)	5.8 (2.5)
	Mc	25.7 (7.6)	13.0 (5.5)	10.0 (10.3)	7.3 (5.4)
Juvenile/total (%)	B1	11%	14%	0	0
	B2	9%	0	0	2%
	Mc	2%	1%	0	12%

Means ($\bar{x}$) and standard deviations (s.d.) are shown.

a B1 and B2 represent the initial and repeat BALB/c groups, respectively, while *Mc* represents the *M. coucha* group.

b 7 animals from each group were studied at the various time intervals, except in the cases of the 12 and 20 week B1 groups and the 20 week B2 and *Mc* groups, which consisted of 8, 9, 10 and 10 animals, respectively.

c Worm recoveries of the B1 group are significantly different from those of the B2 and *Mc* groups, respectively ($P < 0.001$; analysis of covariance), while those of the B2 and *Mc* groups do not differ significantly from one another.

d See text (Section 2.2.6) for definition of juvenile worms.

good: virtually all the worms recovered at six weeks of infection were adults, and the percentage recovery at this time was markedly higher than those from the equivalent BALB/c group (42% versus 24%). However, whereas worm burdens in mice remained relatively stable during the subsequent period of study, those in *M. coucha* declined dramatically, being reduced to 21% by 8 weeks of infection and eventually to 12% at 20 weeks; 2 out of 7 animals perfused at 12 weeks and 2 out of 10 perfused at 20 weeks were found to have no worms. The sex ratio of the worms in *M. coucha* at six weeks reflected that seen in mice, but thereafter did not follow any consistent pattern.

As regards the second study with *S. leiperi* (Table 2.3), comparison of worm recovery data from mice with those of the initial study is limited by the fact that worms were recovered on only two occasions (i.e. 8 and 22 weeks of infection). However, from the 8 week data it appears that sex ratios were better balanced than in the first study. *S. leiperi* appeared to develop and survive well in *M. coucha*, with percentage worm recoveries being considerably higher than those from mice (e.g. 41% versus 25% at 8 weeks of infection); there was no evidence of a decline during the study period, and no juvenile worms were recovered at any stage. The ratio of male:female worms in this host showed a slight, but consistent bias towards females.

2.3.2 Rates of Sexual Maturation of Worms and Preferred Sites of Tissue Egg Deposition

The initial study in mice (Table 2.4) revealed a pronounced difference in the rates of sexual maturation of *S. margrebowiei* and *S. leiperi*, corresponding with the differences in worm growth-rate noted above. In the case of *S. margrebowiei*, 60% of potential worm pairs were gravid by 5 weeks of infection, and by 7 weeks all potential pairs were gravid. In contrast, gravid *S. leiperi* females were first detected at only 7 weeks of infection (19%) and it was not until 10 weeks that essentially all pairs (98%) had reached full sexual maturity. Correspondingly, tissue egg deposition by *S. margrebowiei* first occurred between 4 and 5 weeks of infection, whilst that by *S. leiperi* first occurred between 6 and 7 weeks.

TABLE 2.3 Worm recoveries of *Schistosoma leiperi* from BALB/c mice (initial and repeat studies) and *M. coucha*[a].

		Weeks of Infection[b]				
		8	10	12	14	22
		$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)
% Worm recovery[c]	B1	32.4 (11.7)	29.9 (7.9)	32.4 (8.1)	ND	13.7 (7.1)
	B2	25.4 (12.1)	ND	ND	ND	16.1 (7.6)
	Mc	41.0 (16.9)	45.2 (10.0)	ND	36.6 (9.5)	42.9 (8.1)
Adult male worms	B1	9.4 (3.3)	8.9 (3.8)	9.6 (2.5)	ND	3.0 (1.4)
	B2	5.0 (2.3)	ND	ND	ND	3.6 (1.8)
	Mc	9.3 (4.0)	11.6 (3.2)	ND	9.3 (2.9)	10.7 (2.6)
Adult female worms	B1	3.4 (2.3)	4.3 (1.4)	5.0 (3.2)	ND	2.8 (1.8)
	B2	4.4 (3.1)	ND	ND	ND	2.9 (1.6)
	Mc	13.4 (5.9)	13.3 (4.3)	ND	10.9 (2.8)	12.9 (3.5)
Juvenile worms[d]	B1	1.7 (2.1)	0.3 (0.5)	0	ND	0
	B2	0.7 (0.8)	ND	ND	ND	0
	Mc	0	0	ND	0	0
Total worms	B1	14.6 (5.3)	13.4 (3.6)	14.6 (3.7)	ND	5.8 (3.2)
	B2	10.1 (4.9)	ND	ND	ND	6.4 (3.1)
	Mc	22.6 (9.3)	24.9 (5.5)	ND	20.1 (5.2)	23.6 (4.4)
Juvenile/total (%)	B1	12%	2%	0	ND	0
	B2	7%	ND	ND	ND	0
	Mc	0	0	ND	0	0

Means ($\bar{x}$) and standard deviations (s.d.) are shown; ND represents 'not done'.

a B1 and B2 represent the initial and repeat BALB/c groups, respectively, while *Mc* represents the *M. coucha* group.

b 7 animals from each group were studied at the various time intervals, except for the 22 week B1 group, which consisted of 6 animals only.

c Worm recoveries of the *Mc* group are significantly different from those of the B1 and B2 groups ($P < 0.05$ and $P < 0.01$, respectively; analysis of covariance). The B1 and B2 groups do not differ.

d See text (Section 2.2.6) for definition of juvenile worms.

TABLE 2.4 Worm fecundity and tissue egg accumulation patterns of *Schistosoma margrebowiei* (MB) and *S. leiperi* (LP) from BALB/c mice (initial studies).

		Weeks of Infection[a]									
		4	5	6	7	8	9	10	12	20	22
		$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)
Potential worm pairs[b]	MB	3.6 (1.3)	4.0 (1.6)	5.1 (2.0)	5.6 (2.7)	5.6 (0.5)	4.7 (2.1)	3.3 (1.1)	4.4 (2.0)	4.2 (1.6)	ND
	LP	ND	0	2.1 (1.8)	3.7 (1.8)	3.3 (2.0)	4.3 (3.4)	4.4 (0.8)	4.9 (3.1)	ND	2.7 (1.6)
Gravid worm pairs	MB	0	2.4 (1.1)	4.7 (2.2)	5.6 (2.7)	5.6 (0.5)	4.7 (2.1)	3.4 (1.1)	4.4 (2.0)	4.2 (1.6)	ND
	LP	ND	0	0	0.7 (1.1)	2.4 (1.1)	3.9 (3.1)	3.9 (0.7)	4.7 (3.1)	ND	2.3 (1.5)
Gravid/potential worm pairs (%)	MB	0	60%	92%	100%	100%	100%	100%	100%	100%	ND
	LP	ND	0	0	19%	73%	90%	98%	96%	ND	85%
Liver eggs/gravid worm pair[c,d]	MB	0	473 (426)	2 650 (698)	4 755 (2 224)	8 760 (1 872)	15 108 (5 616)	15 967 (6 964)	21 467 (7 563)	38 652 (11 101)	ND
	LP	ND	0	0	156 (72) [4]	338 (226)	1 389 (724) [6]	2 952 (713)	8 368 (1 873) [6]	ND	26 787 (5 577)
GIT eggs/gravid worm pair[c,d]	MB	0	14 (24)	1 915 (867)	5 842 (3 036)	17 326 (5 522)	29 352 (9 062)	33 191 (16 875)	43 034 (17 757)	127 097 (46 171)	ND
	LP	ND	0	0	0	0	40 (29) [6]	282 (216)	485 (250) [6]	ND	5 489 (3 193)
Spleen eggs/gravid worm pair[e]	MB	0	0	0	213 (370)	179 (234) [5]	157 (60) [6]	1 424 (2 222)	538 (383) [7]	338 (184) [8]	ND [5]
	LP	ND	0	0	8 [1]	0	17 (24) [3]	6 (8) [3]	21 (29) [6]	ND	17 (12) [5]

Means ($\bar{x}$) and standard deviations (s.d.) are shown; ND represents 'not done'.

a 7 animals from each group were studied at the various time intervals, except in the cases of the 12 and 20 week MB groups and the 22 week LP group, which consisted of 8, 9 and 6 animals, respectively.

b See text (Section 2.2.6) for definition of potential worm pairs.

c Only animals with bisexual infections and gravid female worms were included in the calculation of group means for liver and GIT egg burdens; the number of animals with gravid females is indicated in square parentheses.

d Rates of tissue egg accumulation in both liver and GIT of the MB and LP groups are significantly different ($P < 0.001$; analysis of covariance).

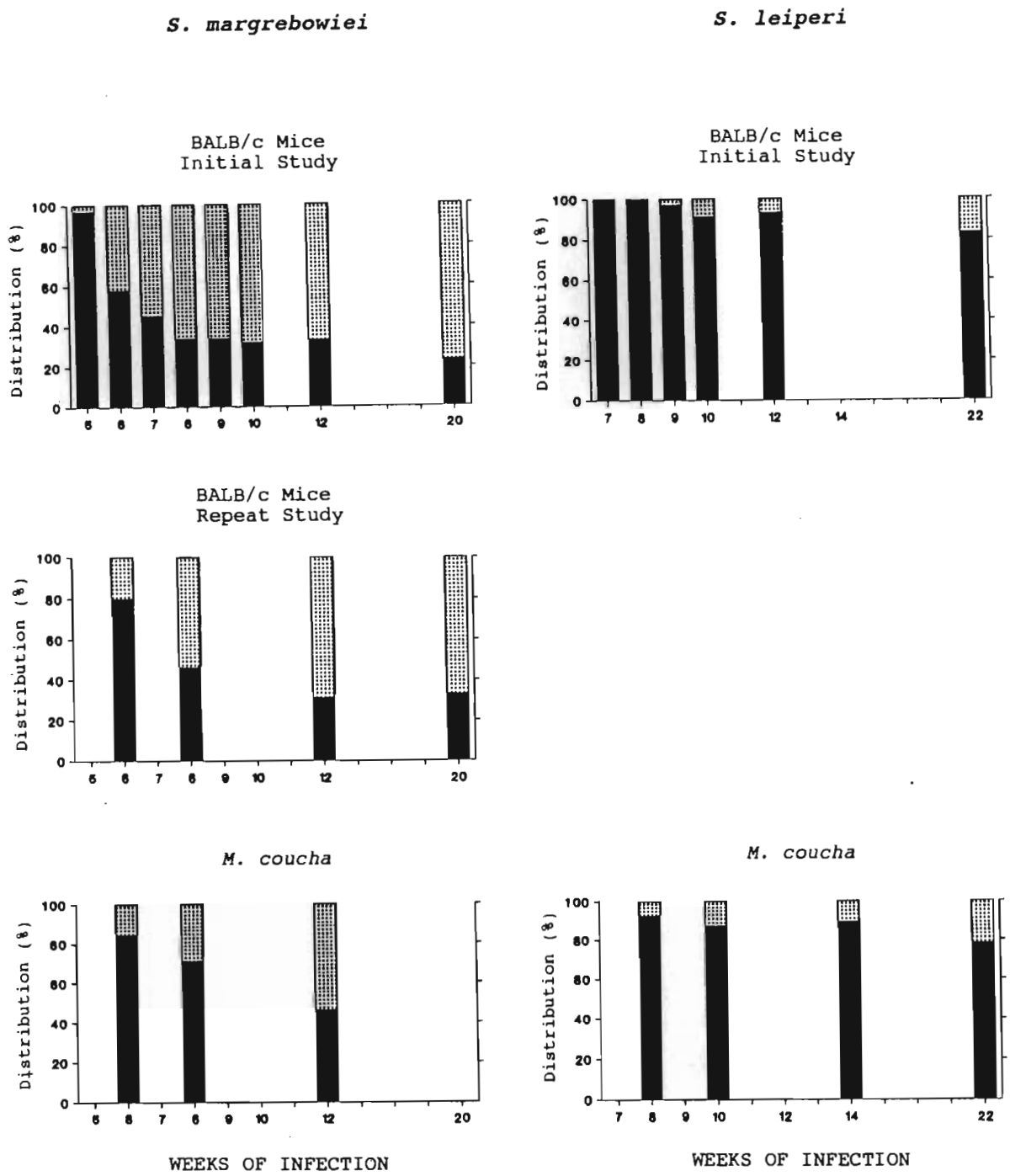
e The mean spleen egg loads/gravid worm pair reported here are based only on data obtained from those animals with eggs in their spleens, where [n] = number of animals.

The pattern of initial egg deposition by both species was similar in that eggs first appeared in the livers of infected animals. However, once the infections became fully patent, a distinct difference emerged, with the majority of *S. margrebowiei* eggs being found in the intestines, in contrast to those of *S. leiperi*, which remained predominantly in the liver. As illustrated in Figure 2.3, the proportion of *S. margrebowiei* eggs found in the intestines increased rapidly, and at 20 weeks of infection represented more than 75% of the total tissue egg burden, whereas that of *S. leiperi* increased to only 17% of the total at 22 weeks. In general, eggs first appeared in spleens about two weeks after the onset of egg laying, and they were seldom found in the spleens of all animals at any particular study interval. Furthermore, the numbers of eggs in spleens were minimal in comparison to those in the livers and intestines.

The results of the subsequent study with *S. margrebowiei* in mice largely confirmed those of the initial study, with virtually all worm pairs having matured by six weeks of infection, and an increasing proportion of eggs being distributed to the intestines as the infection progressed (Table 2.5 and Figure 2.3). This schistosome was also found to be capable of successful pairing and sexual maturation in *M.coucha*. Eggs were found in the tissues of all the animals at each of the study intervals, and, in spite of the steady reduction in overall worm loads with time, gravid females persisted in some of the animals throughout the duration of the study (Table 2.5). At 6 weeks of infection the tissue egg deposition pattern in this host was identical to that in the equivalent mouse group. However, while data pertaining to tissue egg distribution (Table 2.5 and Figures 2.3 and 2.4) and eggs/gravid worm pair (see Section 2.3.3) are presented for the 8 and 12 week intervals, they should be treated with reservation, on account of the marked drop-off in worm loads mentioned in Section 2.3.1. No attempt was made to calculate values for the 20 week interval since 5 of the 10 animals in this group proved not to be harbouring any female worms. Egg deposition in the spleen was negligible in *M.coucha* (Table 2.5).

S. leiperi tissue egg distribution patterns at the corresponding intervals (i.e. 8 and 22 weeks) in the two studies in mice were virtually identical (Table 2.6). Similarly, the patterns of sexual maturation and tissue egg distribution in *M. coucha* corresponded

FIGURE 2.3 Distribution of eggs in the tissues of BALB/c mice and *M. coucha* infected with *S. margrebowiei* and *S. leiperi* at various intervals after the onset of egg-laying.



Each column represents the sum of eggs recovered from the livers and intestines; *solid* (■) and *stippled* (▦) areas indicate the proportions of eggs found in the livers and intestines, respectively.

TABLE 2.5 Worm fecundity and tissue egg accumulation patterns of *Schistosoma margrebowiei* in BALB/c mice (initial and repeat studies) and *M. coucha*[a].

		Weeks of Infection[b]			
		6	8	12	20
		$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)
Potential worm pairs[c]	B1	5.1 (2.0)	5.6 (0.5)	4.4 (2.0)	4.2 (1.6)
	B2	2.9 (1.4)	3.3 (1.1)	3.9 (1.8)	1.7 (1.3)
	Mc	10.6 (4.5)	4.0 (3.7)	3.9 (5.3)	1.2 (1.7)
Gravid worm pairs[d]	B1	4.7 (2.2)	5.6 (0.5)	4.4 (2.0)	4.2 (1.6)
	B2	2.7 (1.5)	3 3 (1.1)	4.1 (2.0)	1.7 (2.3)
	Mc	9.9 (4.4)	4.1 (3.4)	3.7 (5.0)	1.1 (1.6)
Gravid/potential worm pairs (%)	B1	92%	100%	100%	100%
	B2	93%	100%	100%	100%
	Mc	93%	100%	95%	92%
Liver eggs/gravid worm pair[e,f]	B1	2 605 (698)	8 760 (1 872)	21 467 (7 563)	38 652 (11 101)
	B2	3 605 (3 565)	9 036 (3 130)	26 674 (12 154)	61 313 (26 939) [8]
	Mc	3 499 (2 557)	17 762 (8 181)	52 861 (48 300) [5]	NA
GIT eggs/gravid worm pair[e,f]	B1	1 915 (867)	17 326 (5 522)	43 034 (17 757)	127 097 (46 171)
	B2	885 (521)	10 450 (7 371)	60 104 (28 703)	127 093 (46 171) [8]
	Mc	657 (732)	7 418 (3 992)	61 627 (52 336) [5]	NA
Spleen eggs/gravid worm pair[g]	B1	0	179 (234) [6]	538 (383) [7]	338 (184) [8]
	B2	8 (12) [3]	77 (93) [4]	720 (866) [6]	584 (686) [7]
	Mc	0.2 (0.02) [2]	0.3 [1]	7 (3) [5]	NA

Means ($\bar{x}$) and standard deviations (s.d.) are shown; NA represents 'not applicable'.

a B1 and B2 represent the initial and repeat BALB/c groups, respectively, while *Mc* represents the *M. coucha* group

b 7 animals from each group were studied at the various time intervals, except in the cases of the 12 and 20 week B1 groups and the 20 week B2 and *Mc* groups, which consisted of 8, 8, 10 and 10 animals, respectively.

c See text (Section 2.2.6) for definition of potential worm pairs.

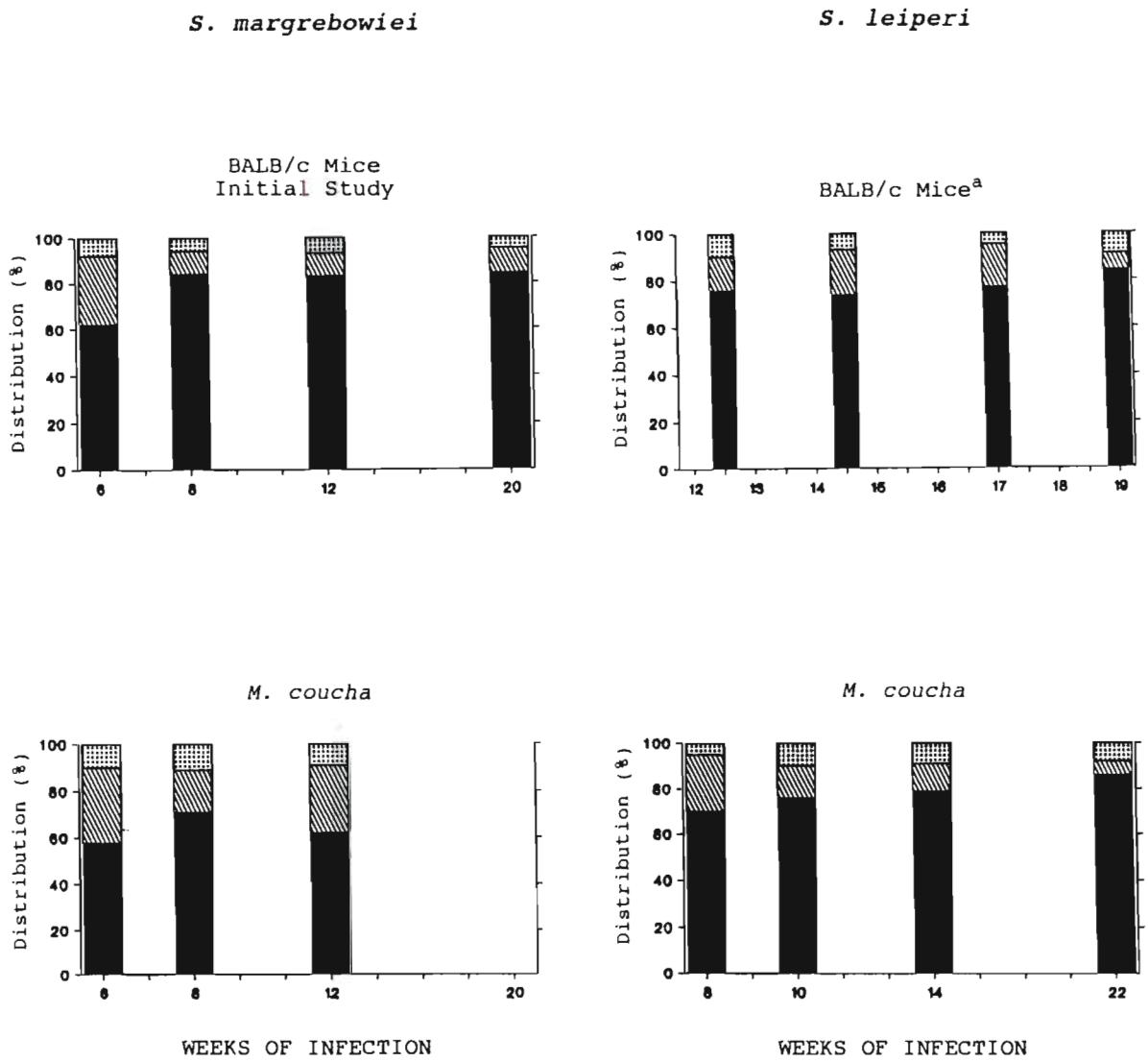
d In some instances the number of gravid females recovered exceeded the number of males; in such cases the number of gravid worm pairs was assumed to be equivalent to the number of gravid females.

e Only animals with bisexual infections and gravid female worms were included in the calculation of group means for liver and GIT egg burdens; the number of animals with gravid females is indicated in square parentheses.

f The rates of tissue egg accumulation of the three groups do not differ significantly (analysis of covariance, using data from the 6 to 12 week study intervals only).

g The mean spleen egg loads/gravid worm pair reported are based only on data obtained from those animals with eggs in their spleens, where [*n*] = number of animals.

FIGURE 2.4 Distribution of eggs in the intestines of BALB/c mice and *M. coucha* infected with *S. margrebowiei* and *S. leiperi* at various intervals after the onset of egg-laying.



Solid (■), *hatched* (▨) and *stippled* (▦) areas indicate the proportion of eggs located in the small intestine, caecum and colon, respectively.

a In this instance data were derived neither from Initial nor Repeat study animals, but rather from a separate experiment (with different aims) in which the estimated cercarial load was 111 cercariae/animal.

TABLE 2.6 Worm fecundity and tissue egg accumulation patterns of *Schistosoma leiperi* in BALB/c mice (initial and repeat studies) and *M. coucha*[a].

		Weeks of Infection[b]				
		8	10	12	14	22
		$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)
Potential worm pairs[c]	B1	3.3 (2.0)	4.0 (0.8)	4.9 (3.1)	ND	2.7 (1.6)
	B2	3.6 (2.3)	ND	ND	ND	2.9 (1.6)
	Mc	8.9 (4.1)	10.3 (2.6)	ND	9.0 (2.5)	10.1 (2.7)
Gravid worm pairs[d]	B1	2.4 (1.1)	3.9 (0.7)	4.7 (3.1)	ND	2.3 (1.5)
	B2	2.0 (0.6)	ND	ND	ND	2.7 (1.3)
	Mc	5.9 (4.2)	9.6 (2.6)	ND	8.3 (2.8)	9.4 (3.1)
Gravid/potential worm pairs (%)	B1	73%	98%	96%	ND	85%
	B2	56%	ND	ND	ND	93%
	Mc	66%	93%	ND	92%	93%
Liver eggs/gravid worm pair[e,f]	B1	338 (226)	2 952 (713)	6 368 (1 873) [6]	ND	26 787 (5 577)
	B2	333 (279)	ND	ND	ND	22 339 (7 606)
	Mc	624 (355)	3 987 (700)	ND	12 344 (2 306)	24 028 (10 720)
GIT eggs/gravid worm pair[e,f]	B1	0	282 (216)	485 (250) [6]	ND	5 489 (3 193)
	B2	8 (12)	ND	ND	ND	3 553 (3 346)
	Mc	55 (30)	583 (249)	ND	1 464 (434)	6 961 (3 726)
Spleen eggs/gravid worm pair[g]	B1	0	6 (8) [3]	21 (29) [6]	ND	17 (12) [5]
	B2	0	ND	ND	ND	1 (1.3) [5]
	Mc	0.4 (0.1) [2]	1 (0.3) [3]	ND	1 (0.1) [5]	2 (2.6) [6]

Means ($\bar{x}$) and standard deviations (s.d.) are shown; ND represents 'not done'.

a B1 and B2 represent the intitial and repeat BALB/c groups, respectively, while *Mc* represents the *M. coucha* group.

b 7 animals from each group were studied at the various time intervals, except for the 22 week B1 group, which consisted of 6 animals only.

c See text (Section 2.2.6) for definition of potential worm pairs.

d Only animals with bisexual infections and gravid female worms were included in the calculation of group means for liver and GIT egg burdens; the number of animals with gravid females is indicated in square parentheses.

e The rates of liver egg accumulation of the three groups do not differ significantly. GIT egg accumulation rates differ only in the case of the B2 and *Mc* groups ($P < 0.05$; analysis of covariance).

f The mean spleen egg loads/gravid worm pair reported here are based only on data obtained from those animlas with eggs in their spleens, where [*n*] = number of animals.

closely with those observed in the initial study in mice (Table 2.6 and Figure 2.3).

The distribution of eggs of both species within the intestines of both hosts (Figure 2.4) was found to be very similar, with the majority being located in the small intestine. In mice the proportion in this region increased to over 80% as the infection progressed. The same pattern was observed in *S. leiperi*-infected *M. coucha*, but in the case of *S. margrebowiei*, no distinct trend was evident in this host, with the proportion in the small intestine remaining around 60% over the period 6 to 12 weeks of infection.

Eggs of *S. margrebowiei* and *S. leiperi* were not detected in the faeces of infected mice until 3 weeks after eggs were first found in the intestines (i.e. 8 and 12 weeks, respectively) (Table 2.7). The number of *S. leiperi* eggs recovered was extremely small, and while the numbers of *S. margrebowiei* eggs were considerably greater, they represent only a minute proportion of the total numbers of eggs present in the intestinal tissues.

2.3.3 Rates of Tissue Egg Accumulation

From Tables 2.4, 2.5 and 2.6 it is clear that the egg output of *S. margrebowiei* in both hosts greatly exceeded that of *S. leiperi*. Tissue egg accumulation rates are shown graphically in Figure 2.5, together with the regression equations which were derived from each set of data (excluding the repeat study on *S. leiperi* in mice). Liver egg accumulation rates in the initial and repeat studies with *S. margrebowiei* in mice were found to differ significantly ($P < 0.01$); however, no significant differences were detected with respect to intestinal or total tissue eggs. Egg accumulation rates in the tissues of *M. coucha* over the period 6 to 12 weeks of infection did not differ statistically from those of either the initial or the repeat studies in mice. A similar picture emerged for *S. leiperi* over the period 8 to 22 weeks of infection, in that no significant differences were detected between the results of the initial study in mice and those of the subsequent study in *M.coucha*.

In all instances the estimated times of onset (t_o) of egg deposition in the liver were earlier than those in the intestines (Figure 2.5),

TABLE 2.7 Egg excretion in the faeces of BALB/c mice infected with *Schistosoma margrebowiei* (MB) and *S. leiperi* (LP) (initial studies).

		Weeks of Infection[a]								
		5	6	7	8	9	10	12	20	22
Total number of ova in faeces[b]	MB	0	0	0	160	450	310	165	400	ND
	LP	0	0	0	0	0	0	30	ND	0
Number of gravid worm pairs[c]	MB	17	33	39	39	33	24	31	21	ND
	LP	0	0	6	17	27	27	33	ND	14
Ova output/gravid worm pair/day in faeces	MB	0	0	0	4.1	13.6	12.9	5.3	19.0	ND
	LP	0	0	0	0	0	0	0.9	ND	0

ND represents 'not done'

a 7 animals from each group were studied at the various time intervals, except in the cases of the 20 week MB and 22 week LP groups, which consisted of 5 and 6 animals, respectively.

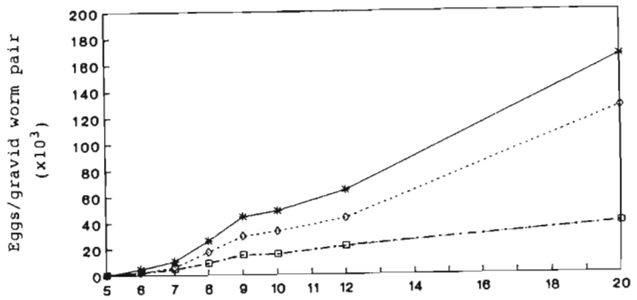
b Faeces were collected over a 24h period immediately prior to perfusion.

c This represents the sum of the gravid worm pairs recovered from all the animals in each group.

FIGURE 2.5 Tissue egg accumulation, expressed in terms of eggs/gravid worm pair, in the livers, intestines (GIT) and livers plus intestines (Total) of BALB/c mice and *M. coucha* infected with *S. margrebowiei* and *S. leiperi*.

S. margrebowiei

BALB/c Mice
Initial Study



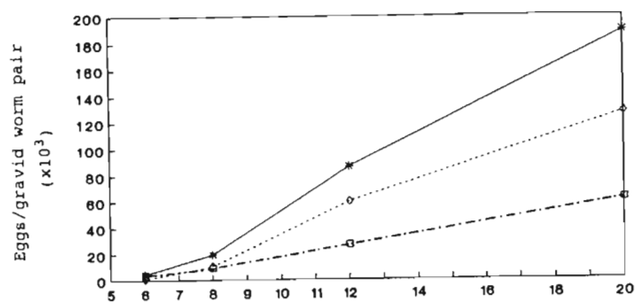
REGRESSION EQUATIONS[a]

TOTAL : y = 11 566t - 67 859
(r = 0.92; t_0= 5.9)

GIT : y = 9 023t - 56 512
(r = 0.89; t_0= 6.3)

LIVER : y = 2 543t - 11 347
(r = 0.90; t_0 = 4.5)

BALB/c Mice
Repeat Study

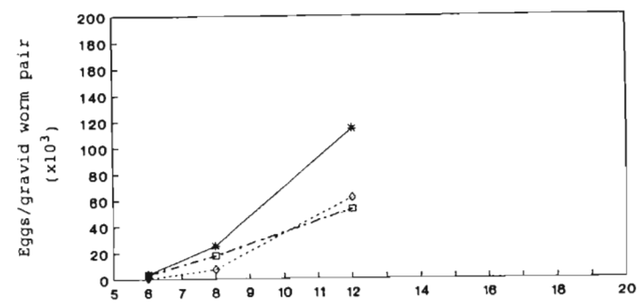


TOTAL : y = 13 295t - 78 314
(r = 0.91; t_0= 5.9)

GIT : y = 9 107t - 55 470
(r = 0.86; t_0= 6.1)

LIVER : y = 4 188t - 22 844
(r = 0.85; t_0= 5.5)

M. coucha[b]



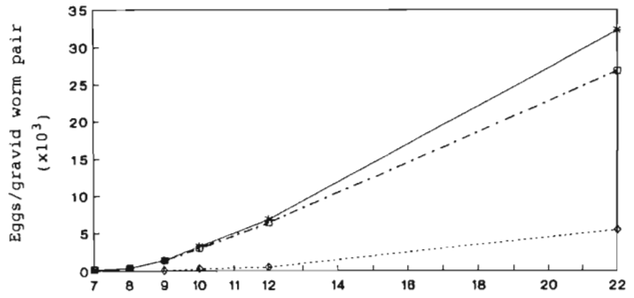
TOTAL : y = 11 136t - 63 159
(r = 0.94; t_0= 5.7)

GIT : y = 6 541t - 41 115
(r = 0.85; t_0= 6.3)

LIVER: y = 4 596t - 22 044
(r = 0.87; t_0= 4.8)

S. leiperi

BALB/c Mice
Initial Study



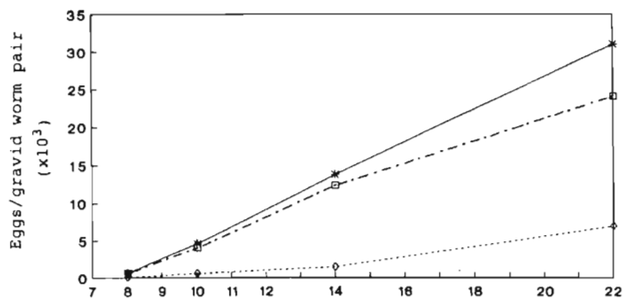
REGRESSION EQUATIONS[a]

TOTAL : y = 2 339t - 19 701
(r = 0.95; t_0= 8.4)

LIVER : y = 1 928t - 15 935
(r = 0.97; t_0= 8.3)

GIT : y = 411t - 3 766
(r = 0.83; t_0= 9.2)

M. coucha



TOTAL : y = 2 179t - 16 905
(r = 0.88; t_0= 7.8)

LIVER : y = 1 679t - 12 414
(r = 0.87; t_0= 7.4)

GIT : y = 501t - 4 491
(r = 0.82; t_0= 9.0)

a t = weeks of infection; r = Pearson's correlation coefficient; t_0 = theoretical time, in weeks, of onset of tissue egg deposition (y = 0). -·-□-·-, liver; --◇--, GIT; —✳—, total.

b In this instance the regression equation is based on data up to 12 weeks of infection only.

corresponding with the fact that eggs were always deposited initially in the livers of the experimental hosts. The liver t_0 values for *S. margrebowiei* in the initial and repeat studies in mice and for the study in *M. coucha* are 4.5, 5.5 and 4.8 weeks, respectively, whilst those for *S. leiperi* in mice and *M. coucha* are 8.3 and 7.4 weeks, respectively.

2.4 DISCUSSION

The percentage recoveries of *S. margrebowiei* worms from mice in the present study (Tables 2.1 and 2.2) are, in general, somewhat lower than those from hamsters (39.8% overall recovery) recorded by Southgate and Knowles (1977). Ogbe (1985) found that worm recoveries from mice and hamsters changed dramatically with time, increasing from around 20% at 3 weeks (20 days) of infection to almost 50% and 60%, respectively, at 7 weeks (50 days), and thereafter dropping to less than 20% by 10 weeks (70 days). This observation is at variance with what was found in the present study, namely that the *S. margrebowiei* recovery rate from mice remained stable for at least 12 weeks and showed relatively little evidence of declining even by 20 weeks of infection. In contrast, Ogbe's findings with respect to infections of ≥40 days duration correspond well with those in *M. coucha*, where a pronounced drop-off in worm returns was seen after 6 weeks of infection (Table 2.2). This finding confirms the observation reported by Visser and Badenhorst (1985), made shortly after the introduction of *S. margrebowiei* into the laboratory by Pitchford (1975) (Visser, personal communication), that the worms of this species die shortly after maturation in *M. coucha*. It is also strongly supported by worm return data from a number of independent, unrelated experiments carried out by the present author prior to the present study, in which it was noted that worm returns from *M. coucha* perfused after 6 weeks of infection were generally high whereas those from various other groups with more long-standing infections (≥10 weeks duration) were consistently considerably lower (unpublished data).

Considering the fact that *S. margrebowiei* appears to be initially so well-adapted to development in *M. coucha*, its characteristic sudden decline shortly after maturation is surprising. It seems possible that this is in some way due to an unfavourable alteration of the

intravenous habitat brought about by the maturation of the parasite itself and possibly linked to the sudden deposition of large numbers of eggs in the tissues (Table 2.5). The ability of a small proportion of the worms to survive and continue egg production for an extended period may reflect genetic heterogeneity on the part of either the host (the *M. coucha* used in this study were not inbred) or the parasite (i.e. a sub-population of the worms possesses the ability to overcome or avoid the factors responsible for the death of the majority of the worms). In some respects the host-parasite relationship of *S. margrebowiei* and *M. coucha* is similar to that of *S. mansoni* and the rat, although in the latter case the initial maturation of worms is less successful and subsequent worm elimination is more severe (Knopf, 1982). Further studies on the *M. coucha/S. margrebowiei* model, similar to those which have been carried out on the rat/*S. mansoni* model (Knopf, 1982) may well be worthwhile; they may provide some valuable additional insights pertaining to the schistosome/host relationship, particularly with respect to the factors which facilitate or militate against long-term survival of schistosomes in the definitive host.

While there have been no previous reports on the infection characteristics of *S. leiperi* in mice, the worm returns reported from this host during the present study (Tables 2.1 and 2.3) compare favourably with those in the hamster (26.1% overall recovery) recorded by Southgate *et al* (1981). The fact that 95% of the worms recovered at 5 weeks of infection in the initial experiment were juveniles suggests that worm migration to the liver was not yet complete, which would provide an explanation for the relatively low worm recovery (21.9%) seen at this time. The much-reduced worm return at 22 weeks in the initial study can possibly be explained in terms of infection-induced mortality (see Chapter 3), in that animals carrying heavier worm loads are likely to die sooner than those with light loads. However, this is not supported by the results of the initial study with *S. margrebowiei* (Table 2.1), where the drop-off in worm loads was much less pronounced, in spite of similar mortality levels (see Chapter 3). It seems possible therefore that the observed reduction in the case of *S. leiperi* may be due, at least in part, to spontaneous elimination of worms at some time between 12 and 22 weeks.

The finding that the percentage worm recoveries of *S. leiperi* from *M.coucha* were significantly higher than those from mice is consistent with observations pertaining to two different strains of *S. mansoni*, where it was similarly found that recoveries from *M.coucha* were consistently higher than those from BALB/c mice (Higgins-Opitz and Dettman, 1991). While this finding clearly reflects a host-related difference in susceptibility to infection, the finding that percentage worm returns from the initial and repeat studies with *S. margrebowiei* in mice were significantly different (Table 2.2) is not as easily explained. It is well known that schistosome worm returns are subject to the effects of numerous environmental and parasite-related influences (Stirewalt and Fregeau, 1965 and 1968; Christensen *et al*, 1979). However, the factors responsible for the differences noted in the present case are not clear, since the two experiments were carried out in the same laboratory under essentially similar conditions. Differences in the ages of the cercariae used in the respective studies has been excluded as an explanation, since the cercariae used in the repeat study were, if anything, slightly younger than those used in the initial study.

There have been numerous reports pertaining to natural and experimental infections with a variety of schistosomes, including *S. mansoni*, *S. japonicum*, *S. haematobium*, *S. mattheei*, *S. intercalatum* and *S. rodhaini* (Liberatos, 1987; Mitchell *et al*, 1990), which strongly suggest that schistosome infections in the definitive host are characteristically biased in favour of male worms. However, in the present author's experience infections with *S. margrebowiei* and *S. leiperi* in mice and *M. coucha* do not conform to any particular pattern. While more males than females were recovered in both the initial and repeat experiments in mice reported here (Tables 2.1, 2.2 and 2.3), sex ratios in *M. coucha* were slightly biased in favour of females. This observation corresponds with those of Southgate and colleagues who similarly observed a preponderance of female worms in their studies with hamsters (Southgate and Knowles, 1977; Southgate *et al*, 1981). Furthermore, results from a substantial number of other experiments carried out by the present author with both of the antelope schistosomes in BALB/c mice have been variable, with approximately equal frequencies of male-dominated and female-dominated infections being encountered. In contrast, the results of numerous experiments involving two different strains of *S. mansoni* (i.e. from Puerto Rico and South Africa, respectively) are in strong

agreement with the above-mentioned literature, with worm loads being characterised either by well-balanced sex ratios or, more frequently, by a dominance of male worms (unpublished data).

In terms of sexual maturation rates, the results of the present study correspond reasonably well with those of previously published reports, in that they confirm the observation that *S. margrebowiei* develops considerably more rapidly than *S. leiperi* (Table 2.1). However, the observations of Southgate and Knowles (1977) and Southgate *et al* (1981) suggest that the maturation of both species in hamsters may be slightly more rapid than that seen in mice, since they found that 95% of paired female *S. margrebowiei* worms were gravid by 35 days of infection, and that 86% and 99% of paired *S. leiperi* females were gravid by 50 and 60 days of infection, respectively. This contrasts with the present study, where only 60% of *S. margrebowiei* pairs were gravid at 35 days, and only 19% and 90% of *S. leiperi* pairs were gravid at 49 and 63 days, respectively (Table 2.4). However, in spite of these differences, there is good correlation between the results of the present and previous studies (including that of Ogbe, 1983) in terms of the time at which tissue egg deposition commenced, viz. between 4 and 5 weeks in the case of *S. margrebowiei* and between 6 and 7 weeks in the case of *S. leiperi*.

The present findings with regard to the relative distribution of eggs in the liver and intestines (Figure 2.3) are again in agreement with those of Southgate and co-workers, who found that in hamsters the majority of *S. margrebowiei* eggs accumulated in the intestines, whereas those of *S. leiperi* were found mostly in the liver (approximately 80% of the total tissue egg burden in each instance) (Southgate and Knowles, 1977; Southgate *et al*, 1981). However, some discrepancies are evident with respect to the intra-intestinal distribution of eggs, since these authors found that well under half of the total intestinal eggs (i.e. about 25% and 40% in the cases of *S. margrebowiei* and *S. leiperi*, respectively) were located in the small intestines; in contrast, the proportion of eggs distributed to this location in BALB/c mice and *M. coucha* was generally found to be in the region of 70-80% (Figure 2.4). The data of Ogbe (1985) also indicate a predilection for intestinal sites of egg deposition in the case of *S. margrebowiei*. However, they are of limited value in terms of assessing the proportional distribution of eggs, either between the liver and intestines, or between the different regions of the

intestinal tract, for the reason that a substantial part of the small intestine, namely the duodenum and jejunum, was apparently excluded from consideration by this author.

Accumulation of the eggs of *S. margrebowiei* and *S. leiperi* in the tissues of mice and *M. coucha* continued at steady rates throughout the duration of each of the studies reported here (Figure 2.5). This finding is similar to those of studies by Cheever (1969) involving *S. mansoni* in mice, in which it was found that eggs accumulated in the tissues in a linear fashion for periods of up to 6 months of infection. On the basis of the regression equations presented in Figure 2.5 the daily rates of *S. margrebowiei* tissue egg accumulation per gravid worm pair in the initial and repeat studies in mice can be estimated at 1652 and 1899, respectively, and that in *M. coucha* at 1590. These values indicate that the rate of egg production by this species is considerably higher than was previously thought. The only previous estimates are those of Southgate and Knowles (1977), who calculated that the mean rate of egg production in hamsters was 837/female/day. However, they suggested that this might be an underestimate, since it was based on data from a fairly small number of animals and included values from a very early stage of infection (30 days). An estimate based on a small group of animals with 50 day-old infections indicated an output of 1450 eggs/day, which is more in keeping with the results of the present study.

The estimated rates of *S. leiperi* egg accumulation in the tissues of mice and *M. coucha*, also based on the regression equations in Figure 2.5, are 334 and 311/female/day. These represent the first estimates of this nature for this schistosome and reveal that in terms of egg productivity in rodent hosts it is similar to the well-characterised Puerto Rican strain of *S. mansoni* (Loker, 1983; Higgins-Opitz and Dettman, 1991) and more prolific than a number of other schistosomes for which data are available, including *S. haematobium*, *S. intercalatum*, *S. mattheei* and *S. bovis* (Loker, 1983), as well as South African *S. mansoni* (Higgins-Opitz and Dettman, 1991).

The somewhat higher estimated rate of egg production in the repeat study with *S. margrebowiei* in mice, compared to that in the initial study, can perhaps be accounted for by the fact that in the former instance, but not the latter, the number of worm pairs recovered at 20 weeks of infection was substantially lower than that at 12 weeks

(Table 2.5). This may have resulted in some exaggeration of the estimated number of tissue eggs/gravid worm pair at the 20 week interval, since some of the ova deposited by female worms prior to their demise may still have been present in the tissues at the time of necropsy. The same applies with respect to the slightly higher value obtained in the initial study with *S. leiperi* relative to that in the study in *M. coucha*; worm pair burdens decreased markedly in mice between 12 and 22 weeks, but remained stable in *M. coucha* (Table 2.6). However, since there are no significant differences between slopes (i.e. rates of tissue egg accumulation) in either of the above instances, it would appear that any such exaggeration is minimal. Although the estimated rate of *S. margrebowiei* egg accumulation in *M. coucha* was found not to differ significantly from that in mice (Figure 2.5), the validity of this estimate seems highly questionable, in view of the unusual dynamics of worm elimination from this host (Tables 2.2 and 2.5).

The considerable delay between the onset of tissue egg deposition by *S. margrebowiei* and *S. leiperi* in mice and the first detection of eggs in the faeces (Table 2.7) is perhaps a reflection of the fact that eggs were initially deposited predominantly in the liver. However, considering how few eggs were excreted in relation to the total daily egg output of these schistosomes, it appears more likely to reflect a poor ability on the part of this host to expel the eggs into the lumen of the intestine. Similar observations have been made with respect to BALB/c mice and *M. coucha* infected with different strains of *S. mansoni*, where it was noted that eggs first appeared in the faeces between one and three weeks after they were first deposited in the intestines, and that the number of eggs excreted represented only a small percentage of the total daily output (Higgins-Opitz and Dettman, 1991). The daily excretion of *S. margrebowiei* eggs by hamsters was also found to be miniscule (Southgate and Knowles, 1981). Interestingly however, with regard to both *S. margrebowiei* and *S. leiperi*, eggs were first detected in the faeces of infected hamsters within just a few days of the onset of egg laying (Southgate and Knowles, 1977; Southgate *et al*, 1981); since egg excretion by these hosts does not appear to be any greater than that by mice, the early detection of faecal eggs by these authors suggests that the method of detection which they used was more sensitive than that used during the present study.

While virtually nothing is known about the infection characteristics of *S. margrebowiei* and *S. leiperi* in their natural definitive hosts, such as lechwe, it seems obvious that their behaviour in rodents is highly misrepresentative, at least in some respects. For example, it can be assumed, on the basis of the need to ensure adequate egg excretion for the purposes of self-propagation, that *S. leiperi* deposits a far larger proportion of its eggs into intestinal sites in the natural host than is seen to be the case in rodents. Similarly, it must be assumed that the ability of antelopes to excrete ova of both species is far superior to that of rodents.

It is possible also that the rates of egg production by the two schistosomes calculated in the present study do not accurately reflect those in their natural definitive hosts. The results of studies on *S. mansoni* by Cheever and Duvall (1974) and Damian and Chapman (1983) indicate that the fecundity of this parasite in non-human primates is two to three times greater than that in rodents. One of the reasons for this discrepancy is that estimates derived from studies in rodents are generally based on tissue egg loads combined with egg excretion data at successive intervals of infection, but fail to take into consideration the numbers of eggs destroyed in the tissues each day (Cheever, 1969). With respect to *S. margrebowiei* and *S. leiperi* in rodents it would appear that excreted eggs can be ignored when calculating worm fecundity, since so few eggs are excreted. However, the rates at which the eggs of these species are destroyed in the tissues of mice and *M. coucha* remain to be assessed. In this regard it is noteworthy that whereas Cheever and Anderson (1971) estimated the half-life of *S. mansoni* ova in mouse tissues to be about 4 weeks, Moloney *et al* (1987b) observed relatively little clearance of *S. japonicum* eggs from the tissues of drug-cured mice over a 20 week period. The latter finding was explained on the basis that tissue-bound *S. japonicum* ova tend to become calcified. Significantly, evidence of what appear to be calcified ova have frequently been observed by the present author in filtrates prepared from the livers of *S. margrebowiei*-infected mice (unpublished observations). Clearly, therefore, it is possible that the rates at which the eggs of *S. margrebowiei* and *S. leiperi* are eliminated from the tissues of infected rodents may differ considerably.

The fact that eggs were found to accumulate at a more-or-less constant rate throughout each of the present studies implies the existence of some form of steady-state relationship between the rate at which eggs are deposited and that at which they are destroyed by mice and *M. coucha*, with the latter occurring at a far lower rate than the former. The inability of these hosts to destroy eggs as fast as they are deposited is easily understood if one considers that an infection with a few worm pairs in a mouse is equivalent on a weight-for-weight basis to a massive infection with many thousands of worms in a large bovine. This 'overloading' effect may also conceivably contribute to a reduced fecundity of the worms in small hosts, since the utilisation of available sites for the deposition of eggs may result in suppression of egg production due to some form of 'negative feedback'.

Evidence that the fecundity of *S. margrebowiei* in ungulates is in fact substantially higher than that seen in rodents was obtained by van Rensburg (personal communication), who successfully infected sheep and goats with this parasite. These animals were observed to pass extremely large numbers of ova in their droppings for periods of up to a year. On the basis of a 24-hour faecal egg excretion estimate and subsequent worm recovery data from a single goat (which yielded a 60% worm return) he calculated that excreted eggs alone (i.e. excluding those trapped in tissues) amounted to approximately 3445 eggs/female worm/24h. He also observed that sheep, goats and cattle experimentally infected with *S. leiperi* excreted substantial numbers of viable ova. Although he did not attempt to determine the daily egg excretion rate per female worm for this species, he found it to be far less pathogenic than *S. margrebowiei* in sheep and goats. Thus, whereas animals infected with *S. leiperi* presented only minimal evidence of disease, those harbouring apparently similar worm loads of *S. margrebowiei* became clearly emaciated and anaemic, and frequently passed bloody droppings. These observations provide strong evidence that the egg-producing capacities of these two species in their natural hosts may differ to a similar degree to that seen in experimental rodent models.

Further studies on the relative egg excretion rates of *S. margrebowiei* and *S. leiperi* in well-adapted hosts would be of considerable interest, since the marked differences in the egg producing capacity of these two parasites are presumably in some way related to the

apparent differences in their respective transmission patterns, as discussed in Section 1.10.4. The observation by Wright *et al* (1979) and Howard *et al* (1981) that *S. margrebowiei* infections in lechwe are rare in animals of more than three to four years of age, being superseded by infections with *S. leiperi*, implies that the life-span of *S. margrebowiei* worms is relatively short, which in turn implies a shorter period over which to seed the environment with miracidia. In the light of this possibility it is conceivable that the ability of *S. margrebowiei* to produce substantially more ova than *S. leiperi* serves to counteract the disadvantages associated with a shorter life-span, and may in fact be the consequence of selection pressure imposed by *S. leiperi*.

CHAPTER THREE

SOME PATHOPHYSIOLOGICAL RESPONSES OF BALB/c MICE TO INFECTION WITH THE ANTELOPE SCHISTOSOMES

3.1 INTRODUCTION

Early experimental studies on *S. mansoni* infections in animal models revealed the development of pronounced hepatosplenomegaly in mice shortly after the onset of egg-laying by the parasites (Moore et al, 1949). Subsequent studies by Warren and DeWitt (1958) demonstrated that this host also develops a number of the other disease syndromes characteristic of human hepatosplenic schistosomiasis, most notably portal hypertension and oesophageal varices. A further similarity to the clinical picture in man was noted in the fact that liver function tests in mice showed relatively little disturbance, in spite of extensive granulomatous reactions to eggs deposited in the liver (DeWitt and Warren, 1959). Shortly thereafter it was shown that the development of typical hepatosplenic schistosomiasis in mice was dependent on the presence of mature, bisexual infections, leading to the conclusion that the schistosome egg is the most important agent in the aetiology of the disease (Warren, 1961).

Subsequent to these early studies, extensive investigations into the pathogenesis of intestinal schistosomiasis in the murine model were carried out (reviewed in depth by Warren, 1982). While the majority of work has focused on *S. mansoni*, a murine model of schistosomiasis japonica was also developed (Warren and Moore, 1966) and has been subjected to considerable study (reviewed by Cheever, 1985). Between them, the many studies in the murine models played a fundamental role in shaping perceptions of the disease process in hepatosplenic schistosomiasis. It was established, for example, that portal hypertension and the development of porta-systemic collateral circulation result from the obstruction of blood flow through the liver, due to the occlusion of hepatic venules by the inflammatory reactions to schistosome eggs and the subsequent fibrosis (Bloch *et al*, 1972). Since the responses to the eggs were seen to be immunologically-mediated, it was thus realized that schistosomiasis should be regarded essentially as a disease of immunological origin (Warren, 1975).

Once the central role of the egg was recognised it became the subject of intensive investigations which have continued virtually unabated until the present. One of the key early observations was that the maximum size of granulomatous lesions around freshly deposited eggs in chronic-stage murine schistosomiasis was substantially less than that around eggs laid in the first few weeks after worm maturation (Andrade and Warren, 1964; Domingo and Warren, 1968), and it was recognised that this modulatory process (also referred to as 'endogenous desensitization') corresponded with a stabilization of the manifestations of disease in long-term infections (Warren, 1966; Warren and Berry, 1972). The immunochemical characteristics of the egg, the molecular and cellular processes of granuloma formation and modulation, and their relationships to fibrosis have been analysed in great depth; the bulk of this work is summarised in the reviews by Warren (1982), Cheever (1985), Boros (1986), Phillips and Lammie (1986), and Stavitsky (1987). While these studies were motivated in part by the idea that it may be possible to promote suppression of the schistosomal granulomatous response at a much earlier stage than normal in human infections, thereby reducing the probability of severe sequelae, they have also been of considerable benefit in enhancing the understanding of granulomatous reactions *per se*.

The studies described in this chapter were aimed at the assessment of some of the features of hepatosplenic schistosomiasis in BALB/c mice resulting from infection with *S. margrebowiei* and *S. leiperi*. They were intended, on the one hand, to provide information which might be of use in the design and/or interpretation of the experiments described in the following chapter. In addition, since there have been extremely few studies of this nature involving non-human schistosomes, it was also considered of interest to determine whether the responses evoked by the antelope schistosomes differ in any significant way from those seen in studies with human schistosomes.

3.2 MATERIALS AND METHODS

3.2.1 Schistosomes

Details regarding the origins and maintenance of the isolates of *S. margrebowiei* and *S. leiperi* used in these studies are presented in Section 2.2.1.

3.2.2 Rodents

Male mice were used throughout; details regarding the origins, housing and maintenance of the animals are presented in Section 2.2.2. Within individual experiments animals were age-matched to within one week of each other; the approximate ages of animals in the different experiments, at the time of infection, ranged between 6 and 11 weeks.

3.2.3 Infection of Mice

Mice were infected with schistosomes according to the method described in Section 2.2.3.

3.2.4 Anaesthesia and Weighing of Animals

Animals were anaesthestised by intra-peritoneal injection of pentobarbitone sodium, essentially as described in Section 2.2.5, although with respect to portal pressure measurements (Section 3.2.5), the aforementioned dosage of 0.20-0.25mg/g body mass was found to be excessive. For this procedure considerable care was therefore taken to determine dosages which produced acceptable levels of anaesthesia and analgesia but did not result in death. On the basis of preliminary trials it was established that dosages of 0.120 and 0.125mg/g body weight were suitable for non-infected and infected animals, respectively.

3.2.5 Measurement of Portal Venous Blood Pressure

Portal pressure was measured using a saline manometer (Cheever, 1985) (Figure 3.1). It consisted of a glass tube (325mm in length; external and internal diameters of 7 and 1,3mm, respectively) joined by means of a 3-way stopcock to a narrow gauge flexible plastic tube (640mm in length; 1,5mm internal diameter) ending in a 23-gauge butterfly needle. The arm of the stopcock was connected by means of a second flexible tube to a saline reservoir. The glass tube was mounted on a wooden board against a graduated backing, marked in millimetres and centimetres over a range of 0-25cm. The board in turn was fixed in

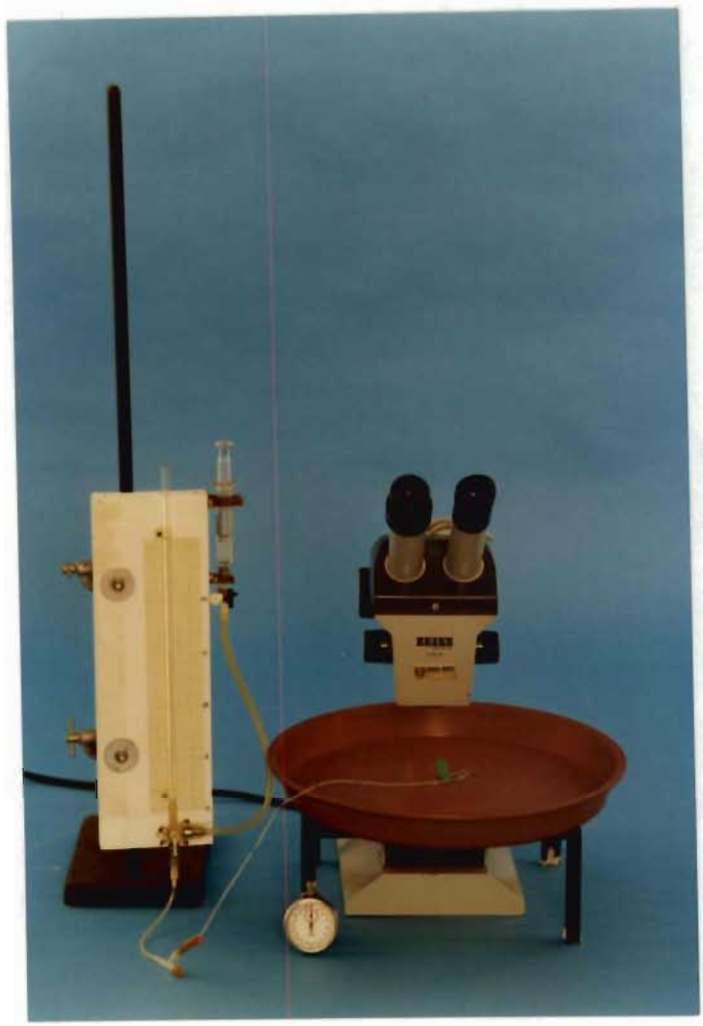


FIGURE 3.1 Saline manometer used for the measurement of portal venous blood pressure.

FIGURE 3.2 Equipment used for the measurement of granulomas.

a vertical position to a retort stand, by means of two rear-mounted horizontal arms. Prior to portal pressure measurement the level of fluid in the glass tube was set between 18 and 20cm of saline and the fluid column was carefully checked to ensure that it was free of air-locks.

Following injection of the anaesthetic the responses of the animal were carefully monitored. As soon as the level of anaesthesia and analgesia was judged to be acceptable for surgical procedures the abdominal cavity was opened. The animal was then placed on the stage of a dissection microscope, positioned at a height relative to the manometer such that when the needle was placed at the level of the portal vein, the saline reached a 'resting' level of 3-3,5cm. The liver was reflected to the side to expose the portal vein, whereafter the stopcock was opened to allow saline to flow from the butterfly needle. As soon as the level of saline reached approximately 8cm the needle was inserted into the portal vein with the aid of the microscope, at which time a stopwatch was started. Manometer readings were taken at 1 minute intervals for 3 minutes after insertion of the needle. The needle was then extracted from and held beside the portal vein for a further 3 minutes, with readings again being taken at 1 minute intervals. The portal blood pressure was taken as the difference between the lowest of the last three readings and the mean of the first three. The respiration rate of the animal was recorded during the first and final 30 second intervals of the procedure, as a guide to the condition of the animal. A sudden or steady decline in the respiration rate was usually accompanied by a rapid fall-off in the portal pressure; in such instances the reading was regarded as unreliable and the data excluded from consideration.

No significant age-related change in portal pressure was detected in non-infected male animals (controls) tested over the period 17 to 29 weeks of age; the mean reading, derived from all control animals (n = 27), was 4.3 cm of saline (s.d. = 0.49). Portal pressure readings from infected animals were expressed in terms of percentage increase relative to the mean control value.

3.2.6 Collection of Blood Samples

Blood samples were taken from the severed subclavian artery, using the axillary pocket method of Chase (1967). It was important to

ensure that this procedure was carried out soon after the induction of anaesthesia, i.e. while cardiac output was still high. In cases where animals were to be perfused after bleeding it was necessary to occlude the severed ends of the artery in order to prevent the excessive loss of perfusion pressure: this was achieved by compressing the surrounding tissues by means of a small crocodile clip.

Blood samples were allowed to clot for 1-2 hours at room temperature, or overnight at 4 C, following which they were centrifuged at 1500*g* for 5 min. Sera were recovered by means of pasteur pipette and stored at -20 C until required for serology (Section 3.2.7).

3.2.7 Serology

Sera were tested for the presence of anti-schistosome IgG antibodies using a modification of the indirect fluorescence antibody technique (IFAT) (Sadun *et al*, 1960), similar to that described by Wolstenholme and Fripp (1981).

Antigen-coated slides were prepared as follows: 12-well multitest slides (Flow Laboratories, Scotland) were briefly soaked in a detergent solution, rinsed thoroughly with tap water followed by distilled water, placed in methanol for 10 minutes, and then allowed to dry. Thereafter, they were immersed for 10 minutes in a 0.2% solution (mass/vol) of sodium metasilicate ($Na_2SiO_3.5H_2O$; Saarchem, Krugersdorp, RSA) and air dried (this treatment is designed to enhance the adherence of the cercarial antigen; Dr R Sher, personal communication). Stock antigen, comprising a suspension of formalin-fixed cercariae, was prepared as described in Appendix C. Prior to coating of slides with antigen the required number of cercariae were withdrawn from the stock suspension, following which they were washed three times with distilled water by sedimentation. They were then resuspended, at a final concentration of 2000-3000 cercariae/ml, in a 0.166% (mass/vol) solution of bovine serum albumin (BSA; Sigma, St Louis, USA). Ten microlitre aliquots of this suspension, each containing 20-30 cercariae, were pipetted into the wells of the Multitest slides, which were then air-dried overnight at room temperature and stored in a dessicator at 4 C.

Test sera (15 microlitres/well), appropriately diluted with phosphate buffered saline (PBS; 0.01M, pH 7.1) containing 2% heat-inactivated normal rabbit serum, were incubated with cercarial antigen in the wells of test slides for 30 minutes at room temperature in a moist chamber. Slides were then subjected to three 5 minute washes with PBS containing 0.2% (v/v) Tween 20 (Sigma Chemical Co., USA), whereupon they were air-dried (with the aid of a domestic hair-drier). Five microlitres of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Sigma Chemical Co., USA), diluted 1 in 10 with PBS containing Evans Blue (used as a counterstain), at a final concentration of 0.005% (mass/vol), were then added to each well. Slides were thereupon incubated, washed and air-dried as above, following which they were examined under a Zeiss fluorescent microscope. Cercarial fluorescence in test wells was compared with that of known positive and negative controls. The intensity of fluorescence was graded visually on a 5-point scale (i.e. -, +/-, +, ++ and +++, with +/- being regarded as negative).

3.2.8 Weighing of Animals and Organs

Animals were weighed to the nearest 0.1g after the induction of anaesthesia (i.e. once they were sufficiently inactive to permit accurate weighing). Livers and spleens were weighed to the nearest 0.01 and 0.001g, respectively. Organ masses were expressed as percentages of body mass.

3.2.9 Measurement of Granulomas

Specimens of liver and intestines were fixed in 10% buffered formalin and processed routinely for paraffin sectioning. Sections (3-5 microns thick) were stained with haematoxylin and eosin. Only granulomas surrounding single eggs containing mature miracidia were considered suitable for measuring. Measurements were carried out with the aid of a drawing tube attached to a Zeiss compound microscope and a Hewlett Packard graphics tablet linked to an HP 86 Hewlett Packard microcomputer (Figure 3.2). Areas of individual granulomas were determined from perimeter measurements, using a computer program devised by Dr A C L Opitz (personal communication). Data were converted from computer units into microns by means of a conversion

factor, calculated from the mean of 10 measurements of a standard (known) length of 200 microns; this calibration procedure was carried out with the aid of a stage micrometer on each occasion that granulomas were to be measured.

Each tissue section was thoroughly scanned in order to locate as many suitable granulomas as possible, and the measurements obtained were used to calculate mean granuloma sizes for each animal. Individual means were in turn used in the calculation of group means. Measurements were done 'blind' (i.e. without knowledge of the duration of infection pertaining to the tissue specimens being viewed), and calculations were performed with the aid of the above-mentioned computer programme.

3.2.10 Determination of Liver Egg Densities

Tissue digestion and egg counts were carried out as described in Section 2.2.7. Liver egg burdens were expressed in terms of eggs per gram of wet tissue.

3.2.11 Assessment of Mortality Rates

Mortality rates were determined by exposing groups of 25 animals to cercariae and thereafter monitoring them for mortalities on a daily basis until 15 weeks after the onset of tissue egg-deposition.

3.2.12 Statistical Methods

When it was necessary to correct for time in comparing groups, the one-way analysis of covariance (Snedecor and Cochran, 1989), at the Bonferroni adjusted level of significance (Neter and Wasserman, 1974) was used.

When data from more than two time intervals within an individual study were compared, a one-way analysis of variance (Montgomery, 1984) was employed to assess whether any differences existed; specific differences were identified from pairwise comparisons, at the Bonferroni adjusted level of significance. Since the data in the

present study indicated the use of non-parametric procedures, the Kruskal-Wallis one-way analysis of variance (Siegel and Castellan, 1989) was used, and pair-wise comparisons were done by means of the Mann-Whitney test (Siegel and Castellan, 1989).

3.3 RESULTS

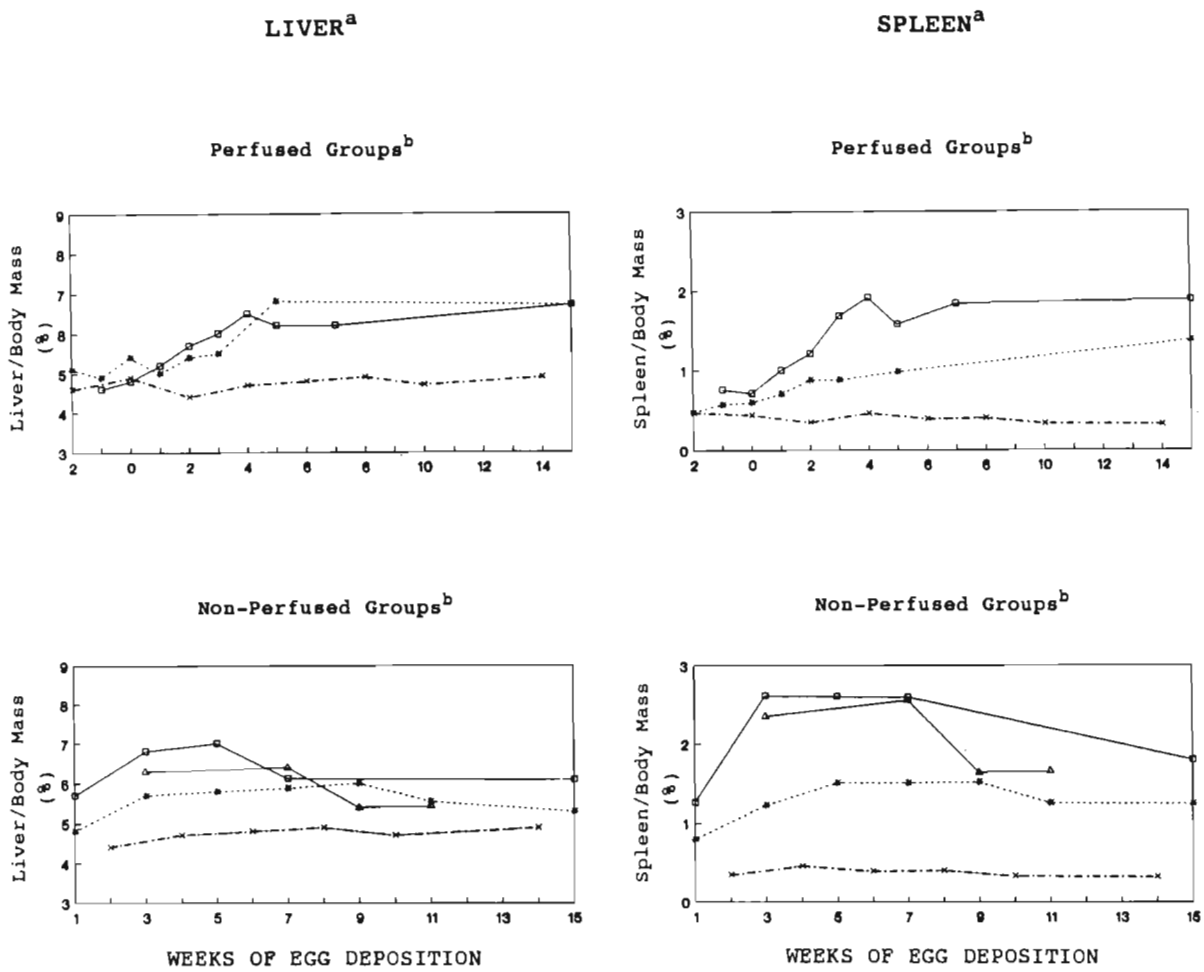
Data pertaining to mice infected with *S. margrebowiei* were obtained from two separate experiments, referred to in this chapter as Studies 1 and 2, the first of which included both perfused and non-perfused animals, and the second only non-perfused animals. In both studies the animals were exposed to approximately 36 cercariae each. Data pertaining to *S. leiperi*-infected mice were derived from a single experiment which included both perfused and non-perfused animals, exposed initially to approximately 45 cercariae each. It should be noted that animals included in the first study with *S. margrebowiei* and the study with *S. leiperi* were infected simultaneously with those of the initial infection characteristics studies discussed in the previous chapter. Worm load data pertaining to these two groups can therefore be obtained from Table 2.1. Worm loads were not assessed in the second *S. margrebowiei* study.

The various parameters assessed in the present studies were, in general, seen to remain within normal limits prior to sexual maturation of the female worms, i.e. before eggs were deposited in the tissues. It was thus decided to express results in terms of period of egg deposition, rather than period of infection (as was used in the previous chapter), assuming pre-maturation phases of 5 and 7 weeks for *S. margrebowiei* and *S. leiperi*, respectively.

3.3.1 Hepatosplenomegaly

Both *S. margrebowiei*- and *S. leiperi*-infected animals developed significant hepatic and splenic enlargement (Figure 3.3). An examination of the data from the perfused groups, which were studied from shortly before sexual maturation of the schistosomes, reveals that the onset of marked organomegaly corresponded approximately with the onset of egg deposition. As a rule it was noted that organ sizes increased rapidly during the first 3-5 weeks after the onset of

FIGURE 3.3 Hepatomegaly and splenomegaly in BALB/c mice resulting from infection with *S. margrebowiei* and *S. leiperi*.



—×—, Controls; —□—, *S. margrebowiei* Study 1; —△—, *S. margrebowiei* Study 2; ···•···, *S. leiperi*.

a Liver and spleen masses are expressed as percentages of body mass. Sample sizes at the various time intervals ranged from 6 to 9 (mostly 7) in the case of perfused groups and from 4 to 5 in the case of non-perfused groups (including controls). Controls comprise non-perfused, non-infected, age- and sex-matched male animals. Longitudinal data are not available from perfused controls. However, comparison of perfused and non-perfused controls at a single time interval (two groups of 15, ten week old males), showed that perfusion has no significant effect on the liver:body mass ratio, but causes a significant reduction in the spleen:body mass ratio ($P < 0.01$, Mann-Whitney test).

b All infected groups, whether perfused or not, differ significantly from controls at the Bonferroni adjusted level of significance ($P < 0.01$; analysis of covariance, with time as cofactor). However, data from perfused and non-perfused animals are presented separately, since analysis of covariance revealed significant differences between equivalent perfused and non-perfused groups (*S. margrebowiei* groups [Study 1], $P < 0.05$ and 0.001 for liver and spleen data respectively; *S. leiperi* groups, $P < 0.05$ and 0.01 for liver and spleen data respectively).

oviposition, but remained more-or-less stable thereafter. Spleen masses in animals infected with *S. margrebowiei* increased by more than 500% in relation to controls, whereas the degree of increase in animals infected with *S. leiperi* remained below 300%.

3.3.2 Liver Egg Densities and Patterns of Egg Deposition

Liver egg burdens in animals infected with *S. margrebowiei* were consistently higher than those in animals infected with *S. leiperi*, at equivalent periods of tissue egg deposition (Figure 3.4). An examination of tissue sections revealed that *S. margrebowiei* ova were often deposited in large clusters, whereas those of *S. leiperi* were typically deposited in smaller, looser groups.

3.3.3 Portal Pressure Measurements and Evidence of Portal-Systemic Collateral Circulation

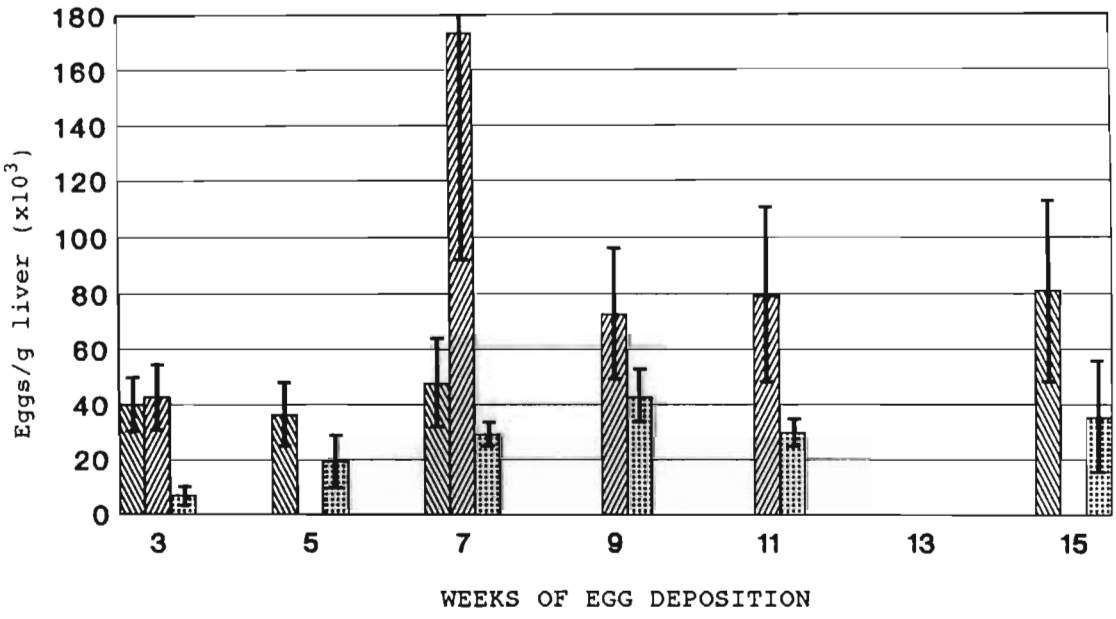
Portal venous blood pressure levels in the infected mice were markedly raised (Figure 3.5). As a whole the degree of hypertension in *S. margrebowiei*- and *S. leiperi*-infected animals was similar, although the highest increases at specific intervals were seen in the latter.

While detailed quantitative records were not kept, clear evidence of the development of portal-systemic collateral circulation was seen in many of the animals used for portal pressure determinations. Extensive enlargement and tortuosity of portal vein tributaries was frequently observed, particularly at the later study intervals, although some measure of these changes was seen in some animals even at the earliest intervals (i.e. 3 and 5 weeks of egg deposition for *S. margrebowiei*- and *S. leiperi*-infected mice, respectively).

3.3.4 Granuloma Measurements

Difficulties were experienced in locating large numbers of suitably discrete granulomas which met the specified selection standards. This was due partially to the fact, as mentioned above (Section 3.3.2), that ova were more often than not deposited in close proximity to one

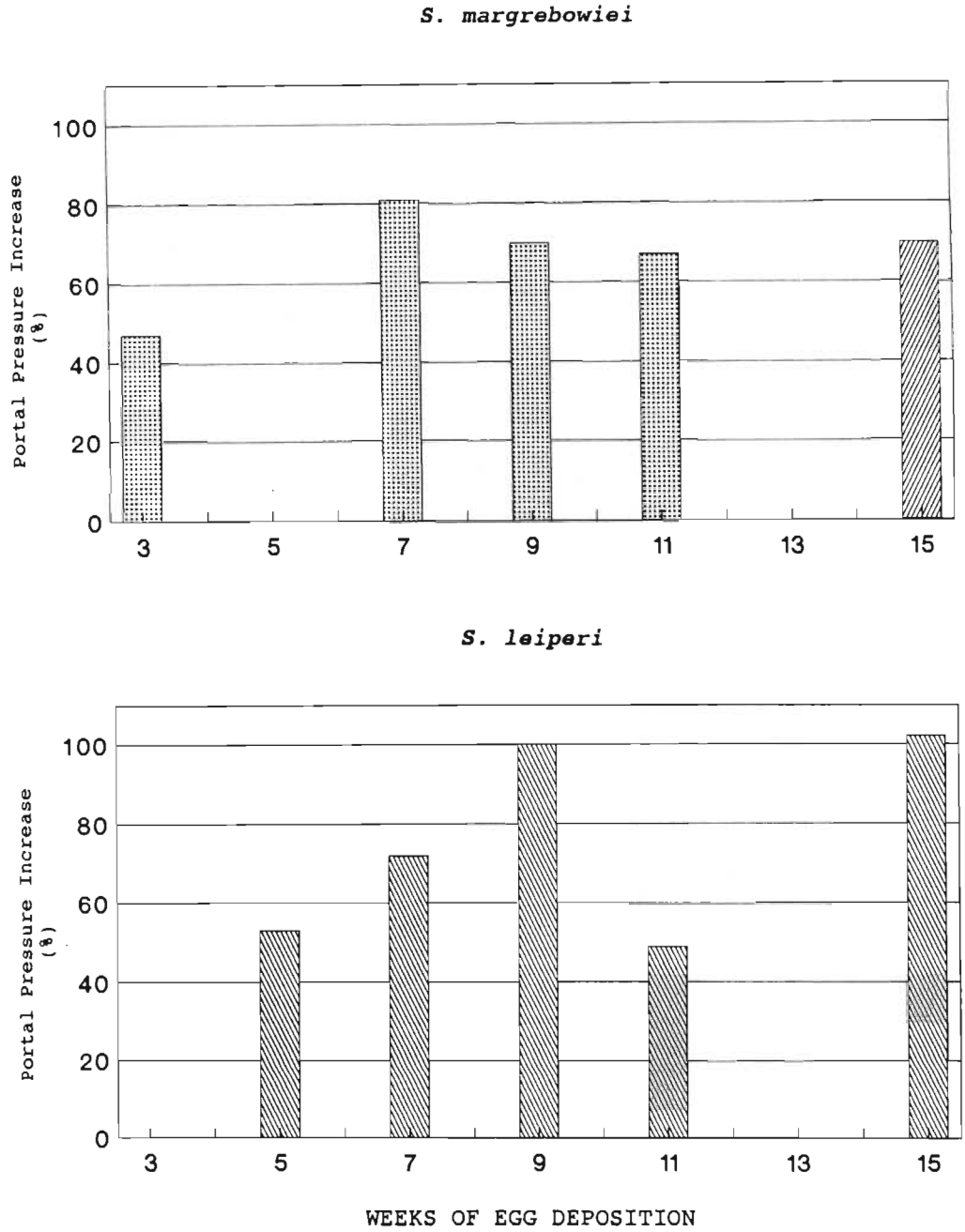
FIGURE 3.4 *S. margrebowiei* and *S. leiperi* liver egg densities (eggs/g wet tissue) in the various groups used for the measurement of portal hypertension and granulomatous responses (Figures 3.5 and 3.6, respectively).



▨, *S. margrebowiei* Study 1; ▨, *S. margrebowiei* Study 2; ▨, *S. leiperi*

Sample sizes ranged from 4 to 15. This variation is in part due to the fact that, where available, data from additional animals belonging to corresponding groups, but used for purposes other than the measurement of portal hypertension or granulomatous responses, were included for the calculation of group means. Vertical bars indicate standard deviations.

FIGURE 3.5 Portal hypertension at various intervals after the onset of egg-deposition in mice infected with *S. margrebowiei* and *S. leiperi*.



, *S. margrebowiei* Study 1; , *S. margrebowiei* Study 2; , *S. leiperi*

The number of infected animals studied at the various intervals ranged from 4 to 12, except in the case of the first *S. margrebowiei* interval, where only 3 animals were tested. Group means are expressed in terms of percentage increase relative to non-infected controls. Portal pressure readings in the infected groups differ significantly from those of controls ($P < 0.01$; analysis of covariance with time as co-factor).

another. Consequently the number of hepatic granulomas measured per animal ranged from 3 to 18 (mostly in the range 4-9) and the number of intestinal granulomas per animal ranged from 3-8 (mostly in the range 4-6). Nevertheless, clear evidence of modulation of the granulomatous responses to *S. margrebowiei* and *S. leiperi* ova was obtained (Figure 3.6).

In both the first and second studies with *S. margrebowiei*, the mean areas of hepatic granulomas increased significantly between 3 and 7 weeks of tissue egg deposition. The results of the first study demonstrated that substantial modulation occurred between 7 and 15 weeks, whilst those of the second study, which was designed to cover the intervening period, indicated that modulation occurred very rapidly after the 7-week interval. Hepatic granuloma areas in *S. leiperi*-infected animals were generally greater than those in their *S. margrebowiei*-infected counterparts, and had already reached peak size by the time of the first study interval (3 weeks of egg deposition). The mean areas of *S. leiperi*-induced granulomas remained stable until 7 weeks of egg deposition, thereafter becoming significantly reduced.

Granulomas surrounding *S. margrebowiei* eggs in the intestinal tissues were characteristically smaller than those in the liver. In the first study the time-related changes in the areas of intestinal granulomas were similar to those in the liver, although the apparent increase in size between 3 and 7 weeks of egg deposition was not statistically significant. While a similar pattern was not evident in the second study, granuloma areas decreased significantly between 3 and 9 weeks of egg deposition. Considerable difficulties were experienced in locating granulomas which met the necessary specifications in the intestinal tissues of *S. leiperi*-infected mice, hence the lack of data in this regard.

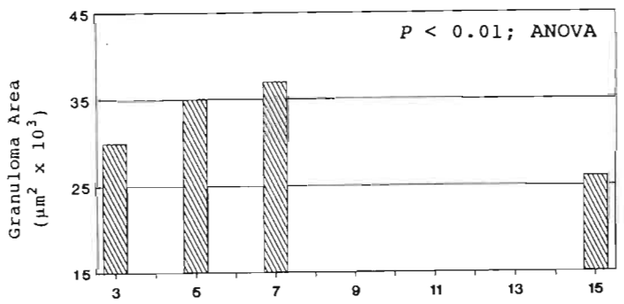
3.3.5 Serum IgG Levels

Virtually no anti-schistosome IgG antibodies were detected in the sera of either *S. margrebowiei*- or *S. leiperi*-infected animals prior to the onset of tissue egg deposition (Table 3.1). However, titres thereafter rose progressively with increasing period of egg deposition.

FIGURE 3.6 Areas of granulomas surrounding mature, embryonated schistosome ova in livers and intestines of mice infected with *S. margrebowiei* and *S. leiperi*, at various intervals after the onset of tissue egg deposition.

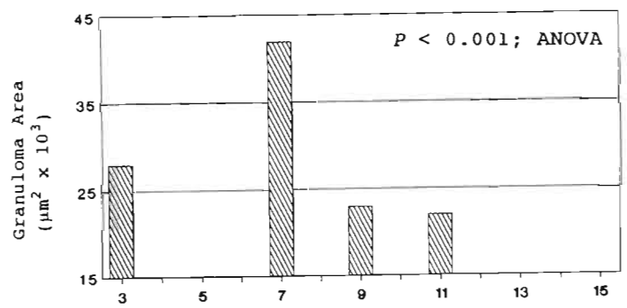
LIVER

S. margrebowiei
(Study 1)



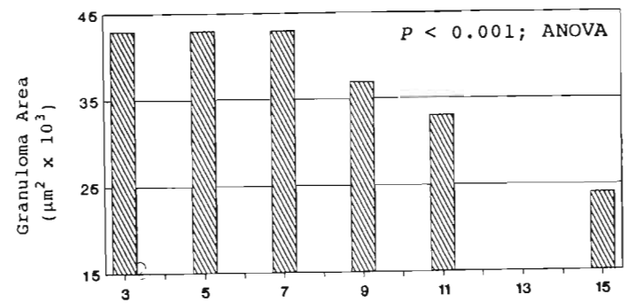
PAIRWISE COMPARISONS	
Intervals showing significant differences	*P*-value
3 versus 5	0.0420
3 versus 7	0.0078*
5 versus 15	0.0016**
7 versus 15	0.0003**

S. margrebowiei
(Study 2)



3 versus 7	0.0000***
3 versus 9	0.0012**
3 versus 11	0.0004**
7 versus 9	0.0000***
7 versus 11	0.0000***

S. leiperi
(Study 1)

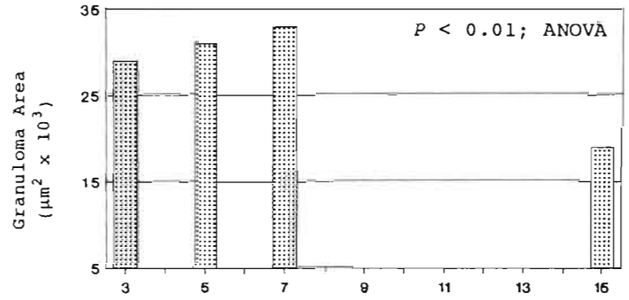


5 versus 9	0.0109
5 versus 11	0.0009**
7 versus 9	0.0084
7 versus 11	0.0007**

WEEKS OF EGG DEPOSITION

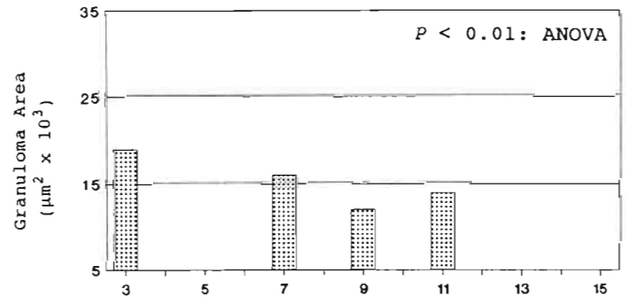
INTESTINES

S. margrebowiei
(Study 1)



PAIRWISE COMPARISONS	
Intervals showing significant differences	*P*-value
3 versus 15	0.0080*
5 versus 15	0.0041*
7 versus 15	0.0017*

S. margrebowiei
(Study 2)



3 versus 9	0.0006**
3 versus 11	0.0065*
7 versus 9	0.0248

WEEKS OF EGG DEPOSITION

Liver granuloma data were obtained from 4 or 5 animals at each time interval, except in the cases of the 11 week *S. margrebowiei* (Study 2) and 15 week *S. leiperi* groups, where acceptable data were only available from 3 animals and a single animal, respectively. Intestinal granuloma areas at the various intervals are based on data from 3 to 5 animals. No data are presented for GIT granulomas in *S. leiperi*-infected mice due to the paucity of ova in the intestinal tissues of these animals. Note that the charts pertaining to liver and intestinal granuloma data differ in terms of the range of sizes on the y-axes.

For each study separately, mean granuloma areas at the various intervals were compared by means of analysis of variance, with pair-wise comparisons. In the case of the *S. leiperi* study the 15 week interval was excluded from the analysis, since only a single measurement was available. Results are shown in tabular form beside each chart; only those comparisons yielding *P* values less than 0.05 are shown. Bonferroni adjusted levels of significance are indicated by means of asterisks, as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 3.1 Total anti-cercarial IgG titres in the sera of BALB/c mice infected with *Schistosoma margrebowiei* and *S. leiperi*.

	WEEKS OF EGG DEPOSITION										
	-1	0	1	2	3	4	5	7	9	11	15
***S. margrebowiei*-infected animals**[a]											
Geometric Mean of Inverse Titres[b]	6	0	2	7	16	23	54	53	47	25	152
Range of Inverse Titres	0-20	0	0-80	0-80	0-80	0-160	10-160	10-320	40-80	0-160	10-640
n	7	7	12	7	16	7	11	17	4	5	13
***S. leiperi*-infected animals**[a]											
Geometric Mean of Inverse Titres[b]	0	0	3	1	11	ND	5	ND	112	47	42
Range of Inverse Titres	0	0	0-20	0-10	0-40	-	0-80	-	80-160	10-80	10-160
n	7	7	7	7	7	-	5	-	4	4	13

a Sera were tested against cercarial antigens derived from a Puerto Rican strain of *S. mansoni* (see Chapter IV). It was established in preliminary tests that the titres obtained with this antigen were little different from those obtained when species-specific cercarial antigens were used (unpublished observations).

b For purposes of serological testing sera were double-diluted, starting at a dilution of 1 in 10.

3.3.6 Mortalities

Mortalities attributable to *S. margrebowiei* (first study) and *S. leiperi* infections were first observed during the fourth and sixth weeks of egg deposition, respectively (Figure 3.7) (the early death in the *S. leiperi*-infected group is regarded as incidental). The majority of deaths in the *S. margrebowiei*-infected group occurred before the end of the eleventh week of egg-deposition, whereas in the *S. leiperi*-infected group deaths were recorded at more-or-less regular intervals throughout the observation period. Fewer survivors remained in the latter group than in the former at the end of the study period.

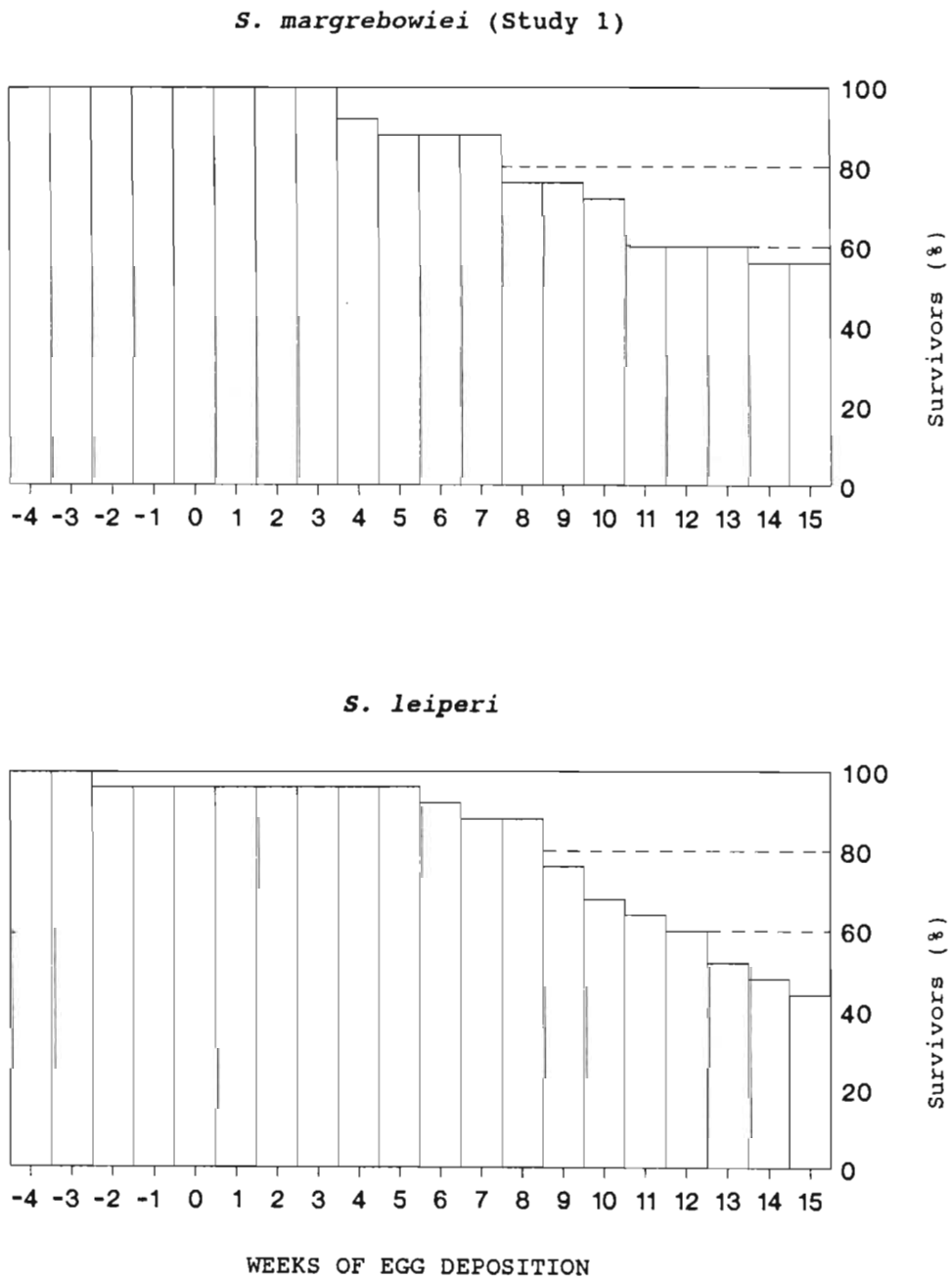
With respect to the second study with *S. margrebowiei*, it should be noted that, while animals were not specifically set aside for monitoring of deaths, losses were far more severe. Thus, by the end of the seventh week of egg deposition more than 50% of the original group of animals had died as a result of the infection, and, due to lack of animals, the experiment could not be continued beyond 16 weeks of egg deposition.

Autopsies were carried out on many of the dead animals and revealed that deaths were often attributable to massive intestinal haemorrhage, almost invariably into the caecum.

3.4 DISCUSSION

The investigations described here demonstrate that BALB/c mice infected with *S. margrebowiei* and *S. leiperi* exhibit essentially identical responses to those observed in a variety of mouse strains infected with *S. mansoni* and *S. japonicum* (Cheever, 1965 and 1985; Dean *et al*, 1981; Warren, 1982), with the development of the major pathological sequelae occurring only subsequent to the onset of oviposition. The large numbers of eggs deposited in the tissues evoked vigorous granulomatous reactions, leading to hepatomegaly, splenomegaly, portal hypertension, portal-systemic collateral circulation and, in some instances, death. Evidence of down-regulation of the granulomatous responses during the more advanced stages of infection was also obtained.

FIGURE 3.7 Cumulative mortalities of BALB/c mice infected with *S. margrebowiei* and *S. leiperi*.



No mortalities were recorded in groups of 10 age- and sex-matched non-infected control animals over the same periods of observation.

The finding that the liver and spleen masses of *S. margrebowiei*- and *S. leiperi*-infected mice rose rapidly following the onset of oviposition and stabilized a few weeks thereafter (Figure 3.3) corresponds with observations made previously with respect to murine schistosomiasis mansoni (Raslavicius, 1965) and schistosomiasis japonica (Warren and Moore, 1967). A similar stabilization of hepatomegaly has also recently been noted in *S. mansoni*-infected hamsters (Morgan *et al*, 1990). The fact that the extent of hepatomegaly in *S. margrebowiei*- and *S. leiperi*-infected animals was similar, in spite of the considerable difference in liver egg loads (Figure 3.4) suggests that maximal levels of hepatic enlargement had occurred in both instances. This is supported by previous investigations on *S. mansoni* and *S. japonicum* which reveal that liver sizes in infected mice are seldom more than about 60-70% greater than those of non-infected controls (Cheever, 1965; Warren and Moore, 1967; Dean *et al*, 1981). During the present study the degree of hepatic enlargement was generally somewhat lower than that reported in these previous studies, i.e. it seldom exceeded 40-45%. This may simply be a reflection of strain-related differences in host responses since the magnitude of hepatomegaly in a variety of mouse strains has been shown to differ substantially (Dean *et al*, 1981). However, this remains to be verified since no references comparing hepatic responses in BALB/c mice with those of other strains could be located in the literature consulted.

As discussed by Dumont *et al* (1975) splenomegaly in schistosomiasis is often assumed to be predominantly a consequence of portal hypertension (i.e. 'congestive splenomegaly'). However, studies by these authors, involving omental (intraperitoneal) and subcutaneous splenic autotransplants in mice, have provided strong evidence that a substantial component of splenic enlargement occurs independently of portal congestion and can instead be ascribed to hyperplasia of the white pulp, presumably resulting from intense antigenic stimulation. Similar observations were also made by Raslavicius (1965) in studies involving the parabiotic union of infected and non-infected mice. In the investigations with the antelope schistosomes described here, it was found that the levels of portal hypertension in *S. margrebowiei*- and *S. leiperi*-infected mice showed no obvious differences (Figure 3.5), whereas those of splenomegaly appeared to reflect the differences in tissue egg burdens (Figure 3.4), being considerably higher in *S. margrebowiei*-infected animals (Figure 3.3).

These observations provide some additional evidence that splenomegaly may be strongly influenced by the magnitude of antigenic exposure and is not simply a function of portal congestion.

The absence of anti-schistosome IgG responses until well after the onset of oviposition (Table 3.1) is further evidence of the apparent lack of host disturbance by the immature stages of *S. margrebowiei* and *S. leiperi* in mice. A distinct relationship between the onset of egg-deposition and the appearance of antibodies which cross-react with antigens expressed on the surfaces of cercariae, schistosomula and adult worms has been demonstrated in murine schistosomiasis mansoni. Thus, Magalhaes-Filho *et al* (1965) found that mice infected with *S. mansoni* only developed detectable titres of cercaria-binding antibodies shortly after the commencement of egg laying. Time course studies by Omer-Ali *et al* (1988) have shown that the production of IgG antibodies which bind to *S. mansoni* schistosomulum carbohydrate epitopes only begins about a week after the onset of oviposition and increases very rapidly thereafter. Virtually the same pattern with respect to the production of IgG_1 and IgM antibodies against adult worm antigens was observed by Bout *et al* (1980); IgA levels increased only some weeks after, and at a slower rate, than those of the other antibody classes.

There have been no previous reports on the dimensions of, and time-related changes in the granulomatous responses to the eggs of the antelope schistosomes. Although the measurements presented in Figure 3.6 are based on small sample sizes, especially by comparison with those in published studies on *S. mansoni* and *S. japonicum* (often 20 or more granulomas per individual organ; Colley and Freeman, 1983; Cheever *et al*, 1984), the results obtained appear to be reasonably credible since, for the most part, they are consistent in showing a well-defined peak, followed by a highly significant reduction in granuloma areas. The reasons for the comparatively ill-defined pattern of size change and the relatively small sizes of the intestinal granulomas in the second study with *S. margrebowiei* are unclear. On the one hand, it may be a reflection of the small sample sizes. Alternatively, it may be related to the fact that measurements were not restricted to any specific regions of the intestines, since it has been shown that modulation of the granulomatous response occurs in certain parts of the intestines (e.g. colon) but not in others (e.g. ileum) (Weinstock and Boros, 1981).

Spontaneous regression of the granulomatous reactions to the ova of *S. mansoni* and *S. japonicum* in mice has consistently been observed to occur after about 3-5 weeks of egg deposition (Colley and Freeman, 1980 and 1983; Weinstock and Boros, 1981; Cheever *et al*, 1984; Cheever, 1985). By comparison, modulation of the responses to *S. margrebowiei* and *S. leiperi* eggs appeared to occur more slowly, not being observed until after 7 weeks of egg deposition.

Granuloma sizes in the murine model have been shown to be subject to considerable host strain-related variation (Colley and Freeman, 1983; Cheever *et al*, 1984 and 1987); there is also some evidence of host gender-related differences (Cheever *et al*, 1987). Furthermore, it has been demonstrated that the size of hepatic granulomas in *S. japonicum*-infected mice decreases with increasing liver egg burden (Cheever, 1986). Direct comparison of granuloma sizes recorded during the present study with those reported in previously published studies, most of which involve *S. mansoni* and *S. japonicum*, is thus considered to be unwarranted. It is clear that in experiments aimed at comparing the granulomatous responses evoked by the ova of different schistosome species, an effort should be made to achieve some measure of standardization in terms of the host strain used, the duration of egg deposition, and the density of eggs in the tissues. This in turn requires the availability of reliable data on infection characteristics, such as those presented in the previous chapter.

The observation that many of the mortalities recorded in the present investigation were attributable to haemorrhage into the caecum corresponds with the findings of Kagan *et al* (1954), who reported this to be the main cause of death in mice infected with *Schistosomatium douthitti*. On the basis of the first study with *S. margrebowiei* in BALB/c mice it appeared that the pathogenicity of this schistosome was similar to that of *S. leiperi* (Figure 3.7). However, the rapid and high mortality rate experienced during the second study, as well as unpublished observations made during the course of other experiments not dealt with here, have led to the conclusion that, at least at similar worm pair loads, *S. margrebowiei* must be regarded as the more pathogenic of the two antelope schistosomes in mice. Since mortalities are generally recognized to be the result of egg-induced pathology, as evidenced by the fact that deaths in the present and other studies (e.g. Cheever *et al*, 1984) were almost invariably seen to occur some time after the onset of

tissue egg deposition, this more than likely reflects the difference in the egg output of these two species.

The observation that *S. margrebowiei* is highly pathogenic in BALB/c mice corresponds with previous reports of high mortalities in other types of laboratory rodents, namely *S. campestris* and *M. coucha* (Pitchford, 1975), hamsters (Southgate and Knowles, 1977; Ogbe, 1985), and gerbils (Ogbe, 1985). Surprisingly, Ogbe (1985) apparently did not observe any mortalities in mice of the TFI strain exposed to approximately 100 cercariae each (i.e. almost three times the cercarial load used in the present investigation) and studied over some 17 weeks of infection. This is probably due, at least in part, to the fact that worm burdens in these animals dropped off rapidly after 8-9 weeks of infection (i.e. about 3-4 weeks after the start of oviposition). However, an identical worm recovery pattern was recorded from hamsters, which did suffer mortalities, suggesting that TFI mice may be particularly resistant to the factors which normally result in deaths. This explanation seems plausible in the light of studies by Dean *et al* (1981) who noted marked differences in the mortality rates of various strains of mice infected with a Puerto Rican strain of *S. mansoni*. There have been no previous reports on the pathogenicity of *S. leiperi* in rodent models.

From the preceding discussion it is clear that the responses elicited by *S. margrebowiei* and *S. leiperi* in BALB/c mice do not differ in any profound way from those seen in previous studies on other schistosomes, at least in terms of the parameters considered in this study. However, it is suggested that further comparative studies on the antelope schistosomes might be worthwhile, since it is possible that either or both species might prove useful as alternative models for studies on disease processes. In the case of *S. margrebowiei*, for example, infections in the mouse bear some similarities to those of *S. japonicum*, in that eggs are produced in much greater numbers than those of *S. mansoni*, and are deposited predominantly in the intestines and in clusters rather than singly. Thus, just as *S. bovis* has been recommended as an analogue of *S. haematobium* for certain types of studies (Agnew and Doenhoff, 1989), *S. margrebowiei* may prove useful as an analogue of *S. japonicum*. In the present author's experience *S. margrebowiei* is a reasonably easy parasite to maintain in the laboratory, largely due to the ease of maintenance and production of the *Bulinus tropicus* snails used as its intermediate

hosts. In contrast, it would appear that the production and handling of *S. japonicum* is somewhat more difficult, especially for the inexperienced worker, since its amphibious intermediate host (*Oncomelania hupensis hupensis*) seems to require fairly specialised maintenance conditions (Moloney *et al*, 1987a).

In-depth analyses of the processes of granulomatous inflammation and of the factors controlling modulation of these processes represents an area in which further animal model investigations on *S. margrebowiei* and/or *S. leiperi* may prove to be of value. The composition of hepatic granulomas, the dynamics of pulmonary granuloma formation, and the immunological processes underlying the modulation of the granulomatous responses induced by the eggs of *S. mansoni* and *S. japonicum* have been shown to differ (summarized by Stavitsky, 1987). There also appear to be some differences in the degree of hepatic fibrosis caused by these two species (Cheever *et al*, 1984). Furthermore, whereas the eggs of *S. mansoni* are known to excrete at least one hepatotoxic antigen, those of *S. japonicum* apparently do not (Dunne and Doenhoff, 1983; Dunne *et al*, 1991). It would be of considerable interest to ascertain the degree of homology or dissimilarity between the eggs of *S. margrebowiei* and *S. leiperi* and those of *S. mansoni* and *S. japonicum* in regard to such parameters.

Garcia *et al* (1982 and 1987) have de-emphasized the role of endogenous immunoregulation in granulomatous modulation, largely on the basis of evidence that immune responses to schistosome eggs are directed in particular against the miracidium. As these responses mature they are thought to increasingly interfere with miracidial development (referred to as 'anti-embryonation immunity'), resulting in a corresponding decrease in antigen excretion. Hence it is suggested that the time-related decrease in the magnitude of granulomatous reactions reflects a decrease in the immunogenicity of ova. More recently, Mitchell *et al* (1990) have extended this hypothesis, suggesting that female miracidia may evoke more vigorous immune responses than males, resulting in a more rapid selective depletion of eggs containing female miracidia. It is thus postulated that decreasing granuloma sizes to some extent reflects an increasing proportion of less immunogenic male eggs. This postulate originates from and is used to explain the observation that sex ratios of worms in both natural (in the case of *S. japonicum*) and experimental (both

S japonicum and *S. mansoni*) infections are often biased towards males. The question arises as to whether these hypotheses are valid for species other than *S japonicum* and *S. mansoni*. In this regard, studies on the antelope schistosomes might provide useful insights and would seem to be appropriate, in view of the aforementioned observation (see Section 2.4) that there is as yet no evidence of a male bias in respect of worms recovered from infected hosts.

CHAPTER FOUR

HOMOLOGOUS AND HETEROLOGOUS CONCOMITANT IMMUNITY STUDIES WITH *SCHISTOSOMA MARGREBOWIEI* AND *S. LEIPERI* IN BALB/c MICE

4.1 INTRODUCTION

There have been numerous experiments in animal models aimed at assessing the ability of primary infections with schistosomes to limit the development of subsequent infections with either the same (homologous) or different (heterologous) schistosome species. The animal hosts most extensively utilised in these investigations include mice, hamsters, rats, rhesus monkeys, baboons, sheep and cattle. For comprehensive coverage of most of this work, which has played a fundamental role in shaping current perceptions of the immunology of schistosomiasis, the reader is referred to the reviews by Smithers and Terry (1969a), Dean (1983), Damian (1984) and Christensen *et al* (1987). As discussed in Section 1.9.4, some of the earliest and most extensive work on interactions between heterologous schistosome species was aimed directly at providing experimental evidence in support of the theory of zooprophylaxis, as applied to schistosomiasis by Nelson *et al* (1962).

One of the most important developments to emerge out of the early work on the immunology of schistosomiasis was the concept of 'concomitant immunity'. This term, adopted from the field of cancer immunology and applied to schistosomiasis by Smithers and Terry (1969b), refers to the observation that whereas animals were unable to eliminate worms established from an initial infection, they were often highly resistant to subsequent superinfection. Although this observation was first made in studies with *S. mansoni* in rhesus monkeys (Smithers and Terry, 1965a), it was subsequently repeatedly confirmed in experiments with a wide variety of other schistosome and host species (see the above-mentioned reviews).

The most widely utilised host in studies based on the concomitant immunity model has undoubtedly been the mouse (Dean, 1983). The majority of experiments in this host have involved initial exposure to *S. mansoni* followed by homologous challenge, although there have also been a number of similar experiments involving *S. japonicum*, *S.*

bovis and *Schistosomatium douthitti* (results summarized by Dean, 1983). In addition, various studies have been conducted in which initial and challenge exposures were carried out with heterologous species; the range of species used in such investigations (i.e. either for initial or challenge infections) includes *S. mansoni*, *S. haematobium*, *S. mattheei*, *S. bovis*, *S. rodhaini*, *S. japonicum*, *S. mekongi*, *S. spindale*, *S. indicum*, *S. incognitum*, *Ornithobilharzia turkestanicum*, *Schistosomatium douthitti*, *Heterobilharzia americana*, and *Trichobilharzia szidati* (Dean, 1983; Christensen *et al*, 1987; Janecharut *et al*, 1988). Perhaps the two most characteristic features to emerge from these many experiments are (i) that acquired resistance is usually only partial - the actual levels of resistance observed in different studies have been found to differ widely, clearly being subject to the influences of numerous methodological and host- and parasite-related variables (reviewed by Dean, 1983), and (ii) that substantial resistance normally only develops some weeks after exposure to a bisexual primary infection - significant reductions in challenge infection worm or egg loads have only rarely been observed in animals previously exposed to unisexual infections or to non-maturing species.

Concomitant immunity studies involving the antelope schistosomes have not previously been carried out. The aim of the experiments described in this chapter was thus to assess and compare the effects of prior infection with *S. margrebowiei* or *S. leiperi* on the subsequent development of homologous or heterologous challenge infections in the inbred BALB/c mouse model. Although Pitchford had suggested that the antelope schistosomes might limit the spread of three other species, namely *S. mansoni*, *S. haematobium* and *S. mattheei* (see Section 1.10.5), for practical purposes it was decided to use only one of these species in heterologous challenge experiments. *S. mansoni* was chosen in preference to *S. mattheei* because of its greater relevance in terms of human disease, and in preference to *S. haematobium* because of its greater compatibility with the mouse. However, since the infection characteristics of a South African (RSA) isolate of *S. mansoni* had been found to differ markedly from those of a Puerto Rican (PR) isolate (Higgins-Opitz and Dettman, 1991), it was decided to carry out challenge exposures with both isolates, in order to determine whether or not they also differ in terms of their ability to develop in previously infected animals. The mouse model was chosen largely because of its availability and ease of handling, and the

fact that it has been extensively utilised in studies of a similar nature, as mentioned above.

4.2 MATERIALS AND METHODS

4.2.1 Schistosomes

Details regarding the origins and maintenance of *S. margrebowiei* and *S. leiperi* are presented in Section 2.2.1. The RSA strain of *S. mansoni* (originally isolated in 1959 from an infected man at Coopersdal in the Eastern Transvaal; P S Visser, personal communication) was obtained from the Bilharzia Field Research Unit (Nelspruit) and the PR strain was obtained from The London School of Hygiene and Tropical Medicine. *Biomphalaria pfeifferi* and *B. glabrata* served as the intermediate hosts for the RSA and PR strains, respectively; *M. coucha* was used as the definitive host for both strains. Specific methods of handling the various schistosomes and their snail hosts are described in Appendix A.

4.2.2 Experimental Hosts

Male mice were used throughout; details regarding the origins, housing and maintenance of the animals are furnished in Section 2.2.2. Within individual experiments animals were age-matched to within one week of each other; the approximate ages of animals in the different experiments, at the time of first infection, ranged from 6 to 9 weeks.

4.2.3 Methods of Infecting Animals and of Assessing Worm and Tissue Egg Loads

Mice were infected and anaesthetised according to the methods described in Sections 2.2.3 and 2.2.5, respectively. Worm loads and tissue egg loads were determined as described in Sections 2.2.6 and 2.2.7, respectively.

4.2.4 Experimental Design

Concomitant immunity experiments followed the conventional design (Smith and Clegg, 1979). Experimental (EXP) groups consisted of those in which animals were exposed to both an initial and a subsequent challenge infection. Each EXP group required two control groups: the Initial Control (IC) group received the initial infection only, while the Challenge Control (CC) group received the challenge infection only. Unless otherwise indicated, challenge infections were carried out at two independent intervals in each experiment, viz. immediately before, and about 4 weeks after the onset of oviposition by the initial infection worms. On the basis of the results presented in Chapter 2 it was assumed that egg-laying by *S. margrebowiei* commenced between 4.5 and 5 weeks of infection, and that by *S. leiperi* between 6.5 and 7 weeks of infection. Similarly, on the basis of previous studies (Higgins-Opitz and Dettman, 1991), it was assumed that oviposition by the PR strain of *S. mansoni* commenced between 4.5 and 5 weeks, and that by the RSA strain between 7 and 7.5 weeks of infection.

In experiments where animals were challenged with either *S. margrebowiei* or PR *S. mansoni*, worm and tissue egg loads were assessed 6 weeks after challenge, whilst in animals challenged with either *S. leiperi* or RSA *S. mansoni*, loads were assessed 8 weeks after challenge. In the case of EXP-group animals which had been exposed to heterologous species, it was possible to differentiate between the eggs deposited by the initial and challenge infections on the basis of morphological characteristics, and they were therefore counted separately. An effort was also made to differentiate between the worms derived from the initial and challenge infections. However, in some instances this could not be achieved, or could only be achieved in terms of gravid female worms, which were distinguished by the quantity and shape of ova *in utero* (sexually-mature *S. margrebowiei* and *S. leiperi* females consistently contain multiple ova *in utero*, whereas those of *S. mansoni* harbour only one ovum at a time and may be egg-free at the time of worm recovery).

For all experiments, irrespective of whether they involved homologous or heterologous challenge procedures, the percentage reduction in

challenge-derived worm loads in the EXP groups was estimated according to the formula (Smith and Clegg, 1979):

$$\% \text{ Reduction} = \frac{CC - (EXP - IC)}{CC} \times 100 \qquad \text{(Formula A)}$$

where IC and CC refer to the mean worm loads in the IC and CC groups and EXP to the mean worm load in the corresponding EXP group prior to species differentiation. For purposes of statistical analysis, the mean number of worms recovered in the IC group was subtracted from the individual recoveries in the EXP group and the mean of the resultant values compared with that of the CC group, using the Mann-Whitney test (Siegel and Castellan, 1989).

In addition, where species differentiation was possible, the mean challenge-derived worm and/or egg loads in EXP groups were compared directly with those in the corresponding CC groups, again using the Mann-Whitney test. In these instances the percentage reduction in challenge loads was estimated according to the formula:

$$\% \text{ Reduction} = \frac{CC - EXP_1}{CC} \times 100 \qquad \text{(Formula B)}$$

where EXP_1 refers specifically to worms or eggs derived from the challenge infection.

For each set of experimental results presented in the following section (4.3), specific details of the cercarial infection loads employed and the times at which infections and perfusions were performed are shown in Appendix D.

4.3 RESULTS

The results of the different experiments are presented sequentially in Tables 4.1 to 4.5.2b. The experiments are grouped into three categories, according to whether the initial infections were carried out with *S. mansoni*, *S. margrebowiei* or *S. leiperi*.

4.3.1 Initial Infection with the PR or RSA Strains of *S. mansoni* Followed by Homologous Challenge

When animals were exposed initially to the PR strain of *S. mansoni* (approximately 27 cercariae/mouse) and challenged after 4.5 or 9 weeks, a significant reduction in challenge worm burden (46.5%; $P < 0.01$) was detected only in the latter instance (Table 4.1). The mean numbers of gravid pairs in the IC groups assigned to the 4.5 and 9 week challenge series were 5.4 and 3.8, respectively (data not shown).

The study on the RSA strain of *S. mansoni* differed from all the other studies in that animals were challenged only at a single time interval (i.e. 9 weeks after initial exposure), corresponding with approximately 2 weeks of egg deposition by the initial infection. Initial infections comprised 42 cercariae/animal and the mean number of gravid worm pairs/animal in the IC group was 5.6 (data not shown). The challenge worm load in the EXP group was reduced by an estimated 75.1% ($P < 0.001$) (Table 4.1).

4.3.2 Initial Exposure to *S. margrebowiei* Followed by Homologous or Heterologous Challenge

Two experiments involving initial exposure to *S. margrebowiei* were carried out (Appendix D; Tables D.2 and D.3): in the first, the animals were challenged with either the PR or RSA strains of *S. mansoni*, and in the second they were subjected to homologous challenge only.

Prior exposure of mice to *S. margrebowiei* (approximately 26 cercariae/mouse), resulting in worm returns of approximately 31% and gravid worm pair burdens of 3-3.5/animal (Table 4.2a), caused only mild reductions in PR *S. mansoni* challenge worm loads, even when challenge was administered 9 weeks after initial infection. By comparison RSA *S. mansoni* worm loads were considerably more reduced, particularly in the group challenged at the latter interval. Table 4.2a shows the levels of resistance to challenge in the EXP groups estimated without differentiating between worms of the initial and challenge infections (Formula A). PR *S. mansoni* total worm loads in the groups challenged at 4.5 and 9 weeks were reduced by 21.2% ($P <$

TABLE 4.1 Reduction of homologous challenge induced by the PR and RSA strains of *Schistosoma mansoni* : total worm loads.

Pre-challenge Interval	GROUP[a]	TOTAL WORM LOADS					
		PR Strain[b]			RSA Strain[c]		
		n	$\bar{x}$(s.d.)	Reduction[d] (%)	*n*	$\bar{x}$(s.d.)	Reduction[d] (%)
4.5 weeks							
	IC	13	11.2 (3.3)	-			
	EXP	13	40.9 (6.2)	**11.6**		Not Done	
	CC	11	33.6 (6.8)	-			
9 weeks							
	IC	10	10.8 (2.1)	-	10	13.5 (3.5)	-
	EXP	10	30.5 (11.6)	**46.5****	9	20.4 (8.6)	**75.1*****
	CC	12	36.8 (7.4)	-	10	27.7 (4.3)	-

a IC (initial infection control) groups were subjected to the initial infection only, EXP (experimental) groups to both initial and challenge infections, and CC (challenge infection control) groups to the challenge infection only. Details of the estimated cercarial infection loads used for the initial and challenge infections, and of the intervals between infection and perfusion applicable to the various groups are shown in the experimental plan (Appendix D; Table D.1).

b These data published previously in Dettman and Higgins-Opitz (1989).

c Note that in this instance the challenge infection was carried out at only a single time interval, corresponding with a period of about 2 weeks of egg deposition by the initial infection.

d Levels of statistical significance are indicated as follows: **, $P < 0.01$; ***, $P < 0.001$.

TABLE 4.2a Reduction of heterologous challenge (PR and RSA strains of *Schistosoma mansoni*) following initial infection with *S. margrebowiei*: gravid worm pair and total worm loads.

Pre-challenge Interval	GROUP[a]	*n*	WORM LOADS			
			Gravid pairs		Total	
			$\bar{x}$(s.d.)	Reduction[e] (%)	$\bar{x}$(s.d.)	Reduction[e] (%)
4.5 weeks						
	IC[b]	22	3.5 (1.9)	-	8.9 (3.5)	-
	EXP-PR	9	14.1 (2.1)	**33.3****	37.9 (3.7)	**21.2***
	CC-PR	10	15.9 (3.8)	-	36.8 (7.5)	-
	EXP-RSA[c]	8	ND	-	25.9 (5.2)	**33.6****
	CC-RSA	9	8.0 (2.8)	-	25.6 (4.8)	-
9 weeks						
	IC[b]	14	3.0 (2.4)	-	7.5 (4.0)	-
	EXP-PR[d]	10	12.3 (5.8)	**29.0**	36.4 (18.7)	**25.3**
	CC-PR	10	13.1 (2.8)	-	38.7 (7.4)	-
	EXP-RSA	8	4.5 (1.7)	**87.3*****	18.1 (10.0)	**66.6****
	CC-RSA	10	11.8 (4.6)	-	31.7 (7.9)	-

a IC (initial infection control) groups were subjected to the initial infection only, EXP (experimental) groups to both initial and challenge infections, and CC (challenge infection control) groups to the challenge infection only. The suffixes -PR and -RSA indicate which strain of *S. mansoni* was used for the challenge infection. Details of the estimated cercarial infection loads used for the initial and challenge infections and of the intervals between infection and perfusion applicable to the various groups are shown in the experimental plan (Appendix D; Table D.2).

b The mean IC group values for the 4.5 week challenge series are based on pooled data from animals perfused at the 10.5 and 12.5 week intervals, together with data from a small number of additional animals perfused a few days before or after scheduled dates, on the grounds that they were clearly moribund. Values for the 9 week challenge series are similarly based on pooled data from the 15 and 17 week intervals, but without data from any additional animals.

c The mean gravid worm pair load is not indicated for this group because, in some instances, difficulties were experienced in determining which female worms were gravid.

d Four animals of this group were perfused about 10 days prematurely, since they were moribund. Substantial numbers of sexually-immature *S. mansoni* worms were clearly evident in the perfusates from these animals, and for this reason the mean total worm load for the group is based on data from all 10 animals. However, the mean gravid worm pair value is based only on data from the 6 animals perfused at the scheduled time.

e Levels of statistical significance are indicated as follows:
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

0.05) and 25.3% (non-significant), respectively; the corresponding reductions in the case of the RSA strain were 33.6% and 66.6% ($P < 0.01$ in both cases).

In this study it was possible in most instances to differentiate between *S. margrebowiei* and *S. mansoni* worms recovered from EXP-group animals, due to the distinctive differences in the sizes of the two species; only in the case of the group challenged with the RSA strain at 4.5 weeks could this not be satisfactorily achieved. Levels of resistance based on *S. mansoni* worms alone were found to be very similar to those calculated using undifferentiated worm data. Thus, worm loads of the PR strain in the groups challenged at 4.5 and 9 weeks were reduced by 21.7% ($P < 0.05$) and 30.0% (non-significant), respectively, while in the group challenged at 9 weeks with the RSA strain, the level of reduction was 66.9% ($P < 0.01$) (Table 4.2b).

S. mansoni tissue egg loads were found to be considerably more reduced than was the case for worms (Table 4.2b). Eggs of the PR strain were reduced by 75.5% ($P < 0.001$) and 67.7% ($P < 0.01$) in the groups challenged at 4.5 and 9 weeks respectively. The corresponding reductions in the RSA-challenged groups were 58.1% ($P < 0.05$) and 95% ($P < 0.001$). This was shown to correlate with a reduction in the number of eggs recovered per gravid female in the EXP groups: thus, whereas the reductions in gravid worm pair loads were generally similar to those for total worms, the number of eggs recovered per mature female was reduced by about 55-60%.

In the homologous challenge study (Table 4.3) initial infection worm returns (approximately 25%, based on an infection load of 25 cercariae/animal) and gravid worm pair loads (2.3-2.6/animal) were somewhat lower than those in the previous experiment. Resistance in animals challenged after 4.5 weeks was negligible (16.6%; non-significant), while in animals challenged after 9 weeks it was substantial (60.5%; $P < 0.001$).

4.3.3 Initial Exposure to *S. leiperi* Followed by Homologous or Heterologous Challenge

Two studies involving initial infection with *S. leiperi* were carried out (Appendix D; Tables D.4 and D.5). In the first, the intention was

TABLE 4.2b Reduction of heterologous challenge (PR and RSA strains of *Schistosoma mansoni*) following initial infection with *S. margrebowiei*: *S. margrebowiei* tissue egg loads and comparison of the *S. mansoni* worm loads and egg output in the experimental and challenge control groups.

	S. margrebowiei	*S. mansoni*	
Pre-			

TABLE 4.3 Reduction of homologous challenge induced by *Schistosoma margrebowiei*: gravid worm pair and total worm loads.

Pre-challenge Interval	GROUP[a]	*n*	WORM LOADS			
			Gravid pairs		Total	
			$\bar{x}$(s.d.)	Reduction[b] (%)	$\bar{x}$(s.d.)	Reduction[b] (%)
4.5 weeks						
	IC	8	2.6 (1.2)	-	6.4 (2.1)	-
	EXP	12	9.0 (2.6)	**1.5**	31.5 (5.9)	**16.6**
	CC	10	6.5 (3.6)	-	30.1 (7.3)	-
9 weeks						
	IC	27	2.3 (1.4)	-	6.1 (2.5)	-
	EXP	23	6.2 (3.4)	**62.5*****	17.2 (8.8)	**60.5*****
	CC	22	10.4 (3.6)	-	28.1 (6.4)	-

a IC (initial infection control) groups were subjected to the initial infection only, EXP (experimental) groups to both the initial and challenge infections, and CC (challenge infection control) groups to the challenge infection only. Details of the estimated cercarial infection loads used for the initial and challenge infections are shown in the experimental plan (Appendix D; Table D.3). Perfusions were carried out 6 weeks after challenge infections.

b Levels of statistical significance are indicated as follows: ***, $P < 0.001$.

to assess the levels of resistance to challenge with the two strains of *S. mansoni* in animals harbouring similar total and gravid worm pair loads to those of *S. margrebowiei*, in the experiments reported above. The second study had two objectives: (i) to assess levels of resistance to homologous challenge in animals with initial infections of similar magnitude to those in the first study, and (ii) to assess levels of resistance to both homologous and heterologous challenge in animals with *S. leiperi* infection loads designed to produce similar liver egg burdens at the 11 week challenge interval to those at the 9 week interval in the first experiment with *S. margrebowiei*. With respect to the latter objective, worm and tissue egg-accumulation data from a number of sources, including the infection characteristics studies as reported in Chapter 2, and the first *S. leiperi* concomitant immunity study, were carefully considered in an attempt to determine the infection load that would be required in order to ensure the appropriate liver egg loads.

In the first study it was found that mice initially exposed to an estimated 37 cercariae each and harbouring approximately 2.4-2.9 gravid pairs (worm returns approximately 25%) showed virtually no resistance to challenge after either 6.5 or 11 weeks with the PR strain of *S. mansoni* (Table 4.4a). In contrast, worm loads of the RSA strain were reduced by 31.1% ($P < 0.05$) and 45.2% ($P < 0.01$) in the groups challenged at the first and second intervals, respectively. In this study it was not possible to confidently differentiate between *S. leiperi* and *S. mansoni* worms recovered from twice-infected mice. However, an examination of tissue egg loads revealed that, as in the study with *S. margrebowiei*, *S. mansoni* egg loads were, for the most part, considerably more reduced than was estimated to be the case for worm loads (Table 4.4b). While PR egg loads in animals challenged after 6.5 weeks were not significantly lower than controls, those in the 11 week group were reduced by 56.6% ($P < 0.01$). Reductions in the corresponding RSA-challenged groups were 64.7% and 89.9% ($P < 0.001$ in both cases), respectively.

With respect to the first aspect of the second study with *S. leiperi*, as defined above, the worm loads resulting from the initial infection (estimated load approximately 40 cercariae per mouse) compared favourably with those in the preceding study, particularly in terms of gravid worm pairs (a mean of 2.8/mouse) (Table 4.5.1). However, levels of resistance to homologous challenge were found to be

TABLE 4.4a Reduction of heterologous challenge (PR and RSA strains of *Schistosoma mansoni*) following initial infection with *S. leiperi*: gravid worm pair and total worm loads.

Pre-challenge Interval	GROUP[a]	*n*	WORM LOADS			
			Gravid pairs		Total	
			$\bar{x}$(s.d.)	Reduction[c] (%)	$\bar{x}$(s.d.)	Reduction[c] (%)
6.5 weeks						
	IC[b]	20	2.9 (1.5)	-	9.6 (2.7)	-
	EXP-PR	10	16.9 (3.4)	**-13.8**	47.7 (8.9)	**18.7**
	CC-PR	10	12.3 (3.9)	-	32.1 (9.5)	-
	EXP-RSA	11	7.9 (1.9)	**39.0***	27.1 (5.7)	**31.1***
	CC-RSA	10	8.2 (3.2)	-	25.4 (7.1)	-
11 weeks						
	IC[b]	21	2.4 (1.0)	-	9.1 (3.0)	-
	EXP-PR	10	13.5 (4.9)	**20.7**	44.0 (10.7)	**6.9**
	CC-PR	10	14.0 (5.5)	-	37.5 (8.5)	-
	EXP-RSA	13	5.2 (3.4)	**73.8*****	26.2 (11.1)	**45.2****
	CC-RSA	10	10.7 (3.8)	-	31.2 (6.1)	-

a IC (initial infection control) groups were subjected to the initial infection only, EXP (experimental) groups to both initial and challenge infections, and CC (challenge infection control) groups to the challenge infection only. The suffixes -PR and -RSA indicate which strain of *S. mansoni* was used for the challenge infection. Details of the estimated cercarial infection loads used for the initial and challenge infections and of the intervals between infection and perfusion applicable to the various groups are shown in the experimental plan (Appendix D; Table D 4).

b The mean IC group values for the 6.5 week challenge series are based on pooled data from animals perfused at the 12.5 and 14.5 week intervals, and those of the 11 week challenge series on pooled data from the 17 and 19 week intervals.

c Levels of statistical significance are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 4.4b Reduction of heterologous challenge (PR and RSA strains of *Schistosoma mansoni*) following initial infection with *S. leiperi*: *S. leiperi* tissue egg loads and comparison of the *S. mansoni* tissue egg loads in the experimental and challenge control groups.

Pre-challenge Interval	GROUP[a]	*n*	*S. leiperi* Tissue Egg Loads		*S. mansoni* Tissue Egg Loads	
			Liver	Total[b]	Total[b]	
			$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	Reduction (%)
6.5 weeks						
	IC[c]	10	21 134 (11 243)	22 910 (13 009)	-	-
	EXP-PR	10	14 145 (6 388)	14 903 (6 852)	16 293 (4 183)	**15.1**
	CC-PR	10	-	-	19 189 (4 778)	-
	IC	10	29 540 (15 293)	33 564 (18 390)	-	-
	EXP-RSA	12	25 708 (13 856)	27 771 (15 469)	4 876 (2 438)	**64.7*****
	CC-RSA	10	-	-	13 805 (6 414)	-
11 weeks						
	IC[c]	8	30 469 (19 138)	33 963 (22 273)	-	-
	EXP-PR	10	27 910 (21 173)	29 305 (22 144)	10 472 (12 241)	**56.6****
	CC-PR	10	-	-	24 138 (11 727)	-
	IC	12	41 658 (17 619)	45 279 (18 951)	-	-
	EXP-RSA	13	32 169 (11 492)	35 119 (13 558)	942 (1 710)	**89.9*****
	CC-RSA	10	-	-	9 347 (3 868)	-

a IC (initial infection control) groups were subjected to the initial infection only, EXP (experimental) groups to both initial and challenge infections, and CC (challenge infection control) groups to the challenge infection only. The suffixes -PR and -RSA indicate which strain of *S. mansoni* was used for the challenge infection. Details of the estimated cercarial infection loads used for the initial and challenge infections and of the intervals between infection and perfusion applicable to the various groups are shown in the experimental plan (Appendix D; Table D.4).

b Total egg load comprises the sum of eggs in the liver and GIT.

c Tissue egg loads are dependent on the duration of infection. Thus, in contrast to the worm data (Table 4.4a), egg data of IC group animals perfused at the 12.5 and 14.5 week intervals were not pooled, nor were those of animals perfused at the 17 and 19 week intervals.

d Levels of statistical significance are indicated as follows: **, $P < 0.01$; ***, $P < 0.001$.

TABLE 4.5.1 : Reduction of homologous challenge induced by *Schistosoma leiperi*: gravid worm pair and total worm loads.

Pre-challenge Interval	GROUP[a]	*n*	WORM LOADS			
			Gravid pairs		Total	
			$\bar{x}$(s.d.)	Reduction[b] (%)	$\bar{x}$(s.d.)	Reduction[b] (%)
6.5 weeks						
	IC	11	2.8 (1.4)	-	9.0 (2.7)	-
	EXP	11	7.2 (2.2)	**38.0**	30.3 (12.1)	**44.4****
	CC	11	7.1 (3.2)	-	38.3 (6.9)	-
11 weeks						
	IC	12	2.8 (1.3)	-	7.1 (2.6)	-
	EXP	12	4.4 (2.5)	**70.9****	16.2 (10.6)	**72.8*****
	CC	11	5.5 (3.9)	-	33.4 (5.5)	-

a IC (initial infection control) groups were subjected to an initial infection only, at a load of approximately 40 cercariae/animal. EXP (experimental) groups received the equivalent initial infection as well as the challenge infection, and CC (challenge infection control) groups received the challenge infection only. Details of the estimated cercarial infection loads used for the challenge infections are shown in the experimental plan (Appendix D; Table D.5). Perfusions were carried out 8 weeks after challenge infections.

b Levels of statistical significance are indicated as follows: **, $P < 0.01$; ***, $P < 0.001$.

considerably greater than those to heterologous challenge indicated in Table 4.4a. To wit, *S. leiperi* challenge worm burdens were reduced by 44.4% ($P < 0.01$) and 72.8% ($P < 0.001$) in the groups challenged 6.5 and 11 weeks after the initial exposure, respectively.

As regards the second aspect of this experiment, it was estimated that an *S. leiperi* infection load of 95-100 cercariae/mouse should result in liver egg loads similar to those in the study with *S. margrebowiei*. As it happened, animals were inadvertently exposed to somewhat higher numbers of cercariae (approximately 111 cercariae/mouse) than was intended, but this was offset by the fact that worm returns were considerably lower than expected (<20%) (Table 4.5.2a). The total worm burden in the IC group assigned to the 11 week challenge series was observed to be significantly lower than that in the group assigned to the 6.5 week challenge series (means of 10.5 and 19.9, respectively; $P < 0.01$) (Table 4.5.2a). A corresponding decrease in gravid worm pair loads (from 7.1 to 4.9) was also noted, but this was not statistically significant. Assuming an inverse relationship between intensity of infection and survival, this difference in worm loads may reflect the fact that the overall mortality rate in the second IC group was considerably greater than that in the first.

With regard to the attempt to match *S. leiperi* liver egg burdens to those of *S. margrebowiei*, it should be noted that insufficient animals were available to permit direct comparison of loads in the two experiments at the actual times of challenge. For this reason comparisons can only be made using data obtained from the various IC and EXP groups, at corresponding periods of egg deposition (e.g. animals perfused 12.5 weeks after an initial infection with *S. leiperi* correspond with those perfused 10.5 weeks after an initial infection with *S. margrebowiei*). Comparison of the data presented in Tables 4.2b and 4.5.2b reveals that, in the case of the first challenge interval, *S. leiperi* liver egg loads were very similar to those of *S. margrebowiei*, whilst in the case of the second interval, they were noticeably lower. The latter finding presumably reflects the reduction in *S. leiperi* gravid worm pair loads mentioned above.

Extremely high levels of resistance to reinfection were seen in animals exposed to increased *S. leiperi* initial infection loads. Worm recovery data indicated that previously-infected animals were

TABLE 4.5.2a Reduction of homologous and heterologous challenge (PR and RSA strains of *Schistosoma mansoni*) following an initial infection with *S. leiperi* in which liver egg loads were made equivalent to those in the study with *S. margrebowiei* presented in Table 4.2b : gravid worm pair and total worm loads.

Pre-challenge Interval	GROUP[a]	*n*	WORM LOADS			
			Gravid pairs		Total worm load	
			$\bar{x}$ (s.d.)	Reduction[c] (%)	$\bar{x}$ (s.d.)	Reduction[c] (%)
6.5 weeks						
	IC[b]	11	7.1 (3.0)	-	19.9 (6.9)	-
	EXP-LP	11	7.5 (2.9)	**94.4*****	17.3 (4.9)	**100**
	CC-LP	11	7.1 (3.2)	-	38.3 (6.9)	-
	EXP-PR	10	13.3 (7.5)	**56.0****	36.6 (20.6)	**57.2***
	CC-PR	10	14.1 (2.5)	-	39.0 (7.1)	-
	EXP-RSA	12	8.4 (4.1)	**89.9*****	21.1 (12.9)	**96.8*****
	CC-RSA	10	12.9 (3.3)	-	37.6 (6.3)	-
11 weeks						
	IC[b]	13	4.9 (2.5)	-	10.5 (4.7)	-
	EXP-LP	13	5.1 (1.7)	**96.4*****	10.5 (3.6)	**100**
	CC-LP	11	5.5 (3.9)	-	33.4 (5.5)	-
	EXP-PR	13	5.2 (3.0)	**98.3*****	14.2 (4.9)	**91.9*****
	CC-PR	11	17.8 (3.0)	-	45.6 (5.8)	-
	EXP-RSA	12	5.3 (3.6)	**95.9*****	12.7 (7.6)	**93.0*****
	CC-RSA	10	9.8 (2.7)	-	31.5 (6.5)	-

a IC (initial infection control) groups were subjected to an initial infection only, at a load of approximately 111 cercariae/animal. EXP (experimental) groups received the equivalent initial infection as well as the challenge infection, and CC (challenge infection control) groups received the challenge infection only. The suffixes -LP, -PR and -RSA indicate the type of schistosome used for the challenge infection. Details of the estimated cercarial infection loads used for the challenge infections, and of the intervals between infection and perfusion applicable to the various groups are shown in the experimental plan (Appendix D; Table D.5).

c The mean IC group values for the 6.5 week challenge series are based on pooled data from animals perfused at the 12.5 and 14.5 week intervals, and those of the 11 week series on pooled data from the 15 and 17 week intervals.

d Levels of statistical significance are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 4.5.2b Reduction of heterologous challenge (PR and RSA strains of *Schistosoma mansoni*) following an initial infection with *S. leiperi* in which liver egg loads were made equivalent to those in the study with *S. margrebowiei* presented in Table 4.2b : *S. leiperi* tissue egg loads and comparison of the *S. mansoni* gravid worm pair loads and egg output in the experimental and challenge control groups.

Pre-challenge Interval	GROUP[a]	*n*	*S. leiperi*		*S. mansoni*					
			Liver egg load	Total egg load[b]	Gravid pairs		Total egg load[b]		Tissue eggs/ gravid worm pair[c]	
			$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	Reduct.[f] (%)	$\bar{x}$ (s.d.)	Reduct.[f] (%)	$\bar{x}$ (s.d.)	Reduct.[f] (%)
6.5 weeks										
	IC[d]	6	59 350 (27 536)	76 982 (41 923)	-	-	-	-	-	-
	EXP-PR	10	60 240 (14 736)	76 188 (20 325)	8.2 (6.9)	**37.4***	6 582 (9 111)	**68.6****	684 (400)	**53.2****
	CC-PR	10	-	-	13.1 (3.4)	-	20 960 (7 070)	-	1 463 (355)	-
	IC[d]	5	62 520 (25 586)	89 744 (48 031)	-	-	-	-	-	-
	EXP-RSA[e]	12	69 133 (19 163)	105 140 (40 382)	0.3 (0.5)	**97.7*****	88 (176)	**99.1*****	ND	-
	CC-RSA	10	-	-	12.9 (3.3)	-	9 426 (3 520)	-	NA	-
11 weeks										
	IC[d]	6	90 725 (18 991)	143 438 (44 013)	-	-	-	-	-	-
	EXP-PR	13	72 000 (22 126)	101 805 (45 194)	0	**100**	0	**100**	NA	-
	CC-PR	11	-	-	17.8 (3.0)	-	23 345 (7 079)	-	NA	-
	IC[d]	7	74 514 (17 822)	106 177 (35 711)	-	-	-	-	-	-
	EXP-RSA	12	88 240 (17 585)	135 945 (42 343)	0	**100**	0	**100**	NA	-
	CC-RSA	10	-	-	9.8 (2.7)	-	13 410 (3 881)	-	NA	-

a IC (initial infection control) groups were subjected to an initial infection only, at a load of approximately 111 cercariae/animal. EXP (experimental) groups received the equivalent initial infection as well as the challenge infection, and CC (challenge infection control) groups received the challenge infection only. The suffixes -LP, -PR and -RSA indicate the type of schistosome used for the challenge infection. Details of the estimated cercarial infection loads used for the challenge infections, and of the intervals between infection and perfusion applicable to the various groups are shown in the experimental plan (Appendix D; Table D.5).

b Total egg loads comprise the sum of eggs in the liver and GIT.

c ND = not done; NA = not applicable.

d Tissue egg loads are dependent on the duration of infection. Thus, in contrast to the worm data (Table 4.5.2a), egg data of IC group animals perfused at the 12.5 and 14.5 week intervals were not pooled, nor were those of animals perfused at the 17 and 19 week intervals.

e *S. mansoni* gravid pair and tissue egg load values represent means for the whole group (n = 12). However, single gravid *S. mansoni* females were recovered from only 4 animals; one of these had no *S. mansoni* eggs in the tissues, another had eggs in the liver only, while the remaining two had eggs in the GIT only.

f Levels of statistical significance are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

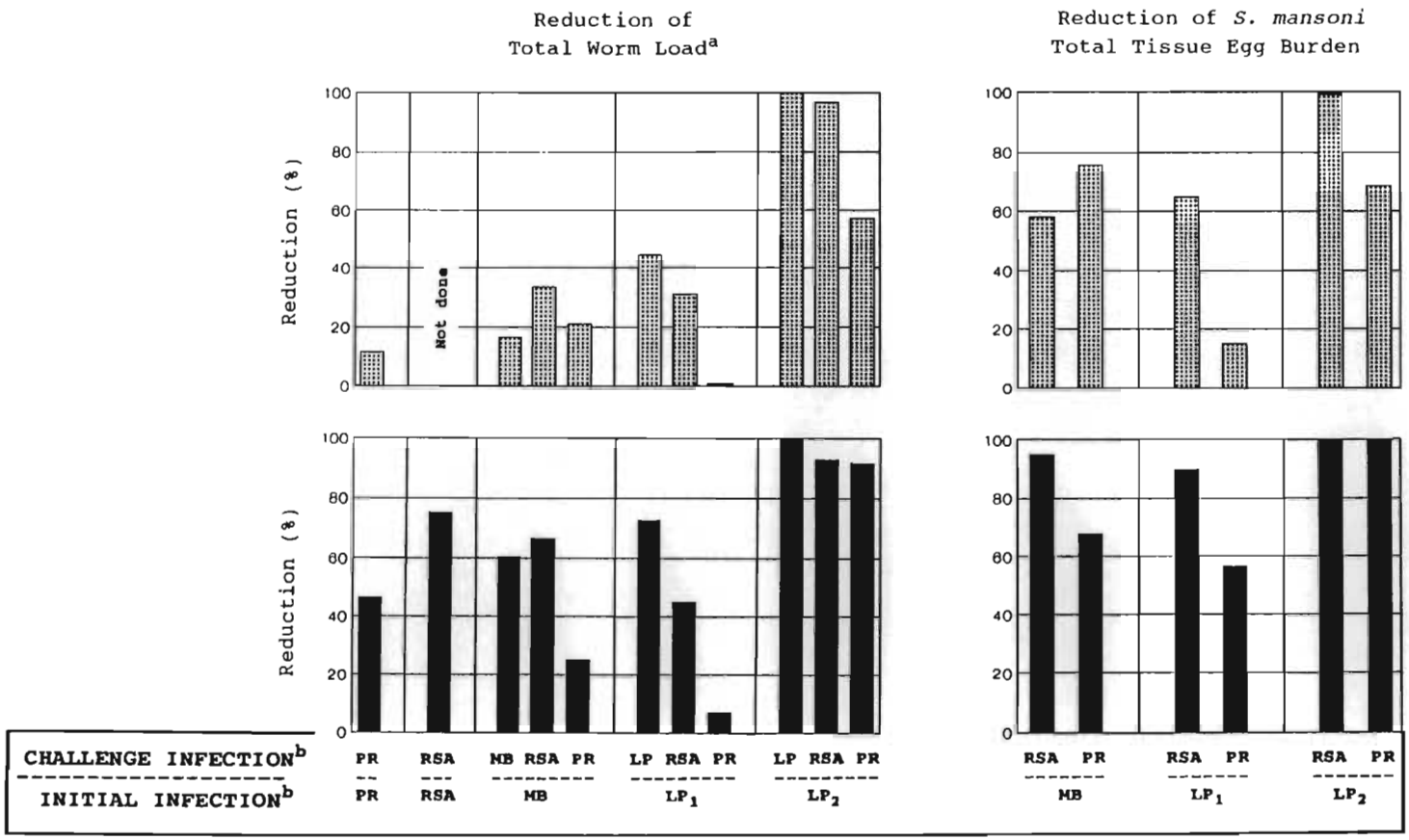
completely resistant to homologous challenge, irrespective of the pre-challenge interval (Table 4.5.2a). With respect to the groups challenged with *S. mansoni*, it was only possible to reliably differentiate between gravid females of the initial and challenge infections. In the case of the RSA strain, the estimated reductions in challenge total worm burdens were 96.8% and 91.9% ($P < 0.001$ in both cases) in the groups challenged at 6.5 and 11 weeks, respectively, and in the case of the PR strain, the corresponding estimates were 57.2% ($P < 0.05$) and 93.0% ($P < 0.001$), respectively (Table 4.5.2a). In animals challenged after 6.5 weeks, egg production by the RSA strain was almost completely curtailed (Table 4.5.2b), and in the case of the PR strain, although the number of gravid females was reduced by only 37.4% ($P < 0.05$), tissue egg loads were reduced by 68.6% ($P < 0.01$). Neither gravid females nor eggs of *S. mansoni* were detected in either of the EXP groups subjected to heterologous challenge at 11 weeks.

4.4 DISCUSSION

Levels of concomitant immunity in the mouse model have frequently been observed to differ substantially from one experiment to the next, even under apparently comparable conditions (Dean, 1983). Nevertheless an attempt was made to facilitate some measure of comparison between the various experiments included in the present investigation by standardising the challenge intervals such that some animals were challenged immediately prior to, and others approximately 4 weeks after the onset of oviposition by the initial infection. The only exception to this rule was the experiment involving homologous challenge with the RSA strain of *S. mansoni*, where challenge was administered at only one time interval, i.e. after about 2 weeks of oviposition. The results of the experiments detailed in Tables 4.1 to 4.5.2b are summarised graphically in Figure 4.1. This serves to illustrate a number of trends which became apparent during the course of these investigations, although in the experiment involving increased *S. leiperi* initial infection loads these were largely obscured, due to the fact that very high levels of resistance were seen at both challenge intervals.

With respect to worm loads, it is clear, firstly, that levels of resistance to challenge were generally substantially greater in the

FIGURE 4.1 Summary of results from concomitant immunity experiments.



Challenge infections were carried out before (▨) or after (■) the onset of egg laying by the initial infection.

a In the case of the study involving initial infection with *S. margrebowiei*, followed by heterologous challenge, the levels of resistance shown here are those from Table 4.2a (based on Formula A, Section 4.2.4) rather than Table 4.2b.

b PR, RSA, MB and LP refer to PR *S. mansoni*, RSA *S. mansoni*, *S. margrebowiei* and *S. leiperi*, respectively; LP_1 and LP_2 refer to experiments in which initial infection cercarial loads were either 37-40 or 111/animal, respectively.

groups challenged at the later time intervals. This observation concurs with the findings of previous homologous (*S. mansoni* and *S. japonicum*) and heterologous (*S. mattheei* versus *S. mansoni*) acquired resistance studies in which it was shown that resistance increased to high levels only once animals had been infected for 6 or more weeks (Dean *et al*, 1978; Long *et al*, 1978; Moloney *et al*, 1984; Amin and Nelson, 1969). Secondly, a marked difference was noted between the PR and RSA strains of *S. mansoni* in terms of their ability to survive and mature in previously-infected mice. This observation provided further evidence of distinctive differences between these two strains (Higgins-Opitz and Dettman, 1991) and prompted additional concomitant immunity studies aimed at assessing the ability of these two strains to induce and withstand acquired resistance in BALB/c mice (Higgins-Opitz and Dettman, unpublished data). In one experiment animals were exposed initially to the PR strain and challenged after either 4.5 weeks or 9 weeks, and in another they were exposed initially to the RSA strain and challenged after either 6.5 or 11 weeks: challenge infections were carried out with both strains. The results of these experiments, which are summarised in tabular form below, served to strongly confirm the impression that the RSA strain is far more susceptible to the factors which result in the elimination of challenge worms (discussed in more detail below).

INFECTIONS		**REDUCTION IN CHALLENGE WORM BURDEN (%)**	
Initial	Challenge	1st Challenge Interval	2nd Challenge Interval
PR	PR	20%	44%
PR	RSA	39%	89%
RSA	PR	20%	20%
RSA	RSA	43%	65%

[Note: The approximate numbers of gravid pairs resulting from the initial infections with the PR and RSA strains were 4 and 3, respectively.]

In the experiments where animals were first exposed to either *S. margrebowiei* or *S. leiperi*, then challenged with *S. mansoni*, it was observed that challenge egg loads were considerably more reduced than worm loads (Figure 4.1). In those instances where it was possible to

differentiate between gravid females originating from the initial and challenge infections (see Tables 4.2b and 4.5.2b) it was found that gravid pair loads and total worm loads were reduced to similar degrees. In other words, the relatively greater reductions in tissue egg loads were apparently due to a reduction in the number of eggs recovered per gravid worm pair, rather than to a disproportionately large depression of pair formation. With respect to the observation that total tissue egg loads of the RSA strain tended to be more reduced than those of the PR strain, the results presented in Table 4.2b are noteworthy, since they reveal that at the level of tissue eggs per gravid pair, reductions were similar for both strains; it thus follows that the differences at the level of total egg loads were essentially a function of the differences in the reductions in worm loads *per se*.

The observation that challenge egg loads may be reduced more than worm loads has been reported in a number of other studies involving initial exposure to animal schistosomes followed by heterologous challenge. Nelson *et al* (1968) found that in mice initially exposed to *S. bovis* and challenged 16 weeks later with *S. mansoni*, challenge worm loads were reduced by 35% whilst egg loads were reduced by 74%. In a subsequent study Amin and Nelson (1969) reported that a 9 week old unisexual male infection with *S. mattheei* caused *S. mansoni* challenge worm loads to be reduced by only 29%, whereas egg loads were reduced by 90%. In a further study in which mice were initially exposed to *Ornithobilharzia turkestanicum* (which deposited only small numbers of eggs in the tissues) and challenged with either *S. mansoni*, *S. haematobium* or *S. bovis* it was again observed that challenge worm loads tended to be considerably less reduced than egg loads (Massoud and Nelson, 1972). An additional, striking example of this phenomenon was also reported by Pedersen *et al* (1982), who found that in mice exposed initially to the avian schistosome *Trichobilharzia szidati* (which failed to mature), *S. mansoni* challenge worm burdens were not noticeably reduced, whereas egg loads were reduced by more than 50%.

The reasons for these disproportionate reductions in tissue egg burdens remain to be elucidated. As noted by Dean (1983), a number of possibilities must be considered. On the one hand the sexual maturation of the challenge parasites may be delayed, or their egg production capacity may be reduced; alternatively, the rates of egg

destruction or faecal egg excretion by previously-infected hosts may be increased. Some clues as to the relative importance of at least some of these suggested alternatives could be obtained via studies aimed at simultaneously assessing challenge infection development rates, worm loads, tissue egg loads and faecal egg excretion rates at successive intervals over an extended period (rather than at a single interval, as is normally the case). An experiment involving initial infection with *S. margrebowiei* followed by challenge with *S. mansoni* would be particularly suitable for such an investigation, since, as noted in the series of investigations described here, both worms and ova of these two species can be differentiated with relative ease.

During the past decade the mechanisms underlying the development of concomitant immunity in the mouse model have been investigated in considerable depth. Much of the early work which led to the concept of concomitant immunity was carried out in the rhesus monkey, in which it was shown that whereas the presence of live adult worms was essential for the development of resistance, the deposition of eggs in the tissues was not (Smithers and Terry, 1969b). However, evidence to suggest that the development of resistance in the mouse required the presence of bisexual, egg-producing infections had been reported as early as 1953 by Olivier and Schneidermann, who observed that mice harbouring bisexual *S. mansoni* infections exhibited substantial resistance to homologous challenge, whereas those harbouring unisexual (male) infections were largely non-resistant. In general, subsequent studies have convincingly confirmed the inability of unisexual infections with *S. mansoni* (Dean *et al*, 1978a; Bickle *et al*, 1979a) or *S. japonicum* (Moloney *et al*, 1986) to elicit protection against subsequent superinfections, although there is limited evidence that moderate resistance may develop in mice with relatively long-term (17-18 weeks) unisexual *S. mansoni* infections (Smithers, 1982). While there have been some reports of heterologous resistance being induced in mice by unisexual infections or by species which produced no or few eggs (see Christensen *et al*, 1987), the actual levels of resistance, as measured in terms of reduction of challenge worm loads, were in most instances quite low.

As a consequence of their observations, both Dean *et al* (1978b) and Bickle *et al* (1979a) recognised the possibility that resistance in the *S. mansoni*/mouse model might not be due exclusively to

schistosome-specific immune responses, but instead could be mediated by non-specific mechanisms arising from the intense inflammatory responses to eggs in the tissues. In this regard, compelling evidence has been obtained, in particular by Wilson and colleagues, that much of the observed resistance to challenge infection in the mouse model is directly related to abnormalities in both the intra- and extra-hepatic vasculature resulting from egg-induced portal hypertension (reviewed by Wilson, 1990). In brief, it has been demonstrated that the dilation of the hepatic sinusoids and the development of portal-systemic anastomoses cause the efficiency of schistosomule trapping in the liver to be markedly reduced, with the consequence that challenge-derived parasites are more likely to become lodged in sites (in particular the lungs) which are unfavourable for their survival.

As discussed in the preceding chapter, infections with *S. margrebowiei* and *S. leiperi* in mice cause similar profound vascular and haemodynamic changes to those seen in studies with *S. mansoni* and *S. japonicum*. It must therefore be assumed that a substantial portion of the resistance to challenge observed in the present concomitant immunity studies was attributable to non-immunological mechanisms, of the type outlined above. The observation that levels of resistance increased when animals were challenged after the onset of oviposition is certainly consistent with such an assumption. In a related vein, it seems possible that the differences in the abilities of the PR and RSA strains of *S. mansoni* to become established in previously-infected hosts may, at least to some extent, reflect a dynamic interplay between the development of egg-induced alterations in the hepatic vasculature and the differences in the infectivity and rates of development of these two strains. It has been shown that the PR strain matures some two weeks in advance of the RSA strain, and yields considerably higher adult worm returns (Higgins-Opitz and Dettman, 1991), indicating a more efficient and rapid completion of the pre-hepatic migration stages. This would suggest a considerable advantage to challenge schistosomula of the PR strain under concomitant immunity conditions: firstly, they would presumably have opportunities to become established in the hepatic sinusoids at a time when vascular changes were less severe, and secondly, they may be inherently better equipped to cope with the stresses associated with such changes. However, while this explanation may partially account for the relatively greater elimination of RSA strain challenge parasites, it seems likely that other factors are involved.

It may be speculated, for example, that molecular processes, such as membrane turnover, which play an important role in the evasion of host immune responses (Capron *et al*, 1987) operate more slowly or less efficiently in the case of the RSA strain.

Vascular changes might conceivably also account, at least in part, for the apparently reduced fecundity of challenge infections, on the basis of delayed maturation, as discussed earlier. As noted by Wilson and Coulson (1989), the establishment of a full complement of liver worms in naive mice requires two to three circuits of the pulmonary-systemic vasculature. However, in chronically infected animals additional circuits may be required, not only increasing the risk of elimination, but also the time taken for the establishment of the final population of liver worms. Furthermore, reduced challenge egg loads in the liver and intestines may be due to increased shunting of eggs to non-systemic sites, in particular the lungs. No attempt was made in the present study to assess lung egg burdens.

Pons *et al* (1989) showed that the level of resistance to challenge infection in mice harbouring a mature infection of *S. mansoni* was greater than that seen in animals in which porto-systemic shunting had been induced by partial portal vein ligation; from this they inferred that at least some of the resistance to reinfection seen in chronic murine schistosomiasis can be ascribed to immunological factors. Additional evidence to support this conclusion comes from a number of other sources. Firstly, James and Cheever (1985) demonstrated that mice of the inbred P strain, which have a genetic defect in macrophage function, develop relatively poor levels of concomitant immunity, in spite of the fact that they exhibit apparently normal levels of egg-related pathology. Secondly, passive transfer of partial resistance has been demonstrated using serum from chronically-infected mice (Sher *et al*, 1975; Goes and Ramalho-Pinto, 1991) and an anti-egg monoclonal antibody (Harn *et al*, 1984). In this regard it is of interest to note that the vast majority of anti-schistosomulum antibodies which are generated in chronic murine schistosomiasis have been shown to be elicited by the eggs, rather than by the schistosomula or adult worms (Omer Ali *et al*, 1988). Thirdly, treatment of mice with a monoclonal antibody which acts against neutrophils and markedly reduces dermal inflammatory responses to challenge schistosomula causes a pronounced suppression of concomitant immunity (McLaren *et al*, 1987). However, as noted by

Doenhoff and Long (1979), it seems likely that the immunological mechanisms which contribute to the elimination of challenge parasites in chronically infected mice represent a combination of both specific (i.e. anti-schistosome) and non-specific (i.e. of a general inflammatory nature) factors. Assessing the relative contribution of each of these in the *in vivo* situation is clearly no simple task.

In the light of the above observations it seems reasonable to assume that at least some of the resistance to challenge observed in the present series of concomitant immunity studies was due to immunological mechanisms. In this regard, some of the features of the present results strongly suggest the need for explanations other than those based on vascular and haemodynamic changes or non-specific immunological factors alone. In particular, it was noticeable that levels of homologous resistance were consistently higher than those of heterologous resistance. If levels of resistance were simply a function of the state of advancement of vascular abnormalities and/or non-specific immunological activity, then the magnitude of the reductions in challenge worm burdens should presumably have been directly related to the maturation rates of the different challenge schistosomes (i.e. the longer the maturation rate, the greater the probability of elimination). Since *S. margrebowiei* and the PR strain of *S. mansoni* are known to develop at similar rates, it follows that the percentage reductions of these two parasites under comparable conditions should have been similar. By the same token, levels of resistance against *S. leiperi* and the RSA strain of *S. mansoni* should also have been similar. Instead, as shown in Figure 4.1, in the case of the studies involving initial exposure to *S. margrebowiei*, homologous resistance was substantially greater than that against challenge with PR *S. mansoni*; likewise, in animals harbouring 2-3 *S. leiperi* worm pairs, homologous resistance was greater than that against challenge with the RSA strain. Along similar lines, it is noteworthy that resistance to the PR strain at 9 weeks was substantially higher in animals infected initially with the homologous parasite than in those with *S. margrebowiei* initial infections, in spite of the fact that liver egg loads at the time of challenge were undoubtedly considerably higher in the latter experiment.

It is clear from the study in which *S. leiperi* liver egg loads were matched with those in the study with *S. margrebowiei* that levels of

resistance are not determined simply by the density of eggs in the liver. However, the reasons for the exceptionally high levels of resistance seen in this experiment remain far from clear. Considering the fact that the ova of *S. leiperi* are much larger than those of *S. margrebowiei* (Table 1.1), and appear to evoke correspondingly larger hepatic granulomas (Figure 3.6), it is conceivable that at equivalent liver egg loads the degree of hepatic damage, and hence the extent of vascular disruption and/or immunological activity induced by *S. leiperi* is proportionally greater than that caused by *S. margrebowiei*. Whatever the mechanisms of resistance operative in this study, it is obvious that they became effective either before or soon after the onset of oviposition, as evidenced by the fact that worm and egg load reductions were extremely high even when animals were challenged at 6.5 weeks.

In conclusion, the results of the studies described in this chapter conclusively demonstrate the ability of the antelope schistosomes to induce concomitant immunity against both homologous and heterologous challenge in BALB/c mice. However, as with all experiments of this nature in the murine model, it is not clear to what extent immunological and non-immunological factors contributed to the total levels of resistance observed in the various experiments. More importantly, since both *S. margrebowiei* and *S. leiperi* reach full maturity in the mouse, and since the development of concomitant immunity in this host is so clearly dependent on the presence of ova in the tissues, it would appear that this model system cannot be considered representative of the situation in man with regard to the proposed immunological interactions between the antelope and the human schistosomes. As noted in Section 1.10.4, the existing evidence indicates that *S. leiperi* is not capable of maturation in man, and that *S. margrebowiei* reaches maturity only on rare occasions. Thus, any form of large-scale immunological protection in humans resulting from exposure to either of these two schistosomes must presumably operate independently of tissue egg deposition.

CHAPTER FIVE

STUDIES ON THE ABILITY OF RADIATION-ATTENUATED CERCARIAE OF *SCHISTOSOMA MARGREBOWIEI* AND *S. LEIPERI* TO INDUCE RESISTANCE TO CHALLENGE INFECTION IN BALB/c MICE

5.1 INTRODUCTION

In 1959 Standen and Fuller reported that the post-penetration development of *S. mansoni* cercariae could be attenuated in a dose-dependent manner by exposing them to ultraviolet (UV) light before allowing them to infect animals. Thus they found that the proportion of cercariae which could be recovered as adult worms 5-7 weeks after infection decreased progressively with increasing UV dose. Furthermore, they observed that even at dosages well above those which completely precluded the maturation of any worms to the liver stage the skin penetration ability of the cercariae did not appear to be impaired, indicating that the larvae were eliminated at some stage between the skin and the liver. Shortly thereafter dose-dependent attenuation by means of gamma irradiation was demonstrated in studies with *S. mansoni* (Villella *et al*, 1961; Radke and Sadun, 1963; Erickson and Caldwell; 1965), and by means of X-irradiation in studies with *S. japonicum* (Hsu *et al*, 1962) and *S. mansoni* (Smithers, 1962).

In the early 1960s it was shown by various workers that vaccination of experimental animals, including mice, rats and rhesus monkeys, with gamma or X-ray-attenuated cercariae of *S. mansoni* and *S. japonicum* evoked substantial resistance to subsequent homologous challenge infections with normal cercariae (reviewed by Hsu *et al*, 1980). Since then the development of substantial partial immunity following exposure to irradiated schistosome larvae has been repeatedly demonstrated with a variety of other host/schistosome systems (see reviews by Hsu *et al*, 1980, and Taylor *et al*, 1991). In the majority of studies attenuation has been effected by means of gamma or X-rays. However, there have also been a small number of studies in which partial resistance was induced using UV-attenuated larvae (reviewed by Ruppel *et al*, 1990).

The early successes with irradiated vaccines led to the suggestion that it may be possible to develop a live, attenuated vaccine for use in man. However, although considerable progress has been made towards the development of such vaccines for use against *S. bovis* and *S. japonicum* in bovines (Taylor, 1987; Taylor *et al*, 1991; Shi *et al*, 1990), it is now generally agreed that this method of vaccination would not be acceptable in humans, for both ethical and practical reasons (von Lichtenberg, 1985; Taylor and Bickle, 1986). Nonetheless, experimental studies with irradiated larvae have been vigorously pursued, largely on account of the following two factors: (i) until quite recently attempts to induce protective immunity using preparations of schistosome antigens and other 'non-living' vaccines were largely unsuccessful (Clegg and Smith, 1978; Hsu *et al*, 1980; James, 1982); (ii) attenuated vaccines provide a means of inducing resistance, which has been shown to be immunologically-mediated (reviewed by Taylor and Webbe, 1989), in a manner which is apparently not complicated by the involvement of non-immunological factors, such as those associated with egg-induced pathology which so limit the value of the concomitant immunity model (see previous chapter).

As with concomitant immunity studies, the most widely used experimental system for investigating immunity due to attenuated vaccines has been that involving the Puerto Rican strain of *S. mansoni* and the mouse (Dean, 1983). This no doubt reflects the comparative ease of working with this strain of *S. mansoni* and, as noted by Taylor and Webbe (1989), the suitability of the mouse both as a permissive host for *S. mansoni* and as a well-characterised subject for experimental immunology. In some of the early studies with this model, moderate to high levels of resistance were reported to develop following vaccination with cercariae attenuated with relatively low irradiation dosages (≤5 krads) (see Dean, 1983). However, more recent evidence strongly favours the conclusion that optimum resistance is obtained with highly irradiated cercariae or schistosomula. While there has been some controversy as to the ideal attenuation dose, with some workers recommending 20 krads (Bickle *et al*, 1979c) and others 56 krads (Minard *et al*, 1978), the weight of evidence appears to support the former dose (James and Dobinson, 1985; James *et al*, 1985). In the cases of both *S. mansoni* (Dean, 1983) and *S. japonicum* (Moloney *et al*, 1985a) substantial resistance to homologous challenge has been reported to develop in mice vaccinated with as few as 50-100 attenuated cercariae or

schistosomula, although it has become conventional to apply an 'inoculum' of 400-500 larvae (von Lichtenberg, 1985).

Studies with radiation-attenuated schistosomes (especially *S. mansoni*) in the mouse model and, to a lesser extent, in rats and guinea pigs, have yielded important insights regarding the stage and sites at which challenge larvae are eliminated, and the immunological mechanisms responsible for such elimination (Wilson and Coulson, 1989; McLaren, 1989). Furthermore, in conjunction with monoclonal antibody and recombinant DNA technology, they have made a significant contribution towards the identification of protective antigens which might be of use in the development of an effective non-living vaccine (reviewed by Taylor and Webbe, 1989).

Vaccination studies in baboons have produced somewhat equivocal results, particularly as far as resistance to *S. mansoni* is concerned (Taylor *et al*, 1991). However, a study by Damian *et al* (1984) is worthy of specific mention, since these authors observed that even in the absence of significant reductions in challenge worm loads, the majority of vaccinated animals showed highly reduced granulomatous responses to eggs deposited in the tissues, thereby giving credibility to the concept of an 'anti-pathology' vaccine (see Section 1.7.2).

One of the most striking features of the immunity induced by attenuated cercariae and schistosomula is its apparent species specificity. Early evidence of this came from studies by Bickle *et al* (1979d) who found that vaccination of sheep with irradiated *S. mansoni* schistosomula did not confer protection against challenge with *S. mattheei* and vaccination with irradiated *S. mattheei* did not protect against *S. bovis*. In contrast it had previously been demonstrated that attenuated *S. mattheei* cercariae and schistosomula induce strong resistance to homologous challenge in these hosts (Taylor *et al*, 1976b). Since then strong evidence has accumulated to indicate that vaccine immunity in the mouse model is also of a species specific nature. Thus, it has been reported that attenuated *S. mansoni* fails to protect against either *S. japonicum* (Cheever *et al*, 1983; Moloney *et al*, 1985a) or *S. margrebowiei* (Aitken *et al*, 1988), and that neither *S. japonicum*, *S. haematobium* nor *S. bovis* protect against *S. mansoni* (Bickle *et al*, 1985; Moloney *et al*, 1985a). It has even been found that vaccination of mice with a

Chinese strain of *S. japonicum* fails to induce resistance to challenge with a Philippine strain of the same species (Moloney *et al*, 1985b). Interestingly, Moloney *et al* (1989) subsequently found that the former parasite, which has been in laboratory culture for over 50 years, did protect against other, recently collected Chinese isolates of *S. japonicum*. There does not as yet appear to be any evidence of strain specific immunity in the case of *S. mansoni* (Bickle and Doenhoff, 1987; Lewis *et al*, 1987). A lack of cross-protection between *S. mansoni* and *S. japonicum* has also been observed in attenuated vaccine studies in the rat model (Moloney *et al*, 1987c).

The studies reported in this chapter were aimed at assessing the immunising potential of radiation-attenuated cercariae of *S. margrebowiei* and *S. leiperi* in the BALB/c mouse. By artificially truncating their development in this normally permissive host it was intended not only to circumvent the problems associated with egg-induced pathology, as discussed in the previous chapter, but also to simulate their apparently restricted development in man. The use of *S. leiperi* was limited to a single experiment, as this work was carried out during a period when cercariae of this species were in short supply.

5.2 MATERIALS AND METHODS

5.2.1 Schistosomes

The parasites included in the studies described in this chapter were *S. margrebowiei*, *S. leiperi*, and the PR and RSA strains of *S. mansoni*. Details regarding the origins and maintenance of the former two species are presented in Section 2.2.1 and those pertaining to the *S. mansoni* strains in Section 4.2.1.

5.2.2 Experimental Hosts

Male inbred BALB/c mice were used for all experiments; details regarding the origins, housing and maintenance of the animals are presented in Section 2.2.2. Within individual experiments animals were age-matched to within one week of each other; the average ages

of the animals in the various experiments, at the time of first infection, ranged between 6 and 8 weeks, except in the last experiment, where 15 week old animals were used.

5.2.3 Irradiation of Cercariae

Cercariae were recovered from infected snails, as described in Appendix A. When necessary cercarial suspensions were concentrated using the first-stage concentrator, as described in Appendix C. Attenuation was achieved by means of gamma radiation, using one of two ^{60}Co sources, namely that situated in the Medical Physics Unit, Addington Hospital, Durban, or that in the Biology Department, University of Natal, Durban.

In the initial experiment (Section 5.3.1), which was aimed at the determination of the relationship between radiation dose and worm recovery, cercariae were distributed in equal portions into small plastic jars for irradiation. Although the cercarial suspensions were approximately 3cm deep, the irradiation dose rate (a function of source output and distance from source) was calculated at a point 0.5cm below the surface, since the majority of cercariae were seen to remain in the upper centimetre of water; a 6%/cm loss of radiation due to absorption by the water was assumed (Mr M Weller, personal communication). In the case of the vaccination experiments (Section 5.3.2) care was taken to ensure that cercarial suspensions (in glass beakers) were not more than 1.5cm deep and the irradiation dose rate was calculated for a point mid-way through the suspension.

Exposure rates in the various experiments ranged from 205 to 415rads/min, and densities of cercariae in suspension ranged from approximately 25 to 700/ml.

5.2.4 Infection of Animals and Assessment of Worm and Tissue Egg Loads

Infections with both irradiated and normal cercariae were carried out according to the method described in Section 2.2.3. Mice were anaesthetised as described in Section 2.2.5 and worm and tissue egg

loads were determined as outlined in Sections 2.2.6 and 2.2.7, respectively.

5.2.5 Design of Vaccination Experiments

In order to select suitable radiation dosages for the vaccination experiments, a preliminary study was carried out to determine the effects of different radiation doses on the subsequent development of *S. margrebowiei* (*S. leiperi* was not included due to a lack of cercariae). To this end groups of 9-11 mice were exposed to cercariae derived from a common pool which were either non-irradiated or irradiated with 0.5, 1, 1.5, 2, 2.3, 2.5, 3, 4, or 6 krads. This dose range was chosen on the basis of published studies on *S. mansoni* (Villella *et al*, 1961; Smithers 1962; Radke and Sadun, 1963; Erickson and Caldwell, 1965; Minard *et al*, 1978; Bickle *et al*, 1979b) and *S. japonicum* (Hsu *et al*, 1962) which consistently demonstrated that worm returns from rodents infected with irradiated cercariae or schistosomula decreased progressively to around zero as the radiation dose increased to about 3 krads (see Footnote). For comparative purposes a number of groups of mice were simultaneously exposed to normal and irradiated cercariae of the PR strain of *S. mansoni*: in this instance a smaller range of radiation dosages was tested (1, 2, 2.5, and 3 krads). All animals were perfused 8.5 weeks after exposure to cercariae.

In vaccination experiments where initial infections were only partially attenuated (i.e. exposed to a radiation dose which allowed the establishment of a small population of liver-stage worms but prevented sexual maturation), three groups of animals were required, namely an Initial Infection Control (IC) group, a Challenge Control (CC) group and an Experimental (EXP) group. The animals in the IC

Footnote: In the papers published during the 1960s radiation doses were expressed in terms of 'r' (roentgen) or 'rep' (roentgen equivalent physical). Whilst these terms refer to the amount of radiation to which an object is exposed, 'rad' (radiation absorbed dose) refers to the amount of radiation which is actually absorbed. However, for purposes of the present discussion 'r', 'rep' and 'rad' can be considered to be more-or-less equivalent (Weller, personal communication).

group received the initial infection only, those in the CC group received the challenge infection only, and those in the EXP group received both the initial and the challenge infections. In these experiments the degree of reduction in challenge-derived worm loads in the EXP groups was calculated using Formula A (Smith and Clegg, 1979), as described in Section 4.2.4:

$$\% \text{ Reduction} = \frac{CC - (EXP - IC)}{CC} \times 100$$

where IC, CC and EXP refer to the mean worm loads in the IC, CC and EXP groups, respectively.

In experiments where the initial infections were fully attenuated (i.e. exposed to a radiation dose which prevented the establishment of liver-stage worms) only two groups of animals were required, namely an EXP group, which received an initial exposure to attenuated cercariae followed by a challenge infection with normal cercariae, and a CC group, which received only the challenge infection. In this instance percentage reductions in challenge-derived worm loads in the EXP groups were determined using the formula:

$$\% \text{ Reduction} = \frac{CC - EXP}{CC} \times 100$$

Due to the delays associated with the transportation of cercariae to the irradiation venues and with the irradiation process itself, cercariae in some experiments were up to 6.5 hours old at the time of animal exposure. However, in most experiments some idea of the condition of the cercariae was obtained by exposing a separate group of animals to non-irradiated cercariae derived from the same pool and treated in essentially the same manner (and thus of the same age) as those subjected to irradiation; cercarial infectivity was assessed on the basis of subsequent worm recovery.

Challenge infections were administered either 4.5 or 9 weeks after initial exposures. These intervals were chosen primarily on the basis of the review by Dean (1983), which revealed that in other studies of this nature pre-challenge intervals were most commonly in the range of 3-9 weeks. In experiments involving challenge with *S. margrebowiei* or the PR strain of *S. mansoni* animals were perfused 6-

6.5 weeks after challenge, and in the case of challenge with the RSA strain perfusions were carried out 8 weeks after challenge.

5.2.6 Statistical Analyses

In the initial experiment (Section 5.3.1) mean worm loads from groups of animals exposed to irradiated cercariae were compared with that from an appropriate control group (exposed to non-irradiated cercariae) by means of the Mann-Whitney test (Siegel and Castellan, 1989). In order to determine whether or not the sex ratio of the worms recovered was significantly affected by irradiation, the proportions of male worms in the groups exposed to irradiated cercariae were compared with that in the control group using the Z-test, following approximation of the binomial distribution with a normal distribution (Cangelosi *et al*, 1983).

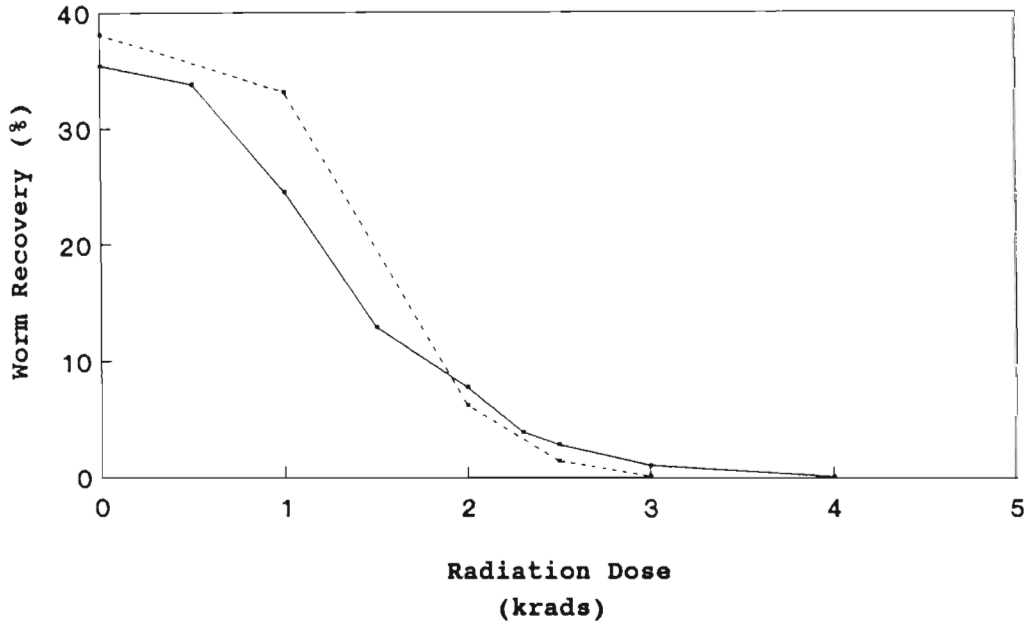
With respect to the vaccination experiments (Section 5.3.2), the mean numbers of challenge-derived worms in the EXP groups were compared with those in the appropriate CC groups using the Mann-Whitney test. In the case of experiments involving initial exposure to partially-attenuated cercariae the mean number of challenge-derived worms in each EXP group was determined only after adjusting for the number of worms assumed to be present from the initial infection: i.e. the mean number of worms in the IC group was subtracted from the individual total worm loads in the EXP group and the mean of the resultant values compared with that of the CC group.

5.3 RESULTS

5.3.1 Relationship between radiation dose and post-cercarial development of *S. margrebowiei*.

In this experiment the animals exposed to non-irradiated *S. margrebowiei* and *S. mansoni* cercariae yielded mean worm returns of 35.4% and 38.1%, respectively. As shown in Figure 5.1, an irradiation dose of 1 krad resulted in a substantial decrease in percentage worm returns of *S. margrebowiei* but had a less marked effect on *S. mansoni*. However, at other comparable irradiation levels the degree of attenuation of the two species was observed to be similar. All

FIGURE 5.1 Comparative effect of increasing cercarial radiation dose on percentage worm recoveries of *S. margrebowiei* and the PR strain of *S. mansoni*.



—•— , *S. margrebowiei*; ····· , *S. mansoni* (PR strain)

For *S. margrebowiei* and PR *S. mansoni* worm recoveries from mice exposed to cercariae irradiated with 1 krad or more were significantly lower than those of control groups ($P < 0.01$ and $P < 0.05$ in the cases of *S. margrebowiei* and *S. mansoni* 1 krad groups, respectively, and $P < 0.001$ in all other cases; Mann-Whitney test)

animals exposed to 2.5 krad-attenuated *S. margrebowiei* cercariae were found to be harbouring worms (1-4/animal), a substantial proportion of which (35%) were stunted; in the 3 and 4 krad groups worms (1-2 per animal) were recovered from 6 out of 10 and 1 out of 9 animals, respectively (data not shown). In the case of *S. mansoni*, 5 out of 10 animals in the 2.5 krad group yielded worms (1-5/animal), whilst only a single stunted female was recovered from the 3 krad group (data not shown).

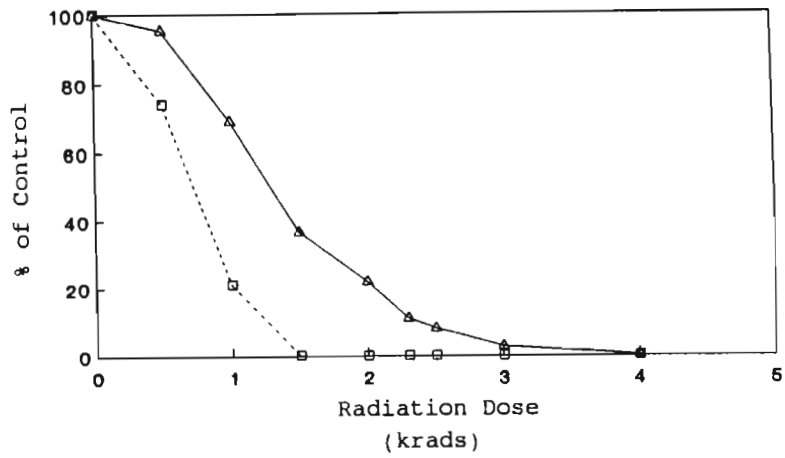
Tissue egg counts from the groups exposed to *S. margrebowiei* cercariae revealed that the fecundity of worms developing from irradiated *S. margrebowiei* cercariae was markedly depressed (Figure 5.2). For example, following irradiation with 1 krad the worm yield was reduced by only 31%, while liver egg loads were reduced by 79% (2 out of 10 animals had no eggs). An increase in the irradiation dose to 1.5 krad resulted in a 63.5% worm load reduction, but an almost complete suppression of sexual maturation, with eggs being found in small numbers in the livers of only 4 of the 10 animals in this group. No eggs were found in any of the other groups. Similar observations were made with respect to *S. mansoni* (data not shown). Thus, in the *S. mansoni* 1 krad group, worm loads were reduced by 13% whilst egg loads were reduced by 26%. Although one animal in the 2 krad group proved to have a few eggs in its liver, no evidence of pairing and sexual maturation was seen in any of the remaining 9 animals, in spite of the fact that 5 of them harboured bisexual infections.

As illustrated in Figure 5.3, irradiation appeared to cause a more severe attenuation of *S. margrebowiei* male cercariae than females. While the ratio of male:female worms in the control group was almost exactly 1:1, the sex-ratios of the worm populations which developed from cercariae irradiated with 1 krad or more were consistently seen to be biassed towards females. A similar, though less marked, trend towards an increased proportion of female worms was observed in the 2 and 2.5 krad *S. mansoni* groups (data not shown).

5.3.2 Vaccination Studies

A series of experiments was carried out involving initial exposures to cercariae which had been irradiated with one of three radiation

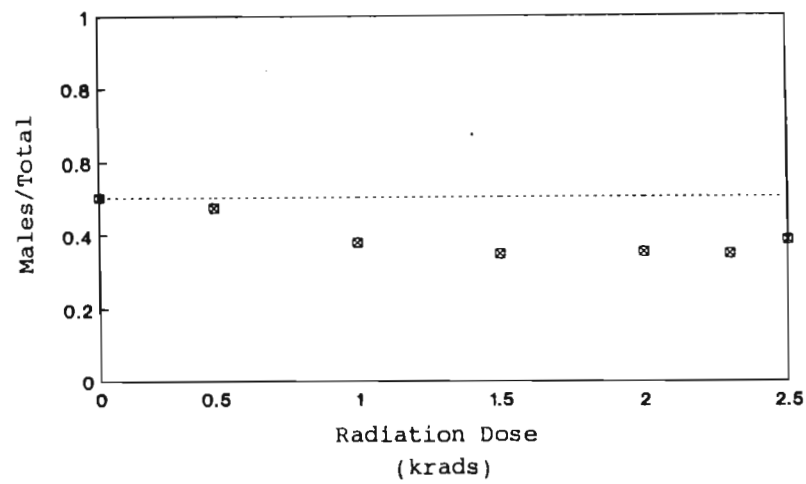
FIGURE 5.2 Comparative effect of increasing cercarial radiation dose on the survival and fecundity of *S. margrebowiei*.



—△— , total worm loads; ···□··· , liver egg loads.

Mean *S. margrebowiei* total worm and liver egg loads in groups of mice exposed to irradiated cercariae are expressed as percentages of those in the group exposed to non-irradiated cercariae (controls).

FIGURE 5.3 Effect of cercarial radiation dose on the sex-ratio of *S. margrebowiei* worms.



The recovery of male worms (⊠), expressed as a proportion of the total, is shown in relation to radiation doses up to 2.5 krad. The dotted line shows the 'expected' proportion of male worms, based on that in the group exposed to non-irradiated cercariae. By making use of the normal approximation of the binomial distribution, it followed from the Z-test that the proportions of surviving males in the 1, 1.5, 2, 2.3 and 2.5 krad groups were significantly lower than the 'expected' proportion.

doses, namely 2.5, 5 or 20 krads. These dosages were selected specifically with a view to creating a spectrum of initial infection conditions, in accordance with the intention of attempting to simulate the infection patterns of *S. margrebowiei* and *S. leiperi* in man. The results of the preceding experiment indicated that a radiation dose of 2.5 krads would permit the development of a small population of sterile liver-stage worms. In terms of worm recoveries and liver egg loads, the dose-related effects of low radiation dosages (i.e. up to 4 krads) on the development of *S. margrebowiei* were seen to be similar to those reported previously for other schistosomes (see Discussion). Hence the assumption was made that the degrees of attenuation resulting from irradiation with 5 and 20 krads would also be similar. Thus, on the basis of observations reported by Bickle *et al* (1979b), pertaining to *S. mansoni*, it was anticipated that a radiation dose of 5 krads would result in infections characterised by essentially normal migration to the lungs, somewhat reduced migration to the liver, and mortalities of schistosomula in both the lungs and livers. A dose of 20 krads was expected to result in delayed and perhaps partially reduced migration to the lungs but little recruitment to the liver, with the lungs therefore being the major site of parasite death (Bickle *et al*, 1979b; Mangold and Dean, 1984; Elsaghier and McLaren, 1989; Cardoso and Coelho, 1989).

Experiment 1 (Table 5.1): In this experiment mice were exposed initially to 2.5 or 5 krad-attenuated cercariae of *S. leiperi*, *S. margrebowiei* or *S. mansoni* (RSA strain) and challenged 9 weeks later with *S. mansoni*; challenge infections with the antelope schistosomes were precluded by a lack of animals. *S. margrebowiei* and *S. mansoni* primary infection loads were in the range of 250-300 cercariae per animal, but in the case of *S. leiperi* cercariae they were limited to approximately 120/animal, due to a shortage of cercariae.

Worm returns from the IC groups showed that there were some differences in the abilities of 2.5 krad-attenuated cercariae of the three species to either develop to or survive at the liver stage. Small numbers of sterile worms were recovered from all the animals in the *S. leiperi* group (mean worm return 3.8%; predominantly females), from 7/9 of the *S. margrebowiei* group (mean worm return 0.9%; virtually all females), and from only 2/10 of the *S. mansoni* group (mean worm return <0.1%). These differences could not be

TABLE 5.1 Effect of initial exposure to 2.5 or 5 krad-attenuated cercariae of *Schistosoma leiperi* (LP), *S. margrebowiei* (MB) or RSA *S. mansoni* (RSA) cercariae on challenge with RSA *S. mansoni* at 9 weeks: total worm loads.

GROUP[a]	*n*	INITIAL EXPOSURE			TOTAL WORM LOAD[b]	
		Species	Cercarial Load	Radiation Dose (krad)	$\bar{x}$ (s.d.)	Reduction[c] (%)
CC	10	-	-	-	27.7 (4.3)	-
IC	10	LP	120	2.5	4.6 (2.0)	-
EXP	10	LP	120	2.5	31.9 (7.4)	**1.4**
EXP	10	LP	120	5	29.3 (5.4)	**-5.8**
IC	9	MB	256	2.5	2.3 (1.6)	-
EXP	10	MB	256	2.5	28.1 (4.7)	**6.9**
EXP	10	MB	298	5	30.2 (5.3)	**-9.0**
IC	10	RSA	273	2.5	0.2	-
EXP	9	RSA	273	2.5	22.6 (5.9)	**19.1***
EXP	10	RSA	248	5	24.2 (9.3)	**12.6**

a CC (challenge infection control) refers to the group which received the challenge infection only and IC (initial infection control) to groups exposed only to an initial infection with 2.5 krad-attenuated cercariae; EXP (experimental) groups were exposed both to initial infection with either 2.5- or 5 krad-attenuated cercariae and to challenge.

b The estimated cercarial load used for the challenge infection was approximately 84 cercariae/mouse; all groups were perfused 8 weeks after the challenge infection.

c Levels of statistical significance are indicated as follows: *, $P < 0.05$.

ascribed to major differences in cercarial infectivity, since worm returns from animals infected with matching non-irradiated cercariae (i.e. derived from the same pools, of the same age, and subjected to the same procedures, excluding irradiation) were 26%, 30% and 32% for *S. leiperi*, *S. margrebowiei* and *S. mansoni*, respectively (data not shown); in all instances, sex-ratios were found to be very evenly balanced.

Evidence of slight but significant resistance to homologous challenge was found in animals exposed initially to 2.5 krad-attenuated *S. mansoni* cercariae. However, there was clearly no resistance in animals vaccinated with either 2.5 or 5 krad-attenuated *S. margrebowiei* or *S. leiperi* cercariae upon heterologous challenge with *S. mansoni*.

Experiment 2 (Table 5.2): In this experiment animals were subjected to initial infections with either 5 or 20 krad-attenuated *S. margrebowiei* or *S. mansoni* (RSA strain) cercariae. For each permutation two cercarial infection loads were applied, i.e. approximately 45 or 250 cercariae per animal. Animals were challenged 9 weeks later with *S. mansoni* (RSA strain). Worm returns from mice exposed at the time of first infection to non-irradiated *S. margrebowiei* cercariae were found to be normal (31%), but those pertaining to *S. mansoni* were considerably lower than expected (11%) (data not shown). However, it is possible that the latter value may represent something of an underestimate since almost half (6/13) of the animals in the group died prematurely - these may well have been animals with relatively heavy worm burdens.

The outcome of this experiment was essentially similar to that of the previous one, with homologous challenge worm loads being slightly and in some cases significantly reduced in animals vaccinated with attenuated *S. mansoni* cercariae, but no resistance to heterologous challenge being evident in those exposed initially to *S. margrebowiei*.

Experiment 3 (Table 5.3): In view of the poor levels of resistance obtained in the first two experiments, it was decided to determine whether or not significant resistance to homologous challenge could be induced in animals vaccinated with the PR strain of *S. mansoni*,

TABLE 5.2 Effect of initial exposure to 5 or 20 krad-attenuated *Schistosoma margrebowiei* (MB) or RSA *S. mansoni* (RSA) cercariae on challenge with RSA *S. mansoni* at 9 weeks: total worm loads.

GROUP[a]	*n*	INITIAL EXPOSURE			TOTAL WORM LOAD[b]	
		Species	Cercarial Load	Irradiation Dose (Krad)	$\bar{x}$ (s.d.)	Reduction[c] (%)
CC	12	-	-	-	27.5 (5.8)	-
EXP	10	MB	46	5	30.4 (8.4)	**-10.5**
EXP	11	MB	250	5	27.5 (5.6)	**0**
EXP	12	MB	45	20	26.3 (6.1)	**4.4**
EXP	12	MB	250	20	31.3 (7.4)	**-13.8**
EXP	12	RSA	45	5	22.4 (5.2)	**18.5**
EXP	12	RSA	255	5	21.3 (4.9)	**22.5***
EXP	12	RSA	46	20	20.6 (6.5)	**25.1***
EXP	12	RSA	255	20	24.3 (9.7)	**11.6**

a CC (challenge infection control) refers to the group which received the challenge infection only and EXP (experimental) to groups which received both initial and challenge infections.

b The estimated cercarial dose used for the challenge infection was approximately 101 cercariae/mouse; all groups were perfused 8 weeks after the challenge infection.

c Levels of statistical significance are indicated as follows: *, $P < 0.05$.

TABLE 5.3 Effect of initial exposure to radiation-attenuated cercariae of the PR strain of *Schistosoma mansoni* on homologous challenge at 4.5 and 9 weeks: total worm loads.

GROUP[a]	INITIAL EXPOSURE		TOTAL WORM LOAD[c]					
	Cercarial Load	Radiation Dose (krads)	*4.5 week Challenge*[b]			*9 week Challenge*[b]		
			n	$\bar{x}$ (s.d.)	Reduct.[d] (%)	*n*	$\bar{x}$ (s.d.)	Reduct.[d] (%)
CC	-	-	11	33.6 (6.8)	-	12	36.8 (7.4)	-
EXP	239	5	11	29.7 (6.1)	**11.6**	10	31.1 (7.0)	**15.5**
EXP	456	5	11	30.7 (8.4)	**8.6**	11	29.5 (5.3)	**19.8***
EXP	246	20	11	23.9 (4.8)	**28.9****	11	28.1 (5.4)	**23.6****
EXP	466	20	11	20.8 (4.1)	**38.1*****	12	21.2 (7.4)	**42.4*****

a CC (challenge infection control) refers to the group which received the challenge infection only and EXP (experimental) to groups which received both initial and challenge infections.

b The estimated cercarial doses used for the 4.5 and 9 week challenge infections were 79 and 87 cercariae/mouse, respectively.

c Perfusions were carried out 6 weeks after challenge infections.

d Levels of statistical significance are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

as has repeatedly been reported in the literature (see Introduction). Cercariae were irradiated with either 5 or 20 krads so as to compare the effects of low and high attenuation doses. In order to assess the importance of the 'inoculum' size, animals were exposed either to approximately 240 or 460 cercariae. Challenge infections were carried out after 4.5 and 9 weeks. The mean worm recovery from animals exposed to non-irradiated cercariae at the time of first infection was 41% (data not shown).

Highly significant, albeit moderate, levels of resistance were demonstrated in this experiment. However, while there was no obvious relationship between levels of resistance and time of challenge, it was found that animals exposed to 20 krad-attenuated cercariae were generally more resistant to challenge than those exposed to cercariae irradiated with 5 krads. Furthermore, within the 20 krad groups levels of resistance were higher in the animals exposed to approximately 460 cercariae (38.1% and 42.4%; $P < 0.001$) than in those exposed to approximately 240 cercariae (28.9% and 23.6%; $P < 0.01$).

Experiment 4 (Table 5.4): Since the results of the third experiment suggested that the numbers of attenuated cercariae used for immunising exposures in the first two studies may have been too low, an experiment was carried out in which mice were exposed to approximately 700 highly irradiated (20 krads) *S. margrebowiei* cercariae and challenged 4.5 or 9 weeks later with either *S. margrebowiei* or the PR strain of *S. mansoni*. The infectivity of non-irradiated cercariae at the time of vaccination was not assessed in this experiment. However, some of the surplus irradiated cercariae were examined microscopically at the end of the exposure period and were found to be highly motile; it was therefore assumed that their infective capacity was satisfactory.

Challenge worm loads in 3 of the 4 vaccinated groups were found to be slightly lower than those in the corresponding control groups. However, no such differences were evident in terms of tissue egg loads.

TABLE 5.4 Effects of initial exposure to a high load of 20 krad-attenuated *Schistosoma margrebowiei* cercariae (approximately 700/mouse) on challenge at 4.5 and 9 weeks with either *S. margrebowiei* (MB) or the PR strain of *S. mansoni* (PR): worm and egg loads.

Pre-challenge Interval	GROUP[a]	*n*	INFECTION LOADS[c]					
			Total worms		Total eggs[d]		Tissue eggs/ gravid worm pair	
			$\bar{x}$ (s.d.)	Reduct.[e] (%)	$\bar{x}$ (s.d.)	Reduct.[e] (%)	$\bar{x}$ (s.d.)	Reduct.[e] (%)
4.5 weeks[b]								
	CC-MB	12	28.8 (4.6)	-	119 435 (59 839)	-	10 572 (3 958)	-
	EXP-MB	12	23.8 (3.8)	17.4*	116 992 (49 217)	2.0	11 400 (3 190)	-7.8
	CC-PR	12	40.9 (5.2)	-	61 742 (10 622)	-	3 828 (394)	-
	EXP-PR	12	35.4 (6.1)	13.4*	56 183 (27 206)	9.0	3 943 (997)	-3.0
9 weeks[b]								
	CC-MB	12	26.8 (4.2)	-	127 733 (73 328)	-	13 044 (6 925)	-
	EXP-MB	12	27.3 (4.7)	-1.9	137 942 (50 881)	-8.0	13 087 (3 434)	-0.3
	CC-PR	12	41.1 (7.1)	-	62 350 (11 881)	-	3 616 (591)	-
	EXP-PR	11	34.7 (6.9)	15.6*	62 705 (27 616)	-0.6	4 058 (879)	-12.2

a CC (challenge control) groups were subjected to the challenge infections only, while EXP groups received both initial and challenge infections; the suffixes -MB and -PR indicate which schistosome was used for the challenge infection.

b The estimated cercarial loads used for the 4.5 and 9 week challenge infections were, in the case of *S. margrebowiei*, 103 and 104 cercariae/mouse respectively, and in the case of PR *S. mansoni*, 95 and 97 respectively.

c Perfusions were carried out 6 weeks after challenge infections.

d Total egg load comprises the sum of eggs in the liver and GIT.

e Levels of statistical significance are indicated as follows: *, $P < 0.05$.

5.4 DISCUSSION

Effect of radiation dose on post-cercarial development: comparison between S. margrebowiei and other species.

The dose-related effects of radiation on the post-cercarial development of *S. margrebowiei*, as shown in Figures 5.1 and 5.2, appear to be generally similar to those observed in published studies on *S. mansoni*. The difference in the effects of 1 krad on *S. margrebowiei* and the PR strain of *S. mansoni* noted in the present study seems unlikely to be of any significance, since some variability is evident in the literature in terms of the degree of attenuation resulting from this low dose. Thus some workers have observed little or no reduction in *S. mansoni* worm loads (Smithers, 1962; Erickson and Caldwell, 1965; Minard *et al*, 1978), while others found that loads were reduced by about 40% compared to controls (Villella *et al*, 1961; Radke and Sadun, 1963). However, all have observed pronounced reductions at irradiation levels of 1.5-2 krads and very low recoveries at a dosage of 2.5 krads (generally about 5% of control worm loads). The occasional recovery of small numbers of worms from animals infected with cercariae or schistosomula attenuated at a dose of around 3 krads, as noted in the present study, has also been reported by Radke and Sadun (1963), Erickson and Caldwell (1965), and Bickle *et al* (1979b).

Egg production, albeit greatly reduced, has been observed on a number of occasions in animals infected with 2 krad-attenuated *S. mansoni* larvae (Smithers, 1962; Radke and Sadun, 1963; Erickson and Caldwell, 1965; Bickle *et al*, 1979b). In contrast, Hsu *et al* (1962) found that an irradiation dose of about 1.2 krads was sufficient to fully prevent sexual maturation of *S. japonicum*. In the present study *S. margrebowiei* egg production was almost fully abrogated by a dosage of 1.5 krads (Figure 5.2). In this respect *S. margrebowiei* appears to show a response intermediate between *S. mansoni* and *S. japonicum*. The finding that irradiation resulted in the establishment of proportionally fewer male than female worms (Figure 5.3) concurs with observations reported in a number of studies on *S. mansoni* (Perlowagora-Szumlewicz and Cannon, 1966; Bickle *et al*, 1979b; Cardoso and Coelho, 1990).

The importance of radiation dose and cercarial infection load in effecting immunity.

The results of the experiment in which animals were vaccinated and challenged with the PR strain of *S. mansoni* (third vaccination experiment), namely that the use of higher attenuation doses (20 krads versus 5 krads) and larger numbers of cercariae (460 versus 240) produced the best levels of resistance (Table 5.3), correspond well with the findings of Bickle *et al* (1979c). These authors observed firstly that schistosomula attenuated with 20 krads consistently induced better protection than those irradiated with 2.3 or 10 krads, and secondly that inoculation with 50-200 schistosomula tended to produce somewhat variable levels of resistance, whereas that with 500 or more schistosomula consistently resulted in optimum protection.

Species-specificity of attenuated S. margrebowiei- and S. leiperi-induced immunity

It is tempting to suggest that the lack of resistance to heterologous challenge in the first two vaccination experiments (Tables 5.1 and 5.2) is further evidence of the species-specificity of attenuated vaccine immunity, especially considering the fact that there was evidence of some, albeit weak, resistance to homologous challenge. However, the results of the two subsequent experiments strongly indicate that such a conclusion is premature. Firstly, as discussed above, it is clear from the homologous study with the PR strain of *S. mansoni* that the elicitation of meaningful levels of protection may require the application of fairly precise experimental conditions. In this regard it is of interest to note that the maximum reduction of challenge worm load achieved in this study (42.4%) appears to be relatively low by comparison with that seen in many comparable studies (Dean, 1983). Although the majority of other attenuated vaccine work has involved the use of mouse strains other than BALB/c, there is some evidence that levels of protective immunity in this strain may be somewhat lower than those in some of the commonly used strains; specifically, Smith and Clegg (1984) reported that the maximum reductions in challenge worm burdens in BALB/c and CBA/Ca mice were 58% and 94%, respectively. However, levels of resistance in the present study appear to be even lower than those in the few published studies involving BALB/c mice, where

challenge worm reductions of the order of 60-65% have been noted (Sher *et al*, 1982; Smith and Clegg, 1984; Kamal *et al*, 1991). This further reinforces the importance of identifying the experimental procedures which promote the development of optimal immune resistance.

With respect to methodology it is perhaps noteworthy that in the present study the paddling method was used for exposing mice to irradiated cercariae, whereas in the overwhelming majority of published studies the tail immersion (Olivier and Stirewalt, 1952) and/or ring (Smithers and Terry, 1965b) methods have been used. With the latter two methods cercarial penetration is by necessity confined to a relatively small area of the body surface, whereas with the paddling method it undoubtedly occurs over a more widespread area of the body. It is conceivable that these differences in penetration patterns may in some way influence the development of protective immunity.

The lack of immunity to homologous challenge in mice exposed to large numbers of 20 krad-attenuated *S. margrebowiei* cercariae (Table 5.4) also makes it clear that it would be premature to regard the results presented in this chapter as evidence of species-specific immunity. Only once the conditions have been identified which result in the development of significant resistance to homologous challenge with *S. margrebowiei* and/or *S. leiperi* will it be possible to investigate the issue of species specificity.

The reasons for the lack of homologous resistance to *S. margrebowiei* are as yet unclear. In this regard it is of interest to note the results of a recent study by Mitchell *et al* (1990) who reported that vaccination of mice with highly irradiated (30 krads) cercariae of *S. japonicum* (Philippine strain) failed to induce protection against homologous challenge, whereas similar vaccination with *S. mansoni* resulted in substantial protection. These authors suggested that the difference in the abilities of the two schistosomes to induce immunity may be related to differences in their migration kinetics, particularly in terms of their respective transit times through the lungs, which are an important site of immune sensitization and challenge parasite attrition (Wilson and Coulson, 1989). It is possible that the failure of *S. margrebowiei* to induce homologous immunity may be explained in similar terms.

Use of attenuated antelope schistosomes in mice to model infection in man.

Since previous attempts to induce cross-specific immunity in rodents with highly irradiated schistosome larvae have consistently been unsuccessful (see Introduction), it seems reasonable to anticipate that the same would ultimately prove to be true of similar attempts with *S. margrebowiei* and *S. leiperi*. However, even if exposure of mice to irradiated cercariae of either of these species can be shown to induce resistance against subsequent infection with *S. mansoni*, it is important, in the context of goals of the present investigation, to consider whether this model system adequately represents the situation in man. Highly irradiated schistosomula are clearly severely physiologically damaged, resulting in abnormally prolonged transit times and/or premature demise in early migration sites, notably the skin, skin-draining lymph nodes and lungs, which are normally negotiated with relative ease. These artificially-induced alterations appear to be the critical factors in the induction of high levels of immune resistance in the mouse model (Bickle, 1982; McLaren, 1989; Wilson and Coulson, 1989; Constant *et al*, 1990). While it is unclear at what stage infections with *S. margrebowiei* and *S. leiperi* abort in man, the apparent ability of *S. margrebowiei* to occasionally reach sexual maturity (see Section 1.10.4) indicates that the early development of at least this species in man is comparatively normal. This suggests that if further experiments in the mouse model are to be relevant to the aims of the present study, they should focus on an approach involving repeated exposure, over extended periods, to cercariae attenuated with low doses of irradiation (1.5-2 krads, i.e. sufficient to fully sterilize worms, but allowing a small proportion to undergo partial development). While the existing evidence suggests that such infections have a low immunization potential (similar to non-attenuated unisexual infections), it is significant to note the view of McLaren and Smithers (1987), who suggest that protective immunity does develop in mice harbouring non-patent infections, but that it takes longer to reach significant levels than is the case in mice infected with normal or highly irradiated cercariae.

CHAPTER SIX

ON THE POTENTIAL OF THE RAT AND THE GUINEA PIG AS MODELS IN WHICH TO TEST THE ABILITY OF THE ANTELOPE SCHISTOSOMES TO INDUCE CROSS-PROTECTION AGAINST *S. MANSONI*

6.1 INTRODUCTION

During the course of the studies described in the previous two chapters it became increasingly apparent that the mouse is of limited value as an animal model host for investigations of the interactions suggested to occur between the antelope schistosomes and *S. mansoni* in man. This is traceable to the fact that the mouse is a permissive host for both *S. margrebowiei* and *S. leiperi*. Thus, while patent infections of these species cause substantial concomitant immunity, this is clearly due in part to non-immunological mechanisms (e.g. portal shunting), which are the consequence of egg-induced immunopathology (see Section 4.4). And although their normal development may be attenuated by means of gamma irradiation, this results in modifications to the parasites and their migration behaviour (see Section 5.4) which possibly do not reflect the development of non-attenuated schistosomes in non-permissive hosts (which is the situation applicable to *S. margrebowiei* and *S. leiperi* in man). Hence consideration was given to the possible use of other laboratory rodents as alternative models for further investigations.

All three of the other common laboratory rodents, namely rats, hamsters and guinea pigs, have been used for experimental schistosomiasis research. The hamster (Maddison, 1982) was regarded as inappropriate for the purposes of the present study for the obvious reason that it is a fully permissive host for both *S. margrebowiei* and *S. leiperi* (see Section 2.4). However, it was decided that both the rat and guinea pig were worthy of investigation, for the reasons elaborated below.

After the mouse, the laboratory rat (*Rattus norvegicus*) is the most extensively utilised model for experimental studies on the immunology of schistosomiasis (Capron and Capron, 1986). In spite of the fact that it is a non-permissive host for both *S. mansoni* and *S. japonicum*, spontaneously eliminating the bulk of worms resulting from

a primary infection some 4-6 weeks after exposure to cercariae (Ho, 1963; Smithers and Terry, 1965c; Knopf *et al*, 1977; Moloney *et al*, 1987c), it develops strong resistance to reinfection following primary exposure to either normal or radiation-attenuated cercariae (Smithers and Terry, 1965c; Usawattanakul *et al*, 1982; Ford *et al*, 1984a; Moloney *et al*, 1987). This resistance has been shown, at least in the case of *S. mansoni*, to be immunologically-mediated (reviewed by Phillips *et al*, 1991). The rat/*S.mansoni* system has yielded important insights into mechanisms of anti-schistosomulum immunity, in particular those involving antibodies (IgG2a and IgE) acting in collaboration with various components of the cellular immune system (eosinophils, macrophages and platelets) (Capron *et al*, 1987). There is considerable indirect evidence to suggest that mechanisms of this type (referred to as 'antibody dependent cellular cytotoxicity' [ADCC] mechanisms) may be operative in human immunity to schistosomes (Capron *et al*, 1987; Damian, 1989). In view of this it was considered that the rat might provide an appropriate model in which to assess the capability of the antelope schistosomes to elicit cross-protection against *S. mansoni*.

Until quite recently the guinea pig was largely disregarded as a host for experimental schistosomiasis research. However, as a result of studies by McLaren and colleagues this animal is now recognised as an important alternative to the mouse and rat as a model for investigations on immunity to schistosomes (McLaren and Smithers, 1987). Infection of these animals with either *S. mansoni* (Pearce and McLaren, 1983a) or *S. japonicum* (Ho, 1963) results in the establishment of stable, sexually-mature adult worm populations and substantial tissue egg deposition. Moreover, they have been shown to develop strong resistance to homologous challenge following exposure to either normal or radiation-attenuated *S. mansoni* cercariae (Pearce and McLaren , 1983b), and moderate homologous resistance following vaccination with attenuated *S. japonicum* schistosomula (Xu *et al*, 1991). However, there is evidence of significant variation in the ability of guinea pigs to support different species of schistosomes, since it has been reported that they are poor hosts both for *S. spindale* (Dutt, 1962) and *S. intercalatum* (Wright *et al*, 1972). It was thus speculated that the guinea pig might prove to be a host in which the development of one or both of the antelope schistosomes is naturally curtailed, thereby simulating the situation in man.

This chapter describes (1) initial experiments aimed at assessing the abilities of *S. margrebowiei* and *S. leiperi* to develop in rats and guinea pigs; (2) a subsequent investigation into the induction of resistance against *S. mansoni* challenge in guinea pigs by repeated prior exposure to cercariae of the antelope schistosomes.

6.2 MATERIALS AND METHODS

6.2.1 Schistosomes

The schistosomes used in these studies were *S. margrebowiei*, *S. leiperi* and the PR strain of *S. mansoni*. Details regarding the origins and maintenance of these parasites are presented in Sections 2.2.1 and 4.2.1.

6.2.2 Experimental Hosts

Male animals were used throughout. Wistar rats, 6-10 weeks of age at the time of infection, were obtained either from the Natal Institute of Immunology (NII; Pinetown) or the South African Institute for Medical Research (SAIMR; Johannesburg). They were housed in groups of 3-4 under conventional conditions in polypropylene cages (dimensions 445 x 280 x 125mm; Labotec, Johannesburg) on woodshavings. Tap water and commercially-produced rat pellets (Epol, Pietermaritzburg) were provided *ad libitum*.

Two types of guinea pig were used during the present studies: (i) Pied-coated animals of undefined lineage, bred in the animal facility of the Research Institute for Diseases in a Tropical Environment (RIDTE) in Durban. The colony from which these animals were derived was originally established from stocks supplied by the Natal Institute of Immunology. It comprised animals with predominantly smooth coats but also included a small proportion with whorled coats, indicating a mixture of animals of the English and Abyssinian varieties (Cooper and Schiller, 1975). (ii) Animals of the Dunkin-Hartley variety (Dunkin *et al*, 1930), obtained from the SAIMR. Guinea pigs ranged in size from 200-400g (at the time of first infection), although within individual experiments they were size-matched to within 100g of each other. They were housed under conventional

conditions in fibreglass cages (810 x 610 x 250mm; Labotec, Johannesburg) suspended on mobile racks. Bedding consisted of woodshavings covered by a layer of straw. Tap water, supplemented with 0.7% (mass/vol) ascorbic acid, and rabbit pellets (Epol, Pietermaritzburg) were provided *ad libitum*.

Details regarding the origins, housing and maintenance of BALB/c mice and *Mastomys coucha*, which were also used during the course of the present studies, are presented in Section 2.2.2.

All animals were maintained under the environmental conditions described in Section 2.2.2.

6.2.3 Infection of Rodents

The infection procedure for rats and guinea pigs was essentially the same as that used for mice and *M. coucha* (Section 2.2.3), except that the duration of the pre-soaking intervals was increased from 3 to 6 minutes, the period of exposure to cercariae was increased from 40-45min to 50-60min, and, for obvious reasons, larger infection vessels were used. Rats and young guinea pigs (up to 400g) were individually exposed to cercariae in 2 litre plastic kitchenware containers (internal dimensions, 120mm diameter x 175mm height) and adult guinea pigs in 5 litre plastic buckets (internal dimensions, 180mm diameter x 210mm height); the volumes of cercarial suspension required to ensure adequate levels of partial immersion (i.e. immersion of the posterior third of the animal) in each type of vessel were 300ml and 800ml, respectively. Mice and *M. coucha* were infected according to the method detailed in Section 2.2.3.

6.2.4 Anaesthesia

Rats were anaesthetised routinely by intra-peritoneal injection of 1.5ml of a 50% (v/v) Sagatal solution (see Section 2.2.5) containing tri-sodium citrate at a final concentration of 0.75% (mass/vol).

Methods of anaesthetizing guinea pigs varied according to the procedures to be carried out subsequently. Where animals were to be bled via the renal vein immediately prior to worm recovery (see

Section 6.2.6), anaesthesia was routinely achieved by intra-peritoneal injection of 2.5ml of the same solution as used for rats. When reversible anaesthesia was required for purposes of cardiac puncture (see Section 6.2.6), a method was used which involved a combination of injectable and inhalational anaesthetics. Thus animals were first injected intra-peritoneally with 1ml of a 1-in-6 solution (v/v) of Sagatal followed by 0.5ml of Rompun (xylazine, 20mg/ml; Bayer Miles [Pty] Ltd, South Africa), then connected to a methoxyflurane rebreathing bag (Austin, personal communication). The latter device was constructed by taping a rigid plastic tube (inner diameter, 55mm; length, 60mm) into the end of a polyethylene bag, covering the open end of the tube with a piece of thin latex rubber sheeting and making a 2cm slit across the middle of the rubber closure. The bag was designed in such a way that its expanded volume was approximately 1 litre. Following the introduction of 0.2ml of methoxyflurane (Penthrane; Abbott Laboratories, North Chicago, Illinois), it was filled with oxygen, whereupon the nose and mouth of the guinea pig were immediately pressed firmly into the vent in the rubber membrane. Methoxyflurane is particularly recommended as an inhalational anaesthetic for guinea pigs (Flecknell, 1987), and was used for the reason that it eliminated spasmodic movements of the animals which tended to occur when only the pentobarbitone sodium/xylazine combination was used, and which increased both the difficulty of blood sampling and the risk of injury to the animal. When animals were to be perfused after cardiac puncture they received an additional intra-peritoneal injection of 1ml of 50% Sagatal solution subsequent to the completion of the bleeding procedure.

The plane of anaesthesia in rats and guinea pigs was evaluated by observing their responses to toe-pinching. Procedures pertaining to mice and *M. coucha* were as described in Section 2.2.5.

6.2.5 Dissection of Animals

Animals were dissected according to the method of Jackson *et al* (1982) in order to expose the heart and portal vein. Rats and guinea pigs were routinely flushed with citrated saline at intervals during the dissection procedure so as to prevent excessive bleeding into the abdominal cavity, since this resulted in the formation of blood clots which tended to trap schistosome worms. In some instances it was

necessary to clamp the ends of severed blood vessels using small artery forceps.

6.2.6 Collection of Blood Samples from Rats and Guinea Pigs

Blood samples were collected from one of two different sampling sites into 5ml disposable plastic syringes equipped with 23 gauge hypodermic needles. Firstly, during the preliminary experiments aimed at assessing the development of the different schistosomes in the rat and guinea pig (Section 6.3.1) samples were recovered from the left renal vein immediately prior to worm recovery (Section 6.2.8). After withdrawal of blood into the syringe and before extraction of the needle the vein was clamped on either side of the sampling site. This served to prevent bleeding into the abdominal cavity as well as the unnecessary loss of perfusion fluid (and a possible reduction in perfusion pressure) during the worm recovery procedure. Secondly, during the heterologous immunity experiment (Section 6.3.2) bleeding was achieved by means of cardiac puncture (Bivin and Smith, 1984).

Blood samples were transferred into test tubes and allowed to clot either at room temperature for 1-2 hours, or overnight at 4 C, following which they were centrifuged at 1500*g* for 5 min. Sera were recovered by aspiration using pasteur pipettes and stored at -20 C until required for serology (see below).

6.2.7 Serology

Rat and guinea pig sera were tested for the presence of anti-schistosome IgG antibodies by means of the indirect fluorescent antibody test described in Section 3.2.4. The method differed only in terms of the specificity of the fluorescent labels used (goat anti-rat and anti-guinea pig IgG fluorescein isothiocyanate conjugates; Sigma Chemical Co., USA).

6.2.8 Recovery and Counting of Schistosome Worms

Worms were recovered from infected rodents by means of the portal perfusion technique essentially as described in Section 2.2.6.

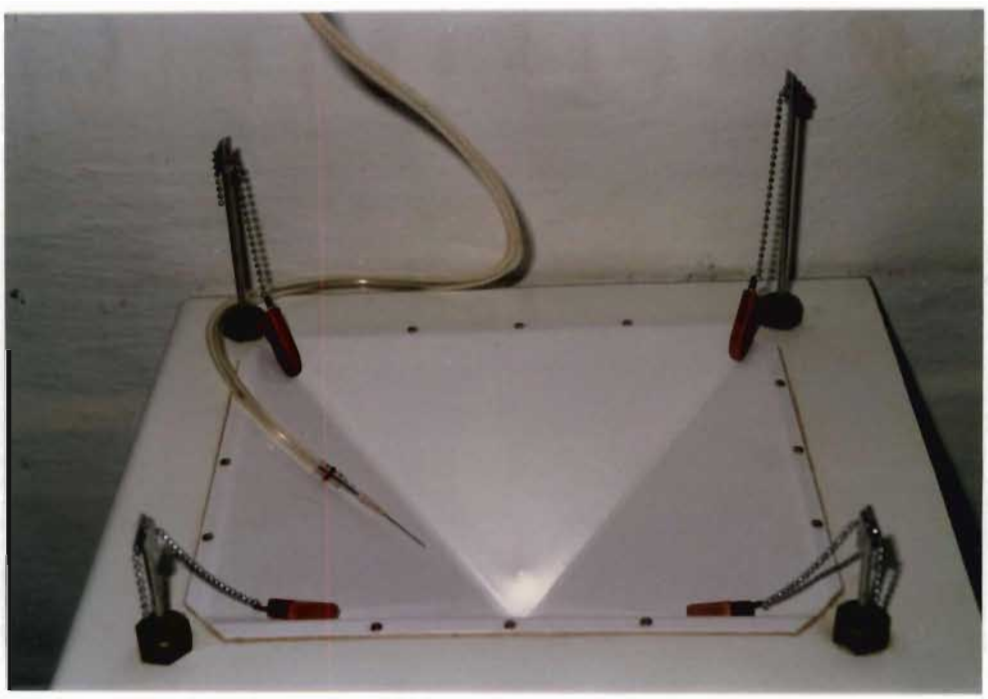
However, for rats and guinea pigs a larger apparatus (Figure 6.1) was employed and perfusion fluid was delivered to the left ventricle by means of a rotary peristaltic pump, at a rate of approximately 200ml/min; rats were perfused for 2-3 min and guinea pigs for 3-4 min. The intestines and mesenteries of guinea pigs were massaged during the course of perfusion as this was found to enhance the clearance of blood from the mesenteric veins.

Sexing, counting and descriptive classification of worms was performed as outlined in Section 2.2.6.

6.2.9 Determination of Guinea Pig Tissue Egg Loads

On completion of perfusions livers and intestines were dissected out. Adipose tissues were stripped off the intestines and discarded. The contents of the small and large intestines were extruded with the aid of a suitably-shaped glass rod and also discarded; similarly, the caecum was slit open longitudinally and gently flushed with tap water to remove the contents. The livers and intestinal tissues were weighed, following which they were digested in a 4% (mass/vol) potassium hydroxide solution (2.5-3ml/g of tissue) at 37 C for 16 hours (Cheever, 1968). In order to partially solubilise fats released from the tissues, digests were treated with detergent ('Down To Earth' dishwashing liquid; Reckitt Household Products, Elandsfontein) at an approximately 5% (v/v) final concentration for 45 min at 37 C. Intestinal digests were then transferred into 1 litre separating funnels and allowed to settle for a minimum of 30 min, whereupon they were run off into beakers, with the upper fat layers being discarded. Each digest was then poured into a helminth filter (Visser and Pitchford, 1972) with inner and outer mesh apertures of 50 and 80 microns and flushed repeatedly with tap water. The internal dimensions (diameter x length) of the inner and outer filters were 64 x 230mm and 70 x 280mm, respectively. After washing, the egg bearing filtrate was run off into a plastic jar, fixed with formalin and stored. Between digests the filter was immersed for a few minutes in a detergent solution at 50-60 C; this served to remove fatty deposits which otherwise accumulated in the filter mesh. Thereafter it was thoroughly flushed with tap water, using a hose spray-gun, to prevent the possibility of 'carry over' of eggs from one sample to the next. On most occasions two helminth filters were employed in

FIGURE 6.1 Apparatus used for recovery of schistosome worms from rats and guinea pigs. Tray dimensions (length x width x vertical depth) are 350 x 300 x 150mm.



rotation; thus while one was in use, the other was soaking in the detergent solution.

The number of eggs in each filtrate was determined by means of the procedure described in Section 2.2.7.

6.2.10 Statistical Analyses

Group means were compared using the Mann-Whitney test (Siegel and Castellan, 1989).

6.3 RESULTS AND DISCUSSION

6.3.1 Development of *S. margrebowiei* and *S. leiperi* in Rats and Guinea pigs

A series of experiments was performed aimed at obtaining data on the infection characteristics of the antelope schistosomes in rats and guinea pigs. In the case of guinea pigs, infections were also carried out with the PR strain of *S. mansoni*, for comparative purposes. The details of the various experiments are summarised as follows:

S. margrebowiei: 4 experiments

MB-I: Rats exposed to approximately 198 cercariae each; perfused 8 weeks after exposure.

MB-II: Rats and guinea pigs exposed to approximately 500 cercariae each; perfused 14.5 weeks after exposure.

MB-III: Rats exposed to approximately 472 cercariae each; perfusions at 4, 6 and 10 weeks of infection.

MB-IV: Guinea pigs exposed to approximately 496 cercariae each; perfusions at 8 and 12 weeks of infection.

S. leiperi: 3 experiments

LP-I: Rats and guinea pigs exposed to approximately 534 cercariae each; perfused 11.5 weeks after exposure.

LP-II: Rats exposed to approximately 512 cercariae each; perfusions at 6, 8 and 12 weeks of infection.

LP-III: Guinea pigs exposed to approximately 461 cercariae each; perfusions at 12 and 16 weeks of infection.

S. mansoni: 2 experiments

PR-I: Guinea pigs exposed to approximately 499 cercariae each; perfused at 14.5 weeks of infection.

PR-II: Guinea pigs exposed to approximately 510 cercariae each; perfused at 8 weeks of infection.

Infection load data and serology results from rats are shown in Tables 6.1 and 6.2, respectively, and those from guinea pigs are shown in Tables 6.3 and 6.4, respectively. For comparative purposes worm load data were also obtained from mice or *M. coucha* infected simultaneously with rats and/or guinea pigs; these are shown in Table 6.5.

Infections in Rats

S. margrebowiei worm recoveries from rats were consistently below 5% (Table 6.1) and there was evidence of a difference in the establishment and survival patterns of male and female worms. Thus, in the first study (MB-I; perfused at 8 weeks of infection) single male worms were recovered from only 3 out of 10 animals, and in the second study (MB-II; worms recovered after 14.5 weeks) no males were found. In the third experiment (MB-III) a pronounced drop-off in worm recoveries over the period 4 to 10 weeks of infection was observed. Male worms were particularly severely affected, dropping from a mean of 12.9 at 4 weeks to 0 at 10 weeks; in contrast the mean number of

TABLE 6.1 Worm recoveries of *Schistosoma margrebowiei* and *S. leiperi* from Wistar rats.

Schistosome	*S. margrebowiei*					*S. leiperi*			
Experiment Number[a]	MB-I	MB-II	MB-III			LP-I	LP-II		
Cercarial Load	198	500	472			534	512		
Number of animals	10	10	7	7	7	8	7	7	8
Weeks of Infection	8	14.5	4	6	10	11.5	6	8	12
% Worm recovery	4.0 (2.8)	1.5 (1.1)	4.2 (3.1)	2.1 (0.4)	0.4 (0.2)	9.4 (3.6)	6.5 (2.9)	6.7 (3.6)	3.0 (2.1)
Male worms[b]	0.3 (0.5)	0	12.9 (8.7)	3.6 (1.7)	0	15.1 (6.9)	19.3 (9.3)	18.7 (10.0)	9.4 (8.5)
Female worms[b]	7.7 (5.4)	7.5 (5.6)	6.3 (6.7)	6.0 (1.9)	2.6 (1.4)	34.8 (15.5)	13.7 (8.6)	15.4 (9.1)	5.9 (4.1)
Total worms[b,c]	8.0 (5.6)	7.5 (5.6)	19.7 (14.8)	10.0 (1.8)	2.6 (1.4)	49.9 (18.6)	33.0 (14.9)	34.1 (18.6)	15.3 (10.7)
Males/Total (%)	4%	0	65%	36%	0	30%	58%	55%	61%
Potential worm pairs[d]	0.3 (0.5)	0	6.3 (6.7)	2.9 (1.6)	0	15.1 (6.9)	11.3 (8.0)	14.9 (9.0)	5.3 (3.3)
Gravid worm pairs	0	0	0	0	0	0	0	0	0

Mean values are shown, with standard deviations in parentheses.

a Rat infections were carried out in the first three of the four experiments involving *S. margrebowiei*, and the first and third of the three involving *S. leiperi*. No *S. mansoni* infections were carried out in the rat model.

b Group means were found to differ significantly (Mann-Whitney test) in the following instances:
(i) MB-III study ($P < 0.01$ in all cases): Male worms - 4 weeks versus 6 weeks; Female worms - 6 weeks versus 10 weeks; Total worms - 4 and 6 weeks versus 10 weeks. (ii) LP-II study ($P < 0.05$ in all cases): Male and female worms - 8 weeks versus 12 weeks; Total worms - 6 and 8 weeks versus 12 weeks.

c Total worm comprises the sum of male and female worms, together with highly stunted worms of uncertain sex.

d See text (Section 2.2.6) for definition of potential worm pairs.

females dropped from 6.3 to 2.6 over the same period. Nonetheless, all the animals perfused at the 4 and 6 week intervals harboured bisexual infections (data not shown). The ratios of male:female worms in rats in the various experiments did not reflect those seen in mice infected at the corresponding times (Table 6.5). For example, in the third study approximately equal numbers of male and female worms were recovered from mice, whereas in rats the male:female ratio changed from 2:1 at 4 weeks to 1:1.7 at 6 weeks. Although worms were not measured, those recovered from rats were consistently found to be substantially smaller than those from mice. Correspondingly, there was no evidence of pairing or sexual maturation of *S. margrebowiei* worms in rats.

S. leiperi worm recoveries from rats were generally somewhat higher than those of *S. margrebowiei* (Table 6.1). Bisexual infections were observed in all instances (data not shown), although worms were again considerably smaller than their mouse-derived counterparts and clearly had not achieved sexual maturation. In the first experiment (LP-I) animals were perfused at 11.5 weeks of infection and yielded a worm return of 9.4%, with the mean number of female worms recovered being more than twice that of males; in simultaneously-infected mice the proportions of male and female worms were observed to be similar (Table 6.5). In the second rat study (LP-II), groups of rats were perfused at 6, 8 and 12 weeks of infection. The decision to initiate worm recoveries at 6 rather than 4 weeks of infection, as in the corresponding study on *S. margrebowiei* (MB-III), was based on the consideration that the slower development rate of *S. leiperi* observed in the mouse model (see Chapter 2) might also apply in the rat. Worm returns at 6 and 8 weeks of infection remained stable at 6.5-6.7%, but by 12 weeks had dropped to 3.0%. No obvious difference was noted in the degree to which male and female worms were eliminated. In this study the sex ratio of worms in rats was seen to be biased in favour of males (55-61% of total) whereas that in mice (Table 6.5) favoured females (59% of total).

Both of the antelope schistosomes were observed to elicit pronounced anti-schistosome IgG responses in the rat (Table 6.2). These exhibited patterns which closely mirrored those of worm returns, with titres being highest at the earliest study intervals and declining progressively with increasing duration of infection. At the same time there was some evidence of an inverse relationship between the

TABLE 6.2 Total anti-cercarial IgG titres in the sera of rats infected for different periods of time with *Schistosoma margrebowiei* and *S. leiperi*.

Schistosome	*S. margrebowiei*			*S. leiperi*		
Experiment Number	MB-III[a]			LP-II[a]		
Cercarial Load	472			512		
Weeks of Infection	4	6	10	6	8	12
Geometric Mean of Inverse Titres[b]	290	160	80	119	108	36
Range of Inverse Titres	160-320	80-320	10-320	40-320	20-320	10-160
n	7	7	7	7	7	7

a Details of the infection loads in these two groups at the various intervals are shown in Table 6.1.

b Sera were tested against cercarial antigens derived from the Puerto Rican strain of *S. mansoni*.

magnitude of antibody responses and the degree of host/parasite compatibility, since titres were generally higher in *S. margrebowiei*-infected animals than in those infected with *S. leiperi*. However, this may simply reflect the difference in the time scales of the respective experiments.

The finding that the antelope schistosomes developed poorly in rats was in no way unexpected in view of the known inability of these animals to support the normal development of either *S. mansoni* (Smithers and Terry, 1965c; Knopf *et al*, 1977) or *S. japonicum* (Ho, 1963; Moloney *et al*, 1987c), both of which are compatible with a wide range of other mammalian hosts (Knopf, 1982; Maddison, 1982; Rollinson and Southgate, 1987). However, the maximum percentage worm returns obtained in the present studies (<5% and <10% for *S. margrebowiei* and *S. leiperi*, respectively) are low by comparison with peak recoveries reported in studies with *S. mansoni* and *S. japonicum*. In the case of *S. mansoni*, percentage worm yields of 15-25% at 3 to 4 weeks of infection have regularly been reported (Smithers and Terry, 1965c; Knopf *et al*, 1977; Ford *et al*, 1984a and 1984b; Vignali *et al*, 1988), and in some instances yields in the range 35-45% have been obtained (Moloney *et al*, 1987c). Similar returns, spanning the range 14-47%, have been recorded for *S. japonicum* (Moloney *et al*, 1987c). This suggests that the intervals selected for worm recovery in the present studies with the antelope schistosomes did not coincide with the times of peak liver worm burdens specific to these two parasites.

The results obtained in the present investigation suggest that there may be both quantitative and qualitative differences in the infection characteristics of *S. margrebowiei* and *S. leiperi* in the rat. Firstly, percentage worm returns of *S. leiperi* were somewhat higher than those of *S. margrebowiei*. Secondly, there were indications of differences in the dynamics of worm elimination, with the attrition of *S. margrebowiei* infections being characterised by a selectively greater depletion of male worms, in contrast to that of *S. leiperi* which displayed no obvious sex bias. Moreover, *S. margrebowiei* appeared to undergo earlier and more severe attrition than *S. leiperi*. These differences are perhaps not surprising, considering the differences in the behaviour of these two parasites in the mouse model (Chapter 2). However, their patterns of development and decline (i.e. timing and magnitudes of peak liver worm loads and rates of

worm elimination) in the rat remain to be fully elucidated. This would necessitate a series of more comprehensive longitudinal studies covering a wider range of study intervals than those used during the present investigation. Since Wistar rats are poorly-defined rodents (Festing, 1979) and appear to have been used rarely for experimental schistosmiasis, it would be preferable to use a better characterised, genetically stable host for further studies. In this regard, the inbred Fischer F344 rat, which has apparently been widely utilized (Capron and Capron, 1986) would seem to be the most appropriate choice.

There are extremely few data on the infection characteristics in the rat of schistosome species other than *S. mansoni* and *S. japonicum* with which to compare the results of the present study. Wright *et al* (1972) observed that *S. intercalatum* worms recovered approximately 9 weeks after exposure were all highly stunted and amounted to only 6% of the cercarial infection load, suggesting that the development of this schistosome in rats may be similar to that of *S. leiperi*. Kagan *et al* (1954) considered the rat to be a poor host for *Schistosomatium douthitti*, a North American schistosome which is highly compatible with hamsters and mice. However, they provide no specific details on the host/parasite relationship, apart from mentioning that the worms tend to be stunted by comparison with those from mice. Contrasting findings were reported with respect to *S. incognitum* by Sinha and Srivastava (1965), who detected eggs in the faeces and recovered normal adult worms from rats infected with this parasite. Similarly, it has been reported that *S. bovis* is capable of successful pairing in this host (Coluzzi *et al*, 1965). However, it is not clear what species of rats were used in the latter two cases, and since it is known that certain species (e.g. *R. rattus*) are permissive hosts for *S. mansoni*, it seems possible, if not probable, that these studies may have involved hosts other than *R. norvegicus*.

Infections in Guinea Pigs

In the first experiment with *S. margrebowiei* (MB-II; perfused 14.5 weeks after exposure) a mean worm return of 17.4% was recorded (Table 6.3), with male worms constituting approximately 24% of the total. The corresponding figures from mice infected at the same time were

TABLE 6.3 Worm recoveries of *Schistosoma margrebowiei*, *S.leiperi* and PR *S. mansoni* from guinea pigs.

Schistosome	*S. margrebowiei*			*S. leiperi*			*S. mansoni* (PR)	
Experiment Number[a]	MB-II	MB-IV		LP-I[b]	LP-III		PR-I[b]	PR-II
Cercarial Load	500	496		534	461		499	510
Number of animals	7	7	7	6	6	7	9	5
Weeks of Infection	14.5	8	12	11.5	12	16	14.5	8
% Worm recovery	17.4 (8.2)	17.9 (8.9)	15.8 (6.8)	38.8 (7.4)	30.0 (2.2)	31.0 (6.1)	26.6 (5.3)	22.0 (10.2)
Male worms	20.6 (12.6)	54.4 (26.2)	53.9 (24.2)	60.7 (15.1)	65.2 (5.8)	61.4 (6.9)	90.2 (14.6)	51.6 (28.2)
Female worms	66.3 (32.4)	33.7 (20.7)	24.6 (17.0)	146.2 (28.3)	73.2 (8.7)	81.3 (25.3)	42.7 (14.3)	60.6 (25.3)
Total worms[c]	86.9 (40.9)	88.6 (43.9)	78.4 (33.6)	206.8 (39.7)	138.3 (10.3)	142.7 (28.3)	132.9 (26.1)	112.2 (52.1)
Males/Total (%)	24%	61%	69%	29%	47%	43%	68%	46%
Potential worm pairs[d]	20.6 (12.6)	35.6 (19.6)	24.4 (16.9)	60.7 (15.1)	63.8 (3.7)	59.9 (8.7)	42.7 (14.3)	48.0 (27.4)
Gravid worm pairs[e,g]	0	0.7 (1.1)	0	3.8 (3.4)	18.0 (13.8)	6.3 (5.4)	42.4 (14.0)	31.8 (28.0)
Gravid/potential worm pairs (%)	0	2%	0	6%	28%	11%	99%	66%
Liver egg burden[f,g]	0	4 829 (4 191)	0	1 866 (2 724)	13 101 (17 638)	4 732 (5 949)	50 040 (27 331)	19 826 (21 925)
GIT egg burden[f]	0	260 (413)	0	27 (39)	59 (102)	66 (82)	27 212 (15 131)	4 803 (5 210)

Mean values are shown, with standard deviations in parentheses.

a Guinea pig infections were carried out in the second and fourth of the four experiments involving *S. margrebowiei*, the first and second of those involving *S. leiperi*, and both of those involving PR *S. mansoni*.

b LP-I comprised 6 animals of the smooth-haired, pied-coated English variety, and PR-I comprised 4 animals of this type plus 5 of the Dunkin Hartley variety; statistical analysis of the data from the latter two sub-groups, by means of the Mann-Whitney test, revealed no significant differences, hence they were pooled. All other groups comprised Dunkin-Hartley guinea pigs only.

c Total worms comprises the sum of male and female worms, together with highly stunted worms of uncertain sex.

d See text (Section 2.2.6) for definition of potential worm pairs.

e Mean gravid worm pair loads are in all instances based on data from all animals in each group, although in the cases of the 8 week MB-IV group, the LP-I group and the 16 week LP-III group, gravid females were present in only 3/7, 4/6 and 6/7 females, respectively.

f In the case of the 12 week LP-III group the mean is based on n=3, due to the inadvertent loss of samples from 3 animals. The mean number of gravid females from the 3 animals for which egg data were available was 12.7, while that from the other 3 animals was 23.3, suggesting that had egg load data been available for all 6 animals a considerably higher mean value would probably have been obtained.

g Mean values at 12 and 16 weeks of infection in the LP-III study do not differ significantly (Mann-Whitney test).

28.5% and 30%, respectively (Table 6.5). During the second *S. margrebowiei* study (MB-IV) it was observed that worm returns remained more-or-less stable between 8 and 12 weeks of infection. The results of this study were generally similar to those of the first, in that percentage worm returns were in the same range (16-18%) and were again about 10% lower than those from the corresponding mouse group (Table 6.5). In addition, the proportion of male worms in guinea pigs (61-69% of total) was again similar to that in mice (56% of total). A notable feature of this experiment was the finding that 3 of the 7 guinea pigs perfused at 8 weeks harboured small numbers (1-3/animal) of gravid female worms. Evidence of partial vitelline gland development was also noted in a few of the other female worms, both in the animals with gravid females and in some of those without. This may have reflected regressive changes, since ova were found in the tissues (predominantly liver) not only of the 3 animals which yielded egg-bearing females, but also in those of 3 others. Neither gravid females nor eggs were detected in any of the guinea pigs perfused at 12 weeks of infection.

In contrast to *S. margrebowiei*, worm returns of *S. leiperi* from guinea pigs (Table 6.3) were considerably higher than those from simultaneously-infected mice (Table 6.5). Thus, in the first and second guinea pig experiments respectively (LP-I and LP-III), yields of 38.8% and 30-31% were obtained, compared with yields of 28.8% and 19.6% from mice. Notwithstanding the differences in percentage worm yields, the proportions of male and female worms in guinea pigs were in both studies extremely similar to those in the corresponding mouse groups. In the second study it was observed that guinea pig worm loads remained unchanged between 12 and 16 weeks of infection.

Schistosome ova were found in the tissues of all 6 guinea pigs in the first *S. leiperi* experiment (LP-I), although only 4 animals yielded gravid female worms (3-8/animal), indicative of either elimination or degeneration of sexually-mature females. Strong evidence of a regression of sexual development was obtained in the second experiment (LP-III), where it was observed that whereas 28% of potential worm pairs were gravid at 12 weeks of infection, only 11% were gravid at 16 weeks. In addition, while all 7 animals in the latter group had eggs in the tissues, gravid females were recovered from only 6. Tissue egg loads reflected the drop off in gravid worm pairs. In both experiments tissue egg deposition was confined almost

entirely to the liver, with some of the animals in each of the groups studied having no eggs in the intestines.

S. mansoni worm returns from guinea pigs (26.6% and 22.0% in the first and second experiments [i.e. PR-I and PR-II], respectively) were intermediate between those of *S. margrebowiei* and *S. leiperi* (Table 6.3), and were considerably lower than those from the corresponding mouse (37.8%) and, in particular, *M. coucha* (68.3%) groups (Table 6.5). Nevertheless the proportions of male and female worms in guinea pigs were found to match those in the mice and *M. coucha* very closely. In contrast to the antelope schistosomes, *S. mansoni* was observed to be capable of highly successful sexual maturation and oviposition in guinea pigs. However, the rate of sexual maturation in these hosts appeared to be somewhat slower than that in small rodents, since in the second experiment (PR-II) only 66% of potential worm pairs in guinea pigs were gravid at 8 weeks of infection, compared to 99% in *M. coucha* perfused at the same time. Furthermore, substantial numbers of the female worms recovered from this group of guinea pigs showed evidence of vitelline gland development, although they were not classified as gravid.

Moderate levels of anti-schistosome IgG were detected in the sera of *S. margrebowiei*-infected guinea pigs (Table 6.4). In *S. leiperi*-infected animals consistently high titres were recorded both at 11.5 weeks in the first experiment and at 12 weeks in the second. However, in the latter study titres were found to be greatly reduced by 16 weeks of infection. No obvious relationship was observed between antibody titres and *S. leiperi* tissue egg burdens, since the highest titres were recorded in the animals with the lowest egg loads. IgG levels in guinea pigs infected with *S. mansoni* were generally high in the first experiment and low to moderate in the second, possibly reflecting the differences in the states of maturation of the respective infections, as discussed above.

The results obtained in the two experiments with the PR strain of *S. mansoni* compare favourably with those reported from an extensive series of experiments with this schistosome by Pearce and McLaren (1983a and 1983b). These authors frequently obtained worm returns within the range of 20-30% and observed also that worms were capable of long-term pairing and oviposition. It is of interest to note that whereas Pearce and McLaren infected their animals by means of the

TABLE 6.4 Total anti-cercarial IgG titres in the sera of guinea pigs infected for various periods with *Schistosoma margrebowiei* and *S. leiperi*.

Schistosome	*S. margrebowiei*			*S. leiperi*			*S. mansoni*	
Experiment Number[a]	**MB-II**	**MB-IV**		**LP-I**	**LP-III**		**PR-I**	**PR-II**
Cercarial Load	500	496		534	461		499	510
Weeks of Infection	14.5	8	12	11.5	12	16	14.5	8
Geometric Mean of Inverse Titres[b]	50	36	66	453	320	17	219	57
Range of Inverse Titres	10-160	10-160	20-640	320-640	320	0-40	20-640	20-160
n	9	7	7	6	6	9	9	4

a Details of the infection loads in the various groups are shown in Table 6.3.

b Sera were tested against cercarial antigens derived from the Puerto Rican strain of *S. mansoni*.

TABLE 6.5 Worm recoveries from BALB/c mice or *Mastomys coucha* infected with *Schistosoma margrebowiei* (MB), *S. leiperi* (LP) or *S. mansoni* (PR strain) on occasions corresponding with the infections of rats and/or guinea pigs.

Schistosome	*S. margrebowiei* BALB/c mice				*S. leiperi* BALB/c mice			*S. mansoni* (PR)	
Host								BALB/c	*M.coucha*
Experiment Number[a]	**MB-I**	**MB-II**	**MB-III**	**MB-IV**	**LP-I**	**LP-II**	**LP-III**	**PR-I**	**PR-II**
Cercarial Load	66	52	59	64	41	68	66	51	50
Number of Animals	7	5	10	5	5	10	15	4	7
Weeks of Infection	8	9	8	8	11.5	10	12	9	8
% Worm recovery	27.1 (8.3)	28.5 (8.8)	30.5 (7.3)	27.1 (6.3)	28.8 (9.7)	30.7 (6.0)	19.6 (5.4)	37.8 (6.5)	68.3 (14.9)
Male worms	7.7 (3.0)	4.4 (3.0)	8.7 (3.1)	9.7 (2.1)	3.2 (2.5)	8.6 (2.9)	5.7 (2.1)	12.8 (1.7)	15.4 (5.7)
Female worms	10.0 (4.2)	10.2 (4.0)	9.3 (2.9)	7.7 (2.2)	8.6 (4.2)	12.3 (2.9)	7.1 (3.1)	6.5 (3.9)	18.7 (5.3)
Total worms[b]	17.9 (5.5)	14.8 (4.5)	18.0 (4.3)	17.3 (4.0)	11.8 (4.0)	20.9 (3.9)	12.9 (3.5)	19.3 (3.3)	34.1 (7.5)
Males/Total (%)	43%	30%	48%	56%	27%	41%	44%	66%	45%
Potential worm pairs[c]	6.7 (2.4)	4.2 (3.1)	7.3 (2.8)	7.3 (2.3)	3.2 (2.5)	8.2 (2.5)	4.7 (1.8)	6.3 (3.4)	14.3 (4.7)
Gravid worm pairs	6.6 (2.4)	4.0 (3.7)	7.1 (2.8)	7.2 (2.3)	2.6 (2.9)	7.7 (2.9)	3.9 (2.1)	6.3 (3.4)	14.1 (4.6)
Gravid/potential worm pairs (%)	99%	95%	97%	99%	81%	94%	83%	100%	99%

Mean values are shown, with standard deviations in parentheses.

a The total number of experiments involving infections of rats and/or guinea pigs was four in the case of *S. margrebowiei*, three in the case of *S. leiperi* and two in the case of *S. mansoni*. These are designated MB-I to MB-IV, LP-I to LP-III, and PR-I and PR-II, respectively.

b Total worms comprises the sum of male and female worms, together with highly stunted worms of uncertain sex.

c See text (Section 2.2.6) for definition of potential worm pairs.

ring method (Smithers and Terry, 1965b), the paddling method was used in the present study. In the former instance the cercarial suspension is placed directly in contact with a small area of shaved skin, while in the latter the posterior third of the animal (unshaved) is immersed in a volume of water containing the required number of cercariae. The similarity in worm returns obtained using the two methods indicates that the coat of the guinea pig does not represent a significant obstacle to cercariae in terms of their access to skin penetration sites. However, it seems likely that the preparatory pre-soaking procedure used routinely in the present author's laboratory, which has been shown to greatly enhance the success of mouse infections (Dettman *et al*, 1989), plays an important role in ensuring satisfactory infection rates. The use of the paddling method in the present investigations was preferred as it was considered to most closely simulate the 'natural' mode of exposure.

In marked contrast to *S. mansoni*, the antelope schistosomes exhibited poor sexual development in guinea pigs, even though substantial numbers of adult worms became established and were presumably available for pairing. In the case of *S. margrebowiei*, the presence of a few sexually-mature females, representing just 2% of the potential worm pairs, was observed only in some of the animals perfused at 8 weeks of infection (second study; MB-IV). While as many as 28% of potential pairs were found to be gravid at 12 weeks of infection in the second *S. leiperi* experiment, it was evident that this reflected a short-term phenomenon, since the numbers of gravid females and eggs in the tissues was found to decrease substantially over the ensuing 4 weeks. The fact that egg loads diminished in spite of the continued presence of gravid females suggests that the tissue half-life of schistosome eggs in the guinea pig is more like that in monkeys (about 8 days) (Cheever and Powers, 1971; Cheever and Duvall, 1974; Damian and Chapman, 1983) than that in the mouse (at least 4 weeks) (Cheever and Anderson, 1971). It appears that the worms of both species occupied an essentially hepatic rather than mesenteric habitat since only small numbers of ova were recovered from the intestinal tissues.

As with the rat, there is very little detailed information on the development of schistosomes other than *S. mansoni* in the guinea pig with which to compare the results of the present studies on the antelope schistosomes. *S. japonicum* is apparently highly compatible

with this host (Ho, 1963), with worm returns as high as 57% having been reported by Xu *et al* (1991). There is also some evidence that *S. bovis* is capable of successful development. In a study involving only 4 animals, Malek (1969) obtained an adult worm recovery of 27% (when immature worm were included this increased to 35%). Two of the animals were observed to be excreting viable eggs, and since guinea pigs have been documented as extremely poor egg excreters (Pearce and McLaren, 1983a; Damian, 1987), it would appear that the worms had deposited large numbers of eggs into the intestinal tissues. On the basis of the same reasoning it appears that *S. rodhaini* is also capable of successful pairing and substantial egg laying in guinea pigs, since Fripp (1968) was able to detect small numbers of the ova of this schistosome in the faeces of these hosts. Although no attempt was made in the present studies to assess faecal egg excretion, the fact that intestinal tissue egg burdens were very low in animals infected with the antelope schistosomes suggests that little, if any, excretion would have taken place.

The guinea pig has been reported to be an unsuitable host for some schistosome species. Wright *et al* (1972) obtained a 7% worm return from animals infected for 10 weeks with *S. intercalatum*, but found that the worms were somewhat stunted compared to those from mice and that females showed no evidence of uterine development. Infections with *S. spindale* were found to be characterised by very low worm returns (generally <1%) and extremely poor development of female worms, although successful pairing and oviposition was observed in some instances (Dutt, 1962). According to Sinha and Srivastava (1965) *S. incognitum* is capable of reaching the adult worm stage in the guinea pig. However, the worm returns reported by these authors were low (ranging from 0.6% to 10.5%; mean = 5.0%, 10 animals) and it is not clear from the data presented whether the worms became sexually mature.

In conclusion therefore, the infection characteristics of *S. margrebowiei* and *S. leiperi* in the guinea pig appear to be unlike those thus far reported for any other species studied in this host. It is particularly noteworthy that infections with these species in this host provide an experimental system which is intermediate between the mouse/schistosome and rat/schistosome systems. As in both mice and rats, a substantial proportion of invading cercariae reach the liver stage. However, unlike the situation in the mouse, in which

most mammalian schistosomes become fully patent, there is only partial and transient sexual development. Conversely, whereas in the rat there tends to be early, precipitous elimination of worms, *S. margrebowiei* and *S. leiperi* populations in the guinea pig remain relatively stable (i.e. according to the present study, for at least 3-4 months).

Since there is evidence that *S. margrebowiei* is capable of occasionally attaining sexual maturity in man (see Section 1.10.4) it is suggested that the development of *S. margrebowiei*, and possibly also *S. leiperi*, in humans is probably more like that in the guinea pig than that in the rat, i.e. exposure results in the establishment of worms which may persist at least for some months, with occasional, short-term, pair-formation and oviposition. In addition, the fact that such a small proportion of antelope schistosome ova were deposited in the intestines of guinea pigs demonstrates that the failure to detect *S. leiperi* ova in human faeces is not necessarily proof that this species cannot mature in man, but may rather be due to the failure of worms to migrate to the mesenteric veins.

6.3.2 Effect of Trickle Infections with *S. margrebowiei* and *S. leiperi* on the Ability of Guinea Pigs to Resist Challenge Infection with *S. mansoni*

The results of the experiments discussed above revealed that the guinea pig is analogous to man in the sense that it supports the establishment of fully patent *S. mansoni* infections on the one hand, but fails to support the normal development of the antelope schistosomes on the other. Consequently it was felt that this was a particularly appropriate model in which to assess the ability of *S. margrebowiei* and *S. leiperi* to induce protective immunity against *S. mansoni*.

An experiment based on the 'trickle infection' method (Webbe and James, 1973) was designed, since it was felt that this more accurately represented the 'natural' situation in which humans are presumably repeatedly exposed to cercariae of the antelope schistosomes. Five groups of guinea pigs were employed: the first and second groups were subjected to four successive infections with *S. margrebowiei*, at 6-weekly intervals, and the third and fourth groups

to four successive infections with *S. leiperi*, also at 6-weekly intervals. The numbers of cercariae to which animals were exposed on each occasion was regulated with a view to achieving cumulative total worm loads in the range of 100-125 worms/guinea pig (assuming no spontaneous or immunologically-mediated elimination of worms). This infection load was chosen primarily in order to provide some consistency with the homologous immunity experiments with *S. mansoni* reported by Pearce and McLaren (1983b) in which initial infections comprised 500 cercariae/animal and worm returns were generally in the region of 20-25%. Six weeks after the final exposure to antelope schistosomes the second, fourth and fifth groups were subjected to challenge infection with the PR strain of *S. mansoni*. Worm and tissue egg loads were determined in all five groups 10 weeks after the challenge infection.

The first and third groups (exposed to antelope schistosomes only) are referred to as the Initial Infection Control (IC) groups, the second and fourth (exposed to antelope schistosomes and *S. mansoni* challenge) as the Experimental (EXP) groups, and the fifth (*S. mansoni* challenge only) as the Challenge Infection Control (CC) group. Percentage reductions in challenge-derived worm and egg loads were calculated according to the formulae described in Section 4.2.4.

On each occasion when guinea pigs were exposed to cercariae (4 *S. margrebowiei* infections, 4 *S. leiperi* infections and 1 *S. mansoni* infection) groups of BALB/c mice and/or *M. coucha* were exposed simultaneously, in order to obtain information regarding the infectivity and sex-ratio of the cercariae. These groups of animals are referred to as 'Infectivity Control' groups. On the basis of data accumulated from numerous experiments (including those dealt with in Chapters 2, 4 and 5) the following worm recovery rates were regarded as 'normal': *S. margrebowiei* = 28% from mice; *S. leiperi* = 25% and 42% from mice and *M. coucha*, respectively; *S. mansoni* = 42% from mice. The inclusion of singly-infected guinea pig control groups was precluded by a lack of cage space.

Specific details regarding the cercarial loads employed for each of the various infections are presented in Appendix E (Table E.1). Cercarial loads for the trickle exposures were initially determined on the assumption that *S. margrebowiei* and *S. leiperi* infections in guinea pigs would yield worm returns of approximately 17% and 30%,

respectively. This assumption was based on the results of the experiments described in the preceding section. Thus it was estimated that in order to achieve cumulative loads of 100-125 worms/animal a total of about 600-750 *S. margrebowiei* cercariae and 350-420 *S. leiperi* cercariae/animal would be required (i.e. 150-180 and 90-105 cercariae/trickle exposure, respectively). However, worm recovery data from the Infectivity Control groups indicated that the infectivities of the cercariae used for the trickle infections were in most instances lower than expected (Appendix E, Table E.2), even though the ages of the cercariae at the start of animal exposure did not at any time exceed 4.5h. As a consequence the cercarial loads applied at each of the successive exposures were progressively increased, resulting in cumulative totals of approximately 993 *S. margrebowiei* and 694 *S. leiperi* cercariae/animal.

Worm and tissue egg load data from the various guinea pig groups are shown in Table 6.6. The mean worm returns from the *S. margrebowiei* and *S. leiperi* IC groups were 25.3 and 56.4, respectively. In terms of the total numbers of cercariae to which the animals had been exposed, these values represent percentage worm returns of 2.5% and 8.1%, respectively. In the case of *S. margrebowiei*, male worms represented only 21% of the total (data not shown). This contrasted with the situation in the mouse infectivity control groups (Appendix E, Table E.2) where males consistently constituted 40-46% of the total. In the case of *S. leiperi*, males comprised 66% of the total worms recovered from the guinea pig IC group (data not shown), while in the infectivity control groups the proportion of males ranged from 47% to 66% of the total.

With regard to the sexual maturation of the antelope schistosomes, gravid worm pair and tissue egg load analyses essentially confirmed the findings of the preceding infection characteristics studies. Thus, in the case of *S. margrebowiei*, no evidence of successful pair formation or tissue egg deposition was observed in either the IC or EXP groups, while in the case of *S. leiperi*, only small numbers of ova were recovered. The number of gravid females in the *S. leiperi* IC group ranged from 1 to 19, with a mean of 6.8; this represented 35% of the potentially available worm pairs (data not shown).

The mean worm recovery from the CC group (111.2) represents a yield of 25.7%, while simultaneously-infected mice yielded a return of

TABLE 6.6 Trickle infection study comprising fourfold exposure of guinea pigs to cercariae of either *Schistosoma margrebowiei* (MB) or *S. leiperi* (LP) at six-weekly intervals, followed by challenge with *S. mansoni* (PR strain) at 24 weeks: antelope schistosome gravid worm pair and/or egg loads, total worm loads, and *S. mansoni* egg loads.

GROUP[a]	*n*	Antelope schistosome gravid worm and/or egg loads			TOTAL WORM LOADS		*S. mansoni*					
		Species	Gravid worm pairs	Total egg load[b]			Gravid worm pairs		Total egg load[b]		Tissue eggs/ gravid worm pair	
			$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	$\bar{x}$ (s.d.)	Reduction[c] (%)	$\bar{x}$ (s.d.)	Reduction[c] (%)	$\bar{x}$ (s.d.)	Reduction[c] (%)	$\bar{x}$ (s.d.)	Reduction[c]
IC	9	MB	0	0	25.3 (15.2)	-	-	-	-	-	-	-
EXP	10	MB	0	0	136.5 (47.7)	0	42.0 (18.0)	-4.2	96 541 (44 926)	-21.6	2 466 (998)	-20.6
IC	10	LP	6.8 (5.5)	3 298 (2 301)	56.4 (16.9)	-	-	-	-	-	-	-
EXP	10	LP	3.3 (4.2)	3 990 (4 283)	167.7 (40.3)	0	35.6 (14.0)	11.7	58 244 (29 258)	26.6	1 627 (454)	20.4
CC	9	-	-	-	111.2 (33.2)	-	40.3 (12.4)	-	79 366 (17 824)	-	2 044 (836)	-

a IC (initial infection control) refers to groups exposed to the trickle infections only, EXP (experimental) to groups exposed both to the trickle and challenge infections, and CC (challenge infection control) to the group exposed to the challenge infection only. As regards trickle infections, animals were exposed to a total of either 993 *S. margrebowiei* or 694 *S. leiperi* cercariae each. Challenge infection with approximately 432 *S. mansoni* cercariae per animal was carried out 6 weeks after the last exposure to antelope schistosomes and perfusion was carried out 10 weeks after challenge. Further details are shown in the experimental plan (Appendix E; Table E.1).

b Total egg load comprises the sum of eggs in the liver and GIT. n=9 in the case of the *S. leiperi* IC group, as no egg data were available for one of the animals. Approximately 95% of *S. leiperi* eggs (in both IC and EXP groups) were located in the liver; in the case of the *S. margrebowiei* EXP group, the *S. leiperi* EXP group, and the CC group, the proportions of *S. mansoni* eggs located in the liver were 53%, 58% and 62%, respectively.

c No statistically significant differences were detected (Mann-Whitney test).

29.2% (Appendix E, Table E.2); male worms constituted 45-46% of the total in both host species (data not shown for guinea pigs). In mice 100% of the potential *S. mansoni* worm pairs were gravid, compared with 83% in guinea pigs (data not shown).

With respect to the EXP groups, it was not possible to reliably distinguish male or non-gravid female worms originating from the trickle infections from those of *S. mansoni*. However, in the case of the group exposed initially to *S. leiperi*, it was possible to differentiate between gravid females of the trickle and challenge species, on the basis of the number and morphology of intra-uterine ova, as described in Section 4.2.4.

There was clearly no resistance to *S. mansoni* challenge infection in guinea pigs following repeated exposure to *S. margrebowiei* cercariae. In the case of animals subjected to *S. leiperi* trickle infections followed by challenge, total worm load data indicate that there was no reduction in the number of *S. mansoni* worms which became established. However, there was evidence of a slight, though statistically non-significant, reduction in the fecundity of *S. mansoni* worms, with gravid pairs, tissue egg loads and egg loads per worm pair being reduced by 11.7%, 26.6% and 20.4%, respectively. This was not attributable to a reduction in overall female worm loads.

Titres of IgG capable of binding to surface epitopes of *S. mansoni* cercariae were generally low in the guinea pig IC and EXP groups shortly (3 days) before challenge; no antibodies were detected in animals of the CC group at this time (Table 6.7). Challenge infection resulted in a pronounced augmentation of antibody production in both the EXP and CC groups, although titres in the *S. leiperi* EXP group were generally lower than those in the *S. margrebowiei* EXP group, which in turn were similar to those in the CC group. Antibody levels in the IC groups at the time of perfusion were little different from those at the time of challenge.

It is conceivable that sera collected just prior to challenge may have contained higher titres of antibodies than were detected, but that these possessed species-specific characteristics and hence were incapable of reacting with *S. mansoni*-specific determinants. However, this seems unlikely, for the following reasons: (i) strong antibody responses were detected in the preliminary studies with the antelope

TABLE 6.7 Trickle infection study in guinea pigs: total anti-cercarial IgG titres in groups 3 days before challenge (A) and at the time of worm recovery (B).

	GROUPS[a]									
	IC-MB		EXP-MB		IC-LP		EXP-LP		CC	
	A	B	A	B	A	B	A	B	A	B
Geometric Mean of Inverse Titres[b]	16	14	2	197	34	14	9	67	0	184
Range of Inverse Titres	10-80	0-320	0-20	80-640	0-640	0-160	0-80	20-160	0	80-320
n	9	9	10	10	10	10	10	10	10	10

a IC (initial infection control) groups were exposed to trickle infections only and EXP (experimental) groups to both the trickle and challenge infections; the suffixes -MB and -LP indicate whether trickle infections comprised *S. margrebowiei* or *S. leiperi*, respectively. The CC (challenge infection control) group was exposed to the challenge infection only.

b Sera were tested against cercarial antigens derived from the Puerto Rican strain of *S. mansoni*.

schistosomes, especially in 11-12 week *S. leiperi*-infected animals (Table 6.4), and (ii) high levels of serological cross-reactivity are known to occur between crude *S. mansoni* antigens and sera from humans and animals infected with various parasites, including non-human schistosomes (Sadun and Biocca, 1962; Amin *et al*, 1969; McLaren *et al*, 1978; Correa-Oliveira *et al*, 1988). Evidence of a pronounced drop-off in IgG levels was observed in the second of the preliminary *S. leiperi* studies (LP-III; Table 6.4). The low pre-challenge antibody titres in the trickle-infected animals may therefore reflect a state of low immunological activation.

It is possible that the trickle infection protocol may have resulted in some form of immunosuppression (Sher and Colley, 1989) or immunological tolerance (Li Hsu *et al*, 1973; Pearce and McLaren, 1983a) and hence an inability to resist challenge infection. However, while there do not appear to have been any previous trickle infection studies in the guinea pig, data from other animal models, such as the mouse (Dean, 1983), rat (Moloney *et al*, 1987c) and the baboon (Webbe and James, 1973; Damian *et al*, 1974; Webbe *et al*, 1982; Sturrock *et al*, 1984) do not support this interpretation. Furthermore, it is known from studies in mice, rats and guinea pigs that animals with relatively low antibody levels may be highly resistant to challenge infection (Rogers and McLaren, 1987; Roberts *et al*, 1988). By way of illustration, whereas levels of resistance in guinea pigs vaccinated with highly irradiated (20 krad) cercariae remain high for at least 32 weeks (Pearce and McLaren, 1983b), titres of complement-fixing schistosomulicidal antibodies in the sera of such animals peak within 5 weeks of vaccination, but by 12 weeks have declined to negligible levels (Gordon and McLaren, 1987).

Although the ability of the antelope schistosomes to induce homologous immunity was not specifically assessed in the present study, evidence of its occurrence was seen in the low mean worm recoveries of the *S. margrebowiei* and *S. leiperi* IC groups (Table 6.6). It is extremely unlikely that these poor returns were simply due to poor cercarial infectivity, even though the worm returns from the mouse infectivity control groups were on most occasions substantially lower than normal (for reasons yet to be ascertained). In this regard it is noteworthy that the percentage worm return from the guinea pig *S. mansoni* CC group (25.7%) compared well with those in the preliminary infection studies (22.0% and 26.6%; Table 6.3),

whereas that from simultaneously-infected mice was approximately 30% lower than expected (Appendix E; Table E.2). Thus, it seems reasonable to assume that the infectivity of the antelope schistosome cercariae in guinea pigs on each trickle occasion was proportionally no more reduced than that in mice. On this basis, using the cercarial load information shown in Table E.1 (Appendix E) and the 'relative infectivity ratings' shown in Table E.2, and assuming 'normal' worm recovery rates from guinea pigs of about 17% for *S. margrebowiei* and 30% for *S. leiperi*, it can be estimated that cumulative worm burdens in the *S. margrebowiei* and *S. leiperi* IC groups, in the absence of any worm elimination, would have been in the region of 107 and 159, respectively. Instead, it was found that actual *S. margrebowiei* and *S. leiperi* worm loads were 76% and 65% lower than predicted, respectively.

While a substantial portion of the homologous resistance observed in guinea pigs infected with normal (i.e. non-irradiated) *S. mansoni* cercariae is considered to be related to egg-induced liver pathology and associated vascular changes (Kamiya and McLaren, 1987), there is evidence that at least some immunity develops independently of egg deposition. For example, Pearce and McLaren (1983b) showed that challenge worm burdens in guinea pigs challenged only four weeks after initial exposure (i.e. before significant liver changes had occurred) were reduced on average by 31% in comparison to controls. In addition, it has been demonstrated that unisexual (male) *S. mansoni* infections in these hosts elicit high titres of antibodies which, in *in vitro* cytotoxicity assays, strongly promote eosinophil-mediated killing of schistosomula (Rogers and McLaren, 1987). These observations support the idea that the low worm returns in the IC groups in the present study were due, at least in part, to homologous immunity.

It is equally possible, of course, that worms may have died as a result of non-immunological factors. The fact that neither *S. margrebowiei* nor *S. leiperi* are capable of sustained sexual competency in guinea pigs indicates that these hosts fail to fully satisfy the physiological needs of either schistosome (Damian, 1984). The apparent time-related decline in egg production observed in the preliminary infection characteristics experiments (Table 6.3) suggests a deterioration in the condition of the worms which may predispose them to early senescence. However, this explanation seems

inadequate to account for all of the worm elimination. For example, if one considers firstly that perfusions were carried out 16 weeks after the final trickle exposure, secondly that *S. leiperi* worm burdens were observed in the preliminary infection characteristics experiments to remain stable for at least this long (Table 6.3), and thirdly that *S. leiperi* cercarial infectivity at the time of the final trickle infection was more-or-less normal (Appendix E, Table E.2), then it can be estimated that the worm burden resulting from this infection alone (approximately 350 cercariae/guinea pig) should have been in the range of 95-105, i.e. virtually twice the number actually recovered. The fact that the sex ratio of worms in the *S. margrebowiei* IC group (21% males) differed markedly from that in the corresponding mouse groups (40-46% males) is indicative of selective worm elimination, possibly immunologically-mediated.

Assuming that the low worm returns in the IC groups were the result of immunologically-mediated worm elimination, it follows that the absence of resistance to *S. mansoni* challenge constitutes evidence that immunity in the guinea pig concomitant immunity model is species-specific. Although, as discussed in Section 5.1, immunity in mice and rats vaccinated with radiation-attenuated cercariae has been shown to be species-specific, there do not appear to have been any previous attempts to establish whether or not immunity in the guinea pig exhibits similar characteristics. It is important to note that the present study differs fundamentally from those in mice and rats in that cercariae were not artificially attenuated. Consequently the post-penetration migration of schistosomula would presumably have been normal (Kamiya and McLaren, 1987), and thus more representative of the likely development of non-pathogenic animal schistosomes in humans.

The results of the present investigation serve to illustrate the point that repeated exposure of humans to the cercariae of *S. margrebowiei* and/or *S. leiperi* will not necessarily result in immunological cross-protection against *S. mansoni*. However, extrapolation of these findings to man is at this stage inappropriate, particularly if one considers that it has been shown in other hosts, including rhesus monkeys (Amin *et al*, 1968; Eveland *et al*, 1969), baboons (Taylor *et al*, 1973a), sheep (Preston *et al*, 1972) and calves (Massoud and Nelson, 1972) that prior exposure to poorly- or non-maturing schistosomes may well induce partial

protection against subsequent infection with pathogenic species. In some of these investigations it was observed that while the number of challenge worms was only slightly reduced, their fecundity was markedly depressed. For example, it was observed that in sheep infected with normal *S. mansoni* cercariae and later challenged with *S. mattheei*, challenge worm loads were reduced on average by about 17%, whereas tissue egg load reductions were of the order of 40-50% (Preston *et al*, 1972). Little evidence of anti-fecundity effects was seen during the present study, apart from a slight but insignificant decrease in *S. mansoni* gravid pair and tissue egg loads in animals multiply-infected with *S. leiperi* (Table 6.6). If real, this may well have been related to the pathological reactions to *S. leiperi* eggs deposited in the tissues, rather than to immunologically-mediated interference, as such.

With respect to studies in sheep it is worth mentioning the results of an investigation by Taylor *et al* (1976b), in which it was found that *S. mattheei* challenge worm burdens in animals previously exposed to normal *S. mansoni* cercariae were reduced by only 13%, while in animals vaccinated with irradiated (6 krads) *S. mattheei* cercariae they were reduced by 74%. While this may reflect a difference in the relative efficacies of homologous and heterologous immunisation procedures, it may equally be a consequence of differences in the developmental dynamics and/or immunogenicity of normal and radiation-damaged cercariae.

6.4 GENERAL DISCUSSION

Further studies aimed at assessing the abilities of *S. margrebowiei* and *S. leiperi* to induce homologous immunity in the guinea pig model may be of considerable value. Firstly, they could assist in clarifying whether the lack of heterologous resistance in the present study was due to the absence of protective immune responses or to the species-specificity of such responses. In addition, however, it is felt that the antelope schistosome/guinea pig models may provide a valuable alternative to those involving *S. mansoni*. Exposure of these hosts to equal numbers (i.e. 500) of normal or radiation-attenuated *S. mansoni* cercariae, which exhibit markedly different migration characteristics (Kamiya and McLaren, 1987), evokes comparable levels of resistance to homologous challenge (Pearce and McLaren, 1983b).

Since in the mouse model the numbers of normal and irradiated cercariae routinely used for immunisation differs greatly (20-30 versus 500), McLaren and colleagues have promoted the guinea pig/*S. mansoni* model as 'a unique system for analysing and comparing the antigenicity and immunogenicity of normal versus attenuated parasites' (Kamiya and McLaren, 1987). However, whereas maximal resistance in guinea pigs vaccinated with highly irradiated (20 krads) cercariae develops within 4-6 weeks, that resulting from exposure to normal cercariae develops only after egg deposition has been in progress for some weeks (i.e. after 12 weeks of infection). Thus it is acknowledged that even in the normally-infected guinea pig model, as in the mouse, a substantial proportion of challenge worm elimination may be directly related to egg-induced pathology and the associated changes in the portal-hepatic vasculature (Kamiya and McLaren, 1987). In contrast, the antelope schistosome/guinea pig models offer an alternative option, in which relatively stable, but sexually incompetent (or, at most, partially competent) bisexual infections can be produced, thereby minimising or even eliminating the confounding influences associated with egg deposition.

It is of considerable interest to assess the extent of the species-specificity of anti-schistosome immunity, especially as far as exposure to non-attenuated schistosomes is concerned, since this will provide insights into whether or not incidental human contact with non-pathogenic species is likely to afford any protective benefit. Although, as mentioned above, there is some evidence of partial protection from experimental studies in non-human primates and bovines, these models are not as readily available and not as well characterised as common laboratory rodents. As far as rats are concerned, there appears to have been only a single report thus far on cross-protection between schistosomes. In this instance it was demonstrated that vaccination of animals with ultraviolet-attenuated *S. mansoni* cercariae failed to protect against *S. japonicum* challenge, and *vice versa* (Moloney *et al*, 1987c). Although there have been no comparable studies with non-attenuated cercariae, it is of interest to note that rats infected with the liver fluke, *Fasciola hepatica*, express resistance to *S. mansoni* challenge infection, but that this is believed to be due largely to immunologically non-specific mechanisms such as those seen in chronically-infected mice (Ford *et al*, 1987a and 1987b). As noted previously, there have as yet been no attempts to assess the species-specificity of anti-

schistosome immunity in guinea pigs using either normal or attenuated parasites. There are thus good grounds for the continuation of studies on the potential of the antelope schistosomes to induce heterologous immunity in both the guinea pig and rat models. The use of these models is supported by the fact that each is considered to share certain immunological effector mechanisms in common with man (Capron *et al*, 1987; Gordon and McLaren, 1987).

Numerous opportunities exist for further experimentation. For example, *in vivo* studies aimed at ascertaining whether or not it is possible under any circumstances to induce significant cross-protective immunity against *S. mansoni* using the antelope schistosomes would be of considerable value. And, assuming that this is possible, it would be of interest to determine whether the levels of immunity which can be achieved and the conditions necessary for the expression of such immunity differ in any substantial way from those observed in homologous immunity studies. The observation that *S. margrebowiei* and *S. leiperi* are capable of eliciting marked antibody responses in both rats and guinea pigs (Tables 6.2 and 6.4) suggests that *in vitro* studies, for example on the ability of such antibodies to promote complement- or cell-mediated killing of *S. mansoni* schistosomula (Pearce and McLaren, 1983; Capron *et al*, 1987), would also be worthwhile. Surprisingly, there appear to have been very few studies on the species-specificity of these *in vitro* mechanisms. The only report of this nature located in the literature consulted was that by Webbe *et al* (1979), who demonstrated that sera from *S. haematobium*-infected baboons exerted cytotoxic effects against *S. mansoni* schistosomula.

CHAPTER SEVEN

CONCLUDING REMARKS

7.1 A re-evaluation of the evidence in support of Nelson's theory of zooprophylaxis, with specific reference to schistosomiasis.

As described in Section 1.9.4, Nelson's original formulation of the concept of 'zooprophylaxis' (Nelson *et al*, 1962) was focused on the issue of heterologous immunity between schistosomes. However, in later publications (Nelson, 1974; 1988) he emphasized the ubiquitous importance of exposure to all varieties of non-pathogenic organisms, ranging from viruses to helminths, stressing that this provided a vital, albeit often inconspicuous, means of immunological stimulation, enabling animals (including man) to cope more effectively with infections by pathogenic organisms. He eventually abandoned the use of the term zooprophylaxis, largely due to its general lack of acceptance in this context (Nelson, 1988). In recognition of the seminal demonstration of cross-immunity (between cowpox and smallpox) by Edward Jenner in 1796, he suggested that the phenomenon be referred to instead as 'the Jennerian principle of cross-protection'. The present author suggests that a shorter alternative might be the term 'quotidian cross-protection', or, with specific reference to the potential of zoonoses to induce protective immunity in man, 'zooimmunoprophylaxis'.

There can be little doubt, in principle, about the potential of protozoan and helminth parasites to induce at least partial heterologous immunity in mammalian hosts (Christensen *et al*, 1987). However, as acknowledged by Nelson (1988), no attempts have been made as yet to quantitatively assess the epidemiological significance of such immunity in either human or animal communities. With specific reference to the possibility of schistosome cross-protection, it is notable that even when the notion was first put forward by Nelson *et al* (1962), some skepticism was evident. Thus, in the participatory discussion following Nelson (1962), Wright drew attention to an area in the Gambia where both *S. haematobium* and *S. bovis* were highly prevalent and there was no evidence of cross-protection in either direction. Similar doubts were subsequently expressed by Jordan, on

the basis of his observations on human and bovine schistosomiasis in Tanzania [see General Discussion following Webbe and Jordan (1966)].

In the absence of substantive field data Nelson supported the idea of heterologous immunity between schistosomes primarily by pointing to evidence derived from experimental studies in a variety of hosts, including mice, rhesus monkeys, baboons, sheep and goats (Nelson, 1974). However, a thorough appraisal of published studies on this subject (see reviews by Christensen *et al*, 1987; Taylor, 1987; Taylor *et al*, 1991), in the light of current perceptions regarding immunity to schistosomes in animal models, suggests that this evidence is, at best, equivocal.

As discussed in Chapter 4, heterologous concomitant immunity experiments in the mouse model can hardly be considered representative of the interactions between pathogenic and non-pathogenic schistosomes as they are envisaged to occur under field conditions. As a result of the ubiquitous permissiveness of most commonly-used mouse strains to mammalian schistosomes in general, primary infections in these hosts typically lead to severe disease syndromes, characterised in particular by major modifications to the portal-hepatic vasculature, which markedly affect the development of challenge parasites. Reductions in challenge infection worm and/or egg burdens have occasionally been reported in mice harbouring unisexual or poorly-maturing initial infections. However, these reports appear to represent the exception rather than the rule and the bulk of evidence supports the conclusion that mature, bisexual infections are a prerequisite for the development of substantial resistance in mouse concomitant immunity studies. The hamster, which has only occasionally been used for heterologous immunity studies (Christensen *et al*, 1987) suffers from the same disadvantages as the mouse, in view of its similar high degree of permissiveness to schistosomes (Maddison, 1982).

Rhesus monkeys have been shown, repeatedly, to develop partial immunity to challenge infections with either *S. mansoni*, *S. haematobium* or *S. japonicum* following primary exposures to non-human schistosomes (Christensen *et al*, 1987). However, these animals exhibit immune responses to schistosomes which do not appear to reflect those in man, since they demonstrate partial or complete self-cure when infected with *S. mansoni* and are thereafter solidly

resistant to reinfection (Sturrock, 1986). In contrast, cercopithecine monkeys and baboons infected with *S. mansoni* do not display self-cure and are capable of developing only partial immunity (Sturrock, 1986). Consequently these hosts are considered to be more appropriate models in terms of simulating human responses to schistosomes. In spite of this, there do not appear to have been any published reports to date on heterologous immunity between non-human and human schistosomes in cercopithecine monkeys and apparently there have been only two such studies in baboons. These latter involved repeated exposures to the cercariae of either *S. bovis* or *S. rodhaini*, neither of which mature in the baboon, followed by *S. mansoni* challenge. In the first investigation partial resistance (around 50%) was demonstrated in animals subjected to cumulative infection loads of 51 000 *S. rodhaini* or 18 000 *S. bovis* cercariae each (administered over a period of 17-18 weeks) (Taylor *et al*, 1973a). However, in a subsequent experiment it was found that exposure to three doses of 5000 *S. rodhaini* cercariae (at approximately 6-weekly intervals) failed to induce protection (Taylor *et al*, 1976a). No definite conclusions can be drawn from these contradictory findings, although, as noted by Christensen *et al* (1987), they suggest that the induction of heterologous resistance in baboons requires the use of primary infection loads which are probably well in excess of those to which animals are normally exposed under natural conditions.

The best evidence of the ability of non-pathogenic schistosomes to induce resistance against pathogenic species comes from studies with cattle. Thus, calves exposed to a single dose of 10 000 *S. mansoni* cercariae were shown to develop moderate resistance to a *S. mattheei* challenge administered 9 weeks later (Hussein *et al*, 1970). Surprisingly, the *S. mansoni* infections were found to have become patent, with some animals excreting viable ova and post-mortem tissue digests revealing the presence of eggs in the viscera of all animals. However, the mean number of adult *S. mansoni* worms recovered amounted to less than 1% of the applied cercariae, and the pathological changes associated with the presence of worms and eggs in the tissues were minimal. Subsequently Massoud and Nelson (1972) showed that a single exposure of calves to 8 000 cercariae of *O. turkestanicum*, a mildly pathogenic parasite of ruminants, elicited substantial resistance against *S. bovis*. More importantly, they demonstrated also that 3 successive exposures to 7 000 *S. haematobium* cercariae (at 4-

weekly intervals) induced moderate resistance to challenge with either *S. bovis* or *Ornithobilharzia turkestanicum*. Between 1 and 3% of the *S. haematobium* cercariae were recovered as adult worms, but eggs of this species were found, in small numbers, in the tissues of only 1 out of 4 animals.

There have been only two studies of note in sheep, both of which involved initial infections with 30 000-40 000 *S. mansoni* cercariae, followed by challenge with *S. mattheei*. As in cattle, a small proportion of the *S. mansoni* cercariae were seen to reach maturity, resulting in deposition of low numbers of eggs in the tissues and occasional faecal egg excretion. However, whereas in the first experiment partial protection was evident (manifested mainly as a reduction in *S. mattheei* egg loads) (Preston *et al*, 1972), in the second experiment only slight reductions in challenge worm and egg loads were observed (Taylor *et al*, 1976b). It is possible that these discrepant findings were due to differences in experimental design; *viz*, in the first study the interval between the earliest exposure to *S. mansoni* and the challenge infection was more than a year, whereas in the second it was only 16 weeks.

As discussed in Chapter 5, the results of various studies in mice, rats and sheep strongly indicate that the immunity evoked by vaccination with radiation-attenuated cercariae or schistosomula is strictly species-specific. The only contradictory evidence in this regard comes from experiments in the rhesus monkey by Eveland *et al* (1969) who demonstrated strong reciprocal immunity between *S. japonicum* and *S. mansoni*. However, in terms of the search for appropriate models for assessing the likelihood of heterologous immunity between schistosomes under natural conditions, the issue of whether the immunity induced by irradiated larvae is species-specific is of secondary importance at this stage. Recent work has provided strong evidence that in rodent models, at least, the retarded migration characteristics and premature deaths of irradiated schistosomula are of fundamental importance in the induction of strong protective immunity (see Section 5.4 for references). Thus, it would seem that before extrapolating the conclusions of attenuated vaccine studies with heterologous species to the natural situation, it is necessary first to assess the degree to which the development patterns, antigenic characteristics and immunostimulatory effects of irradiated

schistosomula differ from and/or correspond with those of non-irradiated larvae in non-compatible hosts.

It can be seen from the above summary that the available evidence with regard to the feasibility of natural heterologous immunity resulting from exposure to non-pathogenic or poorly-maturing schistosomes is by no means conclusive. Furthermore, with respect to those studies in which significant immunity was demonstrated, it is debatable whether the experimental conditions applied (e.g. size and number of cercarial exposures, pre-challenge intervals, etc) can really be considered to reflect natural conditions. In most instances initial infections comprised either a single or only a few exposures to many thousands of cercariae. Such massive infections are probably relatively rare in the field situation, where it is likely that hosts experience repeated exposure to low levels of infection (Anderson *et al*, 1986). It is clear therefore that not only is there is a need for further experimental investigations, but also that in formulating further experiments careful consideration should be given to both the selection of appropriate animal model systems and the application of 'epidemiologically-relevant' experimental procedures.

7.2 Some proposals for appropriately-designed animal model studies on interactions between the antelope schistosomes and *S. mansoni*.

As far as the putative interactions between the antelope schistosomes and *S. mansoni* are concerned, the results presented in the preceding chapter suggest that the guinea pig may prove to be a particularly valuable host for future animal model studies. With respect to the design of experiments which reflect natural transmission patterns, it would be of considerable interest to carry out protocols similar to those of Crombie and Anderson (1985), in which mice were exposed repeatedly (i.e at weekly intervals) over extended periods to constant numbers of cercariae. By applying different weekly infection loads to different groups of animals and assessing worm loads at various intervals during the course of the study, those authors were able to obtain some insights into the dynamics of the establishment, stabilization and decline of *S. mansoni* worm populations in relation to different levels of cercarial exposure. In the context of the present investigation, two experimental strategies would perhaps be

worthy of investigation: *viz*, one in which animals are subjected to a protracted series of light trickle exposures to *S. margrebowiei* or *S. leiperi* before infection with *S. mansoni*, and another in which exposures to *S. mansoni* are interspersed with those to the antelope schistosomes. The first of these alternatives equates to the situation which Pitchford believed to apply in East Caprivi and northern Botswana, i.e. the introduction of *S. mansoni* into an area in which the antelope schistosomes had long been present (see Section 1.10.5). Such a scenario is particularly relevant to the issues presently under consideration. The second approach corresponds to a situation in which the transmission of two schistosome species occurs concurrently, such as when humans and their livestock share the same, or closely adjacent, water contact points.

Since effective immunity in human schistosomiasis appears to develop only as a result of repeated exposure to cercariae over a period of many years (Hagan, 1992), long-term studies on the protective effects of *S. margrebowiei* and *S. leiperi* in non-human primates, especially baboons (*Papio* spp.) and vervet monkeys (*Cercopithecus aethiops*), may also be appropriate. These animals are similar to man in terms of their susceptibility to *S. mansoni* infections, in that they support persistent infections and develop only partial immunity (Sturrock, 1986). There seems to be a strong possibility that the antelope schistosomes would not develop well in one or both of these hosts, particularly considering the demonstration by Taylor *et al* (1973b) that the animal schistosomes *S. bovis* and *S. rodhaini* fail to mature in baboons. However, it seems likely that a substantial proportion of *S. margrebowiei* and *S. leiperi* schistosomula would reach the liver stage of migration, at least in baboons, since schistosomulum migration in these animals appears to proceed extremely efficiently (Wilson *et al*, 1990). This speculation is supported by the observation that *S. margrebowiei* is capable of occasional sexual maturation in man (see Section 1.10.4). Interestingly, strong resistance to reinfection in drug-cured humans is associated with the presence of high levels of IgE (and possibly also IgG1 and IgG3) antibodies specific for adult worm antigens (Hagan, 1992). It would be of interest, therefore, to determine whether or not long-term trickle exposure of baboons or vervets to antelope schistosome cercariae leads to stimulation of anti-worm immune responses and, if so, whether these have any *in vitro* and/or *in vivo* cross-protective capabilities.

One of the important advantages of using non-human primates for further investigations is that the influence of prior contact with the antelope schistosomes on the intensity and effects of subsequent *S. mansoni* infections could be monitored over extended periods in individual animals. Thus, parasite loads could be estimated on the basis of faecal egg excretion and the severity of pathological changes could be measured using clinical methodologies. In contrast, the assessment of challenge infection success and its consequences in rodent models usually requires that animals be sacrificed. The major constraint on primate studies, especially those of a long-term nature, is that of cost (Sturrock, 1986). However, this may be offset to some extent if such studies were to be carried out locally, in view of the relative ease of availability of both baboons and vervet monkeys in South Africa.

7.3 Proposed field study on interactions between the antelope schistosomes and *S. mansoni* - identification of a possible study site in Ngamiland, Northern Botswana.

While there is undoubted merit in the continuation of experimental studies on immunologically-mediated interactions between heterologous schistosome species, it is recognised that such studies, particularly those in rodent models, can only provide clues as to the possible types of interactions occurring naturally. Direct extrapolation of the results of animal model investigations to the field situation is unwarranted, since under natural conditions numerous, often ill-defined, variables may influence parasite transmission and its consequences. Clearly, therefore, there is a need for well-designed field studies aimed at investigating the impact of exposure to non-pathogenic schistosomes on the epidemiology of disease due to pathogenic species (Christensen *et al*, 1987). This is particularly so in the case of Pitchford's proposal that the presence of the antelope schistosomes had limited the spread of human schistosomes and of *S. mattheei* into certain parts of southern Africa (see Section 1.10.5).

The finding that *S. mansoni*, *S. haematobium* and *S. mattheei* were absent from areas in East Caprivi and northern Botswana, in spite of the availability of the appropriate definitive and intermediate hosts, led Pitchford (1975b and 1977b) to infer that their entry into

these areas had in some way been blocked. He suggested that this blocking was due to the effects of heterologous immunity in humans and cattle resulting from incidental contact with the cercariae of the antelope schistosomes (Pitchford, 1975b; Pitchford and Wolstenholme, 1977). In this regard it is significant to note that Nelson *et al* (1968) did not favour the view that exposure to non-pathogenic schistosomes would provide complete protective immunity, but rather that it would result in a degree of partial resistance and hence a milder form of disease. Certainly, in the light of current knowledge of immunity to schistosomes in man, the development of sterile immunity seems extremely improbable. Firstly, there is evidence of considerable genetic heterogeneity within communities in terms of the abilities of different individuals to mount protective immune responses against schistosomes (Hagan, 1992). Furthermore, as noted earlier, resistance takes many years to develop, even in individuals with the capacity to express strong immunity (Hagan, 1992). Thus, even if the antelope schistosomes do evoke cross-protective immune responses in humans, it is difficult to imagine how they would cause entire communities to become completely resistant to infection by *S. mansoni* or *S. haematobium*, as implied by Pitchford. This suggests that the absence of human and cattle schistosomes from communities situated close to areas of high lechwe prevalence may have been due to non-immunological factors. One obvious possibility is that these schistosomes had not previously been introduced into these localities. This seems quite feasible if one considers the remoteness of the areas investigated by Pitchford. Indeed, the extremely rapid spread of mansonian schistosomiasis in Maun and many of the villages surrounding the Okavango Delta subsequent to 1965, the unusually steep decline in prevalence with increasing age, and the low prevalence of late stage complications strongly support this interpretation (Andersen *et al*, 1985; Ali *et al*, 1989; Friis and Byskov, 1987 and 1989). An alternative explanation for the increase in schistosomiasis in Maun, proposed by Appleton (personal communication), is that water contact patterns may have changed in recent years due to the withdrawal of crocodiles deeper into the swamp, as a result of the rapid increase in the human population. This suggestion was based on the earlier observation by Appleton and Bruton (1979) that human contact with *Biomphalaria pfeifferi* habitats along the shores of Lake Sibaya (northern Natal) was limited due to the fear of attack by crocodiles and hippopotami,

possibly explaining the absence of *S. mansoni* infections among the local inhabitants.

These considerations prompted the present author to initiate enquiries with a view to identifying a potential site in which further field studies on the effects of human exposure to *S. margrebowiei* and *S. leiperi* could be carried out. Observations made during a preliminary trip to East Caprivi suggested that the likelihood of significant human exposure to the antelope schistosomes in this area was extremely low, since substantial lechwe populations generally appeared to be situated well away from human settlements. This impression was confirmed subsequently by correspondence with the game conservation officer responsible for this region (Grobler, personal communication). For this reason, it was decided to focus attention on the Okavango Delta, which supports a far larger lechwe population than that in East Caprivi (Williamson, 1979) and is the focal point of human activity in northern Botswana. Since a personal visit was not possible, various persons familiar with the Delta were contacted, with the aim of identifying localities where humans live in close proximity to sizeable populations of lechwe. One area in particular, known as the Jao Flats, was consistently recommended as being highly appropriate in this regard. Some of the relevant features of this area are detailed below, on the basis of information contributed by the following individuals: G S Merron, M Murray-Hudson, L Patterson, P A Smith, L J van der Heiden and A Wellwood (personal communications).

The Jao Flats is a somewhat loosely-defined region situated immediately below the pan-handle (Figure 7.1), which is home to some of the highest densities of lechwe in the Delta. On one of the islands within the region is a village named Gidiba (or 'Jedibe'), which has a population of some 200-300 people. This is the only permanent human settlement of significant size, although there are also a number of other small communities scattered around the Jao area. In addition, some localities are occupied intermittently, according to seasonal activities. The inhabitants of Jao are essentially pastoralists-cum-hunter/gatherers. Thus, apart from tending to livestock (cattle, goats and donkeys), they are involved in fishing, hunting, trapping, and collection of palm leaves, grasses and reeds. Their activities necessitate frequent wading through shallowly-inundated floodplains and take them regularly into habitats

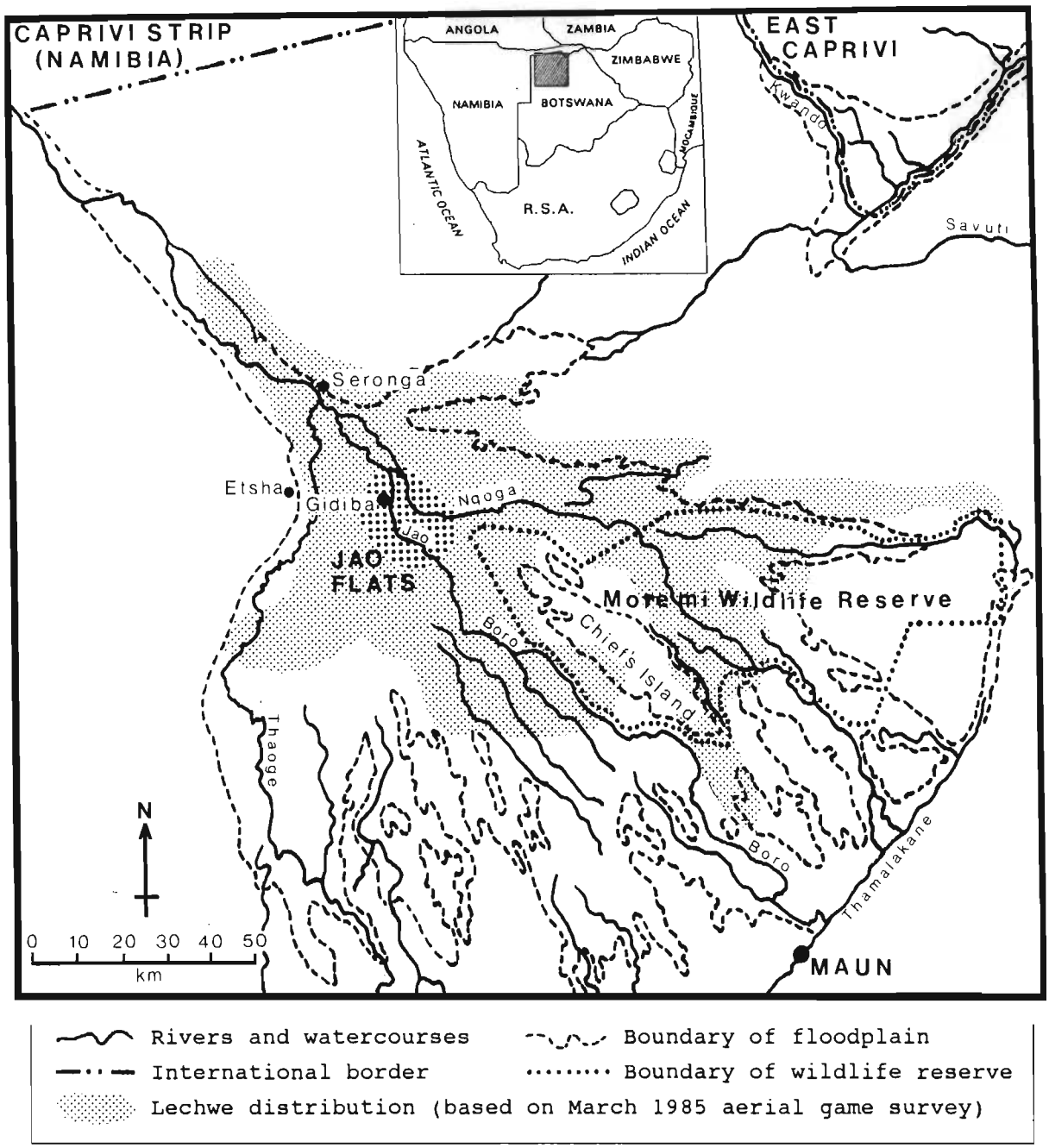


FIGURE 7.1 Map of the Okavango Delta, showing the location of the Jao Flats and human settlements in relation to the distribution of lechwe antelope.

utilised extensively by lechwe, which tend to shy away from the immediate vicinity of human settlements.

There appears to be considerable human movement between the periphery of the Delta and Jao/Jedibe. On the one hand, resident swamp-dwellers travel occasionally to the periphery in order to sell reeds, visit relatives, etc. On the other hand, people on the outskirts regularly journey into the interior for purposes of hunting, fishing and reed cutting. The duration of such trips, which may be seasonally-dependent, can vary from one day to several months. In addition, the traditional route for transfer of cattle from Seronga, at the lower end of the pan-handle, to Etsha on the west of the Delta, apparently passes through Jedibe (Figure 7.1). Apart from long-standing traditional activities, there are also those associated with tourism. A number of hunting, fishing and photographic safari companies operate out of Jedibe, generating a substantial amount of traffic. This includes the transportation of tourists and supply vehicles to and from safari camps, as well as movements of camp employees and work-seekers.

It seems reasonable to assume that a substantial proportion of the lechwe in the Jao Flats harbour *S. margrebowiei* and/or *S. leiperi* infections, although data in this regard are not available. High prevalences of these parasites were previously reported in the Moremi Reserve, directly to the east of the Jao Flats (Figure 7.1) (Pitchford and Wolstenholme, 1977). Furthermore, appropriate intermediate hosts (*Bulinus globous* and snails provisionally identified as *B. tropicus*) are apparently widespread in the Delta (Appleton, personal communication). This suggests that there is a high probability of human exposure to the cercariae of these schistosomes in the lechwe-frequented areas around Jedibe.

There have been no human schistosomiasis surveys in the Jao region to date (Johnsen; personal communication). However, considering the amount of human movement to and from the periphery, together with the fact that *S. mansoni* is firmly entrenched in the villages surrounding the Delta (Andersen *et al*, 1985; Ali *et al*, 1989) there would seem to be a strong possibility that this parasite may have been introduced into the area in recent years, or that it will be in the near future. The probability that it has or will become established is evidently high in view of the abundance of *Biomphalaria pfeifferi*

in the Delta (Appleton, personal communication). Interestingly, schistosomiasis in Ngamiland, although not eliminated, has largely been brought under control, mainly due to an intensive control programme (Ali *et al*, 1989) and the effects of drought (Johnsen, personal communication). However, since the drought is unlikely to affect the potential for transmission within the wetland region of the Delta itself, and since the control programme has not been concerned with resident swamp-dwellers, there may be a real risk of a severe disease outbreak in communities such as that at Jedibe.

In the light of the above observations, it is believed that the Jao Flats may offer a unique environment in which to explore the issue of whether or not incidental exposure to *S. margrebowiei* and *S. leiperi* affects either the susceptibility of humans to infection by *S. mansoni*, or the course of the resultant disease. However, the feasibility and logistics of carrying out a meaningful study in this area remain to be ascertained. Favourable factors in this regard include the possibility of enlisting the help of the local Regional Health Team, which apparently visits Gidiba on a regular basis, and the fact that there is an airstrip close to the village (Smith, personal communcation). Assuming that the opportunity and the necessary resources can be made available, it is proposed to carry out a preliminary survey aimed at determining (a) the prevalence of schistosome infections in lechwe and the availability of suitable snail hosts in the vicinity of Gidiba, (b) the degree of human water contact in lechwe-frequented areas, (c) whether there is any evidence of patent schistosome infections in the residents of the area, either due to antelope or human schistosomes, and (d) whether there is any evidence of schistosome-specific immunological activity in individuals not showing signs of patent infections. To this end, it is envisaged that lechwe droppings and human excreta would be examined for schistosome eggs, snail sampling would be carried out, the water contact patterns of the local residents would be investigated, and blood samples would be recovered for serological testing.

It may prove extremely difficult under field conditions to differentiate between protective effects related to to non-human schistosome exposure and those due to other, as yet unspecified, factors. Nevertheless, some intriguing possibilities for investigations using field-collected material exist. For example, if anti-schistosome antibodies are present in the sera of Jao

inhabitants (in the absence of *S. mansoni* infections), it would be of considerable interest to assess their species-specificity, in terms of their abilities both to differentially recognise antigens and to kill schistosomula of either antelope or human schistosomes.

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APPENDIX A

SCHISTOSOMES AND SNAILS: GENERAL METHODS OF HANDLING AND MAINTENANCE

A.1 ROUTINE MAINTENANCE OF SCHISTOSOMES

During the course of the studies described in this thesis four different schistosome isolates were employed, namely, *Schistosoma margrebowiei* and *S. leiperi* from Chobe National Park (Northern Botswana), *S. mansoni* from the Eastern Transvaal (referred to as the 'RSA' strain), and *S. mansoni* from Puerto Rico (the 'PR' strain). Further details regarding the origins of the different isolates are supplied in Sections 2.2.1 and 4.2.1. The definitive and intermediate hosts used for routine passage were as follows:

SCHISTOSOME	DEFINITIVE HOST	INTERMEDIATE HOST
S. margebowiei	BALB/c mouse	*Bulinus tropicus* (Albino)
S. leiperi	*Mastomys coucha*	*Bulinus africanus* or *Bulinus globosus*
RSA *S. mansoni*	*M. coucha*	*Biomphalaria pfeifferi*
PR *S. mansoni*	*M. coucha*	*Biomphalaria glabrata* (Albino)

It is possible that the different schistosome isolates dealt with here were subjected to various degrees of population bottlenecking (LoVerde *et al*, 1985) prior to their establishment in the present author's laboratory. Nevertheless, an effort was made to preserve as much genetic variability as possible in the respective parasite populations. Thus, as recommended by Fletcher *et al* (1981), a consistent attempt was made to use cercariae from as many shedding snails as possible when carrying out routine definitive host infections; as a general guideline, a minimum of 30 snails was arbitrarily set as a lower limit, although in most instances the number used was substantially higher. Cercarial loads used for rodent infections represented a compromise between the need to ensure

reasonable survival times (so as to minimize the number of animals used for life-cycle maintenance) and the desire to maintain reasonable genetic diversity. As a rule, organs from at least 2-3 animals were used when preparing miracidia for snail infections.

Scheduling of definitive and intermediate host infections was worked out on the basis of monthly assessments of available stocks, considered in conjunction with the requirements of intended experiments. Detailed records were kept of all infections and their outcomes. In an attempt to determine the optimum infection loads for each host-parasite combination, various permutations (in terms of numbers of parasites/host) were tested over a period of some years; the experience and information gained thereby was used to draw up a set of guidelines for routine infections. These guidelines are presented in the following two sub-sections (A.1.1 and A.1.2), whereas the methods used for recovery and handling of miracidia and cercariae and for infecting snails and rodents are detailed in Sections A.3 and A.4.

A.1.1 General Guidelines for Routine Infection of Snails

The recommended miracidial loads for snails of different sizes are indicated in Table A.1, which also includes estimates of the approximate snail survival and infection rates (within one to two weeks of the onset of shedding) applicable to each snail/schistosome combination. The latter were derived from data pertaining to numerous routine infections. As a rule, snails in the smaller size ranges (3-5mm) were used in preference to those of larger size.

Infections were carried out on average every 6 weeks in the cases of *S. margrebowiei* and PR *S. mansoni*, and every 8-12 weeks in the cases of *S. leiperi* and RSA *S. mansoni*.

A.1.2 General Guidelines for Routine Infection of Rodents

The frequency of stock rodent infections was, by necessity, linked to that of snails, with infections usually being carried out within one to two weeks after the onset of cercarial shedding by successive batches of snails (i.e. when the number of shedding snails was

TABLE A.1 Table showing recommended numbers of miracidia for snails of different sizes, as well as approximate prepatent periods, survival rates and infection rates, for each snail-schistosome combination.

SCHISTOSOME	*S. margrebowiei*	*S. leiperi*	*S. mansoni* (RSA)	*S. mansoni* (PR)
SNAIL HOST	*Bul. tropicus* (albino)	*Bul. africanus* group	*Bi. pfeifferi*	*Bi. glabrata* (albino)
Snail size range (mm)[a]	Miracidia/Snail	Miracidia/Snail	Miracidia/Snail	Miracidia/Snail
3.0 - 3.5	4 - 5	4	3	3
3.5 - 4.0	5	4 - 5	4	4
4.0 - 4.5	5 - 6	5	4 - 5	5
4.5 - 5.0	6	5 - 6	5	6
5.0 - 5.5	6 - 7	6	6	7
5.5 - 6.0	7	7	8	8
6.0 - 6.5	8	8 - 9	9	9
6.5 - 7.0		10		10
Prepatent period (weeks)[b]	4.5 - 5	5 - 5.5	4.5 - 5	4 - 4.5
Survival (%)[c]	84	78	70	91
Survivors shedding (%)[c]	76	80	90	98

a Snails were measured to the nearest 0.1mm with the aid of a Vernier caliper. *Bulinus* spp. were measured from the leading edge of the shell aperture to the apex of the spire. In the case of *Biomphalaria* spp., largest diameters were determined (i.e. from the outer edge of aperture, across the centre of the spiral; Frank and Meyling, 1966).

b Snails were maintained at a constant temperature of 25-26 C.

c Estimates based on data collected within 1-2 weeks of patency from numerous routine infections.

maximal). In some instances infections were carried out on more than one occasion after the maturation of specific snail groups, depending on stock levels. On most occasions groups of 5-10 animals were infected. Both male and female animals were used for life-cycle maintenance, largely on the basis of availability; the ages of these animals varied considerably, although care was taken to ensure that they were reasonably young.

S. margrebowiei (BALB/c mouse): Two groups of mice were infected on each occasion, with those of the first group being exposed to 85-90 cercariae each and those of the second, 200 cercariae each. Good yields of miracidia were obtained more reliably from animals exposed to the latter dose; however, deaths were common in these groups from about 8 weeks of infection onwards and it was necessary to use the animals within about 10-11 weeks of infection. The more lightly-infected groups generally served as a backup, in the event that snail infections could not be carried out before all the heavily-infected animals had died.

S. leiperi (*M. coucha*): Standard cercarial load = 250/animal. Mortalities were rare before about 16 weeks of infection, long-term survival was generally good and good yields of miracidia were obtainable even from animals with long-term infections.

PR *S. mansoni* (*M. coucha*): Standard cercarial load = 90/animal. Mortalities were common from 11-12 weeks of infection onwards. Excellent yields of miracidia could be obtained from animals with infections of greater than 9 weeks' duration.

RSA ***S. mansoni*** (*M. coucha*): Standard cercarial load = 250/animal. Mortalities were rare before about 14 weeks of infection and long term survival was moderate. Yields of miracidia were generally very good, especially from animals with infections greater than 12-13 weeks' duration.

A.2 BREEDING AND MAINTENANCE OF INTERMEDIATE HOSTS

A.2.1 Sources

B. africanus and *Bi. pfeifferi* were collected in the vicinity of Durban and *B. globosus* in the vicinity of Kosi Bay in Northern Natal.

B. tropicus (albino strain) and *Bi. glabrata* (albino strain) were supplied by the Bilharzia Field Research Unit (Nelspruit) and the Weizmann Institute (Rehovot, Israel), respectively. Snails are illustrated in Figure A.1.

A.2.2 Bulinid Species and *Biomphalaria pfeifferi*

B. africanus, *B. globosus*, *B. tropicus* and *Bi. pfeifferi* were bred under outdoor conditions in aquaria constructed from green reinforced polyvinylchloride (PVC) sheeting (Figure A.2). This material is amenable to a heat welding process which served to ensure that corner seams were leak-free. Aquaria (dimensions 1000 x 1000 x 160mm; capacity 140-150 litres) were suspended from galvanised iron or stainless steel frames by means of brass eyelets inserted around the upper rim of the PVC, at approximately 200mm intervals. Each aquarium was supplied with an independent shelter, fitted with castor wheels to facilitate easy movement. The sides of each shelter were covered with black 'shadecloth' (70% light occlusion; Alnet (Pty) Ltd., Durban). This served, on the one hand, to ensure that aquaria were well shaded and thus not subject to excessive algal growth, and, on the other hand, to prevent access by birds.

Aquarium water consisted of tap water, dechlorinated with Tetra Aquasafe (TetraWerke, Germany) and supplemented with calcium chloride (60mg/L) and sodium bicarbonate (120mg/L). The latter two agents served to prevent deterioration of the snail shells which otherwise occurred due to the softness of the local water supplies. Aquaria were furnished with various objects in order to provide concealed surfaces for egg laying. These included pieces of slate, clay roof tiles and black or brown plastic 'rafts', consisting of saucers (230mm diameter) from plastic plant pots.

Snails were fed and aquaria inspected twice weekly: *B. tropicus* were fed exclusively on a mixture comprising equal parts of dehydrated lettuce and lucerne (Frank, 1963 and 1965), while *B. africanus/globosus* received this food, as well as occasional supplementation with a mixture comprising equal parts of Tetra Staple and Tetra Conditioning fish foods (TetraWerke, Germany) (recommended by Dr K N de Kock, personal communication). Care was taken to ensure that aquaria were not overfed as this promoted conditions which were

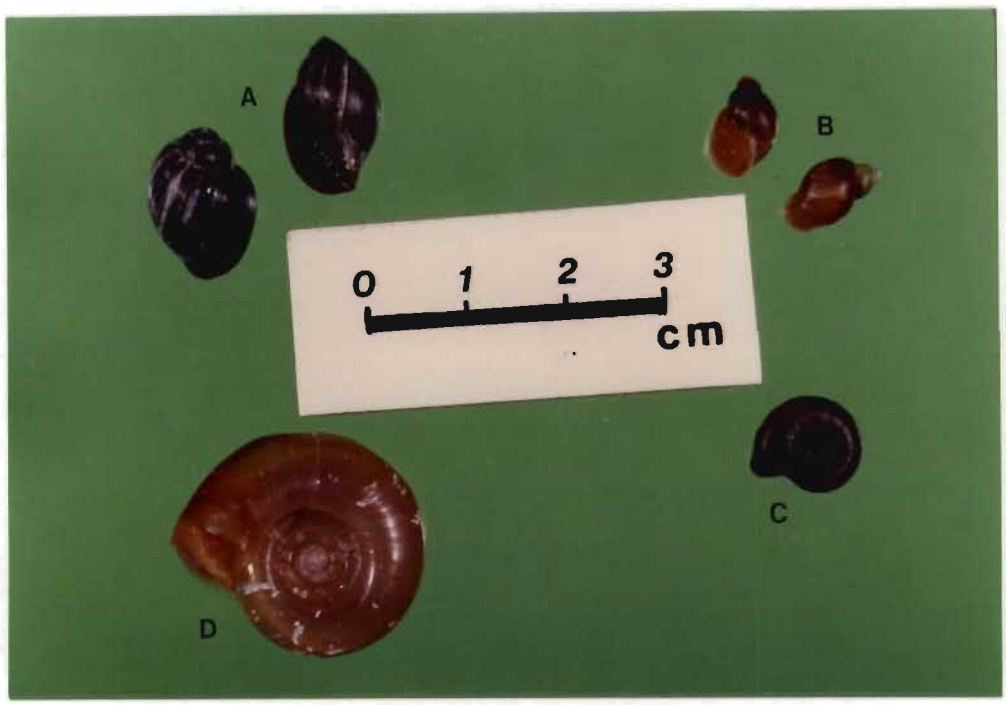

FIGURE A.1 Snail species used in routine maintenance of schistosome life-cycles: **A**, *Bulinus africanus*; **B**, *B. tropicus* (albino); **C**, *Biomphalaria pfeifferi*; **D**, *Bi. glabrata* (albino).

FIGURE A.2 Outdoor snail-breeding aquaria.

unfavourable to the snails. Snail faeces were removed from the aquaria every two weeks, with the aid of a small self-priming pump (Model 15000-303, Gorman-Rupp Industries, Ohio, USA). Every four to six weeks the aquaria were drained to approximately 20% of capacity and refilled with fresh water. Removal of young snails for infection purposes helped to prevent overcrowding, although culling was carried out when necessary. Whenever large numbers of deaths occurred in an aquarium it was treated for a minimum of 24 hours with a strong solution of sodium hypochlorite (household bleach), following which it was scrubbed thoroughly, rinsed copiously, refilled with fresh water and restocked with healthy snails. Regular adherence to all of these practices was the key to ensuring a consistent supply of young snails.

Electricity supply points were distributed to each aquarium so that they could be heated during winter, if necessary. This was achieved by means of domestic aquarium heaters (2 x 200 watt heaters per aquarium) linked to a timer switch, which could be variably set to supply power at specific periods during each 24 hour cycle. Periods of heating could thus be adjusted according to prevailing ambient temperature conditions. Care was taken to ensure that heaters did not lie in direct contact with the floors or sides of the aquaria, as the heat caused the PVC to become brittle and subject to cracking.

This long-term snail culture system provided an adequate supply of snails for infection on a virtually year-round basis. Due to the mild climatic conditions in Durban, heating of aquaria was found to be necessary only occasionally. Although egg laying in unheated aquaria was minimal for two or three months during mid-winter, there were usually sufficient juvenile snails available from the preceding breeding season to meet infection requirements. The quality of the snails produced under outdoor conditions was found to be considerably superior, in terms of general health, infection rates and post-infection survival, to those bred indoors, under constant temperature conditions (25 C) (unpublished observations).

Few problems were encountered with common pest organisms (see Webbe and James, 1971). Snails were free of *Chaetogaster* spp. and troublesome crustaceans, such as ostracods, were seldom seen, except in inadequately maintained aquaria. In contrast, large populations of *Daphnia* spp. were frequently present, indicative of healthy

conditions. However, infestation of the aquaria by the larvae of chironomid midges was a common occurrence. They were observed to cause considerable irritation, especially to the juvenile snails, although they did not appear to attack them directly. Furthermore, they competed aggressively for the food supplied to the snails. Thus, if allowed to proliferate excessively they caused aquaria to become unproductive rapidly. However, they were generally kept in check by the routine cleaning procedures. In cases of severe infestation they were easily eliminated using preparations containing the spores of *Bacillus thuringiensis israelensis* (BTI) (World Health Organization, 1982), which were found to have no adverse effects on the snails, even at concentrations far in excess of those required to kill chironomid larvae (unpublished observations).

A.2.3 *Biomphalaria glabrata*

Biomphalaria glabrata were bred in glass aquaria (inner dimensions, 610 x 320 x 300mm) containing water conditioned as described above. Aquaria were maintained in a constant temperature laboratory at 25-26 C, under a 14/10 hour light/dark cycle. A colony of approximately 100 healthy adult snails was maintained on a continuous basis, as a source of eggs for the production of young snails. Batches of young snails were produced by allowing adults (about 20/aquarium) to lay eggs over a period of a week, removing them from the aquaria, then allowing the eggs to hatch. From each batch of new snails 30-40 healthy juveniles were selected to replace the oldest adults, thereby ensuring regular turnover of the breeding stock. Both adult and baby snails were fed on the fish food mixture described above.

Aquaria were cleaned weekly. Those containing adult snails were emptied, scrubbed and refilled, while those containing juvenile snails were partially (70-80%) drained, following removal of faecal matter by means of a siphon tube. In order to prevent excessive loss of snails during the latter procedure, the siphon tube was fed into a 10 litre bucket, the bottom of which had been replaced with nylon filtration fabric, with a mesh aperture of 400 microns (Nybolt 45 GG-400; Swiss Silk Bolting Cloth Mfg. Co. Ltd., Switzerland). On completion of siphoning, the contents of the bucket were flushed with a stream of water. By adjusting the force of the water appropriately it was possible to break up, and hence flush away, the faecal matter

without damaging the snails, which were subsequently returned to aquaria.

In view of the concern that has been expressed regarding the risks of introducing *Bi. glabrata* into natural habitats in Africa (Pfluger, 1982), great care was taken to ensure that neither live snails nor eggs were permitted to escape from the laboratory. To this end, all aquarium waste water was pooled into a large drum, treated with granular dry chlorine (containing 700g/kg calcium hypochlorite; 70% available chlorine; Olin (Pty) Ltd, Bergvlei) at a dose of approximately 5ml/10L and allowed to stand for at least 24 hours before being discarded.

A.3 METHOD OF PREPARING AND EXPOSING SNAILS TO MIRACIDIA

Eggs recovered from the livers, or occasionally the intestinal tissues (in the case of *S. margrebowiei* only) of infected rodents were used as a source of miracidia for the infection of snails. As a general rule a minimum of 3 weeks from the time of worm maturation was allowed before attempting to recover eggs from stock infected animals.

Tissues were first partially disrupted by brief blending in a small quantity of hypertonic saline [1.2-1.5% sodium chloride solution (mass/vol), prepared in fresh aquarium water]. The blended material was then sieved through a stainless steel screen with a mesh aperture of 425 microns (Figure A.3), using a flat-ended object to facilitate more complete homogenisation of the tissues. The homogenate was then poured into a helminth filter (Visser and Pitchford, 1972) with inner and outer mesh apertures of 50 and 95 microns respectively, and flushed repeatedly with hypertonic saline. Miracidia were obtained by diluting portions of the resultant egg-bearing filtrate with aquarium water in a side-arm hatching flask (Rau *et al*, 1972) (Figure A.4), under ambient temperature conditions of 26-28 C.

Snails harvested from breeding aquaria were sorted according to size, then placed individually into the wells of 24-well tissue culture plates (Costar, Massachusetts, USA) (Figure A.4); each well contained 1-1.5ml of aquarium water. Miracidia were introduced into the wells with the aid of a dissection microscope and a fine-tipped pasteur

FIGURE A.3 Screen used for homogenization of liver tissue (Section A.3).

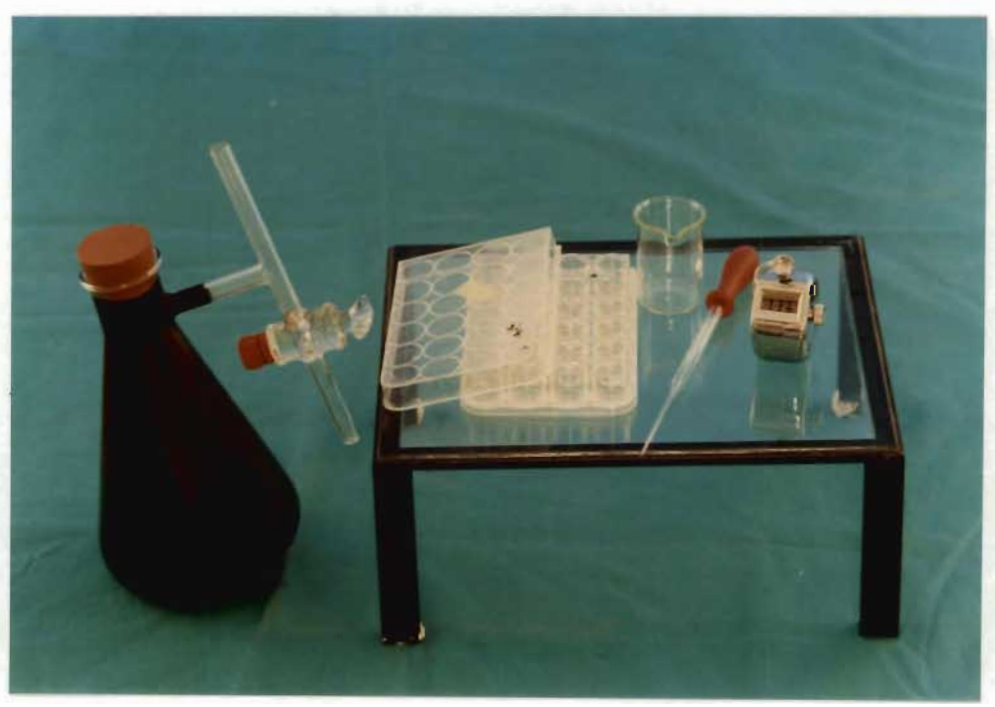

FIGURE A.4 Equipment used for infection of snails (Section A.3).

pipette. The number of miracidia per well was varied on the basis of snail size, according to the guidelines indicated in Table A.1. As a rule they were introduced to the snails within less than 60 minutes after hatching. For this reason, trays of snails were customarily prepared prior to or simultaneously with the recovery of schistosome ova. In addition, when large numbers of snails were to be infected, the egg-bearing filtrate was split into portions so that batches of eggs could be hatched successively. In order to maximise the opportunity for miracidial penetration, a minimum exposure period of 4 hours was allowed before transferring snails to aquaria. Snails were maintained at 25-26 C throughout the period of exposure and thereafter. All surplus eggs and miracidia were discarded into the waste drum used for the elimination of excess *Bi. glabrata*.

Various types of aquaria were used for housing snails after exposure to miracidia, including glass fish tanks (capacities ranging from 10 to 50 litres), 10 litre plastic buckets (diameter 230mm), and 2 and 5 litre plastic tubs (dimensions 150 x 210 x 80mm, and 200 x 300 x 100mm, respectively); the former two types of aquaria were usually provided with aeration. While they were still small, *B. africanus* group snails and *Bi. pfeifferi* were maintained at a density of 4-5/litre; as they approached adulthood this was progressively decreased to 2-3/litre. *B. tropicus* and *Bi. glabrata* were usually maintained at slightly higher densities (5-6/litre); relatively little growth-related reduction in density was found to be necessary in the case of the latter species. All aquaria were emptied, scrubbed and refilled with fresh water once a week.

A.4 RECOVERY AND HANDLING OF CERCARIAE

The approximate durations of the prepatent periods for each of the schistosomes in their respective snail hosts under the above conditions are shown in Table A.1. Snail infection rates were routinely assessed by placing snails individually into glass test tubes and determining the proportion that were releasing cercariae. Snails were subjected to strong illumination and an increased ambient air temperature (26-28 C) during the shedding period, to encourage the release of cercariae. As noted in Section 1.10.6, both *S. margrebowiei* and *S. leiperi* exhibited a distinct early morning shedding peak and, for convenience, animal infections were always

carried out using cercariae shed at this time. Peak shedding of the PR and RSA strains of *S. mansoni* occurred during mid- and late-morning, respectively.

Snail faeces and other debris were removed by filtration of the cercaria-containing water through a nylon filter with a mesh aperture of 180 microns (Nybolt 8P-180; Swiss Silk Bolting Cloth Mfg. Co. Ltd., Switzerland) (Figure A.5); the filter was gently flushed at regular intervals with conditioned water from a wash bottle, to prevent clogging.

The density of cercariae in the pooled cercarial suspension was estimated using the equipment illustrated in Figure A.6. Mixing of the suspension was achieved using a variable-setting, low-speed laboratory rotator, modified by means of gears to reduce its normal speed of operation, and equipped with interchangeable paddles to suit beakers of different sizes. Stirring speed was normally in the range of 35-50 rev/min, and was adjusted according to the size of the beaker and the volume of suspension, the aim being to achieve optimum mixing without damaging the cercariae. A number of 1ml aliquots were withdrawn using a 1-5ml adjustable-volume pipette (Gilson Pipetman, France) and placed into 'Perspex' (acrylic) counting chambers (see also Appendix B), following which the cercariae were stained with a few drops of Lugol's iodine solution and counted with the aid of a dissection microscope. On the basis of the counts obtained the aliquot volume was then adjusted to give the required number of cercariae. If necessary the suspension was diluted with conditioned water, or concentrated according to the method described in Appendix C. As a general rule, an effort was made to adjust concentrations such that the desired number of cercariae could be obtained in a volume of 1-1.5ml. Aliquots containing more than about 150 cercariae were usually distributed into 2 or more counting chambers, as this facilitated greater counting accuracy.

In the case of experimental procedures, in particular, care was always taken to ensure that aliquots contained the desired number of cercariae. Thus, during the course of pipetting cercariae into the infection vessels, a number of aliquots (usually more than 5) were set aside for counting. These counts served to provide a reasonably accurate estimate of the number of cercariae to which the animals were actually exposed.

FIGURE A.5 Filtration of cercarial suspension to remove snail faeces.

FIGURE A.6 Equipment used for estimating and dispensing desired numbers of cercariae.

As a general rule, an effort was made to expose animals to cercariae within 4 hours after the onset of shedding. Infections were carried out by means of the paddling method (Dettman *et al*, 1989).

Surplus cercariae were discarded into the waste drum.

A.5 REFERENCES

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APPENDIX B

EQUIPMENT FOR THE MANUFACTURE OF COUNTING CHAMBERS

Substantial numbers of counting chambers were required for counting schistosome cercariae and ova. However, Sedgewick-Rafter counting chambers, which are commonly used for this purpose, proved to be both expensive and difficult to obtain. For this reason a method was devised whereby counting chambers could be manufactured from small sections of flat, 1.5mm thick, 'Perspex' (acrylic) sheet by means of a heat-forming process. Since sample volumes were often in excess of 1ml, particularly when counting cercariae, it was decided to design chambers with a capacity of up to 1.5ml. Furthermore, they were provided with sides sloping at an angle of 35 , since prototype-testing showed that objects situated close to the sides of chambers with vertical walls were often difficult to detect.

The dimensions of the 'male' and 'female' forming sections are shown in Figure B.1.

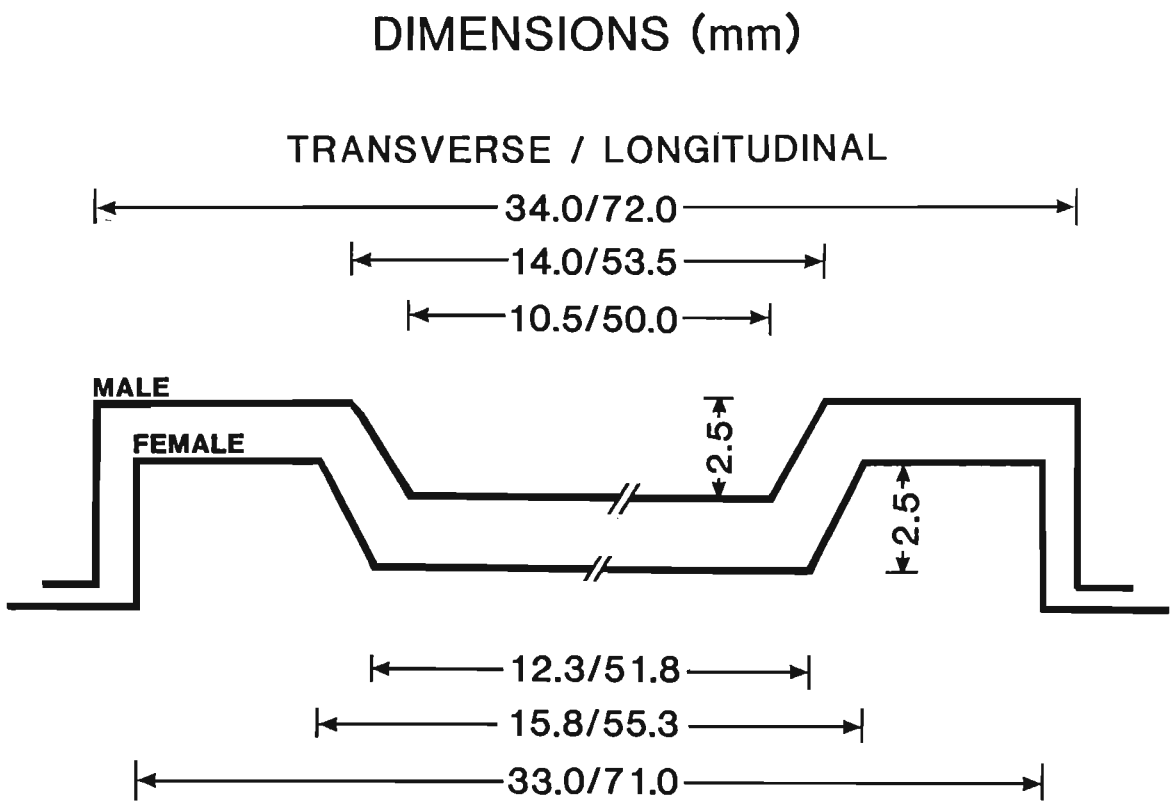

FIGURE B.1 Dimensions of male and female shape-forming sections of counting chamber press. (Note: A clearance of 1.7mm was allowed between the male and female components).

These dimensions served as the basis for the construction, essentially from brass, of a press which facilitated the production of large numbers of counting chambers. This device, illustrated in Figures B.2 and B.3, was manufactured in the Physics Workshop, University of Natal, Durban, under the supervision of Mr W de Beer. It was designed to produce pairs of counting chamber 'blanks' and consisted of a base plate, a 'female' component, and a 'male' component, with a detachable handle.

Description of Counting Chamber Manufacturing Process: The male component was initially preheated for 10min in an oven set at 180 C. Sections of suitably-sized Perspex sheet were then placed into the twin wells of the male component, and it was returned to the oven. After 6-7min (i.e. once the Perspex had become fully malleable) it was removed from the oven and immediately positioned on the base plate, following which the female component was lowered onto the male and pressed down firmly for about 15s. Cold water (20 C) was then poured over the exposed parts of the male component and the base plate. This served to reduce the temperature of the Perspex sufficiently for it to set, whereupon the formed blanks could be removed.

A grid of 3 x 3mm squares was marked onto the bottom of each chamber by means of a guide-grid, a straight edge and a rotary cutter (Model RTY-1, 28mm diameter blade; Olfa Corporation, Osaka, Japan) (Figure B.3). The latter device was found to produce far 'cleaner' lines than could be effected with a scalpel blade. Chambers were fixed into position over the guide-grid with double-sided adhesive tape.

Legs, consisting of narrow strips of 3mm thick Perspex, were then glued onto the counting chambers.

Provided they were treated in such a way as to minimise scratching, these counting chambers (Figure B.4) gave years of useful service.

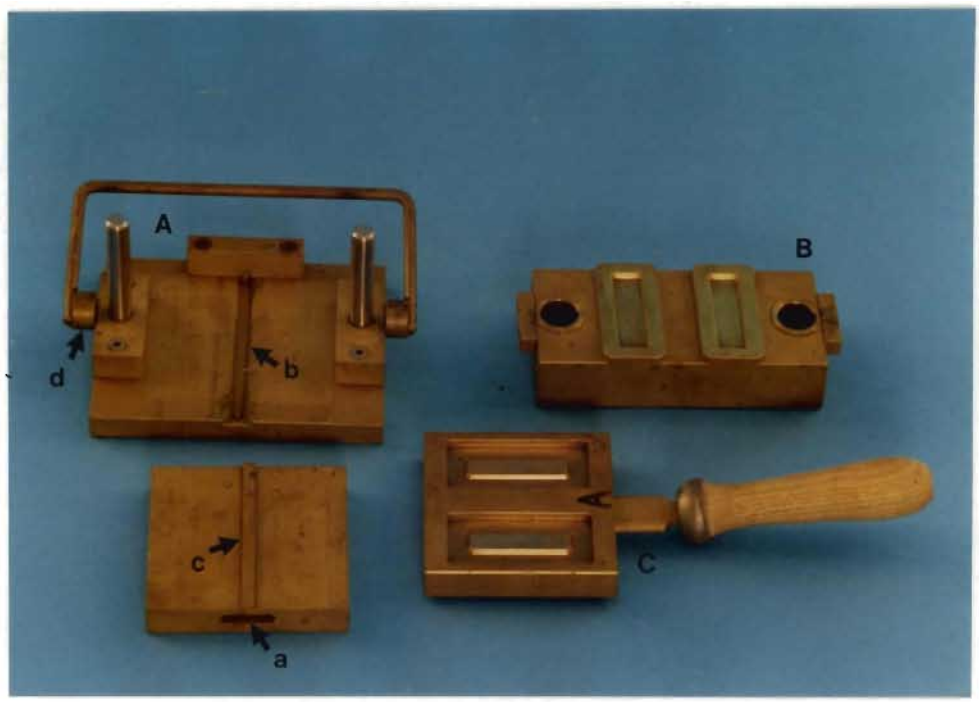

FIGURE B.2 Components of press used for heat forming Perspex counting chambers: (A) Base plate assembly; (B) 'Female' component, inverted to show shape-forming sections; (C) 'Male' component, with detachable handle *in situ*. The blade of the handle fits into a slot in the front of the male component (a). A groove in the base plate (b) and a matching guide on the underside of the male component (c) serve to ensure correct alignment of the forming sections. When in position on the locating columns (see Figure B.2), the female component can be raised and lowered by means of a pair of laterally-positioned cams (d), operated by a common handle.

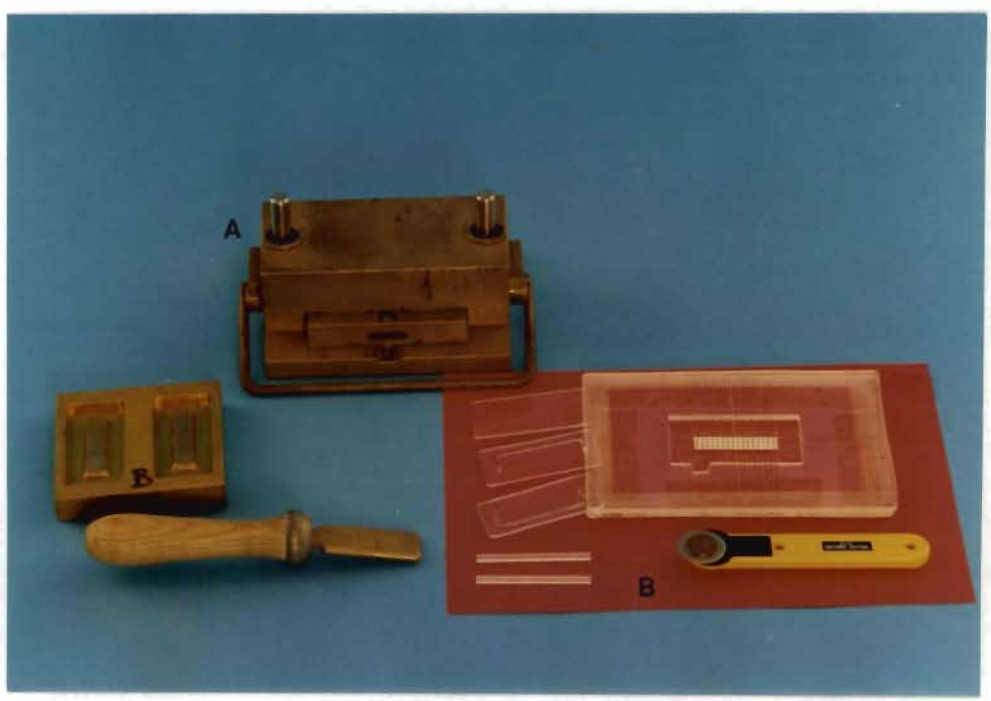

FIGURE B.3 Equipment used for the manufacture of counting chambers: (A) Perspex-forming press, showing the various components in place; (B) Grid-marking equipment.

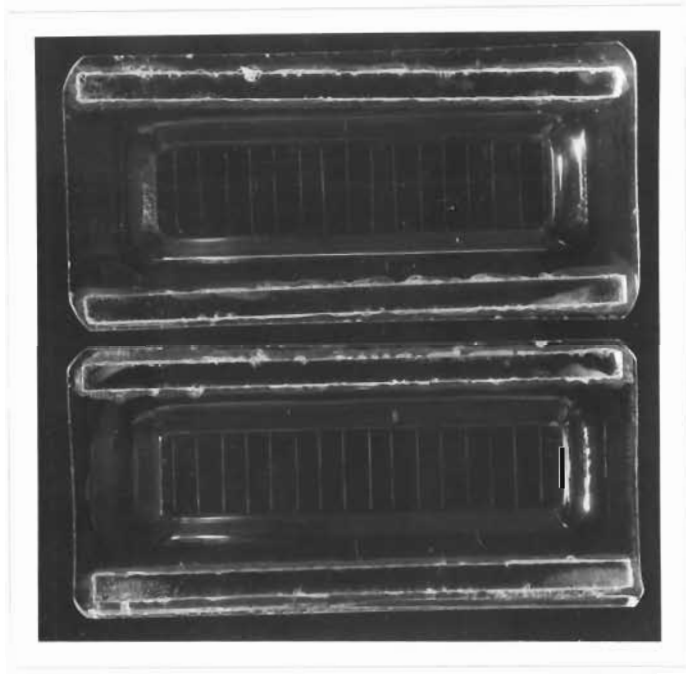

FIGURE B.4 Completed counting chambers.

APPENDIX C

METHODS OF CONCENTRATING CERCARIAE AND OF PREPARING CERCARIAL ANTIGEN

Two pieces of equipment, referred to as first- and second- stage concentrators, respectively, were devised for the concentration of cercarial suspensions (Figures C.1 and C.2). The former was used for increasing the density of cercariae in suspension either for purposes of animal infection or as a first step in the preparation of cercarial antigen. The latter was used in the preparation of highly concentrated suspensions, its major advantage being that it facilitated the washing of live cercariae without the need for repeated centrifugation, and its primary application being in the preparation of cercarial antigen.

C.1 FIRST STAGE-CONCENTRATOR

This consisted of two components: (i) an outer beaker with a basal outlet, to which was affixed a stopcock leading to a 19 gauge hypodermic needle, and (ii) an inner 'Perspex' (acrylic) tube (outer diameter 5-10mm less than the internal diameter of the beaker), the lower opening of which was covered with nylon bolting cloth, with a mesh aperture of 15 microns (Nybolt M-15; Swiss Silk Bolting Cloth Mfg. Co. Ltd., Switzerland). The inner tube was provided with small feet to ensure freedom of fluid movement across the filter interface, as well as graduations indicating the volumes at different levels within the tube. The overall size of this device could be varied by using beakers and inner tubes of different sizes, to accomodate different starting volumes of cercarial suspension.

Prior to introduction of the cercariae, sufficient conditioned water was poured into the beaker to cover the filter. The cercarial suspension (pre-filtered to remove snail faeces) was then poured into the inner tube, following which the stopcock was opened. By restricting the rate of drainage, the needle served to ensure that the drag across the filter was only slight, thus allowing the cercariae to remain in suspension and thereby avoiding clogging of the filter.

FIGURE C.1 Equipment used for concentration of cercarial suspensions: (A) First-stage concentrator; (B) Inner tube of first-stage concentrator; (C) Second-stage concentrator.

FIGURE C.2 Close-up of first-stage concentrator.

Once the desired degree of concentration was achieved, samples could be pipetted directly from the inner compartment. Alternatively, the suspension could be poured off into another beaker, as shown in Figure C.3.

C.2 SECOND-STAGE CONCENTRATOR

This consisted of a plastic conical-bottom tube equipped with two opposing, elongated 'windows' covered with nylon bolting cloth, with a mesh aperture of 10 microns (Nybolt M-10-Super; Swiss Silk Bolting Cloth Mfg. Co. Ltd., Switzerland). The hard plastic storage case of a 60ml disposable syringe (Monoject, Sherwood Medical, Northern Ireland; capacity approximately 110ml; diameter 33mm; overall length 190mm) was found to be ideal for this purpose.

C.3 PREPARATION OF CERCARIAL ANTIGEN

For purposes of antigen preparation the volume of the initial cercarial suspension was first reduced using the first-stage concentrator. The suspension was then poured into the second-stage concentrator and the volume of fluid allowed to decrease to about 10% of the tube capacity; clogging of the filter windows was prevented by frequent back-flushing using a pump-action spray bottle. Immediately thereafter the apparatus was rapidly refilled with phosphate-buffered saline (PBS) (pH 7.1) containing 0.2% (v/v) Tween 20 (Sigma Chemical Co., USA) and the volume allowed to decrease as before. This process was then carried out repeatedly with plain PBS and, once there was no further evidence of foaming due to the Tween (usually 4-5 washes), the highly concentrated cercarial suspension was poured into a measuring cylinder. The inner walls of the tube were briefly rinsed and the windows back-flushed with PBS to recover any cercariae that may have been left behind. Clumping of the cercariae, which sometimes occurred, was dealt with by passing the suspension through a filter identical to that used for preliminary filtration of cercarial suspensions (mesh aperture 180 micons; see Appendix A, Figure A.5); any clumps remaining on the filter mesh were easily dispersed by gentle flushing with PBS from a wash bottle. Finally, the cercariae were fixed by the dropwise addition of formalin to a final concentration of 5% formaldehyde, according to

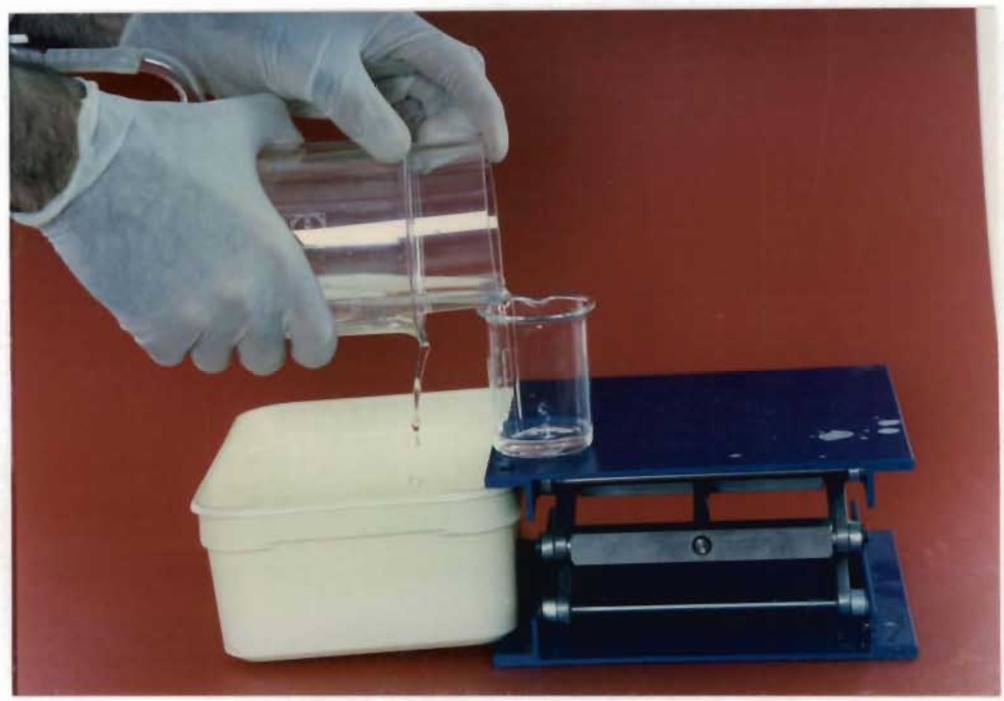

FIGURE C.3 Recovery of cercarial suspension from first-stage concentrator.

the method of Wolstenholme and Fripp (1981). If clumping of the cercariae occurred while they were in the measuring cylinder (i.e. immediately before fixation), they could be easily separated after fixation by filtration and gentle flushing, as described above.

C.4 REFERENCE

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APPENDIX D

EXPERIMENTAL PLANS PERTAINING TO STUDIES BASED ON THE CONCOMITANT IMMUNITY MODEL (SEE CHAPTER 4)

The accompanying tables provide details for each experiment in terms of the type and number of cercariae to which the animals in the various groups were exposed, as well as the times at which infections and perfusions were carried out. The values in square parentheses indicate the estimated number of cercariae to which each animal was exposed. The following abbreviations have been used:

GROUPS

- IC = Initial infection control group; exposed to the initial infection only
- EXP = Experimental group; exposed to both the initial and challenge infections
- CC = Challenge infection control group; exposed to the challenge infection only

SCHISTOSOMES

- MB = *S. margrebowiei*
- LP = *S. leiperi*
- PR = Puerto Rican strain of *S. mansoni*
- RSA = South African strain of *S. mansoni*

(Note that in some tables these abbreviations are used as suffixes, in conjunction with EXP and CC, to indicate the type of schistosome used for the challenge infection)

PROCEDURE

- PERF = Perfusion

In virtually all experiments, challenge infections were carried out a few days prior to, and about four weeks after the expected onset of egg-laying by the initial infection worms. On the basis of studies on infection characteristics, egg-laying was assumed to start at approximately 5 weeks of infection in the cases of *S. margrebowiei* (Chapter 2) and PR *S. mansoni* (Higgins-Opitz and Dettman, 1991) and

7 weeks of infection in the case of *S. leiperi* (Chapter 2). Thus, animals exposed initially to either *S. margrebowiei* or PR *S. mansoni*, were challenged after 4.5 and 9 weeks of infection, and animals exposed initially to *S. leiperi* were challenged after 6.5 and 11 weeks of infection. The only instance where this principle did not apply was the study involving homologous challenge with the RSA strain of *S. mansoni*, where the challenge was carried out 9 weeks after initial infection, i.e. about 2 weeks after the expected onset of egg-laying (Higgins-Opitz and Dettman, 1991). Perfusions were routinely carried out 1 week after the expected onset of egg-laying by the challenge worms, i.e. at 6 weeks for *S. margrebowiei* and PR *S. mansoni*, and at 8 weeks for *S. leiperi* and RSA *S. mansoni*.

REFERENCE

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TABLE D.1 Initial exposure to either the PR or RSA strains of *Schistosoma mansoni*, followed by homologous challenge at 4.5 weeks (PR strain only) and 9 weeks.

TIME (weeks)	PR Strain						RSA Strain		
	4.5 week challenge			*9 week challenge*			*9 week challenge*		
	IC	EXP	CC	IC	EXP	CC	IC	EXP	CC
0	PR [27]	PR [27]		PR [27]	PR [27]		RSA [42]	RSA [42]	
4.5		PR [79]	PR [79]						
9					PR [87]	PR [87]		RSA [84]	RSA [84]
10.5	PERF	PERF	PERF						
15				PERF	PERF	PERF			
17							PERF	PERF	PERF

TABLE D.2 Initial exposure to *Schistosoma margrebowiei*, followed by challenge with the PR and RSA strains of *S. mansoni* at 4.5 and 9 weeks (see Tables 4.2a & 4.2b).

TIME (weeks)	*4.5 week challenge*					*9 week challenge*				
	IC[a]	EXP-PR	CC-PR	EXP-RSA	CC-RSA	IC[a]	EXP-PR	CC-PR	EXP-RSA	CC-RSA
0	MB [26]	MB [26]		MB [26]	MB [26]	MB [26]	MB [26]		MB [26]	MB [26]
4.5		PR [84]	PR [84]	RSA [126]	RSA [126]					
9							PR [92]	PR [92]	RSA [131]	RSA [131]
10.5	PERF	PERF	PERF							
12.5	PERF			PERF	PERF					
15						PERF	PERF	PERF		
17						PERF			PERF	PERF

a The IC groups for the 4.5 and 9 week challenge series were each split into two subgroups, which were perfused at intervals corresponding with the perfusions of the PR- and RSA-challenged groups, respectively. For the purposes of calculating percentage reductions of gravid worm pair and total worm loads (Table 4.2a), the data of the 10.5 and 12.5 week intervals were pooled, as were those of the 15 and 17 week intervals. Tissue egg load data (Table 4.2b) were not pooled in this way, however, in view of their dependence on the duration of infection.

TABLE D.3 Initial exposure to *Schistosoma margrebowiei*, followed by homologous challenge at 4.5 and 9 weeks.

TIME (weeks)	*4.5 week challenge*			*9 week challenge*		
	IC	EXP	CC	IC	EXP	CC
0	MB [25]	MB [25]		MB [25]	MB [25]	
4.5		MB [116]	MB [116]			
9					MB [121]	MB [121]
10.5	PERF	PERF	PERF			
15				PERF	PERF	PERF

TABLE D.4 Initial exposure to *Schistosoma leiperi*, followed by challenge with the PR and RSA strains of *S. mansoni* at 6.5 and 11 weeks (see Tables 4.4a & 4.4b).

TIME (weeks)	*6.5 week challenge*					*11 week challenge*				
	IC[a]	EXP-PR	CC-PR	EXP-RSA	CC-RSA	IC[a]	EXP-PR	CC-PR	EXP-RSA	CC-RSA
0	LP [37]	LP [37]		LP [37]	LP [37]	LP [37]	LP [37]		LP [37]	LP [37]
6.5		PR [82]	PR [82]	RSA [124]	RSA [124]					
11							PR [88]	PR [88]	RSA [118]	RSA [118]
12.5	PERF	PERF	PERF							
14.5	PERF			PERF	PERF					
17						PERF	PERF	PERF		
19						PERF			PERF	PERF

a The IC groups for the 6.5 and 11 week challenge series were each split into two subgroups, which were perfused at intervals corresponding with the perfusions of the PR- and RSA-challenged groups, respectively. For the purposes of calculating percentage reductions of gravid worm pair and total worm loads (Table 4.4a), the data from the 12.5 and 14.5 week intervals were pooled, as were those of the 17 and 19 week intervals. Tissue egg load data (Table 4.4b) were not pooled in this way, however, in view of their dependence on the duration of infection.

TABLE D.5 Initial exposure to *Schistosoma leiperi*, at estimated infection loads of either 40 or 111 cercariae/mouse, followed in the former instance by homologous challenge only, or in the latter instance by either homologous or heterologous (PR and RSA strains of *S. mansoni*) challenge.

TIME (weeks)	*6.5 WEEK CHALLENGE SERIES*									*11 WEEK CHALLENGE SERIES*								
	IC_1	EXP_1 -LP	IC_2	EXP_2 -LP	CC-LP	EXP_2 -PR	CC-PR	EXP_2 -RSA	CC -RSA	IC_1	EXP_1 -LP	IC_2	EXP_2 -LP	CC-LP	EXP_2 -PR	CC-PR	EXP_2 -RSA	CC -RSA
0	LP [40]	LP [40]	LP [111]	LP [111]		LP [111]		LP [111]		LP [40]	LP [40]	LP [111]	LP [111]		LP [111]		LP [111]	
6.5		LP [152]		LP [152]	LP [152]	PR [98]	PR [98]	RSA [152]	RSA [152]									
11											LP [154]		LP [154]	LP [154]	PR [90]	PR [90]	RSA [152]	RSA [152]
12.5			PERF			PERF	PERF											
14.5	PERF	PERF	PERF	PERF	PERF			PERF	PERF									
17												PERF			PERF	PERF		
19										PERF	PERF	PERF	PERF	PERF			PERF	PERF

IC_1 and EXP_1 refer to groups in which the animals were exposed initially to approximately 40 *S. leiperi* cercariae each, while IC_2 and EXP_2 refer to those subjected to initial infections with approximately 111 cercariae/animal. In order to facilitate the interpretation of the results, worm load data of the IC_1/EXP_1 series have been presented separately from those of the IC_2/EXP_2 series (Tables 4.5.1 and 4.5.2a, respectively); this necessitated the duplication of data pertaining to the *S. leiperi* challenge control group (CC-LP), which served as a common control for both series. Animals of the IC_2 group were split into subgroups which were perfused at intervals to correspond with the perfusions of the various challenged groups. For the purposes of calculating percentage reductions of gravid worm pair and total worm loads (Table 4.5.2a), the data from the 12.5 and 14.5 week intervals were pooled, as were those of the 17 and 19 week intervals. Tissue egg load data were not pooled in this way, however, in view of their dependence on the duration of infection.

APPENDIX E

SUPPLEMENTARY INFORMATION PERTAINING TO THE STUDY ON THE POTENTIAL OF TRICKLE INFECTIONS WITH THE ANTELOPE SCHISTOSOMES TO INDUCE RESISTANCE AGAINST *SCHISTOSOMA MANSONI* CHALLENGE INFECTIONS IN GUINEA PIGS (SEE CHAPTER 6)

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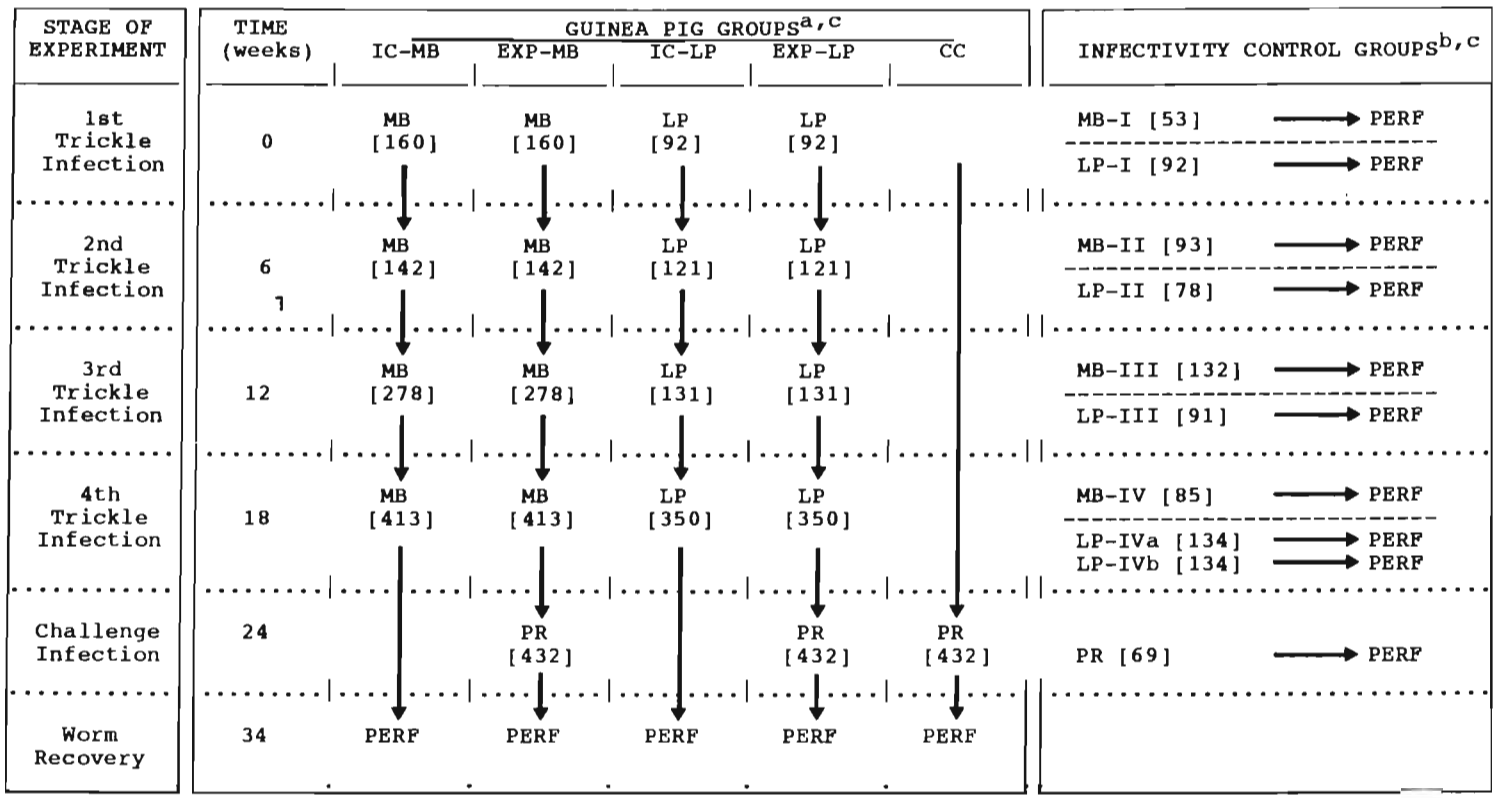
Tables E.1 and E.2 are presented overleaf.

TABLE E.1 Experimental plan for trickle infection study in guinea pigs, showing time schedule, cercarial loads, and groups of BALB/c mice and *Mastomys coucha* infected simultaneously with guinea pigs.



a IC (initial infection control) groups were exposed to trickle infections only and EXP (experimental) groups both to the trickle infections and the challenge infection; the suffixes -MB and -LP indicate whether the trickle infections comprised *S. margrebowiei* or *S. leiperi*, respectively. The CC group was exposed to the challenge infection only. Values in square parentheses indicate the numbers of cercariae to which individual animals were exposed on each of the respective infection occasions.

b All groups consisted of BALB/c mice, except LP-IVb, which consisted of *M. coucha*; all groups comprised 7 animals. Values in square parentheses indicate cercarial loads per animal.

c PERF = perfusion. Worm recovery data from Infectivity Control groups are presented in Table E.2.

TABLE E.2 Trickle infection study in guinea pigs: worm recoveries from BALB/c mouse and *Mastomys coucha* infectivity control groups.

INFECTIVITY CONTROL GROUPS[a]		WORM LOADS[b] % Worm Recovery $\bar{x}$ (s.d.)	Male Worms $\bar{x}$ (s.d.)	Female Worms $\bar{x}$ (s.d.)	Total Worms $\bar{x}$ (s.d.)	R.I.R.[c]
Infected at time of 1st trickle infection	MB-I [53]	12.6 (5.7)	3.1 (2.5)	3.4 (2.0)	6.7 (3.0)	45%
	LP-I [92]	15.4 (3.1)	6.7 (1.9)	7.5 (1.9)	14.2 (2.9)	62%
Infected at time of 2nd trickle infection	MB-II [53]	19.5 (7.5)	7.9 (3.4)	10.3 (5.0)	18.3 (7.0)	70%
	LP-II [78]	14.1 (5.0)	7.3 (4.1)	3.7 (1.5)	11.0 (3.9)	56%
Infected at time of 3rd trickle infection	MB-III [132]	15.7 (7.1)	8.3 (2.8)	12.4 (7.2)	20.6 (9.2)	56%
	LP-III [91]	14.7 (4.6)	6.2 (1.9)	7.2 (4.1)	13.3 (4.2)	59%
Infected at time of 4th trickle infection	MB-IV [85]	20.7 (6.5)	7.1 (3.4)	10.3 (3.4)	17.5 (5.5)	74%
	LP-IVa [134]	23.6 (4.4)	17.1 (4.5)	14.4 (3.5)	31.6 (5.9)	94%
	LP-IVb [134]	42.3 (10.2)	32.3 (9.9)	24.4 (5.7)	56.7 (13.7)	100%
Infected at time of challenge infection	PR [69]	29.2 (7.3)	9.2 (2.6)	10.9 (3.2)	20.1 (5.2)	70%

a MB = *S. margrebowiei*, LP = *S. leiperi* and PR = *S. mansoni*. All groups consisted of BALB/c mice, except LP-IVb, which consisted of *M. coucha*. Groups exposed to *S. margrebowiei* and *S. leiperi* each comprised 7 animals, while that exposed to *S. mansoni* comprised 14 animals. Values in square parentheses indicate the estimated cercarial load/animal.

b All *S. leiperi* groups and the *S. mansoni* group were perfused 10 weeks after infection. With respect to *S. margrebowiei* groups, MB-I and MB-III were perfused 6 weeks after infection, and MB-II and MB-IV 8 weeks after infection. Mean values are shown, with standard deviations in parentheses.

c R.I.R = relative infectivity rating, calculated according to the formula

(Actual % worm recovery Expected % worm recovery) x 100,

assuming the following expected % worm recovery rates:

Mice: MB = 28%, LP = 25%, PR = 42%; *M. coucha*: LP = 42%.