NUTRITIONAL PATHOLOGY DURING EXPERIMENTAL SCHISTOSOMIASIS MANSONI

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DECLARATION

I declare that this thesis describes research carried out by myself unless otherwise cited or acknowledged. It is from my own composition and has not, in whole or part, been presented for any other degree.

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Summary

The relationship between mouse nutrition and *Schistosoma mansoni* infection was investigated under controlled experimental conditions. A Puerto Rican strain of *S. mansoni* and CFLP mice were used for all aspects of this research.

Firstly, the food intake patterns of uninfected, control mice were compared, over a ten week period, with those of mice exposed to a primary infection of *S. mansoni*. The food intake of infected mice was significantly reduced in the first week of infection, and after the sixth week of infection for the duration of the experiment. The largest magnitude in the reduction of food intake was observed between weeks six and seven post infection. Serum samples were taken from mice infected for a corresponding length of time to investigate if the production of tumour necrosis factor (TNF) was linked to the decreased food consumption in infected mice. Serum samples were also taken from mice at 67 days post infection, again corresponding to a time when the food intake of infected mice was less than for uninfected controls. No TNF was detected at either time point by the bioassay or ELISA methods of TNF determination. The growth rates of *S. mansoni*-infected mice did not differ significantly from the growth rates of uninfected control mice, nor was the percentage efficiency of conversion of food eaten into body weight significantly altered by infection.

Hypertrophy of intestinal smooth muscle was found to be a prominent pathological feature in infected mice in the ninth week of *S. mansoni* infection and after 16 weeks of infection. This hypertrophy was observed in all regions of the small intestine that were examined, but was most extreme at the distal end of the bowel. The absence of this response in *S. mansoni*-infected mice after six weeks of infection, and in mice exposed to single-sex cercariae, demonstrated that a patent infection was required for at least two to three weeks before smooth muscle hypertrophy could be observed. The comparison of intestinal smooth muscle mass in infected mice treated with praziquantel with the intestinal muscle mass in untreated mice suggested that

hypertrophy occurred to an increasing degree as the infection progressed and / or that chemotherapy resulted in an abatement of this pathology.

Transmission electron microscopy revealed that the cells of the hypertrophied intestinal muscle had more invaginations in the surface membrane than muscle cells from control mice, and the intercellular gap was wider in infected mice. An investigation of the influence of intestinal muscle pathology on gut motility patterns did not provide conclusive results about the functional significance of muscle hypertrophy because of methodological problems.

The smooth muscle hypertrophy in the small bowel of *S. mansoni*-infected mice in the ninth week of infection, and after 16 weeks of infection, was accompanied by a redistribution of body weight away from skeletal mass and towards the liver and spleen. A similar pattern was observed for infected mice when compared to infected mice that were subsequently treated with praziquantel, but not for mice exposed to single-sex cercariae.

An increase in the villous surface area was observed in the posterior regions of the small intestines of mice in the ninth week of *S. mansoni* infection. This increase in surface area appeared to occur to a smaller degree in infected mice after six weeks of infection, and to be more generalised along the length of the intestine at a later stage of infection (16 weeks). In mice exposed to single-sex cercariae, no change in villous surface area was observed. Treatment of infected mice with praziquantel did not result in a change of villous surface area relative to untreated mice. Accompanying the increase in villous surface area in the small intestines of *S. mansoni*-infected mice was a reduction in microvillus height and density. These changes in the absorptive surface of the gut did not appear to be functionally significant on the basis of results obtained by means of a lactulose / mannitol permeability test.

Goblet cell hyperplasia in the small intestine was also a feature of murine schistosomiasis mansoni. An increase in goblet cell numbers was apparent as early as

six weeks post infection, but was more pronounced in the later stages of infection. Hyperplasia of mucus-producing cells was not observed in the small bowels of mice exposed to single-sex cercariae.

A change in the optical density of goblet cells stained with Alcian Blue was observed in specimens collected from *S. mansoni*-infected mice after 16 weeks of infection when compared to uninfected controls. Transmission electron microscopy revealed the presence of electron dense cores in the mucus secretory granules in both immature and mature goblet cells from infected mice. These electron dense cores were observed only rarely in the immature goblet cells of control mice.

Protein malnutrition was found to be synergistic with *S. mansoni* infection in mice in relation to mouse growth and the efficiency of conversion of protein and food consumption into body weight. Uninfected mice fed on a 4% protein diet maintained body weights similar to those of adequately nourished mice, with an increase in food consumption appearing to account for this observation. The 2% protein dietary regime resulted in weight loss for both infected and uninfected mice. Infected mice on the 4% protein diet consumed significantly less food than their dietary controls for much of the nine-week experimental period, with the reduced food intake appearing to be exacerbated after the infection reached patency. The carcass weights of infected mice fed on the 4% and 8% protein diets contributed less to overall body weight than the carcass weights of dietary controls. Smaller changes in carcass weight were noted for mice fed on the 2% dietary regime. Protein malnutrition did not appear to have any great influence on smooth muscle hypertrophy, goblet cell hyperplasia and villous surface area in the small intestines of mice, with *S. mansoni* infection having a much greater influence on these aspects on intestinal pathology.

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Chapter 1

Introduction

1.1. Life history of *Schistosoma mansoni*

Parasites of the genus *Schistosoma* are dioecious flukes that inhabit the vascular system of their definitive vertebrate hosts. Eighteen species of *Schistosoma* are recognised currently, with five of these species considered to be important pathogens of humans (Rollinson and Southgate, 1987). Of the schistosomes that frequently infect humans, *S. mansoni*, *S. haematobium* and *S. japonicum* are perhaps most important because of their wide-spread distribution and success in many tropical regions; *S. mekongi* and *S. intercalatum* are more localised in their distribution in the Far East and equatorial Africa respectively. Much of the literature reviewed in the following pages deals with *S. mansoni* as this is the parasite of primary concern in this thesis.

Schistosoma mansoni is endemic in more than 50 countries of the world (WHO, 1993). As well as humans, this parasite naturally infects a variety of mammalian species, including certain non-human primates and rodents (Rollinson and Southgate, 1987). Snails of the genus *Biomphalaria* act as the intermediate host for *S. mansoni*, with water providing the common contact site for parasite, mollusc and mammal.

Mature *S. mansoni* inhabit the hepatic-portal system of their mammalian hosts, particularly the superior mesenteric veins. Within these blood vessels, the male and female schistosomes pair; the female lies within a cleft (schistos) in the body (soma) of the male, hence the name schistosome. After pairing, the sexual reproductive phase in the life cycle of the schistosome can begin.

The daily egg output of female *S. mansoni* has been estimated to be approximately 300 according to many textbook sources, although estimates in excess of 1000 ova per female worm have been obtained (Damian and Chapman, 1983). Escape of the eggs from the host vasculature into the external environment is then necessary for dissemination of the parasite.

At least a six-day period elapses between oviposition and egg excretion, allowing embryonation and maturation of ova to occur (Jourdane and Théron, 1987). During this time, the parasite egg must break through a blood vessel wall in the host, pass on through the parenchyma of the intestinal tract and finally be released into the gut lumen. The mechanisms involved in this process are not entirely clear, but the lateral spine of the *S. mansoni* egg, host blood pressure and intestinal peristalsis may all play a role in helping eggs to escape the vasculature. The passage of the ovum through host tissue may be more complex, with the host immune system appearing to be involved intimately in the egg expulsion process (Doenhoff *et al.*, 1978, 1981, 1985, 1986; Dunne *et al.*, 1983). Damian (1987) suggested that *S. mansoni* use the inflammatory response of the host to transport eggs from the serosa to the mucosal surface of the gut. His histological examination of *S. mansoni*-infected baboon guts provides evidence that immotile schistosome eggs can be translocated across the gut wall by the motile cells that constitute the granuloma. Upon reaching the gut lumen, *S. mansoni* eggs can then be carried out of the host in the faeces.

After release from the gastrointestinal tract, the *S. mansoni* egg requires certain environmental conditions to be met before hatching can occur. Of these, the hypotonicity of fresh water and an appropriate temperature range are thought to be the most important factors for the optimal hatching of miracidia (Bair and Etges, 1973; Kassim and Gilbertson, 1976). Hatching appears to be a physical process, with water penetrating the egg through vacuoles; this results in the rupture of the egg shell (Kusel, 1970). The ciliated miracidium, which has developed within the egg, is then free to swim away in search of its molluscan host.

A variety of factors are important in the rather haphazard location of snails by *S. mansoni* miracidia. Firstly, because the life span of this larval schistosome is limited to approximately 8-12 hours (Prah and James, 1978), an element of luck is required for an appropriate snail host to be in the vicinity. The random nature of this process is somewhat reduced by behaviour of miracidia. For example, Chernin and Dunavan

(1962) showed that the miracidia of *S. mansoni* are positively phototactic and negatively geotactic. *Biomphalaria glabrata*, one of the snail hosts of *S. mansoni*, is most often found very near the surface of water (Jourdane and Théron, 1987), thus physical stimuli increase the chance of co-occurrence of snail and schistosome in the same depth of water. Chemical stimuli also appear important to the host location behaviour of miracidia, with *S. mansoni* miracidia being attracted to water in which *B. glabrata* have been present (Chernin, 1970; Sponholtz and Short, 1975). Chernin (1970) suggested that the activity of miracidia is stimulated by water-soluble substances emitted by the snail that he collectively called "miraxone". The "miraxone" appears to be made up of a variety of elements, including fatty acids, amino acids, ammonia and amines (see Jourdane and Théron, 1987).

If a miracidium locates and makes contact with an appropriate molluscan host, infection may then occur. In the infected snail, the miracidium undergoes a series of morphological transformations before development into a mother sporocyst. Differentiation of daughter sporocysts then takes place within the mother sporocyst. The young sporocyst may then migrate to the digestive gland of the snail where cercariogenesis commences (reviewed by Jourdane and Théron, 1987) .

Emission of *S. mansoni* cercariae from the snail follows a circadian rhythm, with maximal emission occurring during the photophase (Pitchford *et al.*, 1969). Light appears to be the most important stimulus in the synchronisation of cercarial emission (Asch, 1972), although thermal stimuli can synchronise the rhythm in the absence of a photoperiod (Valle *et al.*, 1973, cited from Jourdane and Théron, 1987). Once shed, cercariae only have a brief period in which to locate and infect a susceptible host. The life span of cercariae depends on stored glycogen, with the rate of use of this substrate being linked to water temperature. In temperatures ranging from 25-27°C, cercarial glycogen reserves last for 5-8 hours before infectivity is impaired (Lawson and Wilson, 1983).

Wilson (1987) has reviewed the method by which cercariae appear to locate their definitive host. Cercariae remain relatively near the surface of water using a rhythmic swimming behaviour when unstimulated. Turbulence and shadows increase the activity of cercariae, presumably mimicking water disturbance by the host. Skin-derived chemicals also stimulate increased cercarial activity. Attachment to, and penetration of, host skin involves thermal and chemical stimuli.

After penetration of the skin, the schistosome must undergo a series of developmental changes and migrations before maturation into an adult worm can be completed. The transformation of cercariae into schistosomula takes less than one hour (Cousin *et al.*, 1981). Schistosomula then migrate to the lungs after exiting the skin via blood vessels and the lymphatics (Wheater and Wilson, 1979). In mice, schistosomula are first detected in the lungs three days after infection, with numbers peaking on the sixth day of infection (Miller and Wilson, 1978). After this time, schistosomula can be found in the hepatic-portal system (Miller and Wilson, 1980). Here, they begin to feed and increase in mass. The reasons for the selection of the hepatic-portal system as the site for maturation are unclear, but may relate to the distinct physiological characteristics of this region, including physical parameters of blood vessels and the nutrient-rich environment (Wilson *et al.*, 1978; Miller and Wilson, 1980). Pairing of mature worms completes the life cycle.

1.2. *Schistosoma mansoni* and disease

For the sake of clarity, the spectrum of clinical symptoms associated with *S. mansoni* infection can be divided into three phases. These are: (1) an immediate reaction to invading cercariae, (2) acute disease that is associated temporally with the maturation of worms and early egg output, and (3) chronic disease, which frequently develops after five or more years of good health following the initial exposure to this infection.

1.2.1. Cercarial dermatitis

The penetration of host skin by cercariae may induce a papular rash with marked pruritus. "Swimmer's itch," as cercarial dermatitis is often known, is frequently associated with exposure to *S. mansoni* (Hiatt *et al.*, 1979; Mohamed, 1985). The entry of cercariae into the host epidermis is achieved by a combination of muscular activity by the larvae and the secretion of lytic enzymes (Stirewalt, 1974). Histological studies with laboratory rodents have revealed that the invading schistosome invokes a cutaneous inflammatory response by the host (von Lichtenberg *et al.*, 1976; Incani and McLaren, 1984; Ward and McLaren, 1988). This response is associated with the elimination of cast cercarial tails and the migration of schistosomula (Incani and McLaren, 1984; Pearce and McLaren, 1986). Subsequent challenge of the same host with homogeneous cercariae induces a more pronounced cutaneous response (Colley *et al.*, 1972; von Lichtenberg *et al.*, 1976), and cercarial dermatitis appears to be the result of immune sensitisation (Olivier, 1949, cited from Smithers and Doenhoff, 1982).

1.2.2. Acute schistosomiasis mansoni

Acute schistosomiasis, also known as Katayama fever, is a more serious problem that may be associated with the early stages of infection. This syndrome generally presents in adolescents and adults following their first exposure to schistosome-infested water. The range and severity of symptoms associated with this clinical condition are exemplified in a study carried out by Hiatt *et al.* (1979) in Puerto Rico. The majority of patients in this study exhibited the common symptoms of fever, anorexia, weight loss and abdominal pain; headache, dry cough, nausea, vomiting and diarrhoea were also frequently observed. The incubation period of the illness in these patients ranged from 11-58 days after exposure, thus symptoms were present in some patients prior to egg production. Hiatt *et al.* (1979) also observed that the clinical severity of disease correlated closely with the *S. mansoni* faecal egg output.

The main aetiological agent of acute schistosomiasis mansoni appears to be the parasite egg, with evidence for this arising from a variety of sources. The positive

correlation of disease severity with host faecal egg output (Hiatt *et al.*, 1979) provides a clear indication that ova contribute significantly to illness. Furthermore, the granulomatous response to schistosome eggs modulates as the infection progresses, with the size of these inflammatory lesions decreasing (reviewed by Smithers and Doenhoff, 1982). This, when combined with the knowledge that symptoms abate despite the maintenance of faecal egg excretion rates (Hiatt *et al.* 1979), supports the theory that the symptoms of acute schistosomiasis are a consequence of the regulation of host defence mechanisms before they become fully efficient (Phillips and Colley, 1978). The appearance of symptoms prior to schistosome egg excretion (Hiatt *et al.* 1979) demonstrates that the parasite egg is not the only aetiological agent of this disease. However, in the absence of a well-characterised experimental model of acute schistosomiasis, and with the difficulties of obtaining clinical data, this illness is not well understood.

1.2.3. Chronic schistosomiasis mansoni

In 1937, Pons described the intestinal and hepatosplenic diseases associated with schistosomiasis mansoni as two more or less distinct syndromes (cited from Smithers and Doenhoff, 1982). This clinical division of the disease is somewhat arbitrary, however, as a degree of damage in both the liver and the gut occurs almost invariably. Generally, the clinical manifestations of schistosomiasis mansoni depend on the extent of pathology associated with these two organ systems. The chronic phase of this disease is frequently asymptomatic, with infected individuals generally leading a normal life unless the disease is well-advanced, the intensity of infection is particularly heavy or complications exist (reviewed by Chen and Mott, 1988).

Uncomplicated intestinal lesions caused by granulomatous reactions to *S. mansoni* ova are thought to contribute little to disease pathophysiology. Symptoms, if present, are non-specific, and include weakness, abdominal pain and diarrhoea with blood and mucus (Mohamed *et al.*, 1990). A fairly common complication of intestinal schistosomiasis is colonic polyposis (Chen and Mott, 1988). These granulomatous

pseudopolyps have been observed frequently in Egyptian farmers, and contain very high concentrations of schistosome eggs when compared to other regions of the colon, suggesting focal oviposition (Cheever *et al.*, 1978). This observation contrasts with Cheever's post-mortem study in Brazil where schistosomal polyposis was not observed (Cheever, 1968), although colonic egg burdens were similar in both studies (Cheever *et al.*, 1977). Regional variation of the prevalence of schistosomal colonic polyposis has been attributed variously to genetic variation of host or parasite, and the intensity and duration of infection (Chen and Mott, 1988). Schistosomal polyposis may cause blood loss, anaemia and protein-losing enteropathy, and can result in significant morbidity (Lehmen *et al.*, 1970; Abaza *et al.*, 1978).

The disease and pathology of hepatosplenic schistosomiasis are well documented, and have been reviewed recently by Chen and Mott (1988). The granulomatous reaction that destroys parasite eggs also damages host tissue and leads to the formation of fibrous scars. Cheever (1969) estimated that approximately 33% of *S. mansoni* eggs are flushed to the liver in humans. The host response to these enormous numbers of schistosome eggs produced over long periods of time may lead to extensive fibrosis of the liver. The fibrosis around portal radicals may cause presinusoidal portal hypertension by partially blocking blood flow. Compensatory arterial blood flow maintains the total liver blood flow within normal limits.

In advanced cases of hepatosplenic disease, whitish fibrous triads may surround the portal vein. These triads were described by Symmers (1904; cited from Smithers and Doenhoff, 1982) as resembling "white clay pipe-stems". Marked venous obstruction resulting from fibrosis causes portal hypertension, congestive splenomegaly and portal-systemic collateral development, including oesophageal and gastric varices. Oesophagogastric haemorrhage is a severe and frequent consequence of hepatosplenic schistosomiasis. Rupture of oesophagogastric varices may prove fatal, although recurrent haematemesis is more common. Liver function is generally maintained (Chen and Mott, 1988).

1.3. The public health significance of schistosomiasis mansoni

The public health significance of any disease encompasses not only the pain and suffering of individuals, but also the burden on a family for caring for a sick member and the economic losses to a community through disability and death. *Schistosoma mansoni* is estimated to infect at least 57 million people (Peters and Gillies, 1977), although real numbers may be very much higher (De Vlas and Gryseels, 1992). The potential for disease is therefore vast.

The WHO (1985) has described schistosomiasis as "second only to malaria" in terms of the socio-economic and public health significance of parasitic infections in tropical and subtropical regions. Nevertheless, this significance is often thought to be underestimated for two main reasons. Firstly, the distribution of worms within a community is generally overdispersed. Severe chronic disease, that frequently occurs in the heavily infected individuals, is usually found in less than 10% of a population, but this proportion may be as high as 35% (Warren, 1982). In the case of *S. mansoni* this represents some 5.7-20 million seriously ill people. This leads to the second main reason for underestimation of the public health significance of schistosomiasis mansoni. Severe disease usually follows after many years (WHO, 1993), thus although many individuals may be asymptomatic regardless of the intensity of infection (Cook *et al.*, 1974; Siongok *et al.*, 1976; Smith *et al.*, 1979), the potential for disease is high. The WHO (1993) recommends that control programmes should be directed at reducing the intensity of *S. mansoni* infection, especially in children, to diminish the chance of severe disease.

Walsh and Warren (1979) have described the mortality and morbidity of the major schistosomal diseases. Of the 200 million people with schistosomiasis throughout the world, between 0.25% and 0.5% are estimated to die each year. Of the remaining infected population, 20 million people will suffer from clinical disease in a year. Each person with clinical disease will endure, on average, 600-1000 days of disability in their life time, with disability described as "ambulatory" or "minor" (Walsh and Warren,

1979). Any disease that causes half a million deaths each year, and up to 55 million years of disability for the collective 20 million people with clinical disease, represents a disease of major public health significance.

1.4. Investigative approaches to the study of schistosomiasis mansoni

The investigative approaches adopted to study the effects of schistosomes on their hosts take two main directions, human studies and controlled animal experiments. Human studies can be further sub-divided into community-based field studies and the generally smaller scale clinical studies that normally involve hospitalised patients. Each approach has its own set of advantages and limitations.

For animal studies to be of value in the investigation of human disease, two basic criteria must be met by the host-pathogen model selected (Stephenson, 1987). Firstly, the parasite must be able to develop normally within the chosen host and, secondly, the resultant pathology must mimic closely the human disease. In the case of *S. mansoni*, the first criterion is relatively easy to meet as this parasite is not particularly species-specific and will infect a variety of mammals (see Rollinson and Southgate, 1987). Non-human primates are perhaps the most suitable animals in which to simulate patterns of human infection, but difficulties with housing and working with these primates, ethical considerations and the often prohibitive costs, make them an infrequently used option. The relative ease and low cost of working with the laboratory mouse, a generally permissive host, make the *S. mansoni*-mouse system the most frequently used model of infection.

With regard to the second criterion of close mimicry of human disease, the mouse, chosen for study in this thesis, seems relatively suitable to study some aspects of schistosomiasis mansoni. DeWitt and Warren (1959) investigated hepatosplenic schistosomiasis in mice, and observed that the five primary signs of human hepatosplenic schistosomiasis, hepatomegaly, splenomegaly, ascites, anaemia and oesophageal varices, could all be reproduced in mice infected with *S. mansoni* for 8-16 weeks. However, these infections did not produce the periportal fibrosis first described

by Symmers (1904; cited from Smithers and Doenhoff, 1982), but instead the liver lesions consisted of granulomas scattered along the portal veins (Warren and DeWitt, 1958; DeWitt and Warren, 1959). A subsequent study by Andrade and Warren (1964) with mice harbouring low numbers of worms for a 25 week period did demonstrate a degree of fibrosis similar to that observed in humans with early portal fibrosis.

One persistent problem that exists with the *S. mansoni*-mouse model is that the intensity of infection must, almost invariably, be higher than in humans when scaled on an isometric weight basis. In the mouse, a minimum of one worm pair is required for the infection to be patent. In a 50 kg human, the same grade of infection would be in the range of 1000-3000 worm pairs. Little information is available on the schistosome burdens of humans as *post-mortem* studies are required to obtain worm counts. One study by Cheever (1968) demonstrated that over 1600 worm pairs could be found in a human; these worms were recovered from a child with fatal schistosomal colitis. Only around 10% of the individuals in Cheever's study, however, had worm burdens in excess of 90 worms.

Isometric scaling of worm burdens in relation to body mass should not, however, be considered as a true indication of the potential severity of disease. As discussed by Schmidt-Nielson (1984), real organisms can seldom be scaled on an isometric basis as certain biological proportions change in a nonisometric, or allometric, fashion. For example, liver size decreases relative to increasing body size (Stahl, 1965), as does the relative metabolic rate of the liver (Krebs, 1950). Thus a mouse, in comparison to a human, should be able to accommodate relatively more granulomatous damage to its liver without altering the functional capacity of this organ. In a similar manner, the potentially protective role of the liver in detoxicating harmful biproducts of worms or eggs may be relatively greater in the mouse.

Results from animal experiments, however, provide only an indication of the effects of parasites on human communities. Animal experiments are generally designed so that infected animals are exposed to similar numbers of pathogens. This may result in

animals harbouring fairly similar worm burdens, and can lead to disparity with human populations where schistosomes tend to be overdispersed (WHO, 1993). Clinical studies perhaps correlate more closely with experimental systems as they often deal primarily with hospitalised patients with the greatest worm burdens and associated morbidity. Although these studies can provide great insight into disease processes, it is often impossible to gauge what proportion of a population that these individuals represent.

Community-based field studies provide a better overview of how a particular population is affected by *S. mansoni*. However, these field studies are very much more difficult to set up than controlled animal experiments as a whole host of logistical, ethical and cost-related problems must be solved before the investigation can even begin. For a nutritional study, a community would be required to have a relatively high prevalence of *S. mansoni*. Secondly, the community would be required to have low prevalences of other pathogens which might confuse any results obtained. As field studies tend to be costly and labour-intensive, knowledge of parasitic infection in different age and sex profiles may help determine if only a sub-sample of the community is required to be monitored. As "malnutrition is a process; it is not a point in time" (Williams, 1977; cited from Stephenson, 1987), it must be decided whether a longitudinal or cross-sectional study is more appropriate. Environmental, ethical and socio-cultural factors must also be considered.

Obviously, meeting all the above requirements is extremely difficult. Frequently, studies have been carried out under circumstances that are less than ideal from a scientific viewpoint. For example, one report on the small intestine structure in human schistosomiasis surveyed 50 people. Of these, only 13 were excreting ova of *S. mansoni*, and a proportion of these individuals were also excreting ova of *Ancylostoma duodenale* (Halsted *et al.*, 1969). Although this study was not initially designed to look solely at *S. mansoni* infection, it does demonstrate some of the difficulties associated with human investigations.

Overall, the limitations of human and animal studies convey the need for both approaches. Each investigative method can complement the other, contributing to a clearer overview of the significance of schistosome-induced disease and the underlying pathological mechanisms.

1.5. Nutritional implications of helminth infection

That relationships exist between parasitism and host nutrition is now long established (Scrimshaw *et al.*, 1968). There are two main reasons for identifying the effects of helminth infection on host nutritional status. Firstly, any knowledge of alterations in host metabolism, or of changes in patterns of nutrient uptake and use, provides a clearer picture of the pathophysiology associated with infections. This, in turn, allows for a more informed approach to treatment. Secondly, the negative effects of parasites on host nutritional status are important to identify in order to assess the total benefit that may arise from disease control programmes.

A full review of the effects of *S. mansoni* infection on host nutrition would pre-empt much of the discussion in the following chapters of this thesis. Instead, the range of ways in which helminths can disturb host nutritional status are reviewed using selected examples.

1.5.1. Food intake

Parasites can influence both the ability of the host to obtain food and the voluntary food intake of the host. Any infection that interferes with the sensory organs that are involved in food acquisition may have profound effects on the foraging ability of the host. For example, *Diplostomum spathaceum*, a digenean that inhabits the eyes of freshwater fish, may affect host vision. Severe infections can blind fish (Erasmus, 1958), and even moderate infections of *D. spathaceum* can reduce visual acuity to the extent that feeding efficiency is reduced (Crowden and Broom, 1980; Owen *et al.*, 1993). In humans, *Onchocerca volvulus* may severely impair vision (Nelson, 1970). In a co-operative human society individuals are unlikely to starve as a result of blindness.

However, when a significant proportion of a population is affected by river blindness, it is likely that the productivity, and thus the nutritional status, of the overall population will be impaired.

Parasites can also influence the activity of their hosts to the extent that infected animals may appear exhausted when compared to uninfected counterparts (reviewed by Dobson, 1988). Reduced activity and lethargy, in turn, are likely to have negative effects on the foraging behaviour of the host, thereby reducing food intake. Learned taste aversion may change food consumption patterns if certain flavours of food are associated with the trauma of initial parasitic infection (Keymer *et al.*, 1983). Anorexia is also frequently observed during parasitic infection (reviewed by Crompton, 1984; Symons, 1985); this is discussed in more detail in Chapter 3.

1.5.2. Competition for nutrients

Parasites, at some stage in their life cycle, must obtain their nutritional requirements for growth and reproduction from their hosts. However, the competition for resources between host and parasite is seldom detrimental to the nutritional status of the host, as the energy requirements of parasites are frequently very small in relation to the equivalent requirements of their hosts. Obvious exceptions occur when the parasite is large relative to its host. Such a relationship exists between the cestode *Schistocephalus solidus* and its intermediate fish host. Much of the energy intake of the fish is redirected into the production of *S. solidus* plerocercoid tissue (Walkey and Meakins, 1970). This results in the depletion of host energy reserves and a higher metabolic demand on the fish (Lester, 1971; Meakins and Walkey, 1975).

When specific nutrients are in limited supply, the "theft" of these substances by the parasite may also be of significance. For example, *Diphyllobothrium latum* can induce the development of megaloblastic anaemia by obtaining large proportions of vitamin B_{12} before the host intestinal mucosa has a chance to absorb this vitamin (Von Bornsdorff, 1967). It has also been suggested that the presence of large infections of *Ascaris lumbricoides* in malnourished children may represent a significant drain on

scarce supplies of energy and protein (Woodruff, unpublished, cited from Taren and Crompton, 1989).

1.5.3. Nutrient digestion and absorption

Lesion formation in the intestinal mucosa is one of the most striking pathological features of enteric helminth infection. These lesions may take a variety of forms including villous atrophy, muscle hypertrophy, crypt hyperplasia and inflammatory-cell infiltration. As the normal digestive and absorptive functions of the gut depend upon adequate surface area and intact macro- and micro-structure, intestinal functions may be altered dramatically in the parasite-infected host.

Perhaps the forms of intestinal damage that are easiest to explain are the lesions arising as a result of direct physical damage by parasites feeding on the intestinal mucosa. The dog hookworm *Ancylostoma caninum* feeds by drawing mucosal tissue into its buccal cavity (Kalkofen, 1970). Blood loss occurs as a result of these mucosal lesions and damage to intestinal capillaries (Kalkofen, 1970). The human hookworm *Ancylostoma duodenale* secretes an anticoagulant to facilitate feeding (Hotez and Cerami, 1983); this presumably exacerbates blood loss. Direct mucosal damage must increase nutrient excretion through blood loss, and is also frequently associated with iron-deficiency anaemia (Crompton and Stephenson, 1990).

Parasites that do not directly feed upon the intestinal mucosa can also cause major changes in the morphology of the small bowel. For example, experimental studies of *Ascaris suum* infections in pigs have demonstrated that this nematode can induce changes in the mucosal structure of the small intestine that are associated with an increase in the weight of this organ (Stephenson *et al.*, 1980; Forsum *et al.*, 1981). Hypertrophy of the *tunica muscularis* was observed in these pigs, and this was suggested to be the cause of increased intestinal weight. Other structural changes observed in the intestine of *A. suum*-infected pigs include a reduction of the villus height-to-crypt depth ratio and goblet cell hyperplasia (Stephenson *et al.*, 1980).

Accompanying the morphological changes in the small intestine of pigs harbouring *A. suum* infection was an impairment of lactose digestion; this appeared to be due to decreased lactase activity in the mucosa (Forsum *et al.*, 1981). Lactose maldigestion has also been reported in children infected with *A. lumbricoides* (Carrera *et al.*, 1984).

Trichinella spiralis infection also induces a variety of morphological changes in the host intestine. Hypertrophy of small bowel smooth muscle, villous atrophy and crypt hyperplasia are all associated with intestinal inflammation in rodents infected with this nematode (reviewed by Castro, 1989; 1990). These morphological disturbances are accompanied by a variety of functional changes in the small intestine, including gut motility patterns and decreased brush border enzyme activity (Castro, 1989; 1990).

1.5.4. Host metabolism

Parasites sometimes release substances that have a direct influence on host metabolism. Tapeworm plerocercoids of the genus *Spirometra* produce a substance known as plerocercoid growth factor (PGF). The PGF of *Spirometra (=Diphyllobothrium) mansonoides* closely resembles human growth factor in structure (Phares, 1987) and promotes growth in normal and hormone-deficient animals (Mueller, 1974; Shiwaku and Hirai, 1982; Shiwaku *et al.*, 1983). The PGF of *S. erinacei* can also effect host metabolism. This PGF appears to reduce serum glucose in golden hamsters by increasing the utilisation of glucose in peripheral tissue (Hirai *et al.*, 1987).

Examples of parasites producing chemicals that directly affect host metabolism, however, are fairly few. More common are the observations of indirect physiological changes by the host. As the concentrations of metabolites in mammals are under the tight control of a variety of hormones, much attention has been paid to this aspect of host metabolism during parasitic infection. Due to their important influence on carbohydrate metabolism, glucocorticoid hormones are an obvious source of interest. Corticosterone, the predominant glucocorticoid in rats, exerts its effects on carbohydrate metabolism by increasing the rate of gluconeogenesis in the liver and by

decreasing glucose uptake by tissues (Beato and Doencke, 1980). Corticosterone release in response to certain helminth infections appears to be biphasic. This pattern has been demonstrated in rats infected with *Nippostrongylus brasiliensis*, where a temporary elevation in corticosterone concentration is found during the initial stages of infection, followed by a decrease in the plasma concentration of this hormone towards the end of infection (Robertson, 1989). A similar pattern is observed in rats infected with *Strongyloides ratti* (Bailenger and Carcenac, 1984).

The administration of corticosteroids has long been known to increase the activity of the gluconeogenic enzyme alanine-amino-transferase (ALT) (Rosen *et al.*, 1958; Segal *et al.*, 1962). It is therefore not surprising that the rise and fall of plasma corticosterone levels during rat nippostrongylosis is accompanied by an initial elevation of liver ALT, followed by a later trough in the activity of this gluconeogenic enzyme (Robertson, 1989a). Glucocorticoids can also cause a simultaneous increase in the release of amino acids from skeletal muscle and the rate at which these substrates are "trapped" by the liver and converted into carbohydrate (Hardy, 1981). That this is also the case during nippostrongylosis seems probable as skeletal muscle weight is observed to decrease during this infection (Crompton *et al.*, 1978; Ovington, 1986). Another indicator of skeletal muscle proteolysis providing substrates for increased gluconeogenesis is the fall of the serum levels of the gluconeogenic amino acids, alanine and glutamine, in comparison to other serum amino acid levels during the early stages of nippostrongylosis (Ash *et al.*, 1985). Thus glucocorticoid release during *N. brasiliensis* infection in rats appears to have a dramatic influence on overall host metabolism.

1.5.5. The costs and benefits of nutritional disturbance

The preceding sections have described but a few of the host nutritional responses to parasitic infection. The use of selected examples has allowed the variety of disturbances to be exemplified, but has provided little insight into the overall nutritional costs of infection. For example, only a few of the metabolic disturbances in rats infected

with *Nippostrongylus* were described above, whereas at least 35 physiological responses of rats to this nematode are known to occur (Robertson, 1989). Such wide-ranging responses, including anorexia, hormonal changes, skeletal muscle atrophy, changes in gut structure and loss of body weight, must signify an extremely large energy toll for the infected rat. However, in balanced host-parasite populations, parasites tend not to seriously jeopardise the health of the majority of infected individuals. This would suggest there that are underlying defence benefits to the host, although nutritional changes may appear superficially to be detrimental to the host.

Possible benefits to the host may not be evident in individual parasitic infections as the host defence responses have presumably evolved to cope with a wide variety of infectious agents. For example, it has been suggested that the anorexia that occurs in rainbow trout infected with *Cryptobia salmositicia* lowers plasma protein levels, which results in the reduction of the reproductive rate of the parasite, thereby reducing the severity of disease (Li and Woo, 1991). The anorexia that accompanies *N. brasiliensis* infection, on the other hand, does not appear to have any benefit. However, the changes in gut structure in rats with nippostrongylosis may facilitate worm rejection. The increased intestinal permeability of the intestine to intact proteins (Barth *et al.*, 1966) is believed to assist in the expulsion of this nematode by allowing antibodies to come into contact with the worm. Goblet cell hyperplasia also occurs around the time of worm expulsion, resulting in an increase in mucus secretion and mucin turnover (Miller, 1987; Koninkx *et al.*, 1988). This leads to nematode trapping and discharge from the intestine.

All these defence mechanisms are likely to be controlled by the cytokines that co-ordinate the inflammatory and immune responses of vertebrates. The same cytokines responsible for beneficial responses, however, may also produce undesirable alterations in host metabolism. Tumour necrosis factor (TNF), for example, can induce anorexia (Plata-Salamán *et al.*, 1988; Tracey *et al.*, 1988) and can also cause changes in the small intestine (Kunkel *et al.*, 1989). As an elevated production of TNF is observed in bronchoalveolar washings obtained during rat nippostrongylosis (Benbernou *et al.*,

1992), it could be postulated that this cytokine is at least partially responsible for the intestinal changes that result in worm expulsion. The induction of anorexia may simply be a biproduct of a protective response that helps rid the rat of infection.

Thus the nutritional disturbances arising during parasitic infection may represent a trade-off for defence in the majority of life-threatening situations for an overall population. These disturbances, never-the-less, must impose an energy drain on the infected host. The investigation of such nutritional disturbances in the *S. mansoni*-infected mouse, and their consequences for the host, are the subject of this thesis.

Chapter 2

Materials and Methods

2.1. Parasite, host and infection procedure

All procedures employed in this thesis were covered by the Home Office Animals (Scientific Procedures) Act 1986 under the Project Licences PPL 60/00370 and PPL 60/01340 and the Personal Licence PIL 60/03085.

The *Schistosoma mansoni* used during these studies were kindly supplied by Dr. J.R. Kusel and colleagues, Division of Biochemistry and Molecular Biology, University of Glasgow. The parasite is derived from a Puerto Rican strain of *S. mansoni*, and is maintained routinely in *Biomphalaria glabrata* and mice in the Division of Biochemistry and Molecular Biology and the Division of Environmental and Evolutionary Biology at the University of Glasgow. The *S. mansoni* and *B. glabrata* were obtained originally from the National Institute for Medical Research, Mill Hill, London.

Male, outbred CFLP mice (Interfauna Ltd., Huntingdon, Cambridgeshire) were used throughout the course of this work. Mice of similar age and weight were used for each individual experiment. These animals were housed in a constant photoperiod (12 hours light / 12 hours dark), with *ad libitum* food (CRM Rat and Mouse Diet. Labsure) and water supplies unless stated otherwise. Animals, food and water supplies were checked daily.

Mice were infected with *S. mansoni* following the induction of general anaesthesia. After shaving the mouse abdomen, a suspension of cercariae was applied to the abdominal skin and left for 20-25 minutes. The numbers of cercariae used for infecting mice were estimated by aliquot counts of cercarial suspensions diluted with Lugol's iodine. Mice were exposed to *S. mansoni* within two hours of collection of

cercariae. Control mice were treated identically except that they were sham-infected.

The anaesthesia used for the infection procedure was a combination of fentanyl/fluanisone (Hypnorm, Janssen) and midazolam (Hypnovel, Roche). One part of fentanyl/fluanisone mixed with one part sterile water was added to an equal volume of one part midazolam mixed with one part sterile water. Anaesthesia was induced by intraperitoneal injection of this mixture using a dose of 0.1 ml / 20 g body weight.

2.2 Food intake and tumour necrosis factor

2.2.1. Food intake, growth and tumour necrosis factor

The mice used in the experiment monitoring food intake and tumour necrosis factor production in response to infection with *S. mansoni* were fed on a synthetic diet with an 8% protein (casein) content by mass. The composition of this diet has been detailed by Crompton *et al.* (1981), and is shown in Table 2.1.

Mice were caged individually and acclimated to the 8% protein diet for 8-9 days prior to infection or sham-infection. On the day of infection, mice in the weight range of 28.3 - 36.2 g were allocated to one of three groups: (I) *S. mansoni*-infected mice, exposed to 147 ± 18 cercariae from a single pool, allowed to feed *ad libitum*, (II) sham-infected controls pair fed with reference to infected mice and (III) sham-infected controls allowed to feed *ad libitum*. Mice were allocated randomly to Group I (n = 9) and then a partner mouse was selected on the basis of closely matching body weight for each infected mouse to make up Group II (n = 9). The remaining mice were allocated to Group III (n = 7). Animals in Group II were pair fed on the basis of the food consumed during the previous 24 hours by their infected partners in Group I.

Food consumption was monitored on a daily basis for each mouse. Spillage of diet was prevented almost completely by the use of feeding pots similar to those described by Nesheim *et al.* (1977). Briefly, each individually caged mouse was supplied with a circular glass feeding pot, 50 mm in diameter and 30 mm in depth.

Table 2.1. Constituents of the synthetic diet (from Crompton *et al.*, 1981).

	g/kg
Casein	varied as required
Maize starch	makes up to 1000 g
Cellulose	109.5
Maize oil	100
DL-methionine	1.5
L-threonine	1.5
Choline chloride	1.5
Mineral mixture	
$CaHPO_4$	13.24
$CaCO_3$	8.49
Na_2HPO_4	7.62
KCL	5.86
$MgSO_4.7H_2O$	5.51
$KHCO_3$	3.51
$MnSO_4.4H_2O$	0.36
$C_6H_5O_7.5H_2O$	0.33
$ZnCO_3$	0.058
$CuSO_4$	0.0153
KIO_3	0.0025
Butylated hydroxytoluene	0.1
Vitamin mixture	**mg/kg**
d-calcium pantothenate	50
nicotinic acid	50
pyridoxine HCl	15
riboflavin	15
folic acid	10
thiamine HCl	10
menadione	2
biotin	1
vitamin B_{12}	0.02
	IU/kg
vitamin A	5000
vitamin D_3	1000
vitamin E	20

Each pot was secured to a square metal base of approximately 10000 mm^2 , and was fitted with a lid with an aperture of 33 mm to allow mice access to the diet. Before the lid was fastened down, a piece of stainless steel mesh was placed on top of the diet to minimise further the spillage of food.

2.2.2. Growth responses and *post-mortem* measurements

The body weights of mice were recorded at weekly intervals. The amount weight gained by each mouse was expressed as a percentage of food eaten by that individual for a given week. This was used to give an indication of assimilation efficiency for each group of mice from six weeks post infection for the duration of the study.

Mice were sacrificed towards the end of the tenth week of infection (67 days post infection). After mice weights were recorded, blood samples were collected from each mouse by cardiac puncture. Worm burden was estimated following the recovery of adult *S. mansoni* by portal perfusion (Smithers and Terry, 1965). Sham-infected, control mice underwent the same perfusion procedure. The liver, spleen and small intestine were then removed and the gut contents flushed out with phosphate-buffered saline. The weights of these visceral organs were recorded and then subtracted from the terminal body weight to give an estimate of carcass weight.

2.2.3. TNF determination at six weeks post infection

Twenty mice in the weight range of 31.0 - 36.7 g were divided into two groups alternately on the basis of similar body weight, and either infected with 197 ± 10 *S. mansoni* cercariae or sham-infected. These mice were housed in groups of 3 - 4 mice per cage and allowed free access to food and water. The mice were sacrificed at the end of the sixth week of infection (42 days post infection) and blood samples were taken by cardiac puncture. Worm burden was estimated following portal perfusion (Smithers and Terry, 1965).

2.2.4. Determination of TNF

2.2.4.1. Preparation of serum

The blood samples collected from the above mice were transferred to Eppendorf tubes and immediately centrifuged for one minute at 13,000 r.p.m. on an MSE Microcentaur. The plasma supernatants were transferred to clean Eppendorfs and allowed to clot. The samples were then centrifuged again at 13,000 r.p.m. for two minutes and the resulting serum supernatant was frozen prior to cytokine analysis.

2.2.4.2. TNF determination by ELISA

A commercially available ELISA kit, the Factor-Test mTNF Kit (Genzyme, Massachusetts, USA), was used for the determination of TNF in serum samples; this test kit is specific for murine TNF. All procedures were carried out in full accordance with the manufacturers instructions. Briefly, the kit is a solid phase ELISA based on the "antibody sandwich principle". A specific monoclonal anti-mTNF is bound to a microtitre plate to create the solid phase. Samples and standards are then incubated with the solid phase antibody to bind any TNF that is present. The solid phase bound TNF can then be incubated with an anti-TNF antibody and then with a horseradish peroxidase-conjugated antibody. The resulting immune complex is than incubated with the peroxide substrate and o-phenylenediamine. The resulting colour change is directly proportional to the amount of TNF present, and can be determined spectrophotometrically.

2.2.4.3. TNF determination by bioassay

The bioassay determination of TNF used in this study utilised the WEHI 164 subclone 13, a continuous, natural killer cell-resistant murine fibrosarcoma cell line which is highly sensitive to the lytic effects of TNF (Chen *et al.*, 1985). This cell line is sensitive to extremely low levels of TNF in the picogram range (Espevik and Nissen-Meyer, 1986).

Any cell growth inhibition due to lytic effects of TNF was measured using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay (Hansen *et al.*, 1989). This assay is based on the mitochondrial reduction of yellow MTT to an insoluble purple formazan salt. The colour change is measured spectrophotometrically.

The lytic activity of serum samples was analysed using the FLEXIFIT programme developed by Guardabasso *et al.* (1988).

2.3. Intestinal pathology

2.3.1. Pathology time course

The development of smooth muscle hypertrophy was investigated in the sixth, ninth and sixteenth weeks of *S. mansoni* infection in mice. For the six-week time point, mice weighing 31.0 - 36.7 g were exposed to 197 ± 10 cercariae taken from a single pool (n = 10) or sham infected (n = 10). For the later time points, mice in the weight range of 31.5 - 43.8 g were exposed to 167 ± 7 cercariae from a single pool (n = 20) or sham infected (n = 20). Mice were allocated to infection or sham-infection protocols alternately on the basis of similar body weight, and housed in groups of 3 - 4 animals per cage.

2.3.2. Single-sex infections

Single-sex schistosome infections were obtained by infecting *B. glabrata* with a single *S. mansoni* miracidium. Mice, in the weight range of 30.9 - 39.9 g, were allocated to either infection (n = 10) or sham-infection (n = 10) procedures alternately on the basis of similar body weight. These animals were housed in groups of 3 - 4 mice per cage, with free access to food and water. Mice selected for infection with *S. mansoni* were exposed to cercariae from three pools in the following numbers: 198 ± 21 (4 mice), 189 ± 18 (3 mice) and 192 ± 12 (3 mice).

2.3.3. Chemotherapy

The effects of chemotherapy on intestinal pathology were examined in *S. mansoni*-infected mice treated with praziquantel (Bayer AG, Germany). Mice (n = 16) were exposed to 148 ± 16 *S. mansoni* cercariae, and housed in groups of four mice per cage with free access to food and water. After eight weeks of infection, these mice were divided into two groups alternately on the basis of similar body weights. Each mouse in the first group (n = 8) was treated with a single oral dose of 25 mg praziquantel suspended in 0.25 ml of 2.5% Cremaphor EL (Sigma) in water given by gavage. Each mouse from the second group received 0.25 ml of 2.5% Cremaphor EL in water.

2.3.4. *Post-mortem* measurements

Mice from the pathology time course experiment were sacrificed in the sixth (42 days post infection), ninth (59 - 60 days post infection) and sixteenth (112 days post infection) weeks of infection. Mice from the experiments examining intestinal pathology during single-sex infections and after chemotherapy were sacrificed at the end of the eighth week of infection (56 days post infection) and at the end of the sixteenth week of infection (112 days post infection) respectively. The mice were then weighed and portal perfusion (Smithers and Terry, 1965) was performed for both infected and control mice.

The liver, spleen, small intestine, stomach and caecum were removed from each mouse. Small intestine samples were taken for histology, and liver and spleen weights were recorded. The livers from infected mice and the adult *S. mansoni* worms collected by portal perfusion were then fixed in formalin to allow estimation of worm burdens at a later stage. The remaining carcass weights were also recorded.

2.3.5. Histology samples

Following the removal of the small intestine from infected and control mice, the

bowel, from the pyloric sphincter to the ileo-caecal junction, was divided into four equal lengths, and a 20 mm sample was taken from the posterior end of each segment. These four samples, termed the 25, 50, 75 and 100% distances from the pylorus, were then fixed in 10% neutral buffered formalin prior to dehydration and embedding in paraffin wax.

Transverse sections (perpendicular to the mucosal surface and 7 μm thick) were cut from the paraffin wax blocks. Each block contained a control and an infected sample from the same region of the small intestine, allowing control and infected sections to be exposed to exactly the same staining procedures.

2.3.6. Smooth muscle hypertrophy

Smooth muscle area was calculated from measurements recorded from the transverse sections described above after they had been stained with haematoxylin and eosin. The smooth muscle thickness was measured at eight equidistant points around the circumference of the section and the diameter of the section was measured on four equidistant diagonals. These measurements were recorded using a Leica Microvid computer-controlled microscope system. This system allows the computer monitor image to be superimposed onto the microscope image, thereby enabling direct measurements to be made.

Mean values for smooth muscle thickness and diameters were then used to calculate the muscle area using the following equation:

$$A_m = \pi (r_t)^2 - \pi (r_i)^2$$

where A_m is the smooth muscle area, r_t is the radius of the section calculated from the mean diameter, and r_i is the mean smooth muscle thickness subtracted form r_t. That is, the area of smooth muscle was calculated as the total area of the section from which the area of the section not containing smooth muscle was subtracted.

2.3.7. Lumen perimeter

The lumen perimeter was measured for the sections stained with haematoxylin and eosin that are described above. Measurements were made with a Leica Microvid computer-controlled microscope system used in conjunction with a mouse pen. This system allowed the lumen perimeter to be traced directly from the microscope image upon which the computer monitor image was superimposed.

2.3.8. Goblet cell hyperplasia

The transverse sections described above were stained with Alcian Blue to allow the numbers of goblet cells to be quantified. Cell numbers were counted for seven villi for each section, with counts being made only on villi with rounded tips that displayed an intact epithelial covering. Only goblet cells that were resident in the epithelial layer were included in this quantification. The mean values of these counts were used as an estimate of the number of goblet cells at the specified locations of the small intestine for each individual mouse.

2.3.9. Microphotometry

The differential uptake of Alcian Blue by goblet cells in the small intestine of *S. mansoni*-infected mice and control mice was investigated using microphotometry. The simultaneous processing, cutting and staining of paired intestinal samples from infected and control mice allowed the use of photometry to quantify the intensity of stain uptake. The absorbance of light by goblet cells was measured using a Leitz MPV Compact Microscope Photometer with a 592 nm filter. These measurements were made for 40 goblet cells resident in the epithelial layer for each section, with the mean absorbance then used as an estimate of stain intensity in goblet cells at specified locations in the small intestine for each experimental animal. All measurements were recorded in a blacked-out room.

2.3.10. Scanning electron microscopy

The small intestine specimens used for scanning electron microscopy (SEM) were subsampled from the 25% and 100% region specimens described above. The samples, fixed in neutral buffered formalin, were split along their length and pinned to a small piece of cork prior to being washed several times with a 0.1 M sodium coadylate buffer to rinse away gut contents and formalin. The gut samples were then fixed in 2.5% glutaraldehyde in 0.1 M sodium coadylate buffer (pH 7.4) for one hour. Following this initial fixation, the tissue was post-fixed in 1% osmium tetroxide in buffer for one hour. The tissue was then thoroughly rinsed with distilled water and placed in 0.5% uranyl acetate for 1-2 hours in the dark. Following this, the specimens were then dehydrated through a graded series of acetones, dried to critical point in liquid carbon dioxide and mounted on aluminium stubs. The samples were then sputter coated with gold and viewed on a Philips 500 Scanning Electron Microscope.

2.3.11. Transmission electron microscopy

Small intestine samples for transmission electron microscopy (TEM) were taken at a distance of 25% of the length of the small bowel away from the pylorus. The tissue was fixed in 2.5% glutaraldehyde in 0.1 M sodium coadylate buffer (pH 7.4) for 24 hours. After post-fixation in 1% osmium tetroxide for one hour, the specimens were stained in 0.5% aqueous uranyl acetate for one hour in the dark. The tissue was then dehydrated to 70% ethanol then transferred to a 70% ethanol solution containing 1% p-phenylenediamine and placed on a rotator for one hour. The samples were then rinsed, further dehydrated to absolute alcohol and passed through epoxy-1,2 propane. The specimens were then placed on a rotator in a 1:1 mixture of epoxy-1,2 propane and epoxy-resin (Araldite) overnight. The samples were then fresh embedded in Araldite and placed in an oven at 60°C for 48 hours.

Semi-thin sections were cut using a Reichert OmU3 Ultramicrotome, mounted on glass slides and stained with Toluidine Blue. When sections of interest and of

correct orientation were found, a corresponding series of ultrathin sections were cut using the Reichert OmU3 Ultramicrotome with a diamond knife. These sections were mounted on formvar-carbon coated copper grids, stained with 2% uranyl acetate and Reynold's lead citrate, and examined with a Zeiss 902 Transmission Electron Microscope.

2.4. Intestinal pathophysiology

2.4.1. Intestinal motility

The gut transit time for a dose of barium sulphate was used to investigate intestinal motility in *S. mansoni*-infected and control mice. Mice in the weight range of 31.7- 42.3 g were divided randomly into two groups. The first group of mice (n = 10) were exposed to 148 ± 10 cercariae, and the second group of mice (n = 9) were sham infected as controls. Mice were housed in groups of 3 - 4 animals per cage, and allowed free access to food and water.

Pilot experiments were conducted to estimate mouse stomach size (for mice in a similar weight range as those described above) and to gauge the length of time for barium to move halfway along the small intestine. The capacity of the mouse stomach was observed never to be less than 0.6 ml, and the transit time for barium was approximately 20 minutes after mice had been fasting for 12 hours.

In the tenth week of infection (66 days post infection), food was withdrawn from mice at 15 minute intervals. Twelve hours after the withdrawal of food, each mouse was given an oral dose of 0.5 ml 10% barium sulphate solution (Radiopaque, obtained in reconstituted form from the Radiology Department, The Western Infirmary, Glasgow) by gavage. After a 20 minute period, mice were sacrificed and the small intestine removed with great care to avoid any stretching of this organ. The stomach was also removed and fixed in neutral buffered formalin. The small intestines were secured to a perspex sheet and covered by paper towel soaked in neutral buffered

formalin. After all the small intestines had been treated in this way, an X-ray was used to quantify the maximum transit distance of the barium along the bowel of each mouse. The contents of the stomachs and small intestines were then flushed out with phosphate buffered saline. These organs were then placed in an oven at 60°C for 48 hours, following which the dry weights were recorded.

2.4.2. Intestinal permeability

The functional integrity of the small intestine during *S. mansoni* infection was examined using lactulose and mannitol as intestinal markers. Mice in the weight range of 38.6 - 45.3 g were exposed to 198 ± 15 *S. mansoni* cercariae (n = 10) or sham infected (n = 10), and housed in groups of 3 - 4 animals per cage. After 10 weeks of infection (day 70 post infection), each mouse was given an oral dose of 0.1 ml water containing 40 mg of lactulose and 10 mg of mannitol. The mice were then placed individually in sterilised cages with free access to water but no food. Urine was collected over the next five hours, care being taken to avoid any faecal contamination.

The volume of urine collected from each mouse was estimated by weight, following which samples were made up to 250 µl with distilled water if necessary. The samples were then frozen, packed in dry ice, and transported to the MRC Dunn Nutritional Laboratory, Cambridge. Here, the amount of lactulose and mannitol in the urine samples was quantified by Dr. P. Lunn using an automated COBAS BIO centrifugal analyser. Dr. Lunn also supplied the lactulose and mannitol used in this experiment.

2.5. Protein restriction and intestinal pathology

Mice in the weight range of 35.9 - 45.6 g were allocated to six different groups, each containing nine mice, on the basis of similar body weights. These groups consisted of *S. mansoni*-infected mice fed on 8, 4 or 2% protein (casein) diets (I8P, I4P and I2P respectively) and uninfected, control mice fed on 8, 4 or 2% protein diets

(U8P, U4P and U2P respectively). The composition of this diet is noted in Table 2.1., with the maize starch being substituted for casein on a weight for weight basis to vary the protein content of the diet; this allowed all the diets to be theoretically isoenergetic (Crompton *et al.*, 1981). Due to the severe nature of protein restriction for I2P and U2P, these groups of mice were fed on the 8% protein diet for all but the last two weeks of the experiment when the 2% protein diet was substituted.

Animals were housed individually and acclimated to the above dietary regimes for 3 - 4 days prior to infection. Food was supplied in the feeding pots described in section 2.2.1; mice were allowed free access to both food and water. Mice allocated to the infection protocols (I8P, I4P and I2P) were exposed to approximately 200 *S. mansoni* cercariae.

Food intake was recorded daily and body weight was recorded at weekly intervals. An estimation of assimilation efficiency was made using the same method as described in section 2.2.2. Towards the end of the ninth week of infection, mice were sacrificed over three consecutive days (61 - 63 days post infection). *Post-mortem* measurements were identical to those described in section 2.2.2, and the histological investigation of intestinal pathology utilised the methods described in sections 2.3.6 - 2.3.9.

2.6. Statistics

The statistical analyses were carried out using Minitab version 8.2 for the Macintosh unless otherwise stated.

Chapter 3

Food intake and tumour necrosis factor

3.1. Introduction

3.1.1. Host food intake and helminth infection.

The normal food intake patterns of mammals are often disturbed during parasitic infection. The affects of parasites on host food consumption have been reviewed by Crompton (1984) and Symons (1985). In the vast majority of cases, when an *ad libitum* food supply is made available to both infected animals and uninfected controls, reductions of food intake are observed in the infected hosts.

A number of factors have been associated with a reduced nutrient intake by the helminth-infected host. These include worm migration, developmental changes during the parasite life cycle and the intensity of infection. For example, the largest magnitude of reduction in food intake during a primary infection of *Nippostrongylus brasiliensis* infection in rats occurs during the first week of infection (Ovington, 1985), and is temporally associated with larval migration through host tissue (Haley, 1962). Humans infected with *Schistosoma haematobium* show signs of anorexia that are associated temporally with the maturation of these flukes and subsequent onset of oviposition (Edington and Gillies, 1976). Food consumption is also related to intensity of infection, and this has been studied extensively in animals of economic importance. For example, sheep harbouring *Fasciola hepatica* infection and pigs infected with *Oesophagostomum dentatum* show larger reductions of food intake as the intensity of infection increases (Boray, 1969; Pattison *et al.*, 1980). Similar observations have also been made in a variety of laboratory models, such as the *N. brasiliensis*-rat system (Keymer *et al.*, 1983a; Ovington, 1985).

These observations demonstrate that a variety of helminth infections, occupying different microhabitats within their hosts, can induce the common symptom of reduced

food intake. A unifying feature of the these host-parasite relationships is the inflammatory response mounted by the host as a response to the invading organism. Presumably, a larger number of invading parasites would cause more extensive inflammation. Thus intensity of infection, host food intake and inflammation may all be integrated in a dose-dependent manner.

3.1.2. Tumour necrosis factor and its influence on food intake

One of the cytokines involved in orchestrating the inflammatory response is tumour necrosis factor (TNF) (Kunkel *et al.*, 1989), a cytokine that also has wide-ranging effects on many aspects of nutrition (Klasing, 1988). The biological effects of TNF generally seem to be dose-dependent, with low levels co-ordinating tissue repair and inflammatory responses, and high levels causing organ failure and death (Cerami, 1992). Chronic exposure to TNF results in a potentially lethal syndrome of weight loss, lean tissue wasting and anorexia (Tracey *et al.*, 1988). This syndrome is known as cachexia, an ancient Greek word meaning "I have it bad." Thus the term cachexia describes aptly the severe weight loss that affects both muscle and adipose tissue, and that cannot be explained solely by anorexia (Tracey *et al.*, 1988).

The reduction of food intake induced by TNF has been reviewed by Oliff (1988). Inappetance associated with the administration of this cytokine has been observed in both clinical studies and laboratory studies. For example, TNF therapy has been used in human trials as an anti-cancer agent. In these studies, both short and long term administration of this cytokine frequently induces a loss of appetite in patients (Blick *et al.*, 1987; Chapman *et al.*, 1987; Sherman *et al.*, 1988). Intraperitoneal injection of TNF also induces anorexia in rats (Socher *et al.*, 1988; Tracey *et al.*, 1988), as does the introduction of TNF-producing tumour cells into mice (Oliff *et al.*, 1987).

Appetite is thought to be centrally regulated, with the control of satiety and feeding taking place in two centres of the hypothalamus (reviewed by Morley, 1980). The lateral hypothalamic centre appears to initiate feeding, while a ventromedially

situated centre is associated with satiety. These two centres operate in conjunction with each other, and Morley (1980) describes the hypothalamus as a transducer that integrates multiple inputs to maintain nutritional homeostasis by inhibiting or activating feeding behaviour. The intracerebroventricular infusion of TNF into rats suppresses food intake, and electrophoretically applied TNF reduces the activity of glucose-sensitive neurons in the lateral hypothalamus (Plata-Salamán *et al.*, 1988). These observations suggest that this cytokine acts directly on the central nervous system to reduce food consumption.

3.1.3. TNF as a potential mediator of inappetance during parasitic infection.

TNF's key role in co-ordinating inflammation (Kunkel *et al.*, 1989) ranks it high in contention as a mediator that alters host food intake during parasitic infection. The production of this cytokine occurs during a variety of protozoan infections such as those caused by *Plasmodium* spp. and *Leishmania* spp. (reviewed by Titus *et al.*, 1991), with both cerebral malaria and visceral leishmaniasis presenting anorexia as a symptom of infection (Scrimshaw *et al.*, 1968; Maru, 1979).

Elevated serum levels of TNF have been observed in patients with chronic schistosomiasis mansoni (Zwingenberger *et al.*, 1990). This cytokine also appears to be involved in the granulomatous response to the eggs of *S. mansoni* (Chensue *et al.*, 1989; Amiri *et al.*, 1992). As one of the symptoms associated with acute schistosomiasis mansoni in mice is reduced food intake (Vengesa and Leese, 1979), it is possible that TNF may also be involved in nutritional disturbance during this infection.

These observations gave rise to the objectives of the work described in this chapter that were: (1) to quantify accurately the patterns of food intake in mice with a primary infection of *S. mansoni* and (2) to investigate the proposition that any reduction of food consumption might be related to the production of TNF.

3.2. Results

3.2.1. Food intake

The daily food intake patterns for *S. mansoni*-infected mice and sham-infected mice are shown in Figure 3.1. The daily food intake data were analysed on a weekly basis, with a triphasic pattern of food consumption being observed in the *S. mansoni*-infected mice. An initial reduction of food intake by infected mice occurred during the first week of infection (Mann-Whitney, $W = 3165.0$, $P < 0.05$). This was followed by a five week period where consumption of food remained at similar levels for all animals. The largest magnitude in the reduction of food intake occurred between six and seven weeks post infection (Mann-Whitney, $W = 3452.0$, $P < 0.001$), with significantly reduced food consumption being maintained for the duration of the experiment (week 8, Mann-Whitney, $W = 3356.0$, $P < 0.001$; week 9, Mann-Whitney, $W = 3507.0$, $P < 0.001$; week 10, Mann-Whitney, $W = 1140.0$, $P < 0.005$).

3.2.2. Growth responses to infection

Changes in the live body weight of the mice from the three treatment groups were used to follow the growth responses of mice to infection with *S. mansoni* (Fig. 3.2). Only after the first week of infection was there any significant variation in body weight (Kruskal-Wallis ANOVA, $H = 6.0$, D.F. $= 2$, $P = 0.05$).

The percentage efficiency of the conversion of food eaten into body weight after the *S. mansoni* infections reached patency showed no significant variation at the 95% confidence level (infected [*ad libitum*] - 1.4%; control [pair fed] - 2.3%; control [*ad libitum*] - 2.4%) (Kruskal-Wallis ANOVA, $H = 4.45$, D.F. $= 2$, $P = 0.109$).

3.2.3. *Post-mortem* examination

Worm burden, in terms of the number of worm pairs recovered, ranged from 0 - 15 in the experiment examining TNF production at 67 days post infection, with infected

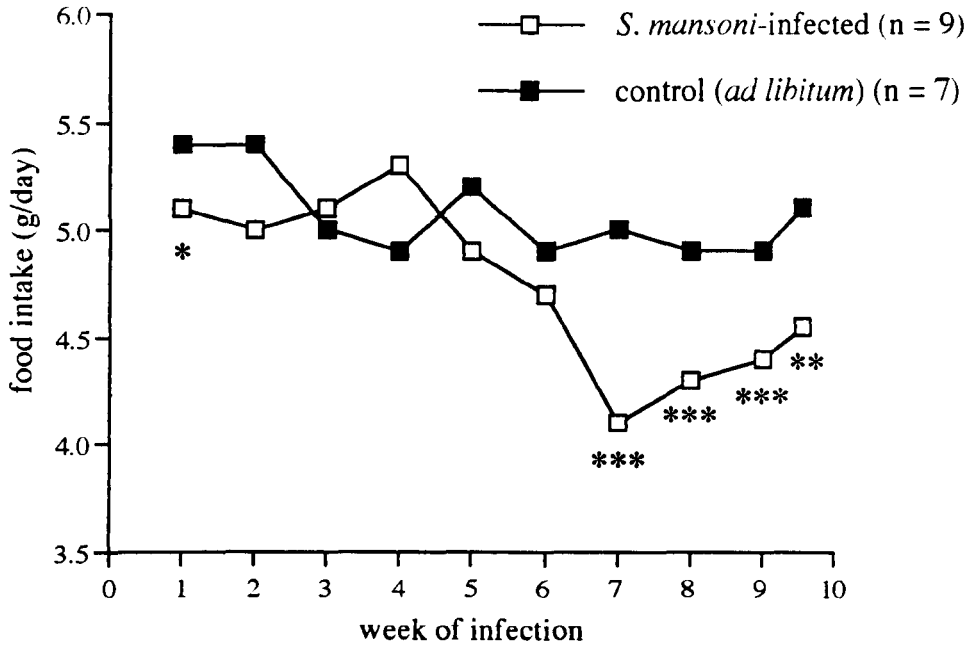


Figure 3.1. Median daily food intake (g) plotted on a weekly basis for *S. mansoni*-infected and control mice fed *ad libitum*.
(* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$)

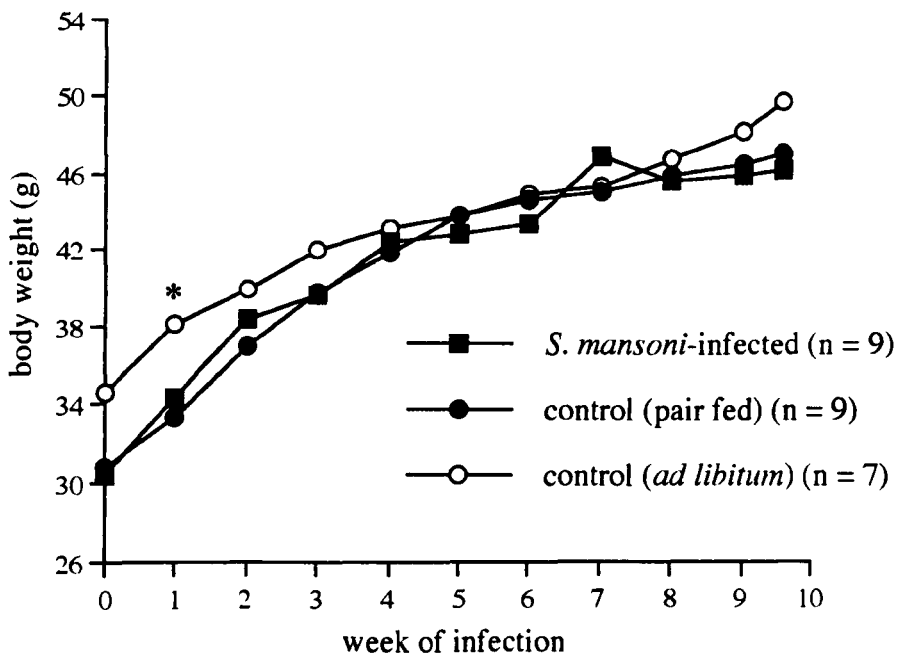


Figure 3.2. Median mouse body weight (g) plotted on a weekly basis for *S. mansoni*-infected mice and control mice (pair fed and fed *ad libitum*).
(* $P < 0.05$)

Table 3.1. Median visceral organ, body and carcass weights for sham-infected, control mice (fed *ad libitum*) (n = 7), sham-infected, control mice (pair fed) (n = 9) and *S. mansoni*-infected mice (fed *ad libitum*) (n = 9).
a: Liver weights significantly greater for infected mice when compared to pair fed control mice ($P < 0.05$).
b: Spleen weights significantly greater for infected mice when compared to control mice ($P < 0.05$).
c: Small intestine weights significantly greater for infected mice when compared to control mice ($P < 0.05$).

Weights (g)	Control *ad libitum*	Control pair fed	*S. mansoni*-infected
Liver	2.23	1.73	3.14 [a]
Spleen	0.12	0.13	0.45 [b]
Small intestine	1.43	1.38	2.83 [c]
Body	49.8	47.1	46.2
Carcass	46.1	43.9	39.7

mice harbouring a median number of 3 worm pairs. In the experiment investigating TNF production at 42 days post infection, the number of worm pairs recovered ranged from 1 - 13, with a median worm burden of 3.5 worm pairs per host.

Visceral organ, carcass and body weights for mice sacrificed at 67 days post infection are shown in Table 3.1. Significant variations in liver (Kruskal-Wallis ANOVA, $H = 11.81$, D.F. = 2, $P < 0.005$), spleen (Kruskal-Wallis ANOVA, $H = 16.74$, D.F. = 2, $P < 0.001$) and small intestine (Kruskal-Wallis ANOVA, $H = 16.62$, D.F. = 2, $P < 0.001$) weights were found between the three treatment groups. Multiple comparison tests (Siegel and Castellan, 1988) revealed that the livers of infected mice were significantly heavier than the livers of pair fed, control mice, and that the spleens and small intestines of infected mice were heavier than these organs in control mice ($P < 0.05$). No significant variation was observed for either body weight (Kruskal-Wallis ANOVA, $H = 1.86$, D.F. = 2, $P > 0.05$) or estimated carcass weight (Kruskal-Wallis ANOVA, $H = 3.10$, D.F. = 2, $P > 0.05$).

3.2.4. TNF determination

Sera from mice infected with *S. mansoni* for 42 and 67 days, and from uninfected controls, were analysed for the presence of TNF using ELISA and bioassay methods. Neither technique detected the presence of TNF in these samples.

3.3. Discussion

3.3.1. Food intake and TNF production

Although reductions in food intake have been detected previously during murine schistosomiasis mansoni (Knauft and Warren, 1969; Vengesa and Leese, 1979), it appears that no information on the longitudinal food consumption patterns has been published. It was necessary to establish such a pattern to test the hypothesis of a temporal relationship between TNF production and the suppression of food intake. Two periods of reduced food intake were observed in *S. mansoni*-infected mice during this

study. These occurred in the first week of infection and after the sixth week of infection for the duration of the experiment. These two reductions can be associated with distinct phases in the parasite life cycle.

In the first week of infection, the *S. mansoni* cercariae penetrate the host skin and transform into schistosomula, a process which takes less than one hour to complete (Cousin *et al.*, 1981). Twenty-four hours after cercarial penetration, the dermis contains a diffuse polymorphonuclear inflammatory infiltrate (Wheater and Wilson, 1979). Leukocytic infiltration is accompanied by oedema which peaks around 48-72 hours after infection, and the skin may not return to its normal structure even as late as seven days post infection (Mastin *et al.*, 1983). Schistosomula then migrate to the lungs after exiting the skin via blood vessels and the lymphatics (Wheater and Wilson, 1979). In mice, schistosomula are first detected in the lungs on day three post infection, and numbers peak on day six (Miller and Wilson, 1980). At this time schistosomula may cause damage to epithelial cells (Crabtree and Wilson, 1986).

The second phase of reduced food intake begins after six weeks of infection, coinciding with the maturation of worms and early egg output. These eggs must pass from the venous habitat of the adult worms to the gut lumen to allow their excretion in host faeces. The schistosome ova invoke granulomatous responses in the gut (reviewed by Weinstock, 1987), with the granulomas appearing to mediate egg excretion (Damian, 1987). Many ova never reach the gut, with up to 63% of eggs in mice being swept to the liver with the direction of hepatic-portal blood flow (Warren, 1973). These eggs become trapped in the liver and, again, invoke a granulomatous response by the host (Warren, 1973).

Thus the first period of reduced food intake is associated with the migration of the schistosome through the skin, and then on to the lungs. This migration is accompanied by cellular damage and inflammation. The second period of decreased food intake can be related to the maturation of worms and the onset of helminth oviposition, with egg

deposition in the liver and intestine inducing granulomatous responses by the host.

The magnitude of the reduction of food intake observed in this study between six and seven weeks post infection is similar to the reduction of food intake observed by Knauft and Warren (1969) in mice exposed to 40 *S. mansoni* cercariae. Vengesa and Leese (1979) observed a larger (50%) reduction of nutrient consumption in mice exposed to 250 - 300 cercariae. This more dramatic reduction may reflect the heavier infection level used by Vengesa and Leese (1979), and provide further evidence of a link between intensity of infection, size of inflammatory response and reduction of host food intake. Alternatively, the differing degrees of food intake may be influenced by the virulence of the parasite strain or by varying host responses to infection.

TNF appears to play an essential role in granuloma formation during *S. mansoni*-infection in mice (Chensue *et al.*, 1989; Amiri *et al.*, 1992). For this reason, the times chosen to test sera for the presence of this cytokine were both after the infection had reached patency. Sera from mice were analysed for the presence of TNF at 67 days post infection, a time when food intake had been reduced for nearly four weeks in the *S. mansoni*-infected mice. A second experiment was set up to allow analysis of sera from mice at 42 days post infection, the time corresponding with the largest reduction of food intake. However, no TNF was detected in the serum of these mice at either time point.

Although no evidence for the production of TNF was obtained from this study, a number of factors, other than the simple non-production of this cytokine, may explain this observation. Firstly, the detection limit of the TNF-ELISA kit was in the nanogram range which would not be sensitive enough to measure the levels of TNF observed by Zwingenberger *et al.* (1990) during human schistosomiasis mansoni. The WEHI 164 is more sensitive to lower levels of TNF (Espevik and Nissen-Meyer, 1986), but circulating receptor fragments that bind TNF may neutralise bioactivity (Ashkenazi *et al.*, 1991; Zee *et al.*, 1992). In addition, the release of TNF may be intermittent rather

than continuous throughout the course of an illness (Tracey and Cerami, 1994).

Evidence to support the production of TNF during murine schistosomiasis is, however, strong. Apart from the production of TNF during human schistosomiasis mansoni (Zwingenberger *et al.*, 1990), Chensue *et al.* (1989) observed that TNF appeared important in the maintenance or effector functions of pulmonary granulomas produced by mice after the intravenous injection of *S. mansoni* eggs. A seemingly essential role for TNF in mediating granuloma formation in response to the schistosome ova has been demonstrated recently by Amiri *et al.* (1992). These researchers studied the granulomatous response to *S. mansoni* infection in severe combined immunodeficient (SCID) mice that lack functional T and B lymphocytes but have normal macrophages. Most eggs trapped in the liver of these mice did not invoke a granulomatous response. However, when the SCID mice received injections of recombinant TNF, the capacity to produce granulomas was restored. Amiri *et al.* (1992) also found evidence to suggest that TNF may act as a reproductive trigger to schistosomes. Thus, although not demonstrated in this study, recent research in other laboratories suggests that TNF may play an important role during murine schistosomiasis mansoni.

3.3.2. Growth responses to infection

Although the greatest reduction in host food intake was observed between six and seven weeks post infection, mouse body weight was only significantly altered after the first week of infection. This significant weight variation may represent a combination of the slightly lower initial weights of mice allocated to the *S. mansoni*-infected and pair fed control treatment groups, and the reduced food consumption by these mice during the first week of infection.

Kavaliers and Podesta (1988) observed that hamsters with patent *S. mansoni* infections displayed decreased patterns of locomotor activity; this appeared to arise from an opioid-mediated alteration in host behaviour. A decrease in host energy

expenditure, brought about by a parasite-induced modification of activity, may explain why the body weights of infected mice do not drop significantly during the patent period of infection in the present study, even though food intake is decreased to its greatest extent at this time.

The observed similarity of growth rate in the pair fed mice when compared to the infected mice would, at first glance, indicate that a parasite-induced modification of host activity plays no role in the maintenance of host body weight. However, the individual variation of appetite in the pair fed and *S. mansoni*-infected mice resulted in some of the pair fed animals receiving a more than adequate food supply. This meant that the pair fed mice ate less food, in total, than the *S. mansoni*-infected group. As growth rates are almost identical for these two groups of mice, the variation of food consumption is reflected in the percentage efficiency of conversion of food eaten into body weight. This efficiency, although lower in the infected mice, was not significantly different at the 95% confidence limit. If the efficiency of food conversion into body weight is indeed lower in *S. mansoni*-infected mice, an altered activity in these animals may help explain the body weight conservation observed in these mice.

When energy intake is restricted, animals can respond by reducing overall rates of protein degradation and synthesis (Millward and Waterlow, 1984). As these are both metabolically demanding processes, energy can be conserved in this way. A different pattern is generally associated with reduced energy intake during infection, where skeletal muscle proteolysis is accompanied by an outpouring of amino acids which are taken up by the liver (reviewed by Fischer, 1991). This increase in the amino acid supply to the liver appears to provide the substrates for the synthesis of acute phase proteins, such as the complement component C3 and α-glycoprotein that may be produced during abdominal sepsis (Sax *et al.*, 1988). This amino acid supply may also provide resources for immune proliferation and wound healing.

Although terminal body weight did not differ significantly between control or

infected mice, increased liver, spleen and small intestine weights were all observed in the infected mice. These changes are due largely to the host granulomatous response to the ova of *S. mansoni* in the liver and small intestine. The inflammatory response in the liver may result in the restriction of blood flow through this organ, with an accompanying redirection of blood to the spleen which may cause congestive splenomegaly (reviewed by Warren, 1973). A diversion of host energy resources into inflammatory responses and associated physiological changes must therefore be required.

Estimated carcass weight, although not significantly varied between control and infected mice, was lowest for the *S. mansoni*-infected mouse group. This hinted that skeletal muscle proteolysis may be providing the energy resources for the granulomatous response, and the production of acute phase proteins such as C3 and serum amyloid P-component that have been observed in *S. mansoni*-infected mice (Peypes *et al.*, 1980). The production of TNF would provide further evidence for this theory, as this cytokine can induce rat skeletal muscle proteolysis *in vivo* in the presence of an intact adrenal response (Mealy *et al.*, 1990). These authors also observed that liver protein content and DNA levels both rise, indicating that liver protein content and hepatocyte numbers increase.

3.4. Conclusions

It seems paradoxical that the periods associated with decreased host food intake are accompanied by inflammatory responses to the invading schistosome or its ova, as these processes must be rely on a diversion of host nutritional resources into defence mechanisms. That granuloma formation around schistosome eggs is an important host defence mechanism was suggested by von Lichtenberg (1964). The capacity of mice to mount a granulomatous response against *S. mansoni* eggs is compromised by T cell deprivation or thymus aplasia, and these animals die earlier as a result of infection than immunologically intact controls (Byram and von Lichtenberg, 1977; Doenhoff *et al.*,

1979). Early mortality in these animals was thought due to an acute hepatotoxicity reaction. This hypothesis is supported by the observations of Amiri *et al.* (1992), who found that hepatotoxic effects were evident around the ova of *S. mansoni* in SCID mice. Thus the granuloma appears to protect the host against toxic substances released from the *S. mansoni* egg.

If TNF plays the important role during schistosomiasis mansoni that is suggested in the discussion of this chapter, although production of this cytokine was not observed, reduced food intake may simply be a pathological biproduct of defence mechanisms that result in overall host protection. That is, decreased food intake might simply represent a trade off between nutritional disturbance and the continued survival of the *S. mansoni*-infected host.

3.5. Summary

1. Food intake by mice, during a primary infection with *S. mansoni*, was decreased during the first week of infection, and after the sixth week of infection for the duration of the experiment.

2. TNF was not detected in sera obtained from *S. mansoni*-infected mice at 42 and 67 days post infection.

3. Growth rates of infected and control mice did not vary significantly except in the first week of infection.

4. The percentage efficiency of conversion of food eaten into body weight was not significantly altered by infection.

5. Although estimated carcass weight was not significantly reduced by infection, increases in liver, spleen and small intestine weights were observed.

Chapter 4

Intestinal pathology: I. Smooth muscle hypertrophy

4.1. Introduction

One of the most striking pathological features that is observed during murine schistosomiasis mansoni is the enlargement of the small intestine, the surface of which is studded by granulomas. This is accompanied by a general induration of this organ, particularly in the posterior region. Domingo and Warren (1969) noted that the fresh weight of the small intestine increased by 20-35% in *S. mansoni*-infected mice when compared to uninfected mice, and that this weight increase was accompanied by an intestinal thickening of 3.8-11.4 times that of normal mice. Tiboldi (1979) also observed a weight gain in the small intestine, and attributed this to three main causes, (1) the weight of the schistosome eggs themselves, (2) the weight of the inflammatory tissue surrounding the eggs and (3) the weight of increased amounts of blood and lymph as a response to diminished blood flow in the liver.

An increase in intestinal weight and thickness frequently accompanies gut inflammation in a variety of enteric helminth infections, including *Nippostrongylus muris* (= *N. brasiliensis*) in rats (Symons, 1957), *Ascaris suum* in pigs (Stephenson *et al.*, 1980) and *Moniliformis moniliformis* in rats (Singhvi and Crompton, 1982). In these infections, the weight gain in the small intestine is attributable partly to an increase in smooth muscle mass. Thickening of alimentary tract muscle has also been observed in the ceca of fish infected with *Leptorhynchoides thecatus* (Buron and Nickol, 1994). Although *S. mansoni* does not inhabit the small intestine, the inflammatory response to the passage of eggs through the gut wall might invoke similar alterations in the bowel of the *S. mansoni*-infected mouse.

Smooth muscle is the major structural component of the gastrointestinal tract. The *muscularis externa* consists of a relatively thick layer of circular muscle with fibres

arranged in a circular pattern around the intestine, and a thinner, outer layer of longitudinal muscle. The isolated contraction of circular muscle either narrows the lumen of the gut or increases the hydrostatic pressure within the lumen; longitudinal muscle produces the opposite effects when it contracts in isolation. The independent or simultaneous contraction of these muscle layers in a single region, or at many loci along the intestine, provides the unique motility patterns of the gut. Thus, as well as providing the structural support for intestinal mucosa, the *muscularis externa* is responsible for producing the forces that move and mix intestinal contents, allowing for (1) the mixing of foodstuffs with digestive enzymes, (2) circulation of contents so that they come into contact with the absorptive cells of the mucosa and (3) the net aboral movement of intestinal contents.

The potential importance of increased intestinal smooth muscle mass as a nutritional cost of infection is therefore two-fold. Firstly, the amount of energy and nutritional reserves diverted into producing more muscle must be considered. Secondly, an intact macro- and micro-structure in the gut is necessary for the maintenance of optimal intestinal function, thus any change in intestinal structure might adversely affect nutrient uptake in an infected animal. These possible influences on mouse nutrition during the course of *S. mansoni* infection gave rise to the objectives of research described in this chapter, which were: (1) to determine if intestinal smooth muscle hypertrophy occurs during murine schistosomiasis mansoni, (2) to investigate the possible aetiology of any such changes in intestinal musculature, (3) to examine the functional significance of any change in muscular structure and (4) to investigate further the changes of body weight redistribution that were observed in Chapter 3.

4.2. Results

The thickness of the *muscularis externa* was observed to be greater in the small intestines of *S. mansoni*-infected mice in the ninth week of infection than in uninfected controls in the 25% region (Mann-Whitney, $W = 73.0$, $P < 0.05$), 50% region (Mann-Whitney, $W = 28.0$, $P < 0.005$), 75% region (Mann-Whitney, $W = 28.0$, $P <$

0.005) and the 100% region (Mann-Whitney, W = 28.5, P < 0.005) (Table 4.1). This ranged from a two-fold increase in the anterior region of the small bowel up to an approximately four-fold increase in the most posterior region. In only the most distal site of the small intestine was the bowel diameter significantly larger in infected mice (25% region: Mann-Whitney, W = 66.5, P > 0.05, 50% region: Mann-Whitney, W = 37.0, P > 0.05, 75% region: Mann-Whitney, W = 43.0, P > 0.05, 100% region: Mann-Whitney, W = 29.0, P < 0.005) (Table 4.2). This change in diameter does not represent simply the increase in thickness of the *muscularis externa*, as the median submucosal diameter of the small intestine is also greater in infected mice when compared to uninfected hosts (Mann-Whitney, W = 32.5, P < 0.05; Table 4.3).

These measurements of diameter and smooth muscle thickness gave rise to the calculated increase in the area of *muscularis externa* that was noted for mice in the ninth week of infection with *S. mansoni* (Fig. 4.1). This increase was observed in all regions of the small bowel that were examined (25% region: Mann-Whitney, W = 76.0, P < 0.005, 50% region: Mann-Whitney, W = 29.0, P < 0.005, 75% region: Mann-Whitney, W = 28.0, P < 0.005, 100% region: Mann-Whitney, W = 28.0, P < 0.005), ranging from 2.2 times in the most anterior region to 5.7 times in the most posterior region. Smooth muscle area was positively correlated with increasing distance from the pylorus in the infected mice (Spearman rank correlation, $r_s = 0.538$, P < 0.005), but not in uninfected mice (Spearman rank correlation, $r_s = 0.008$, P > 0.05).

No significant changes in the area of the *muscularis externa* in the anterior region (Mann-Whitney, W = 33.0, P > 0.05) or the posterior region of the small intestine (Mann-Whitney, W = 51.0, P > 0.05) were observed between mice infected with *S. mansoni* and uninfected controls at six weeks post infection (Fig. 4.2). Later in the course of infection (16 weeks post infection), the area of intestinal smooth muscle in infected hosts, when compared to control animals, was found to range between a 2.1 times increase in the anterior region (Mann-Whitney, W = 73.5, P < 0.01) up to a 7.6

Table 4.1. Median small intestine smooth muscle thickness (mm) of uninfected, control mice (n = 7) and *S. mansoni*-infected mice (n = 7) mice in the ninth week of infection. (* $P < 0.01$, ** $P < 0.005$).

Distance from pylorus (%)	Uninfected control mice	*S. mansoni*-infected mice
25	0.05	0.10 *
50	0.06	0.12 **
75	0.06	0.20 **
100	0.06	0.26 **

Table 4.2. Median small intestine diameters (mm) of uninfected, control mice (n = 7) and *S. mansoni*-infected (n = 7) mice mice in the ninth week of infection. (* $P < 0.005$)

Distance from pylorus (%)	Uninfected control mice	*S. mansoni*-infected mice
25	3.86	4.46
50	3.83	4.47
75	3.30	4.07
100	3.52	5.27 *

Table 4.3. Median submucosal diameters (mm) of the small intestines of uninfected, control mice (n = 7) and *S. mansoni*-infected mice (n = 7) in the ninth week of infection. (* $P < 0.05$)

Distance from pylorus (%)	Uninfected control mice	*S. mansoni*-infected mice
25	3.76	4.23
50	3.71	4.27
75	3.20	3.61
100	3.40	4.91 *

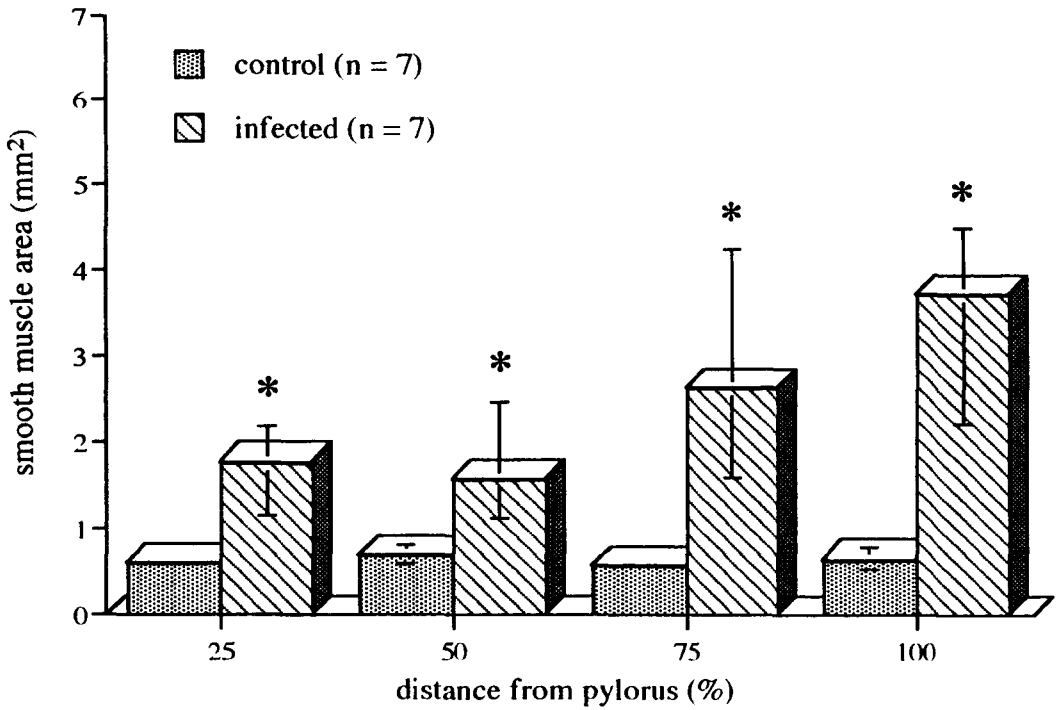


Figure 4.1. *Muscularis externa* area (mm^2) (median values and interquartile ranges) for control and *S. mansoni*-infected mice in the ninth week of infection (* $P < 0.005$).

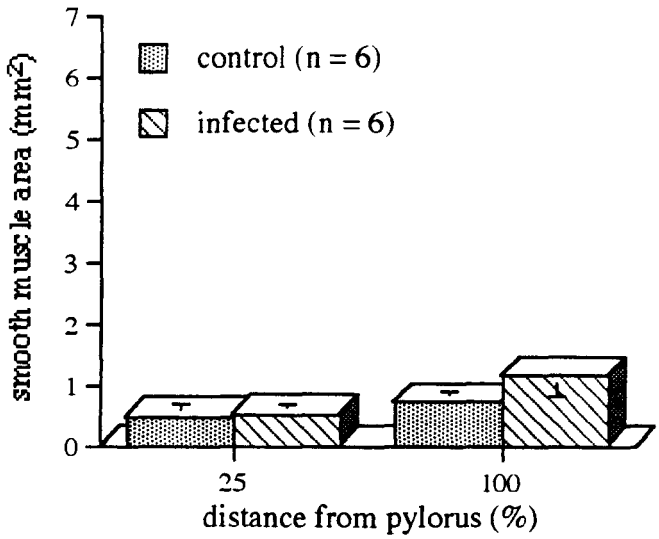


Figure 4.2. *Muscularis externa* area (mm^2) (median values and interquartile ranges) for control mice and *S. mansoni*-infected mice at 6 weeks post infection.

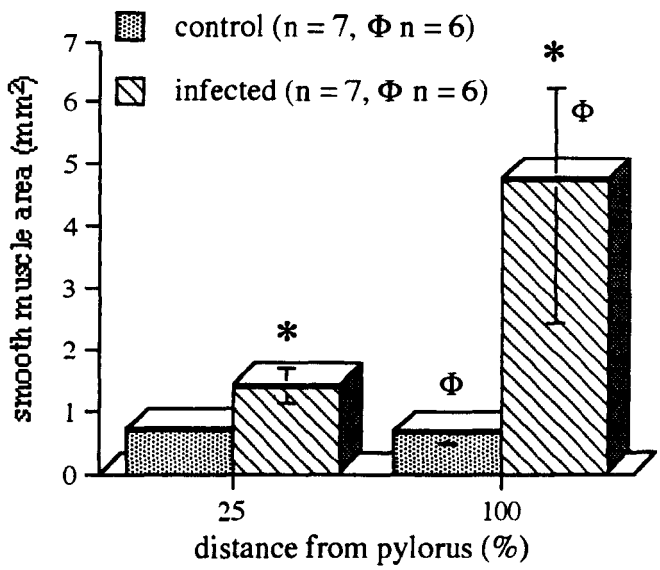


Figure 4.3. *Muscularis externa* area (mm^2) (median values and interquartile ranges) for control mice and *S. mansoni*-infected mice at 16 weeks post infection.(* $P < 0.01$).

Plate 4.1. Transverse section from the distal end of the small intestine from an uninfected, control mouse. —— 50 µm

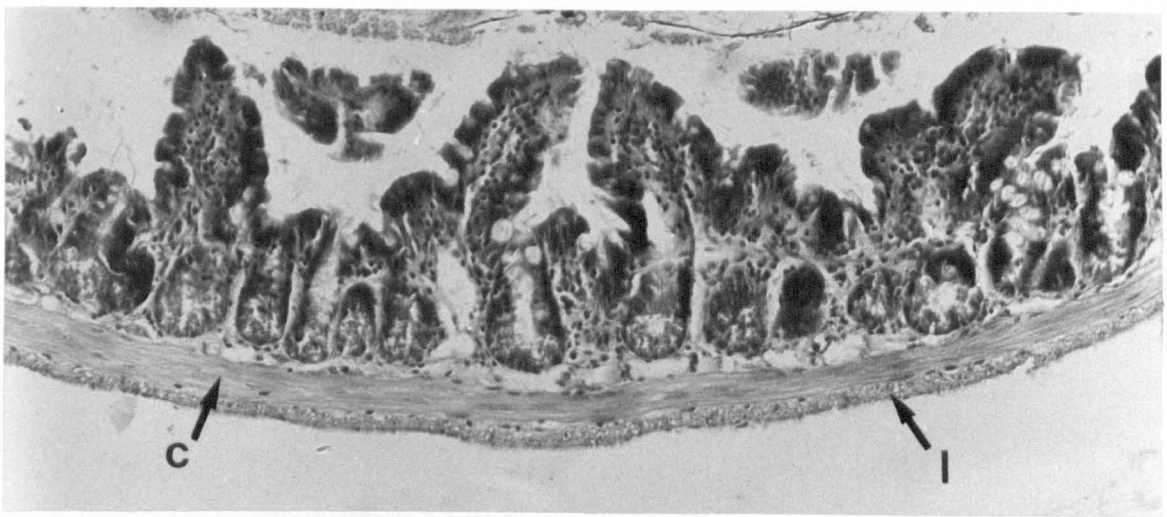
c - circular smooth muscle **l** - longitudinal smooth muscle.

Plate 4.2. Transverse section from the distal end of the small intestine from a mouse infected with *S. mansoni* (ninth week of infection). Thickening of the smooth muscle occurs in the absence of granulomatous reactions. —— 50 µm

c - circular smooth muscle **l** - longitudinal smooth muscle.

Plate 4.3. Transverse section from the distal end of the small intestine from a mouse infected with *S. mansoni* (ninth week of infection). Thickening of intestinal smooth muscle in the presence of granulomatous reactions. —— 50 µm

c - circular smooth muscle **l** - longitudinal smooth muscle **g** - granuloma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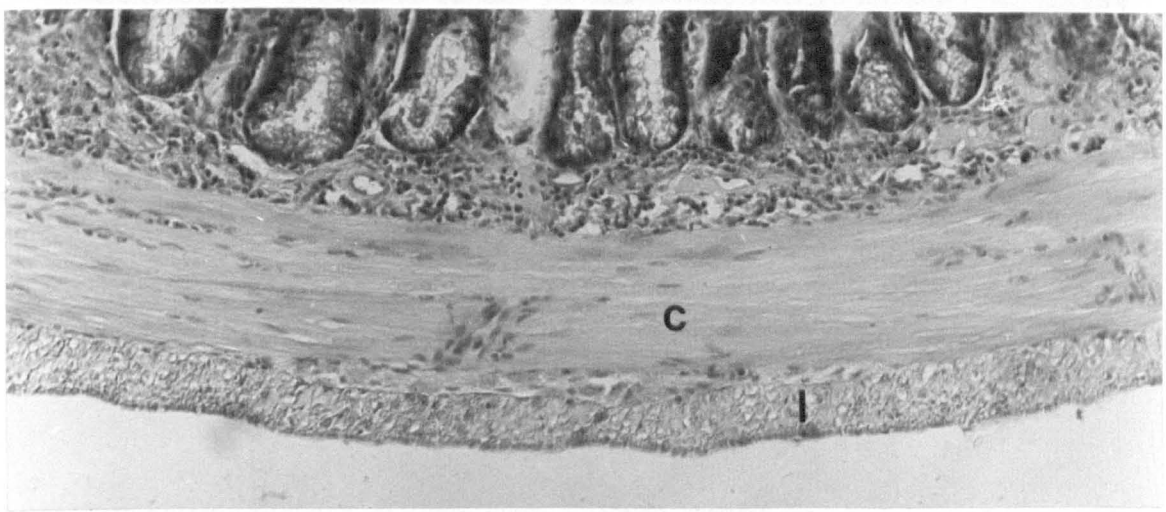
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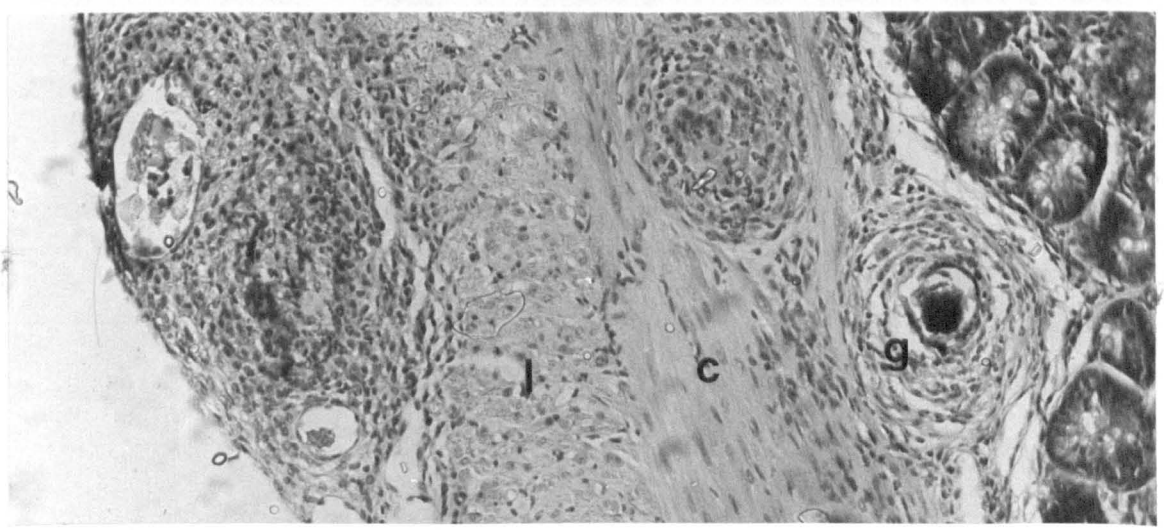
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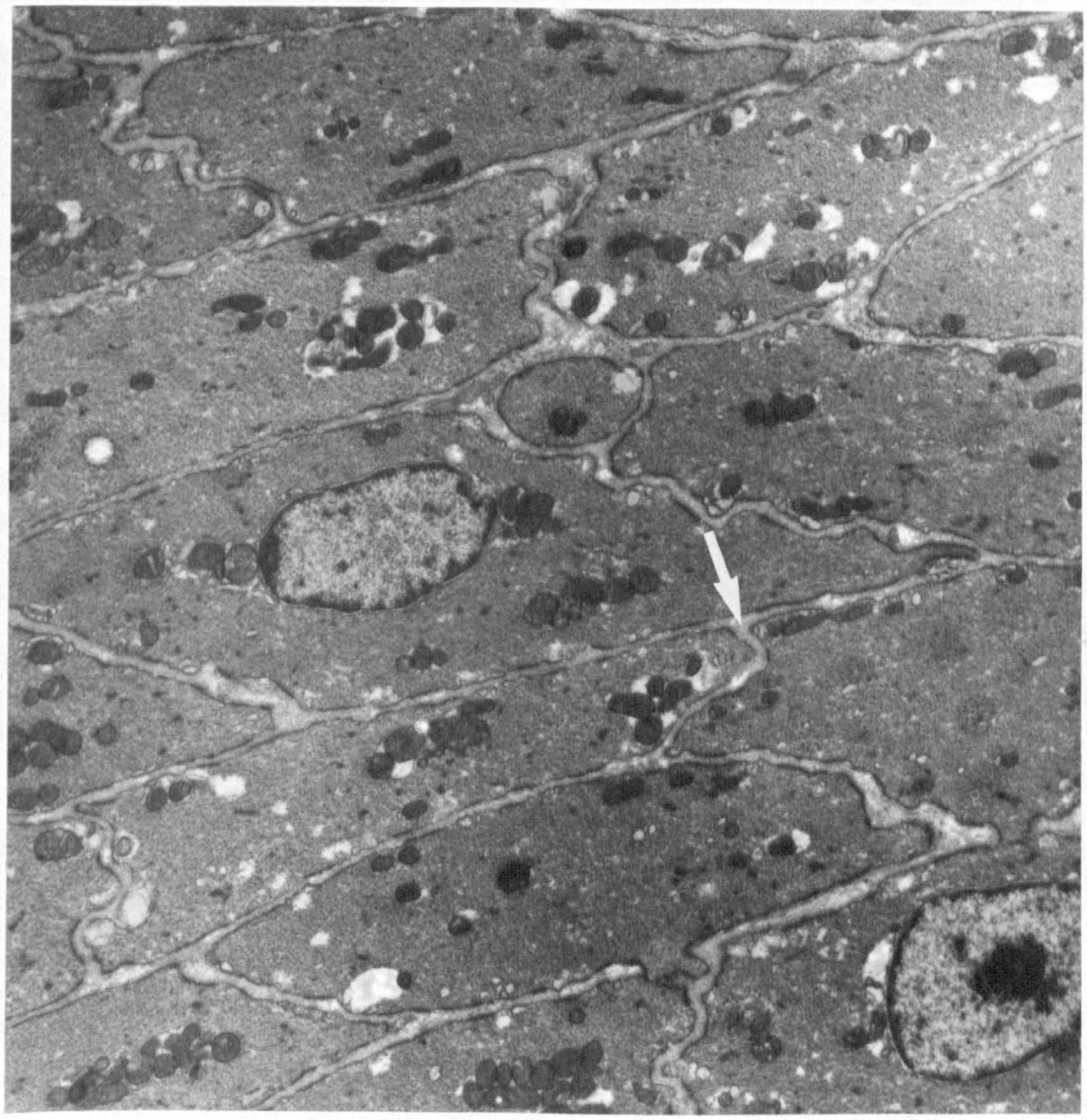


Plate 4.4. Transmission electron micrograph of the longitudinal muscle layer in transverse section from the small intestine of an uninfected, control mouse. Arrow indicates intercellular gap. —— 2.5 μm

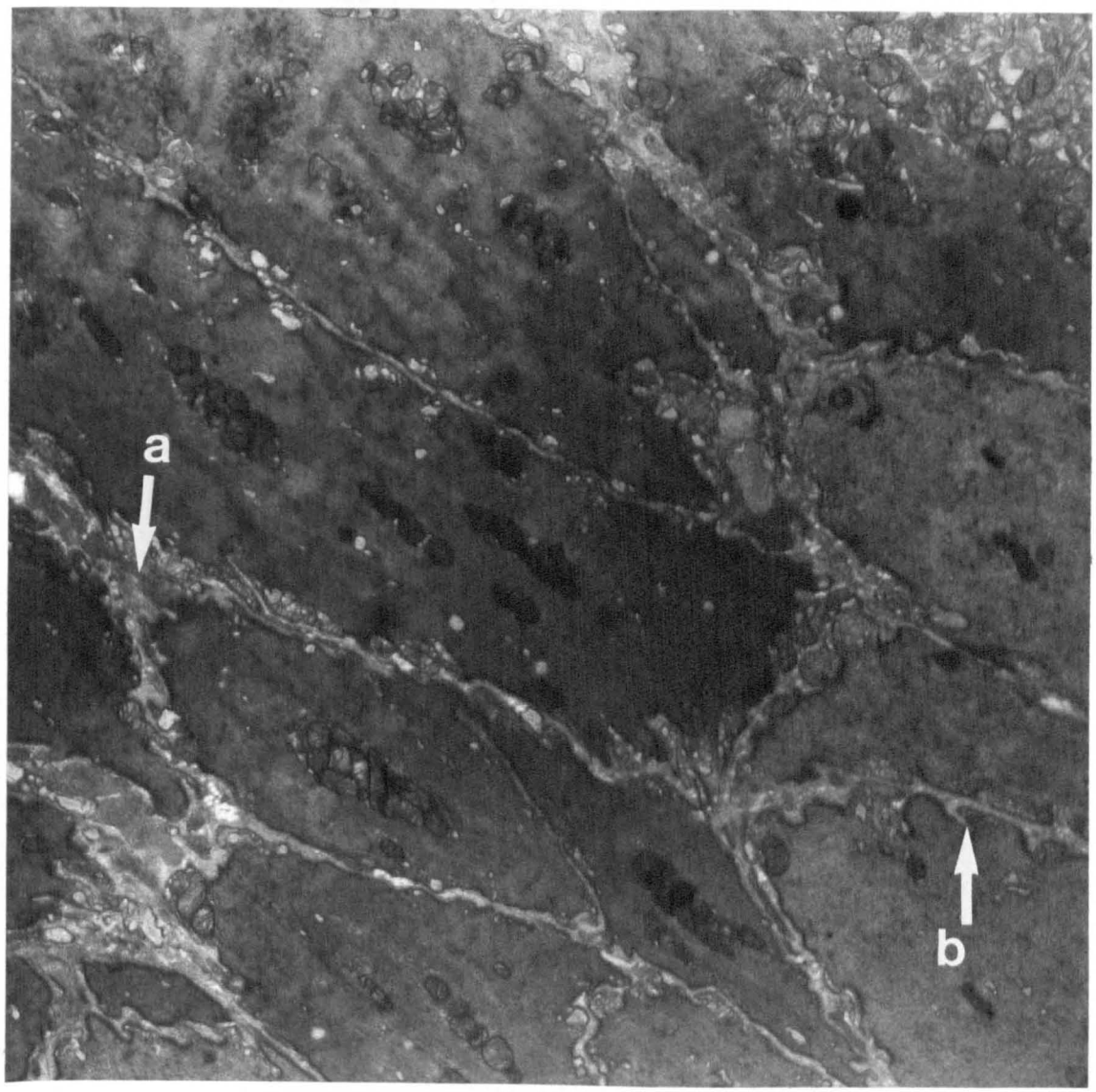


Plate 4.5. Transmission electron micrograph of the longitudinal muscle layer in transverse section from the small intestine of an *S. mansoni*-infected mouse in the ninth week of infection. Arrows indicate **a** - intercellular gap and **b** - invaginations in cell membrane. ——— 2.5 μm

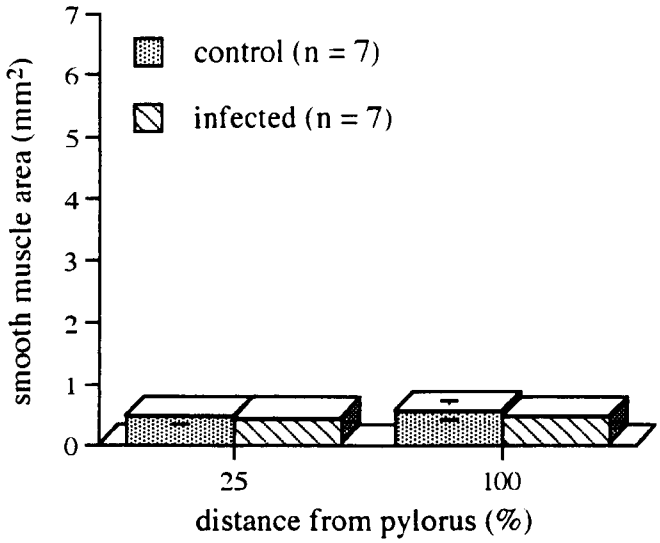


Figure 4.4. *Muscularis externa* area (mm^2) (median values and interquartile ranges) for control mice and single-sex *S. mansoni*-infected mice at 8 weeks post infection.

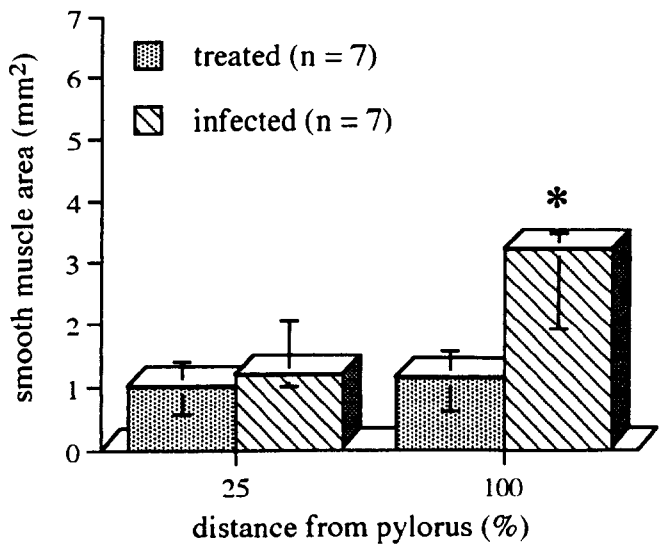


Figure 4.5. *Muscularis externa* area (mm^2) (median values and interquartile ranges) for *S. mansoni*-infected mice at 16 weeks post infection and *S. mansoni*-infected mice treated at 8 weeks post infection with praziquantel. (* $P < 0.01$).

Table 4.4. Barium transit through the small intestine over a 20 minute period, small intestine length and dry weight and stomach dry weight for uninfected, control mice and *S. mansoni*-infected mice at 10 weeks post infection (median values). ϕ Maximum transit distance of barium sulphate from the stomach calculated as a proportion of the small intestine length. (* $P < 0.05$, ** $P < 0.001$)

	Uninfected, control mice	*S. mansoni*-infected mice
No. of mice receiving barium sulphate dose	9	10
No. of mice with barium in the small intestine	6	10
Small intestine length (mm)	463	500
Maximum transit point of barium ϕ	0.471	0.448
Small intestine dry weight (mg)	422	452 *
Stomach dry weight (mg)	52	20 **

Table 4.5. Mouse body, carcass, liver and spleen weights for uninfected, control mice (n = 7) and *S. mansoni*-infected mice (n = 7) in the ninth week of infection. All values are median. Carcass weight was measured after removal of small intestine, caecum, liver and spleen, and after perfusion of both control and infected mice. (* $P < 0.05$ ** $P < 0.01$ *** $P < 0.005$)

	Uninfected control mice	*S. mansoni*-infected mice
Body weight (g)	51.4	52.3
Carcass weight (g)	43.2	38.0*
Liver weight (g)	2.3	4.3***
Spleen weight (g)	0.2	0.6**

Table 4.6. Mouse body, carcass, liver and spleen weights for uninfected, control mice (n = 10) and *S. mansoni*-infected mice (n = 10) at 16 weeks post infection. All values are median. Carcass weight was measured after removal of small intestine, caecum, liver and spleen, and after perfusion of both control and infected mice. (* $P < 0.05$ ** $P < 0.001$)

	Uninfected control mice	*S. mansoni*-infected mice
Body weight (g)	55.5	57.6
Carcass weight (g)	48.4	42.1*
Liver weight (g)	2.8	4.0**
Spleen weight (g)	0.2	0.4**

Table 4.7. Mouse body, carcass, liver and spleen weights for uninfected, control mice (n = 8) and mice exposed to single-sex *S. mansoni* cercariae (n = 9) at 8 weeks post infection. All values are median. Carcass weight was measured after removal of small intestine, caecum, liver and spleen, and after perfusion of both control and infected mice. (* $P < 0.05$)

	Uninfected control mice	*S. mansoni*-infected mice
Body weight (g)	54.0	51.7
Carcass weight (g)	46.5	45.2
Liver weight (g)	2.7	2.5
Spleen weight (g)	0.1	0.2 *

Table 4.8. Mouse body, carcass, liver and spleen weights for praziquantel treated mice (n = 7) and *S. mansoni*-infected mice (n = 7) at 16 weeks post infection. All values are median. Carcass weight was measured after removal of small intestine, caecum, liver and spleen, and after perfusion of both treated and infected mice. (* $P < 0.05$ ** $P < 0.005$)

	Praziquantel treated mice	*S. mansoni*-infected mice
Body weight (g)	60.1	59.4
Carcass weight (g)	52.2	47.0 **
Liver weight (g)	3.2	4.5
Spleen weight (g)	0.3	0.4 *

times increase in the distal region of the small intestine (Mann-Whitney, $W = 57.0$, $P < 0.01$) (Fig.4.3).

The striking increase in the thickness of the *muscularis externa* in the ninth week of *S. mansoni* infection is illustrated in Plates 4.1 - 4.3. Of particular note is the occurrence of muscle thickening both in the presence of granulomas (Plate 4.3) and in the apparent absence of these focal inflammatory responses (Plate 4.2). Difficulty in orienting muscle samples for TEM meant that no firm conclusions could be drawn about the hyperplasic or hypertrophic nature of the smooth muscle cells. A comparison of intestinal muscle structure in control and infected animals (Plates 4.4 - 4.5) did, however, reveal some minor differences. These included a broadening of the intercellular gap and invaginations in the membranes of smooth muscle cells in infected mice.

The area of smooth muscle in the small intestine of mice exposed to single-sex cercariae was not significantly altered in comparison with that of uninfected, control mice (25% region: Mann-Whitney, $W = 56.0$, $P > 0.05$; 100% region: Mann-Whitney, $W = 31.0$, $P > 0.05$) (Fig. 4.4). Mice infected with *S. mansoni* that were treated subsequently with praziquantel had a smaller area of intestinal smooth muscle in the distal region of the small intestine than untreated mice (Mann-Whitney, $W = 74.0$, $P < 0.01$). Treatment with praziquantel had little affect on muscle area in the proximal region of the small bowel (Mann-Whitney, $W = 64.5$, $P > 0.05$) (Fig. 4.5).

The maximum transit of barium sulphate away from the stomach over a 20-minute period in the small intestine was calculated as a proportion of the intestine length. This accounted for the significant lengthening of the small intestine in *S. mansoni*-infected mice when compared to uninfected controls (Mann-Whitney, $W = 128.5$, $P < 0.05$) (see Table 4.4). The transit distance of barium in the small intestine of infected mice did not appear to differ significantly from the transit distance in uninfected mice (Mann-Whitney, $W = 82.0$, $P > 0.05$) (Table 4.4). Stomach dry weight was found to be higher in control mice (Mann-Whitney, $W = 55.0$, $P < 0.001$) and small intestine dry

weight was increased in infected mice (Mann-Whitney, W = 125.0, P < 0.05) (Table 4.4).

Mice in the ninth week of *S. mansoni* infection had significantly increased liver (Mann-Whitney, W = 76.5, P < 0.005) and spleen weights (Mann-Whitney, W = 74.0, P < 0.01) when compared to their uninfected counterparts (Table 4.5). Similarly, after 16 weeks of *S. mansoni* infection, liver (Mann-Whitney, W = 174.0, P < 0.001) and spleen weights (Mann-Whitney, W = 167.5, P < 0.001) were greater for infected mice than for uninfected control mice (Table 4.6). After removal of these organs, the small intestine and the caecum, infected animals were also found to have significantly lower carcass weights than control mice (week 9: Mann-Whitney, W = 36.5, P < 0.05; week 16: Mann-Whitney, W = 76.5, P < 0.05) although terminal body weight did not differ between these two groups (week 9: Mann-Whitney, W = 52.0, P > 0.05; week 16: Mann-Whitney, W = 139.0, P > 0.05) (Tables 4.5 and 4.6). Only spleen weight was found to be elevated in mice exposed to single-sex cercariae (Mann-Whitney, W = 118.5, P < 0.05) (Table 4.7.). When infected mice were treated with praziquantel, spleen weight was lower (Mann-Whitney, W = 36.5, P < 0.05) and carcass weight was greater (Mann-Whitney, W = 75.0, P < 0.005) than in untreated animals (Table 4.8).

4.3. Discussion

4.3.1. Smooth muscle area

The area of the *muscularis externa* has been shown to increase in all regions of the small intestine of mice in the ninth week of infection with *S. mansoni*. This increase appears to be a function of both small intestine diameter and smooth muscle thickness. An increase in smooth muscle thickness is consistent with observations made in trichinized guinea pigs (Lin and Olsen, 1970), in pigs infected with *Ascaris suum* (Stephenson *et al.*, 1980), and in rats infected with *Moniliformis moniliformis* (Singhvi and Crompton, 1982) or *Nippostrongylus muris* (= *N. brasiliensis*) (Symons, 1957). The increase in intestinal diameter in *S. mansoni*-infected mice is also consistent with the

dilation of the small intestine that was observed to accompany *N. muris* infection in rats (Symons, 1957).

The increase in the area of the *muscularis externa* in *S. mansoni*-infected mice occurred with greater severity at increasing distances along the small intestine from the pyloric sphincter. Although granuloma area was not corrected for when calculating muscle area, the physical size of these inflammatory reactions cannot be the sole cause of increased muscle area as this hypertrophy also occurred in the absence of any local granulomas. The area occupied by granulomatous reactions may, however, contribute to the correlation of muscle area with increasing distance from the pylorus as inflammatory damage appeared to be more severe in posterior regions. This increased proportion of muscle thickening in distal sites of the small bowel appeared to reflect a preference of adult worms for mesenteric vessels leading from the posterior region of the small intestine, although neither worm location nor the area occupied by granulomatous reactions were quantitatively assessed. These observations are consistent with the finding that *S. mansoni* worm pairs occupy more retrograde sites in the mesenteric vessels of humans (Cheever, 1968).

An increase in intestinal smooth muscle area was maintained with a longer duration of infection (16 weeks). Treatment of infected mice with praziquantel did reduce this muscle mass when compared to untreated mice, at least in the most distal region of the small intestine. This suggested that muscle thickening occurred to an increasing degree as the infection progressed and / or that treatment resulted in an abatement of this pathological feature within an eight-week period.

The similarity of intestinal muscle area in *S. mansoni*-infected mice when compared to uninfected, control mice at six weeks post infection indicated that a patent infection was required for at least a couple of weeks before an increased mass of smooth muscle could be observed. No increase in muscle area was observed in response to single-sex schistosome infections. This suggested that egg-associated pathology was responsible for the growth of the *muscularis externa* beyond normal physiological

bounds. However, as female worms do not reach full sexual maturity until paired with a male worm (see Erasmus, 1987), it is possible that a new antigenic repertoire produced by adult worm pairs stimulates this smooth muscle enlargement.

The increase in area of intestinal smooth muscle can be considered to be hypertrophic, but it is not clear, however, if muscle growth is brought about by a change in cell size, cell number, or a combination of both. Unlike skeletal muscle cells in which no mitoses occur after development of myofilaments (Moss and Leblond, 1971), mitoses are observed in the gastric musculature (Jurukova and Atanassova, 1974; McGeachie, 1971; Gabella, 1979). Symons (1957) broached this question of increased size or number of intestinal myocytes in *Nippostrongylus*-infected rats by counting muscle nuclei in formalin-fixed sections. Although an increased number of nuclei were observed in the intestinal muscle of infected rats, evidence for hyperplasia of muscle was considered to be doubtful due to a higher degree of tissue contraction in these animals (Symons, 1957). One of the original aims of the present study was to investigate cellular hyperplasia and hypertrophy as components of muscle growth in *S. mansoni*-infected mice using TEM. However, the difficulty of exact specimen orientation for accurate measurement of fibre length and diameter, which was compounded by a shortage of fixed tissue, meant that no conclusive results relating to cell size could be obtained.

Some minor changes were observed in the structure of the *muscularis externa* of *S. mansoni*-infected mice using TEM. These changes included a broadening of the intercellular gap and a more irregular cell membrane surface. Invaginations in the membrane of muscle cells are also observed in intestinal muscle that has hypertrophied as a response to surgically induced stenosis (Gabella, 1979). Invagination of the cell surface may help counteract the reduction of surface-to-volume ratio that occurs with an increase in cell volume (Gabella, 1979). The invaginations observed in the cell membrane of intestinal smooth muscle in *S. mansoni*-infected mice may therefore be suggestive of increased cell size in these animals.

The nature of TEM makes the extent of these changes difficult to evaluate. The samples in this study were taken from the anterior region of the small intestine in which a smaller degree of muscle hypertrophy was observed. This suggests that similar changes in distal sites could be more severe, but further research would be required for confirmation.

The mechanisms underlying smooth muscle hypertrophy during helminth infection remain to be elucidated. Castro (1989) has suggested that increased muscle mass may be linked to the physical presence of parasites in the gut. In healthy guinea pigs, surgically induced stenosis in the small bowel causes an increase in the circumference of the surrounding gut and a thickening of the muscle wall (Gabella, 1979). Presumably this hypertrophy follows a period of intestinal distension as luminal contents are forced through the constricted bowel. The presence of worms that form a relatively large mass in the small intestine may increase the pressure on the gut wall and induce hypertrophy in a similar manner. For example, Symons (1957) noted an apparent dilatation of the upper jejunum that was "markedly congested" in *Nippostrongylus*-infected rats on the fifth day of infection. It was then observed that "towards the fifteenth day the jejunal wall appeared flabby and thickened rather than distended" (Symons, 1957). Although no worms are present in the small intestine of *S. mansoni*-infected mice, an analogous pressure change affecting the bowel wall might be caused by an increased amount of inflammatory tissue associated with the parasite ova.

Chemical mediators of inflammation may also be involved in the hypertrophy of gut muscle (Castro, 1989). PDGF (platelet-derived growth factor), in particular, is thought to play a role in the proliferation of smooth muscle cells and fibroblasts at sites of wound repair (reviewed by Deuel *et al.*, 1991). This growth factor is also strongly implicated in uterine smooth muscle hypertrophy during pregnancy (Mendoza *et al.*, 1990).

That PDGF plays an integral role in hypertrophy of the *muscularis externa* in *S. mansoni*-infected mice seems very possible. Platelets and inflammatory cells are known to aggregate around schistosome eggs in mesenteric vessels after release from the adult worm, and it has been suggested that lytic products released from these inflammatory responses may facilitate transport of the egg from the mesenteric vessels to the intestinal lumen (Doenhoff *et al.*, 1986). Release of PDGF at this stage would attract a variety of cells including monocytes, neutrophils and fibroblasts (Deuel *et al.*, 1982; Seppa *et al.*, 1982; Siegbahn *et al.*, 1990). Some of the cellular constituents of the granuloma may therefore be present prior to schistosome eggs leaving the mesenteric vessels, and PDGF may play an important part in co-ordinating the first stages of ova excretion. Entry of ova into the gut wall causes further damage, which would attract more platelets to the site of injury. As PDGF is also a chemoattractant for smooth muscle cells (Grotendorst *et al.*, 1981), hyperplasia of intestinal musculature may be part of the host repair mechanism in response to granulomatous damage.

A recent study of rabbits infected with a Baling strain of *S. malayensis* would seem to confirm the intimate association of granulomatous reactions with the thickening of smooth muscle. Shekhar and Pathmanathan (1992) found numerous miliary granulomas in the lungs of infected rabbits, and demonstrated pathological changes in the pulmonary arterioles, with many of the blood vessels showing medial hypertrophy. On the rare occasion that granulomata encroached upon the pulmonary artery, muscular thickening of this vessel was also observed.

The above hypothesis for smooth muscle hyperplasia in the gut of *S. mansoni*-infected mice would explain localised thickening in the intestinal wall. The more generalised thickening of intestinal smooth muscle observed in mice with *S. mansoni* might be brought about by stretching of adjacent tissue, perhaps inducing hypertrophy by a non-immune mechanism such as a paracrine response to pressure changes in the local cellular environment. This may be similar to the surgically induced hypertrophy in healthy guinea pigs, where the eventual hypertrophy develops over most

of the length of the small intestine, but remains maximal in regions nearest the stenosis (Gabella, 1979). As the schistosome-damaged gut must be more rigid due to the presence of granulomas and resulting fibrosis, the general thickening of the small intestine may allow the dynamic mixing and propulsive forces of this organ to be maintained, at least to a certain extent.

4.3.2. Intestinal motility

The structural changes in the intestinal musculature of *S. mansoni*-infected mice observed in this study may be associated with an impairment of motor function in the small intestine. Although a variety of parasites induce changes in the smooth muscle structure of the small intestine, motility patterns associated with this hypertrophy appear only to have been examined in trichinized rats (Castro *et al.*, 1976). In these animals, intestinal transit was increased significantly by infection. This would have the effect of decreasing the time available for digestion and absorption of nutrients, and may therefore influence the nutritional status of the host.

There is an indication that intestinal transit time may be related to the selective malabsorption of nutrients in humans with schistosomiasis mansoni (Pucci *et al.*, 1978). Previous studies of intestinal function in *S. mansoni*-infected mice appear to have been limited to the absorption of nutrients (DeWitt, 1957; Domingo and Warren, 1969; Vengesa and Leese, 1979). However, Varilek *et al.* (1991) observed that schistosome-induced granulomas caused extensive damage to the myenteric plexus and nerves supplying the circular muscle layer in the ileum of mice, and the authors concluded that impairment of motor function was a strong possibility in these mice.

Intestinal motility was investigated in the present study by measuring the transit distance of barium through the small intestine of infected and control mice. The transit distance of barium in the infected mice did not differ significantly from that of uninfected mice, suggesting that motility patterns were not affected by gut pathology. These results may, however, be invalid for two main reasons. Firstly, although a pilot

investigation revealed that a 20-minute period resulted in barium passing approximately half way along the small intestine in uninfected mice, three control mice had no trace of barium within this organ, and had only a trace of barium within the stomach. Although mice received equal doses of the barium sulphate test solution, it appeared that they did not consume equal amounts of barium. It was felt likely that these observations resulted from uneven distribution of barium in the test solution, although every attempt had been made to avoid this problem. Secondly, the measurement of stomach dry weights demonstrated that infected mice had a significantly reduced tissue mass in this organ. If the lower stomach weight reflects a decrease in stomach capacity, this may influence the stomach emptying rate (reviewed by Low, 1990) and therefore have an affect upon intestinal transit time. In light of the neural damage observed in the small intestine of *S. mansoni*-infected mice (Varilek *et al.*, 1991), and the hypertrophy of the *muscularis externa* observed in this study, this aspect of gut function merits further investigation.

4.3.3. Body weight distribution

In all the experiments described in this chapter, mice were selected on a similar body weight basis for alternate allocation to control or infected experimental protocols. This allowed for further investigation of the body weight redistribution in response to infection that was first examined in Chapter 3.

The terminal body weights of mice did not differ significantly in any of the experiments described, confirming the observation that overall growth rate does not differ between infected and uninfected mice (see Chapter 3). Carcass weight was measured directly after removal of spleen, liver, small intestine and caecum, rather than the indirect estimation described in the previous chapter, and a variety of changes were observed. Mice in the ninth and sixteenth weeks of *S. mansoni* infection had significantly reduced carcass weights, and elevated spleen and liver weights. The degree of hypertrophy observed in the *muscularis externa* of these mice would presumably indicate an increase in the weight of this organ as well, although no quantification was made.

The reduced carcass weight of infected mice demonstrates a distinct redistribution of body weight away from skeletal mass towards certain visceral organs. Evidence suggests that this reduction of skeletal mass may be due to proteolysis of skeletal muscle. During sepsis, proteolysis of skeletal muscle provides a massive outpouring of amino acids to supply the liver with amino acids for acute phase reactant synthesis (Fischer, 1991). That this is also the case in parasitic infection seems very probable. During rat nippostrongylosis, a decrease in gastrocnemius muscle weight is observed (Ovington, 1986). An elevation of serum amino acid concentrations, including alanine and glutamine, is also observed during the course of this infection (Ash *et al.*, 1985), as is an increase in liver weight when infected rats are compared to pair-fed controls (Ovington, 1986). Increased biosynthesis in the liver in *Nippostrongylus*-infected rats at a cost to other tissues such as skeletal muscle was thought "part of a purposeful redistribution of a host's resources" (Ovington, 1986).

The same mediator may be responsible for the postulated muscle proteolysis in *S. mansoni*-infected mice as in sepsis or rat nippostrongylosis. Tumour necrosis factor (TNF) appears to be an important regulator of muscle proteolysis during sepsis (Fischer, 1991). The administration of TNF to rats reduces carcass nitrogen content and increases liver protein content (Mealy, 1990), increases the net efflux of alanine and glutamine from muscle and appears to stimulate their uptake by other tissues (Warren *et al.*, 1987). The remarkably similar responses of rats to administration of TNF or infection with *Nippostrongylus* suggests that this cytokine plays an important part in protein metabolism during a variety of infections, and TNF may well be responsible for reduced carcass weight in the *S. mansoni*-infected mouse.

No change in carcass weight was observed in mice exposed to single-sex infections. As the increase in visceral organ mass in infected animals appears to be due largely to the granulomatous response to schistosome ova, this result was expected. The significant increase in spleen weight is probably due to antigenic stimulation by adult worms that causes lymphoreticular hyperplasia (Dumont *et al.*, 1975). The greater

carcass weight in mice treated with praziquantel indicates either that carcass weight is reduced to a greater degree as the infection progresses, or that chemotherapy rapidly corrects the redistribution of body mass seen during infection. Liver weights for infected and treated mice were not significantly different, perhaps indicating that some aspects of pathology take longer to recede.

4.3.4. Conclusions

Smooth muscle in the small intestine of *S. mansoni*-infected mice becomes hypertrophied a few weeks after the infection reaches patency. From a nutritional perspective, the diversion of resources into the synthesis of this extra protein must be costly. The increased cost of tissue synthesis may, however, be outweighed by the maintenance of mixing and propulsive functions in the small intestine, although motility patterns may be affected. The source of reserves directed into the formation of this extra muscle, and into the inflammatory reaction to eggs in the liver, appears to be skeletal muscle. The costs and benefits of the diversion of energy resources into granulomatous reactions have been discussed previously (Chapter 3).

The implications of these results in relation to human disease must be treated with caution, but it is certainly possible that similar observations might apply. For example, hepatosplenomegaly and granulomatous damage in the gut are pathological features of human schistosomiasis mansoni. Energy reserves must be directed into these responses, and it is likely that skeletal muscle is the source in both humans and mice. A wasting of skeletal muscle might help to explain the symptoms of fatigue and weakness in clinical disease. In particular, a reduction of skeletal muscle mass might explain why a reduction in physical fitness has been noted in boys infected with *S. mansoni* that was not accompanied by anaemia, or a difference in weight for height measurements between infected and uninfected children (Hiatt and Gebre-Medhin, 1977). In addition, weight for height measurements should be treated with caution if taken as a direct comparison of

health and nutritional status, as they do not take into account any redistribution of body weight that may accompany infection.

Thickening of the gut wall may have a further implication for human studies of schistosomiasis. The only feasible method of estimating prevalence and intensity of infection is to measure the concentration of eggs in faeces. Thickening of the gut wall in heavy infections may decrease the number of eggs released in stool, thus resulting in a misleading estimation of intensity of infection.

4.4. Summary

1. In the ninth week of *S. mansoni* infection in mice, the smooth muscle area of the *muscularis externa* was greater than in uninfected, control mice. This increase in muscle area was due largely to muscle thickening, but was also a product of increased intestinal diameter in the most posterior region of the small bowel. The hypertrophy of smooth muscle in the intestines of infected mice occurred in all regions of the bowel that were examined, but was most severe in distal sites.

2. The absence of smooth muscle hypertrophy in the intestines of *S. mansoni*-infected mice after six weeks of infection indicated that a patent infection was required for two to three weeks before this aspect of pathology developed.

3. Hypertrophy of intestinal smooth muscle did not occur in mice exposed to single-sex cercarial infections.

4. Smooth muscle hypertrophy was a prominent feature of pathology later in the course of *S. mansoni* infection in mice (16 weeks post infection).

5. Treatment of infected mice with praziquantel reduced intestinal muscle hypertrophy relative to untreated mice in the posterior region of the small intestine.

6. Transmission electron microscopy revealed that invaginations were present in the membranes of intestinal muscle cells in *S. mansoni*-infected mice.

7. The transit times for barium to pass through the intestines of infected and control mice were compared, but problems with the experimental technique meant that no conclusions could be made about motility patterns.

8. A redistribution of body weight away from skeletal mass towards certain visceral organs was observed in *S. mansoni*-infected mice in the ninth week of infection and after 16 weeks of infection. A similar pattern was observed for infected mice when compared to infected mice that were subsequently treated with praziquantel, but not for mice exposed to single-sex cercarial infection.

Chapter 5

Intestinal pathology: II. The absorptive surface

5.1. Introduction

The structure of the small intestine is integral to its success as an organ that digests and absorbs nutrients. The luminal surface of the small bowel is organised to maximise the surface area available for contact with the intestinal contents. This is achieved through the presence of numerous mucosal villi which amplify the absorptive surface to a 7 - 14 fold increase over that of a simple tube structure (Madara and Trier, 1987). The surface of a villus is covered mainly by enterocytes, the columnar absorptive cells of the small intestine. These cells are covered with microvilli that further enhance the absorptive area of the small bowel by approximately 14 - 40 fold (Madara and Trier, 1987).

Microvilli do more than simply increase the absorptive area of the small intestine. Associated with these plasma membrane extrusions is a surface coat or glycocalyx. A variety of digestive processes are localised in the glycocalyx-microvillus complex, with enzymes including disaccharidases and peptidases being associated with the cell membrane (Eichholz, 1968; Kim and Perdomo, 1974; Crane, 1975).

Disruption of the intestinal architecture frequently accompanies enteric helminth infections. The parasites capable of inducing intestinal smooth muscle hypertrophy (see Chapter 4) may also initiate changes in the mucosal properties of the gut. For example, *Nippostrongylus brasiliensis* infection in rats causes a flattening of the mucosa and disturbance of microvillus structure (Symons *et al.*, 1971; Symons, 1976; Martin, 1980; Cheema and Scofield, 1982). These changes in mucosal structure are accompanied by functional disturbances including altered intestinal permeability (Cobden *et al.*, 1979; Nawa, 1979), and malabsorption (Scofield, 1980; Nolla *et al.*, 1985). *Trichinella spiralis* infections in rodents may cause villous atrophy

(Manson-Smith *et al.*, 1979) and a shortening of microvilli, which may also be reduced in number (Lin and Olsen, 1970). This infection may also depress intestinal disaccharidase activity and cause malabsorption (Castro *et al.*, 1979).

During human schistosomiasis mansoni, parasite eggs may be deposited in the small intestine in large numbers (Cheever, 1969; Cheever *et al.*, 1977). Despite this observation, small-bowel disease is not commonly reported (Chen and Mott, 1988), and few studies have investigated the structure of this organ during schistosomiasis. One biopsy study in Sudan indicated that a significant broadening and shortening of villi occurred in the jejunum of subjects with active *S. mansoni* infection (Fedail and Gadir, 1985). However, these researchers concluded that it was unlikely that egg-associated pathology would significantly affect nutrient uptake because parasite ova occupied only small areas of the intestine. Jejunal structure has also been examined in Egyptian farmers infected with *S. mansoni* (Halsted *et al.*, 1969). The villi from several of the biopsy specimens were wider and shorter, but the villous structure from other samples appeared entirely normal (Halsted *et al.*, 1969). Again it was concluded that a lack of specific abnormality in intestinal structure would result in a maintenance of bowel function.

Cheever (1969), with reference to *S. mansoni* infections, has noted that "there are no "light" infections in mice, but only less massive and more massive infections." In light of the changes observed in intestinal architecture during human schistosomiasis, it is therefore surprising that relatively "massive" infections of *S. mansoni* in mice appear to have little affect upon the small intestine structure. In the small bowel of mice infected with 1-3 worm pairs for 20 weeks, villi adjacent to granulomas occasionally appeared flattened, but were mostly unaffected (Domingo and Warren, 1969). No significant change in the absorptive capacity of the small bowel was observed in these mice, and this maintenance of function was attributed to the focal nature of granulomatous damage. However, changes in the absorptive capacity of the small intestine of mice infected with *S. mansoni* have been reported, albeit in mice with

heavier infections (DeWitt, 1957; Vengesa and Leese, 1979). This would be suggestive of villous damage with higher intensities of infection.

This anomaly, with the human small intestine having a lower concentration of *S. mansoni* ova (Cheever, 1969) but signs of villous damage, and mice having a higher concentration of parasite eggs in the small bowel (Cheever, 1969) but with few signs of a disturbed villous architecture, prompted the present investigation. The objectives of this study were: (1) To quantify the absorptive surface area of villi in *S. mansoni*-infected mice, (2) to investigate the aetiology of any changes in villous architecture in infected mice, (3) to examine enterocyte structure in the small intestine of infected mice, and (4) to assess the functional significance of any intestinal damage in relation to intestinal permeability.

5.2. Results

When compared to uninfected, control mice, *S. mansoni*-infected mice in the ninth week of infection were found to have larger small intestine lumen perimeters. These increases were significant in the posterior regions of the gut (75% region: Mann-Whitney, $W = 77.0$, $P < 0.005$; 100% region: Mann-Whitney, $W = 55.0$, $P < 0.05$) but not in the proximal regions (25% region: Mann-Whitney, $W = 50.0$, $P > 0.05$; 50% region: Mann-Whitney, $W = 56.0$, $P > 0.05$) (Fig. 5.1). There was no significant increase in lumen perimeter when this parameter was expressed as a unit of the submucosal perimeter (25% region: Mann-Whitney, $W = 47.0$, $P > 0.05$; 50% region: Mann-Whitney, $W = 48.0$, $P > 0.05$; 75% region: Mann-Whitney, $W = 34.0$, $P > 0.05$; 100% region: Mann-Whitney, $W = 33.0$, $P > 0.05$) (Table 5.1). At six weeks post infection, the lumen perimeters of infected mice were slightly, but significantly, increased in the most distal region of the small bowel (Mann-Whitney, $W = 70.0$, $P < 0.05$) but not in the anterior region (Mann-Whitney, $W = 61.0$, $P > 0.05$) (Fig. 5.2). A longer duration of infection (16 weeks) resulted in a significant increase of small intestine lumen perimeter in both the anterior (Mann-Whitney, $W = 69.0$, $P < 0.05$) and the posterior (Mann-Whitney, $W = 73.0$, $P < 0.05$) regions of the bowel (Fig. 5.3).

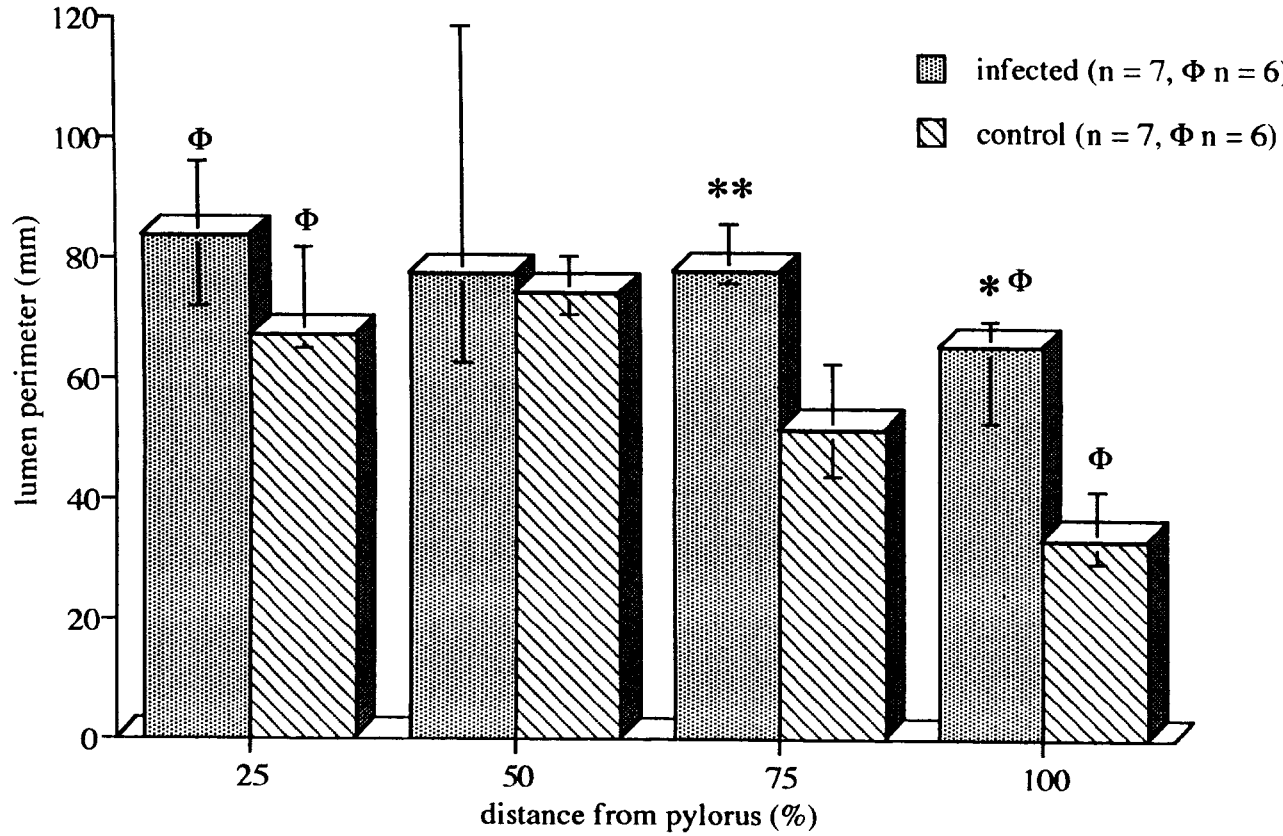


Figure 5.1. Lumen perimeter (mm) (median values and interquartile ranges) for mice in the ninth week of *S. mansoni* infection and for uninfected, control mice. (* $P < 0.05$, ** $P < 0.005$)

Table 5.1. Lumen perimeter (mm), expressed per millimeter of small intestine submucosal perimeter, for uninfected, control mice (n = 6) and *S. mansoni*-infected (n = 6) mice in the ninth week of infection (median values).

Distance from pylorus (%)	Uninfected control mice	*S. mansoni*-infected mice
25	5.27	6.27
50	6.29	6.75
75	6.36	5.6
100	6.69	5.99

Table 5.2. Lactulose / mannitol ratios for *S. mansoni*-infected (n = 8) and uninfected, control mice (n = 8) at 10 weeks post infection. (median values)

	Uninfected control mice	*S. mansoni*-infected mice
Lactulose/ mannitol ratio	1.58	1.75

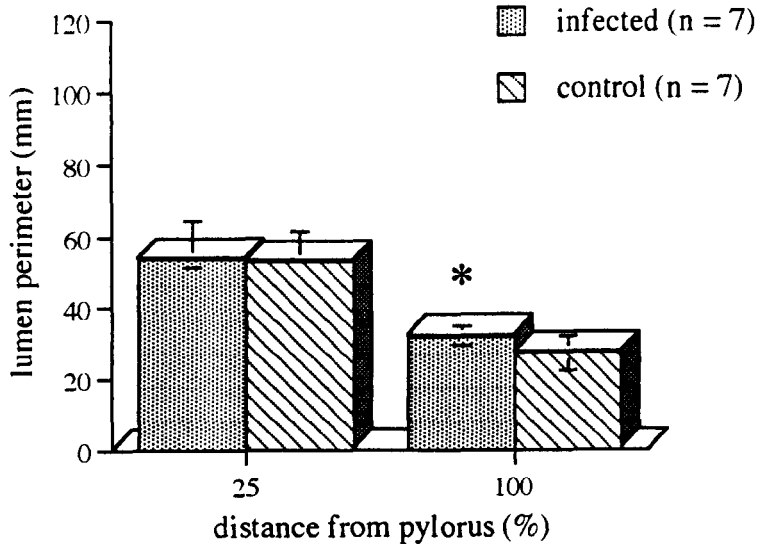


Figure 5.2. Lumen perimeter (mm) (median values and interquartile ranges) for *S. mansoni*-infected mice at 6 weeks post infection and uninfected, control mice. (* $P < 0.05$)

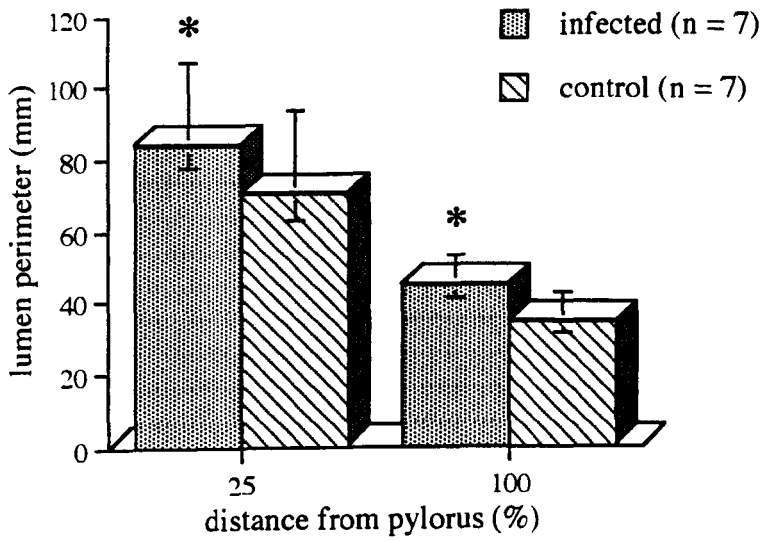


Figure 5.3. Lumen perimeter (mm) (median values and interquartile ranges) for *S. mansoni*-infected mice at 16 weeks post infection and uninfected, control mice. (* $P < 0.05$)

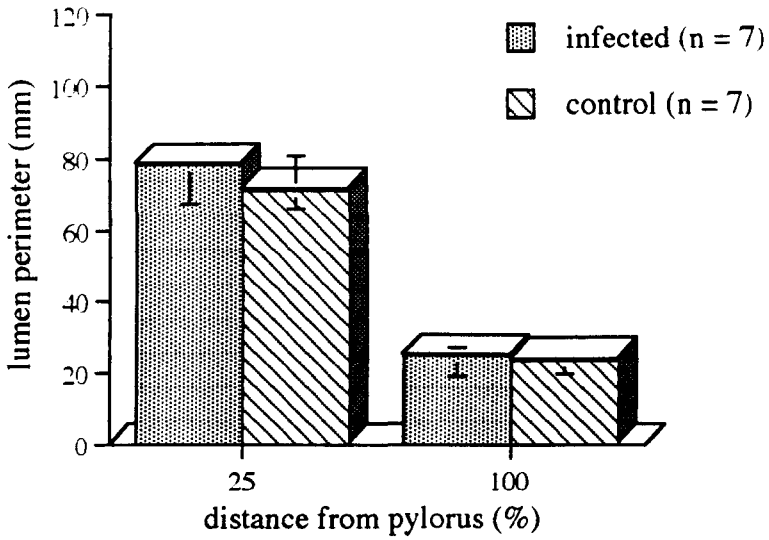


Figure 5.4. Lumen perimeter (mm) (median values and interquartile ranges) of the small intestines of uninfected, control mice and mice exposed to single-sex *S. mansoni* cercariae.

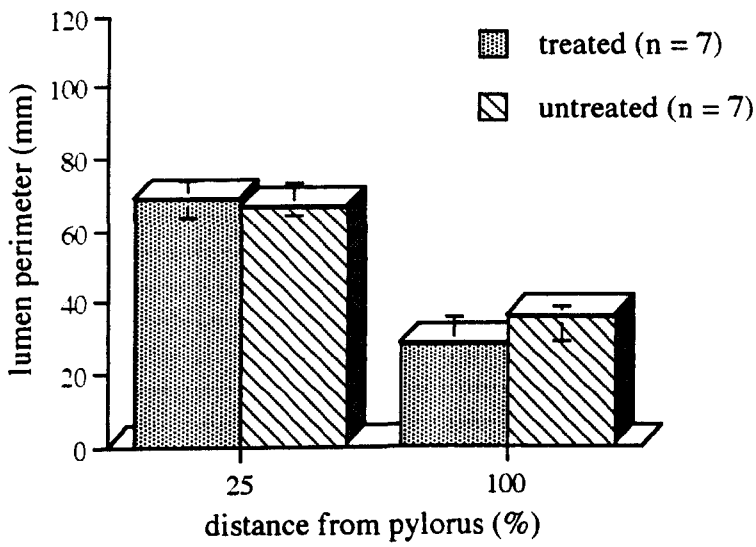
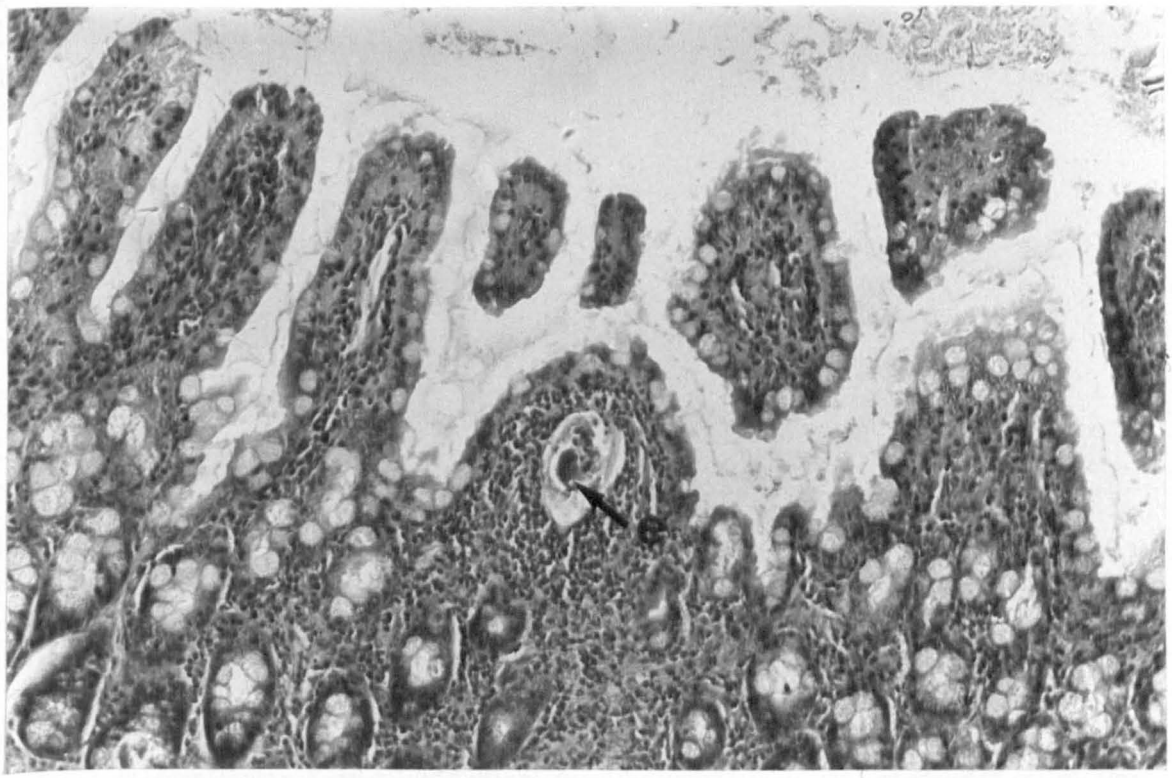


Figure 5.5. Lumen perimeter (mm) (median values and interquartile ranges) of the small intestines of *S. mansoni*-infected mice, and *S.mansoni*-infected mice subsequently treated with praziquantel.

Plate 5.1. Transverse section from the distal end of the small intestine from an uninfected, control mouse showing the typical villous structure for this region of the gut. ——— 50 μm

Plate 5.2. Transverse section from the distal end of the small intestine from an *S. mansoni*-infected mouse in the ninth week of infection. The increase in villous length is representative of the villous hypertrophy that was observed to occur in the posterior region of the bowel in infected mice.
——— 50 μm

Plate 5.3. Transverse section from the distal end of the small intestine from an *S. mansoni*-infected mouse in the ninth week of infection showing the broadening and/or fusion of villi.
e - *S. mansoni* egg in the intestinal mucosa. ——— 50 μm



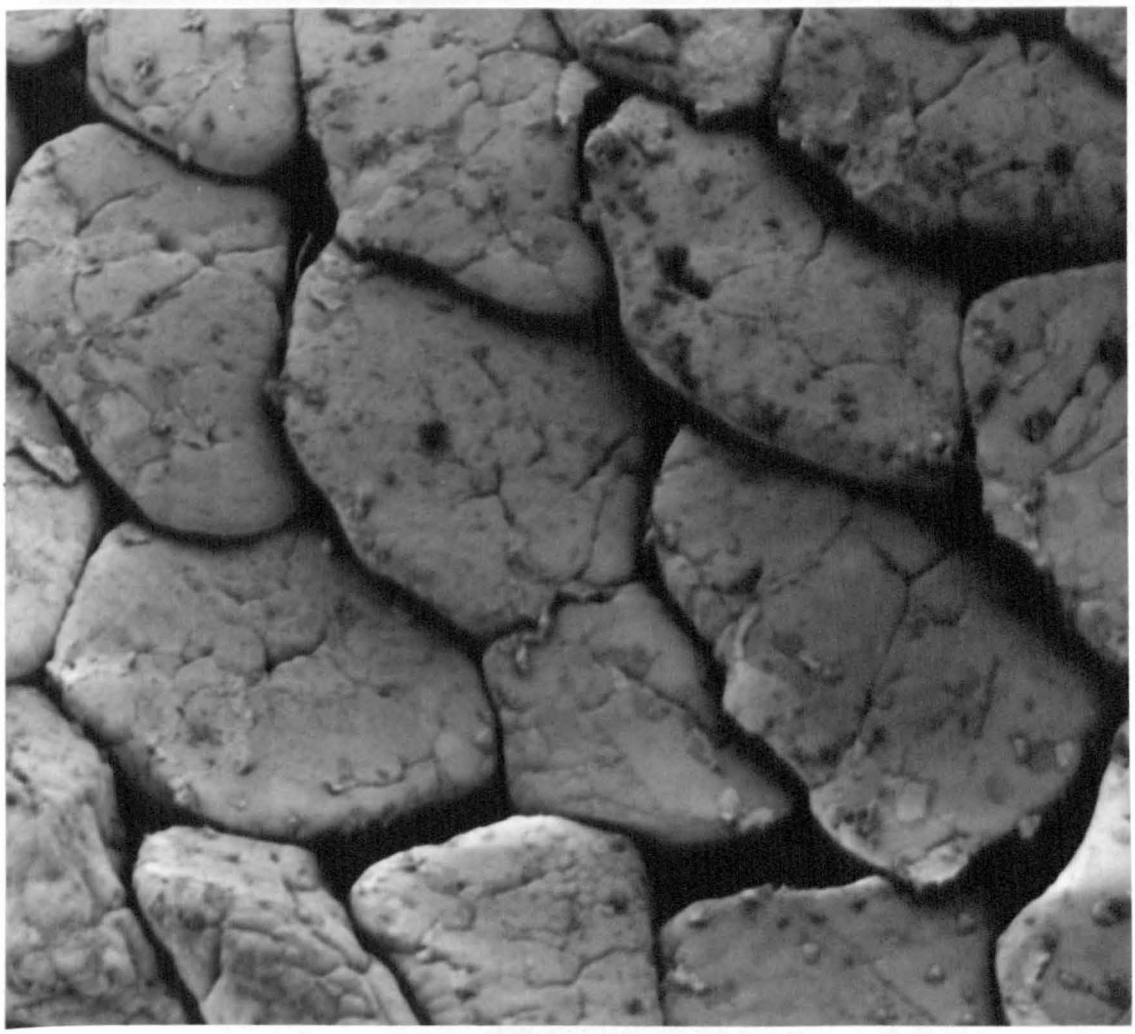


Plate 5.4. Scanning electron micrograph showing the mucosal surface of the distal region of the ileum from an uninfected mouse.

——— 25 μm

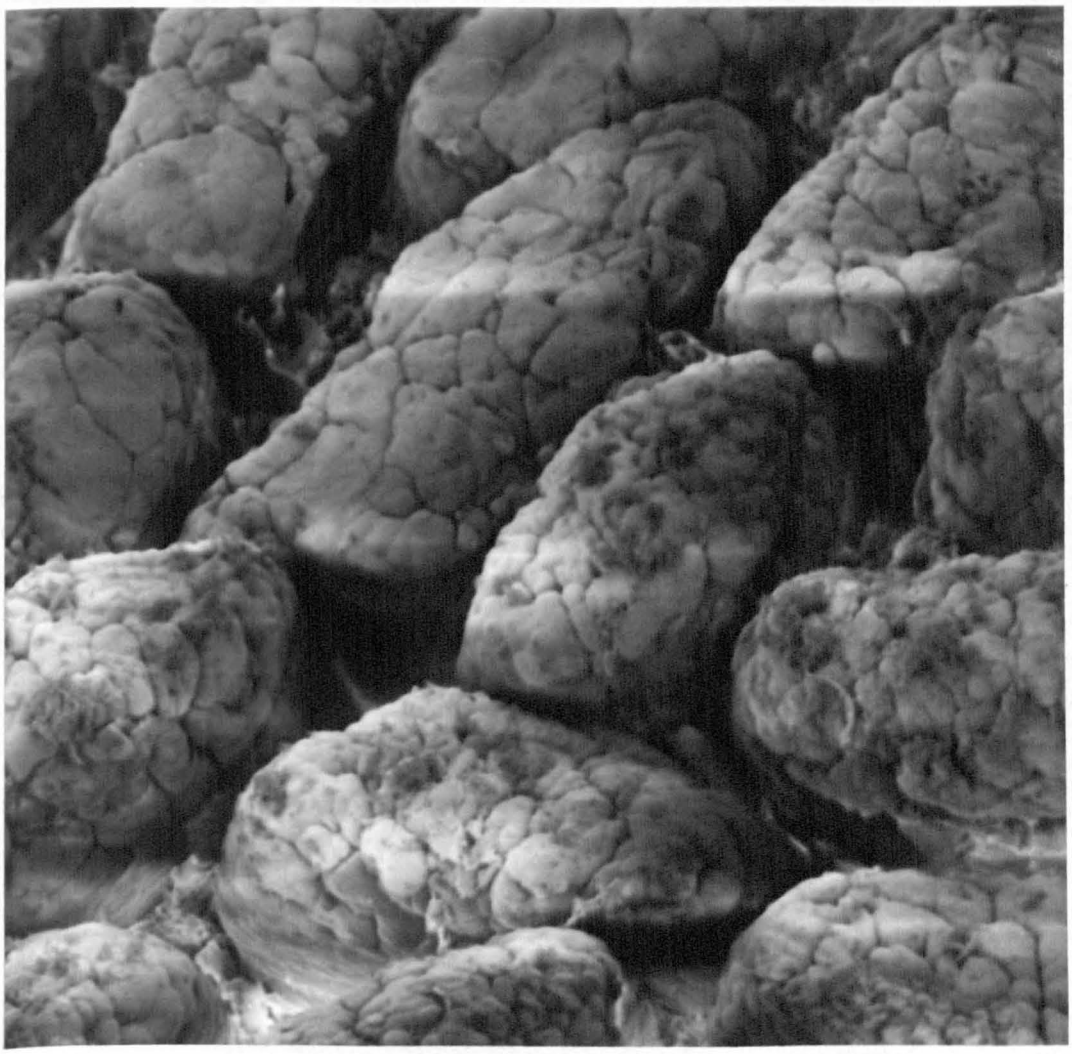


Plate 5.5. Scanning electron micrograph showing the mucosal surface of the distal region of the ileum from a mouse in the ninth week of *S. mansoni* infection.

——— 25 μm

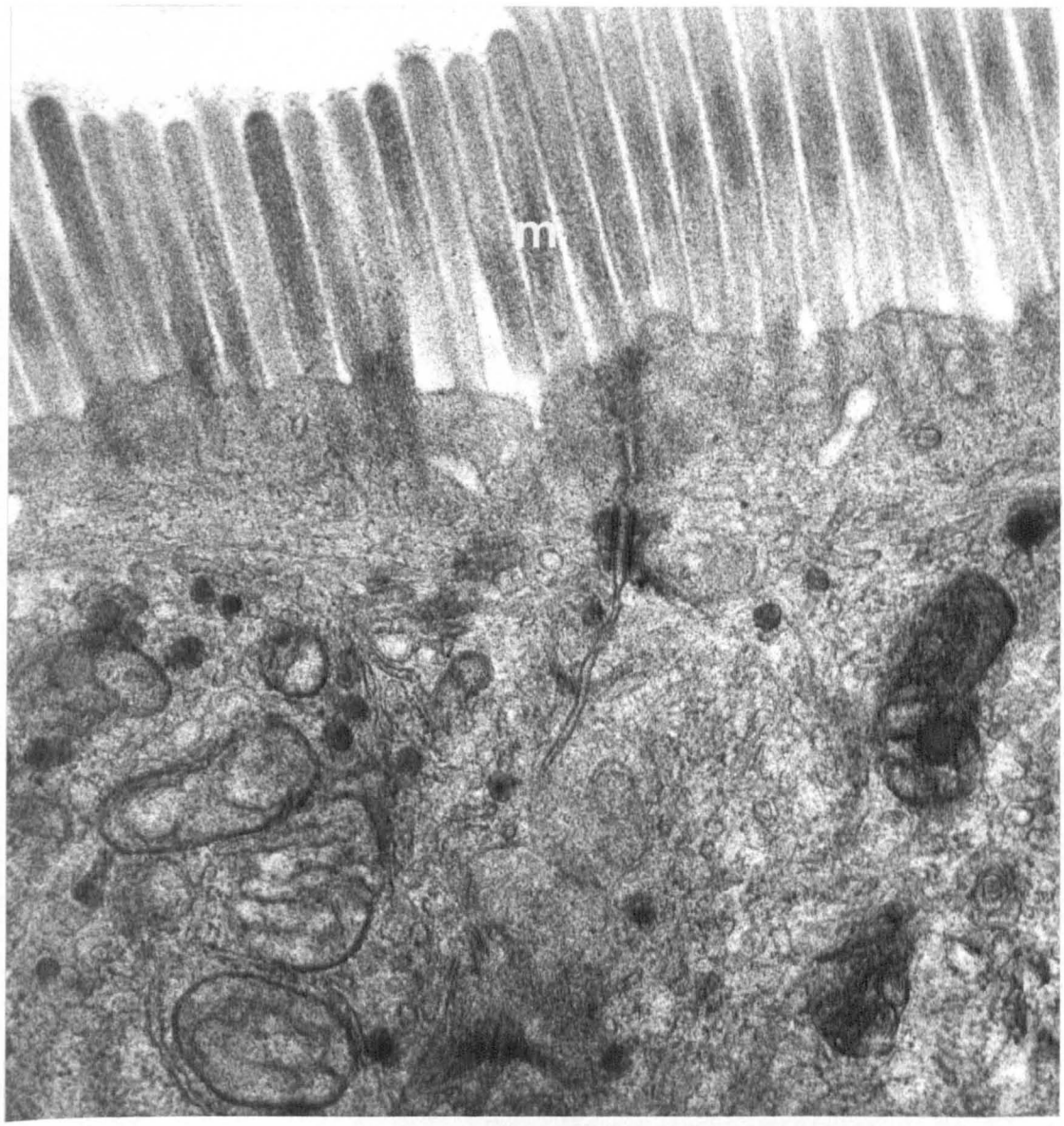


Plate 5.6. Transmission electron micrograph of the surface of two adjacent enterocytes from the intestine of an uninfected, control mouse.
(**m** - microvillus) ——— 0.5 μm

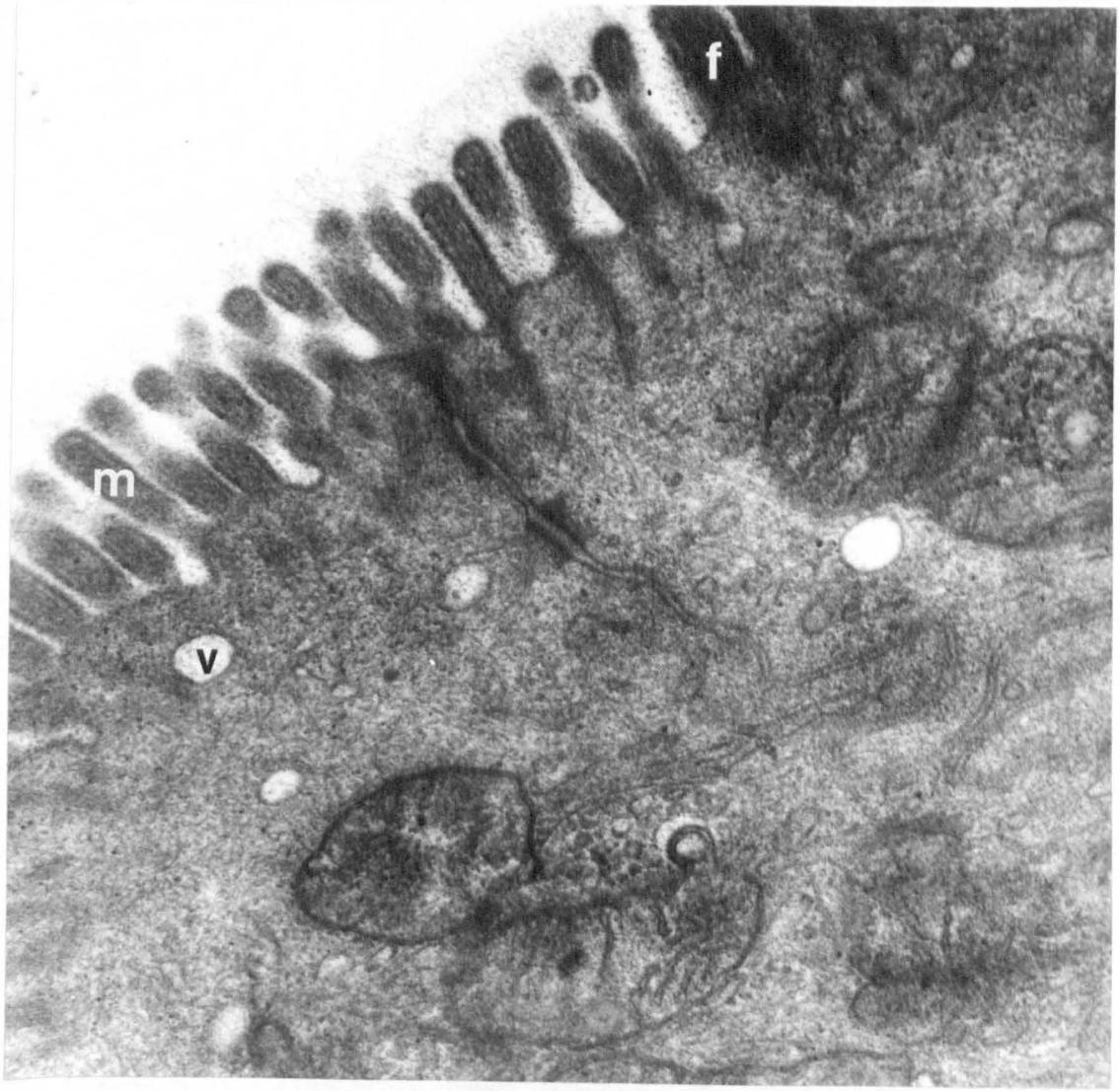


Plate 5.7. Transmission electron micrograph of the surface of two adjacent enterocytes from the intestine of an *S. mansoni*-infected mouse in the ninth week of infection.
(**m** - microvillus, **f** - possible fusion of microvilli, **v** - vacuole)
——— 0.5 μm

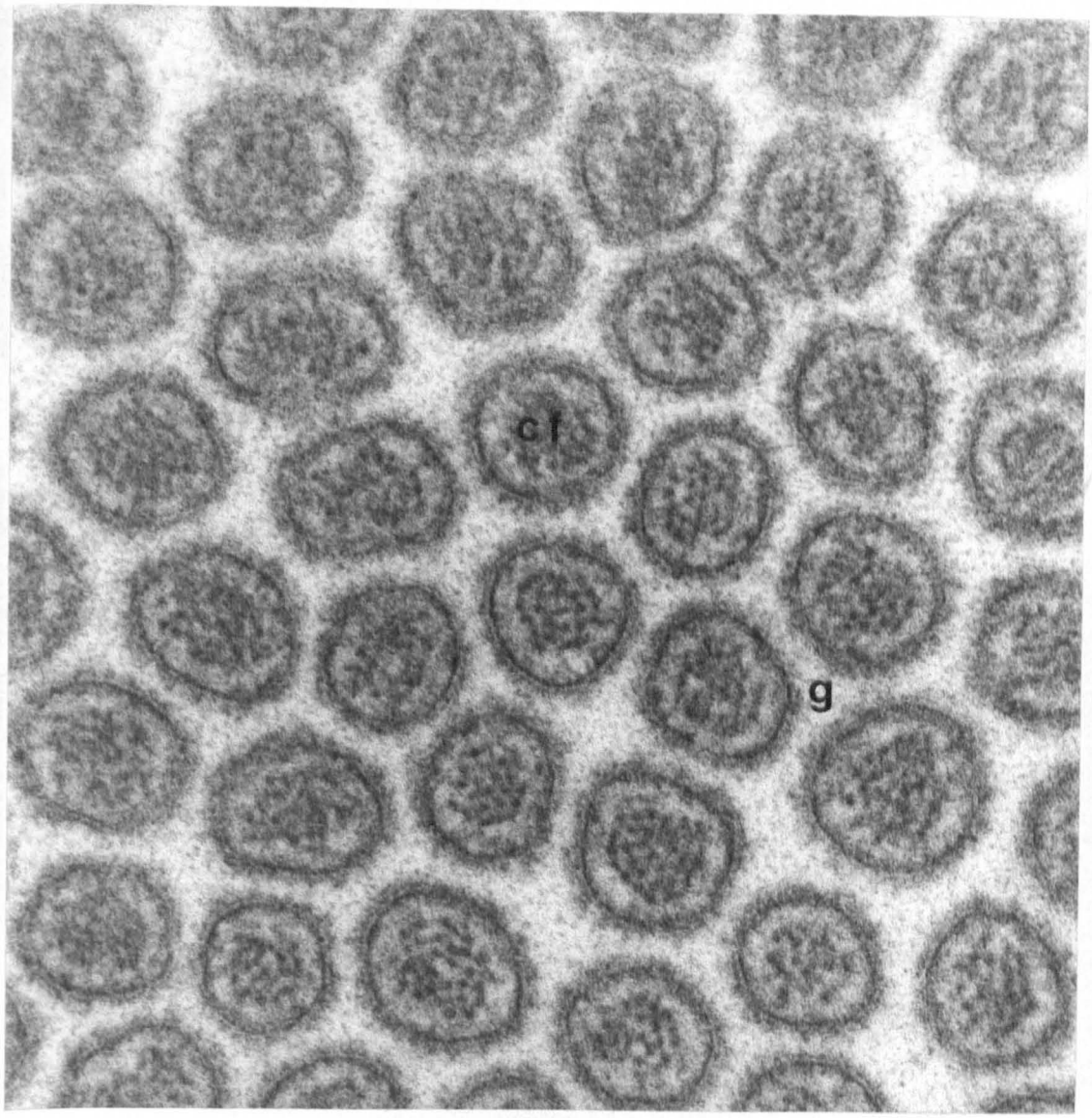


Plate 5.8. Transmission electron micrograph showing the cross section of microvilli from the intestine of an uninfected, control mouse.
(**g** - glycocalyx, **cf** - core filaments of microvillus) ——— 0.1 μm

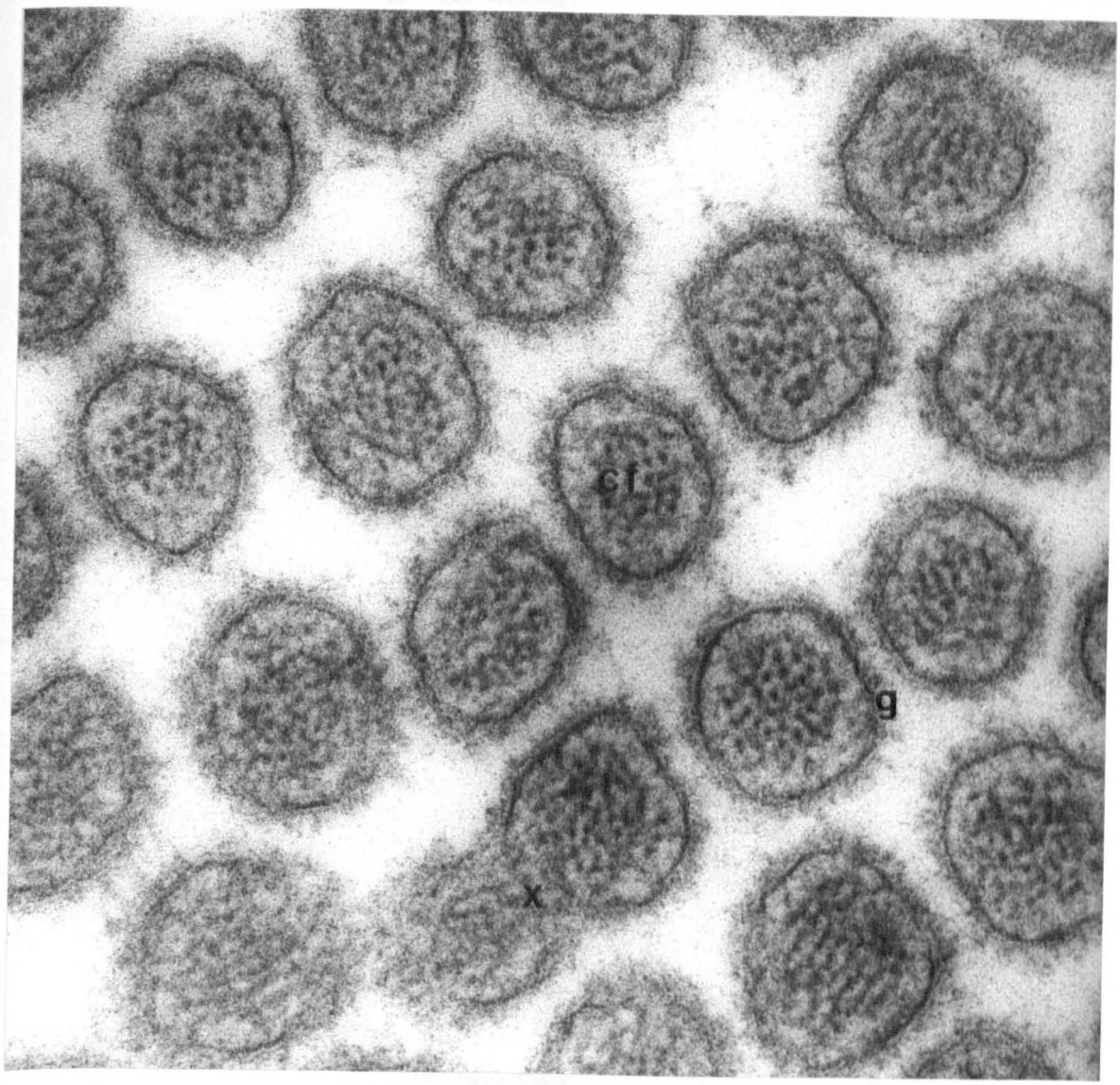


Plate 5.9. Transmission electron micrograph showing the cross section of microvilli from the intestine of an *S. mansoni*-infected mouse in the ninth week of infection. (**g** - glycocalyx, **cf** - core filaments of microvillus, **x** - possible fusion of microvilli) ——— 0.1 μm

Table 5.3. Total urinary lactulose recovered from uninfected, control mice (n = 8) and *S. mansoni*-infected mice (n = 8) at 10 weeks post infection. The percentage dose recovery is also shown. Values are medians, shown with interquartile ranges (Q1 and Q3).

	Total urinary lactulose (μg) (% dose recovered)	Q1	Q3
S. mansoni infected mice	28.5 (0.075)	7.0	54.8
Uninfected, control mice	12.5 (0.03)	4.3	21.0

Table 5.4. Total urinary mannitol recovered from uninfected, control mice (n = 8) and *S. mansoni*-infected mice (n = 8) at 10 weeks post infection. The percentage dose recovery is also shown. Values are medians, shown with interquartile ranges (Q1 and Q3).

	Total urinary mannitol (μg) (% dose recovered)	Q1	Q3
S. mansoni infected mice	10.5 (0.105)	4.0	28.5
Uninfected, control mice	7.5 (0.075)	2.8	23.3

These observations appeared to reflect an overall increase in the villous surface area of the small intestine of infected mice, as intestine length was not observed to differ between control (median length = 555 mm) and infected mice (median length = 583 mm) at 16 weeks post infection (Mann-Whitney, W = 138.5, P > 0.05). Small intestine length was not measured at other stages of infection.

Infection of mice with single-sex *S. mansoni* cercariae did not result in an increase in lumen perimeter in the small bowel (25% region: Mann-Whitney, W = 58.0, P > 0.05; 100% region: Mann-Whitney, W = 58.0, P > 0.05) (Fig. 5.4). Treatment of infected mice with praziquantel at eight weeks post infection had no significant affect upon lumen perimeter when treated and infected mice were compared 16 weeks after infection (25% region: Mann-Whitney, W = 55.0, P > 0.05; 100% region: Mann-Whitney, W = 43.0, P > 0.05) (Fig. 5.5).

Light microscopy revealed villous hypertrophy in the small intestine of infected mice in the ninth week of infection when compared to uninfected mice (Plates 5.1 and 5.2). Areas of villous fusion and / or broadening were also observed in infected mice at 16 weeks post infection (Plate 5.3). The fine detail of the villous surface in the region of the small intestine adjacent to the caecum is shown for infected and control mice in the ninth week of infection (Plates 5.4 and 5.5). In uninfected mice. the fine grooves that cross the villi form a mosaic pattern, and partially outline the luminal surface of the enterocytes. The villous surface of infected mice was irregular, with the grooves between the enterocytes appearing more pronounced. The villi of infected mice appeared larger, again indicating hypertrophy of these structures, and appeared to be less compact in their spacing.

Transmission electron micrographs show the longitudinal enterocyte structure in infected and control mice in detail (Plates 5.6 and 5.7). The most obvious difference is the stunted and irregular appearance of the microvilli in infected animals. Additional abnormalities included the presence of vacuoles in the cytoplasm and an apparently diminished glycocalyx. The irregularity in microvillus structure can also be seen

clearly in cross section (Plates 5.8 and 5.9). The distribution of microvilli was less dense in infected mice, and the glycocalyx did not form a continuum between microvilli as in control animals. There was also some evidence of microvillus fusion in infected mice.

The lactulose / mannitol ratio was similar for infected and uninfected mice at ten weeks post infection (Mann-Whitney, $W = 77.0$, $P > 0.05$) (Table 5.2). As the total recoveries of lactulose and mannitol did not differ significantly between the two experimental groups (lactulose: Mann-Whitney, $W = 79.5$, $P > 0.05$, mannitol: Mann-Whitney, $W = 72.0$, $P > 0.05$) (Tables 5.3 and 5.4), this indicated that no significant alteration of intestinal permeability was induced by *S. mansoni* infection in mice.

5.3. Discussion

5.3.1. Murine schistosomiasis mansoni

The results presented in this chapter indicate that the villous surface area of the small intestine increases as a response to patent *S. mansoni* infections in mice. This effect was observed locally in the posterior region of the small intestine during the early stages of patency, but appeared to become more generalised along the length of this organ later in the course of infection.

The observed increase in villous surface area was not directly related to villous hypertrophy, as the lumen perimeter did not increase significantly when expressed as a function of the submucosal perimeter. This suggested that the enhancement of villous surface area was related to a dilatation of the of the small bowel (see Chapter 4). Although villous hypertrophy would act to increase the surface area of the gut, it appears that this effect was offset by villous fusion and / or broadening which would have the opposite effect upon surface area.

Egg-associated damage in the small intestine of infected mice would appear to

be linked to the enlargement of villous surface area, as no change in lumen perimeter was observed in single-sex *S. mansoni* infections. The slight increase in villous surface area in infected mice at six weeks post infection, when compared to the much larger increase in surface area in the distal small intestine of mice infected for longer periods, provides further evidence for this idea. Treatment of infected animals with praziquantel did not bring about a reduction of surface area within the time scale of the study.

Electron microscopy demonstrated further changes in the structure of the small intestine in infected mice. Scanning electron microscopy (SEM) provided evidence for a reduced density of villi, and indicated that swelling of individual enterocytes was a possibility, as denoted by their more pronounced outline. Transmission electron microscopy (TEM) revealed further changes in enterocyte structure. The most notable changes were a shortening of microvilli, and a widening of these membranous structures that seemed to be caused, at least in some areas, by microvillus fusion. The glycocalyx appeared reduced, both by the width of this surface coat, and through the reduced surface area of the microvilli. The samples for TEM were taken from the proximal end of the small intestine. As smooth muscle hypertrophy (Chapter 4) and the increase in intestinal surface area associated with *S. mansoni* infection were found to be more pronounced in the distal region of the small bowel, this might suggest that enterocyte structure could be more extensively altered in the posterior regions of the small intestine.

The observations made using TEM in the current study are similar to changes noted in the small intestines of *Nippostrongylus*-infected rats (Symons *et al.*, 1971). The decreased microvillus surface area observed during rat nippostrongylosis, however, is accompanied by villous atrophy and fusion (Symons and Fairbairn, 1962; Martin, 1980; Cheema and Scofield, 1982), rather than the increase in the surface area of villi that was observed in *S. mansoni*-infected mice.

The decrease in surface area of microvilli in *S. mansoni*-infected mice may be compensated for, at least in part, by the observed increase in surface area of the villi.

The small bowel is known to have the capacity to adapt readily in a variety of situations. For example, villi may increase in height during lactation (Lichtenberger and Trier, 1979) and after partial resection of the bowel (Hanson *et al.*, 1977; 1977a; Williamson, 1978). Diet may also influence the architecture of villi, with high-fibre diet inducing a widening and possible fusion of these structures in humans (Tasman-Jones *et al.*, 1978).

The small intestine also appears to adapt in response to a variety of enteric parasites. The length of the small bowel has been observed to increase during *N. brasiliensis* and *Nematospiroides dubius* (= *Heligmosomoides polygyrus*) infections in rats; this may allow for a longer period of time, and a larger surface area, for the absorption of nutrients (Scofield, 1974; 1975; 1980). In rabbits infected with *Nematodirus spathiger*, a reduction of villous length in the jejunum is accompanied by signs of villous hypertrophy in the distal region of the small intestine (Hoste *et al.*, 1993). A similarly adaptive response appears to occur in rabbits infected with *Trichostrongylus colubriformis* (Hoste *et al.*, 1988).

Physiological adaptation to enteric helminth infection also appears to occur in the small intestine. An accelerated glucose-stimulated transport in the small intestine is observed in rats infected with *Hymenolepis diminuta*; this is accompanied by an increase in the area over which bicarbonate-stimulated fluid absorption occurs (Podesta and Mettrick, 1976). This finding may help to explain why this infection is not accompanied by diarrhoea or other common manifestations of gastrointestinal disease.

The increase in villous surface area in *S. mansoni*-infected mice is unlikely to compensate fully for the decrease in microvillus area. However, the reserve capacity of the small intestine might enable the maintenance of adequate gut function, even in the presence of fairly severe disruption to the intestinal architecture. Under normal conditions, this reserve capacity of the small bowel is known to exceed vastly the capacity that is required for sufficient nutrient absorption. It has been estimated that the

human small intestine has the ability to absorb more than 10 kg of fructose and glucose in a single day (see Castro, 1990).

Domingo and Warren (1969) observed no change in small intestine absorption during *S. mansoni* infection in mice. It is possible that changes in the intestinal architecture were so minor that a reserve capacity of the bowel was adequate to offset any significant change in absorptive function. Equally, as no estimation of villous surface area was made, and microvillus structure was not examined, it is possible that adaptive responses in the bowel may have played a role in the maintenance of gut function.

The method chosen to examine the function of the small bowel in *S. mansoni*-infected mice in this study measures the relative permeability of the intestine to lactulose and mannitol. These probe molecules have been used frequently to assess intestinal integrity during gastrointestinal disease. During Crohn's disease and coeliac disease, for example, mannitol uptake is decreased and lactulose uptake is increased (Pearson *et al.*, 1982; Ukabam *et al.*, 1983). This method has also been used to examine intestinal permeability in rats infected with *N. brasiliensis*, with a decrease in mannitol absorption and an increase in lactulose uptake again being observed (Cobden *et al.*, 1979). This method is perhaps particularly useful for measuring permeability during infection where non-mucosal factors, such as transit time, may influence uptake. Lactulose and mannitol are administered simultaneously, allowing the relative intestinal permeability to be the main determinant of the urinary excretion ratio of these molecules.

The success of the simultaneous administration of lactulose and mannitol as a method to evaluate permeability depends on the properties of these markers. Both molecules are rapidly cleared from the plasma by the kidney at similar rates, and both are absorbed by unmediated diffusion (reviewed by Elia *et al.*, 1987). Mannitol, a monosaccharide, is thought to be small enough to be absorbed through the same transcellular pores as water, and the reduced uptake of this molecule in disease is

thought to reflect a reduction in surface area of the small intestine (Cobden *et al.*, 1979). Lactulose, a larger disaccharide, cannot cross healthy enterocytes, and is probably absorbed across tight junctions between adjacent enterocytes (Menzies, 1983). The increase in permeability of the bowel to this sugar during disease may represent a change in the structure of cellular junctions.

The lactulose / mannitol ratio appeared to be unchanged by infection, and no significant difference in the uptake of either lactulose or mannitol was observed between *S. mansoni*-infected and control mice. This demonstrated a maintenance of intestinal function during this infection in mice, despite evidence of altered microvillus structure and focal regions of villous fusion. The observed increase in villous surface area may help counteract the reduction of microvillus surface area. Such an adaptive response may also occur, but to a lesser extent, in rats infected with *N. brasiliensis*, where a slight increase in epithelial cell numbers is observed in the small intestine due to bowel dilatation (Symons and Fairbairn, 1962). Villous atrophy and erosion during rat nippostrongylosis (Symons and Fairbairn, 1962; Martin, 1980; Cheema and Scofield, 1982) may explain the altered permeability to lactulose and mannitol during this infection (Cobden *et al.*, 1979), whereas the villous hypertrophy observed in this study would appear to explain normal permeability patterns in *S. mansoni*-infected mice.

Malabsorption has, however, been observed in mice following exposure to large numbers of *S. mansoni* cercariae (DeWitt, 1957; Vengesa and Leese, 1979). This may suggest that mice infected with this parasite have finite limits to both intestinal adaptation and reserve capacity. Alternatively, a minimum length of time may be required before the adaptive responses can develop maximally. Sugar absorption was impaired in mice seven weeks after infection with 250 - 300 *S. mansoni* cercariae (Vengesa and Leese, 1979), and protein and fat absorption appeared to be impaired eight weeks after exposure to 150 cercariae (DeWitt, 1957). Permeability of the small intestine to lactulose and mannitol was found to be within normal limits at ten weeks

post infection in this study with mice exposed to approximately 200 cercariae, and absorptive function was found to be conserved in mice 20 weeks after infection of mice with 40 cercariae (Domingo and Warren, 1969). As this investigation used a similar infection level to that used by DeWitt (1957), the time to respond adaptively would appear to be the most important factor for the maintenance of intestinal function. In the current investigation, villous surface area was only slightly increased at six weeks post infection, and the increase in surface area was observed in both anterior and posterior regions of the gut only after 16 weeks of infection. Alternatively, parasite strain differences and the variability in host responses to infection might influence the pathophysiology of disease.

5.3.2. Human schistosomiasis mansoni

The difficulties of studying the small intestine in humans with schistosomiasis mansoni are numerous. *Post-mortem* investigation of bowel structure is frequently impossible due to the rapid decay of this enzymatically rich tissue after death. Invasive sampling is totally unethical unless used as part of a diagnostic procedure, and small biopsy samples can provide only limited information about such a large organ. Schistosomes seldom exist in isolation from other infections within their hosts, so the precise role of this parasite as a causative agent of gastrointestinal disease in humans would be extremely difficult to define. The paucity of studies investigating intestinal structure and function presumably reflects these difficulties, rather than a lack of interest in the symptoms such as abdominal pain and diarrhoea that are reported in people living in regions where the prevalence of this parasite is high (WHO, 1993).

Protein-losing gastroenteropathy does not appear to be a generalised feature of human schistosomiasis mansoni (El-Sayed and Fikry, 1964), although it has been reported in patients with schistosomal colonic polyposis (Lehman *et al.*, 1970). Glucose intolerance has been observed in individuals with heavy intensities of *S. mansoni* infection (Ghanem *et al.*, 1971), and in advanced cases of bilharzial fibrosis of the liver, a delay in the absorption of D-xylose has been reported (Fikry *et al.*, 1962). In

severe cases of schistosomiasis mansoni with ascites, abnormal intestinal absorption has also been observed, although this did not appear to be accompanied by villous damage (El-Rooby, 1963, cited from Stephenson, 1987). Malabsorption of fat also appears to be associated with this infection (Pucci *et al.*, 1978). However, as Stephenson (1987) has noted, most of the studies on intestinal function during schistosomiasis mansoni have been carried out with fairly small numbers of infected patients, often with the complications of later disease. The precise role of *S. mansoni* in gastrointestinal disturbance is therefore unclear. This aspect of pathology deserves further investigation in humans to allow a more complete assessment of the benefits of control programmes.

Given that mice infected with *S. mansoni* have a higher concentration of parasite eggs in the small intestine than humans (Cheever, 1969), the maintenance of intestinal function observed in this investigation, and by Domingo and Warren (1969), suggests strongly that adequate function should also be maintained in humans. Biopsy studies in humans infected with *S. mansoni* indicate focal damage rather than a more generalised response (Halsted *et al.*, 1969; Fedail and Gadir, 1985). If damage is more widespread in the small intestine of humans, there appears to be no reason why adaptive changes in the gut structure could not compensate for this aspect of disease in a way similar to the apparently adaptive changes in the mouse. Although the energy cost of synthesising extra gut tissue would be significant, the maintenance of an adequate supply of essential nutrients would be more important in a chronic infection such as schistosomiasis mansoni.

5.4. Summary

1. An increase in the villous surface area in the small intestines of mice in the ninth week of *S. mansoni* infection was observed, although there was no significant increase in lumen perimeter when this parameter was expresses as a function of submucosal perimeter.

2. The increased villous surface area in the small bowels of *S. mansoni*-infected mice appeared to occur to a smaller extent during the early stages of patent infection, and to become a more generalised response along the length of the small intestine after 16 weeks of infection.

3. A lack of increase in the villous surface area in the small intestines of mice exposed to single-sex *S. mansoni* cercariae indicated that egg-associated pathology was an important factor in the initiation of this response to infection.

4. Transmission electron microscopy showed a reduction of microvillus height and density in *S. mansoni*-infected mice.

5. The lactulose / mannitol ratio, used as a measure of intestinal integrity, was not significantly influenced by infection.

Chapter 6

Intestinal pathology: III. Mucus

6.1. Introduction

Mucus is a viscous and slippery gel that consists of a complex mixture of water, glycoproteins, peptides, lipids, electrolytes, micro-organisms, cellular debris and a variety of serum and cellular macromolecules (Neutra and Forstner, 1987; Tse and Chadee, 1991). The production of mucus in the intestine is carried out by goblet cells. These cells are found interspersed amongst enterocytes, and acquire their name from a distension of mucin granules in the apical region of the cell which gives rise to a wine goblet appearance. Although goblet cells are present throughout the intestine, their relative contribution to the total number of intestinal cells increases from the proximal jejunum to the distal ileum (Kemper and Specian, 1991).

Secreted from goblet cells by exocytosis (Neutra and Forstner, 1987), mucus seems to form a continuous layer over the surface of enterocytes (Allen *et al.*, 1990). This gel-like layer appears to have a variety of functions. It acts as a lubricant to allow foodstuffs to slide easily through the intestine with a minimum of abrasion, and also provides a distinct barrier between the intestinal surface and digestive juices (Neutra and Forstner, 1987; Tse and Chadee, 1991). In addition, mucus acts as a diffusion medium for solutes and nutrients such as lactose, sucrose and small peptides (Smithson *et al.*, 1981; Proust *et al.*, 1984).

Mucus also provides protection against a variety of micro-organisms, toxins and antigens that may be present within the intestinal lumen (reviewed by Walker and Owen, 1990). Mucins can inhibit microbial overgrowth by binding and trapping pathogens. For example, mucin carbohydrate moieties may mimic epithelial cell glycoproteins that are recognised and bound by the adherence lectin of *Entamoeba histolytica*, thereby acting as a competitive inhibitor (Chadee *et al.*, 1987). Interactions

between mucins and secretory immunoglobulin A (IgA) can trap IgA-coated bacteria within the mucus layer (Magnusson and Stjernstrom, 1982), with the subsequent expulsion of this continually regenerating layer ridding the host of the pathogen. Cholera toxin may elicit mucin discharge (Forstner *et al.*, 1981; Roomi *et al.*, 1984) and the toxin may subsequently be bound to the mucin (Strombeck and Harrold, 1974).

Similar protective roles for mucus during helminth infection have been observed. Approximately two weeks after experimental infection of rats with *Nippostrongylus brasiliensis*, goblet cell hyperplasia and increased mucin synthesis coincide temporally with the expulsion of these worms (reviewed by Miller, 1987). The structure of the mucin is also altered at this time, with an increase in the number of goblet cells containing acid mucin (Koninkx *et al.*, 1988). Mucus also appears to act as a trap for immature worms prior to their establishment in the host following a secondary exposure to this infection (Miller *et al.*, 1981).

The intestinal parasite *Trichinella spiralis* also stimulates mucin synthesis and goblet cell hyperplasia in the rat (Bell *et al.*, 1984; Miller, 1987). The rapid expulsion of *T. spiralis* larvae from intestinal epithelia appears to be mediated by the coating of the parasite in surface antigen-reactive antibodies (Carlisle *et al.*, 1990). The antibody-coated larvae are then entrapped in mucus prior to expulsion from the intestine (Carlisle *et al.*, 1990).

Evidence for hypersecretion of mucus and goblet cell hyperplasia during *S. mansoni* infection in mice is conflicting. Domingo and Warren (1969) indicated that goblet cell hyperplasia was not a feature of this infection, although no data were presented in relation to this phenomenon. However, it has been noted that the villi of *S. mansoni*-infected mice are covered with pronounced strands of mucus when compared to uninfected controls (Vengesa and Leese, 1979). A similar observation was made when examining the absorptive surface of the small intestine using scanning electron microscopy.

Because mucus appears to provide effective protection for the small intestine by providing a barrier to physical, chemical and biological attack, the present study sought to establish if goblet cell hyperplasia was a feature of murine schistosomiasis mansoni. As all nutrients must be transferred through this mucus blanket, any change in secretion or structure of mucin may have implications for mouse nutrition during the course of *S. mansoni* infection.

6.2. Results

An increase in the number of goblet cells was observed in all the regions of the small intestine that were examined from mice in the ninth week of *S. mansoni* infection when compared to uninfected controls (25% region, Mann-Whitney, $W = 52.0$, $P < 0.05$; 50% region, Mann-Whitney, $W = 64.0$, $P < 0.05$; 75% region, Mann-Whitney, $W = 56.0$, $P < 0.01$; 100% region, Mann-Whitney, $W = 76.0$, $P < 0.005$)(Fig. 6.1). This increase in goblet cell number was positively correlated with increased distance from the pylorus (Spearman rank correlation, $r_s = 0.45$, $P < 0.025$), while the number of goblet cells per villus did not correlate with distance from pylorus in control animals (Spearman rank correlation, $r_s = 0.075$, $P > 0.5$). Qualitatively, the goblet cells in the gut of infected mice appeared larger and to stain more densely with Alcian Blue (Plates 6.1 and 6.2). A thick layer of mucus was often observed to cover the villi of infected mice; a similar but much thinner layer was observed only occasionally on the surface of villi in control mice (Plates 6.1 and 6.2).

As early as six weeks after exposure to *S. mansoni* infection, goblet cell hyperplasia was observed in the distal region of the small intestine of infected mice (Mann-Whitney, $W = 76.0$, $P < 0.005$), but not in the anterior region (Mann-Whitney, $W = 66.0$, $P > 0.05$) (Fig. 6.2). The numbers of goblet cells per villus were not quantified at 16 weeks post infection because of the frequently fused structure of villi in infected mice. At this time, however, an increase in staining density of goblet cells was observed in infected animals when compared to controls (25% region, Mann-Whitney, $W = 65.0$, $P > 0.05$; 100% region, Mann-Whitney, $W = 71.0$,

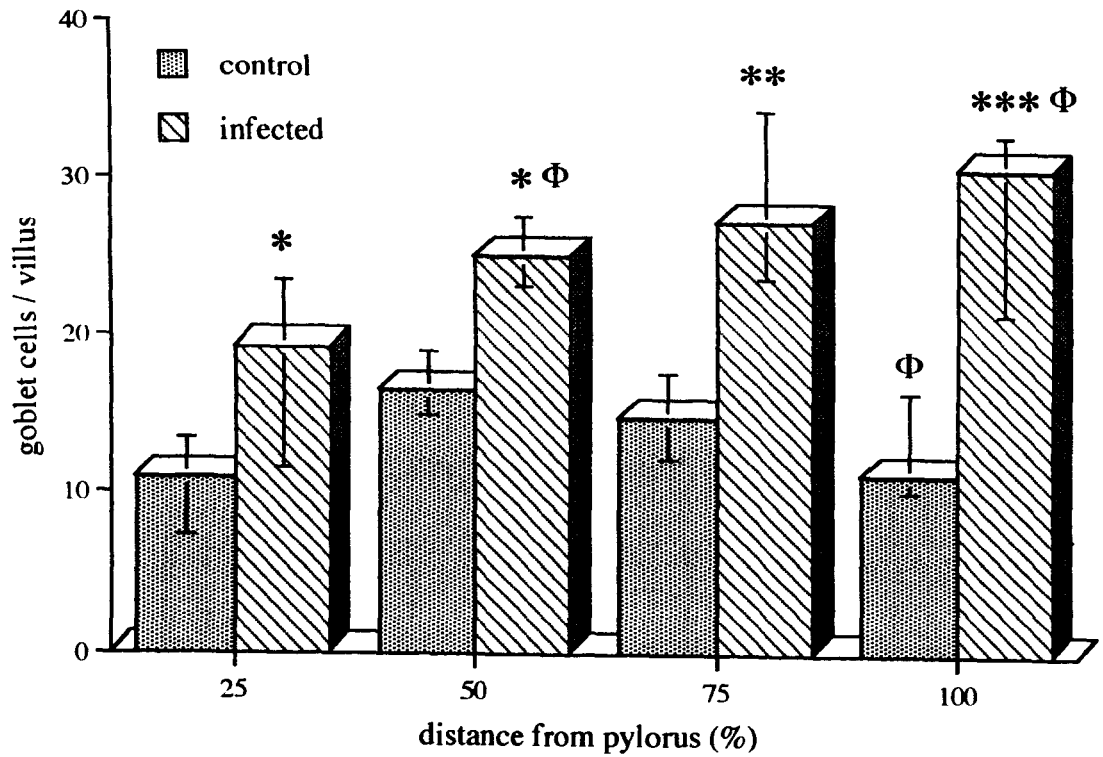
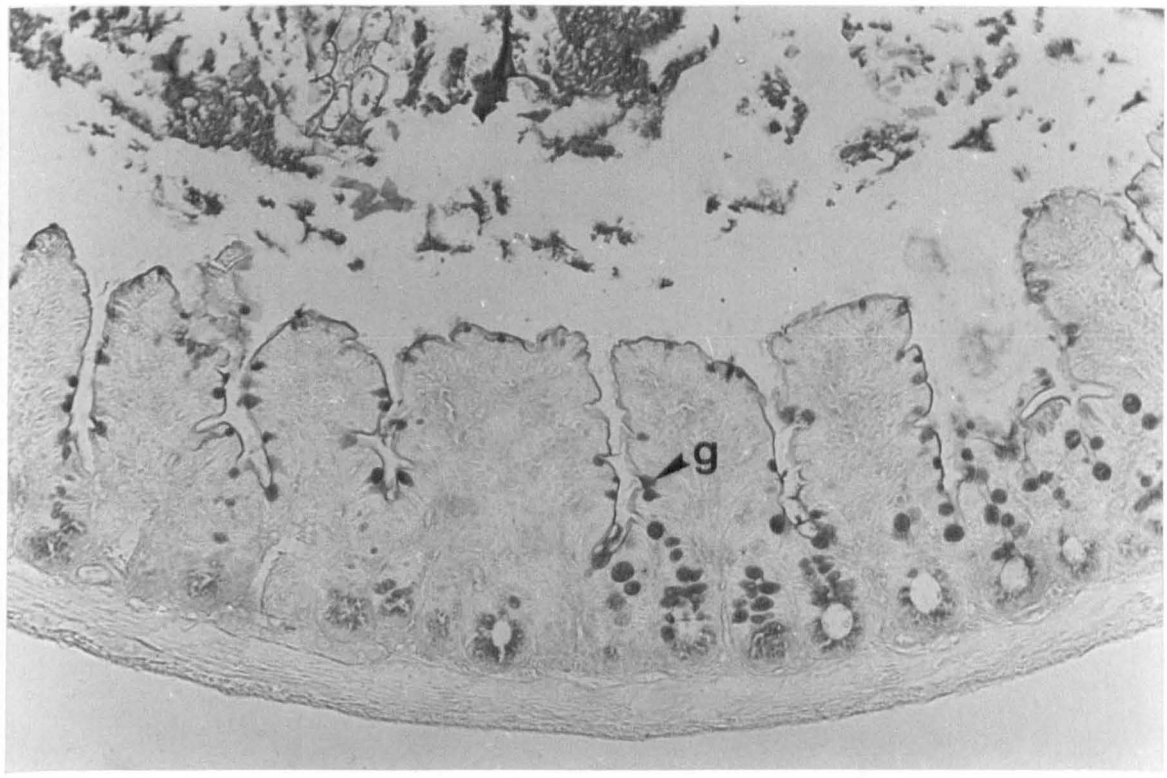
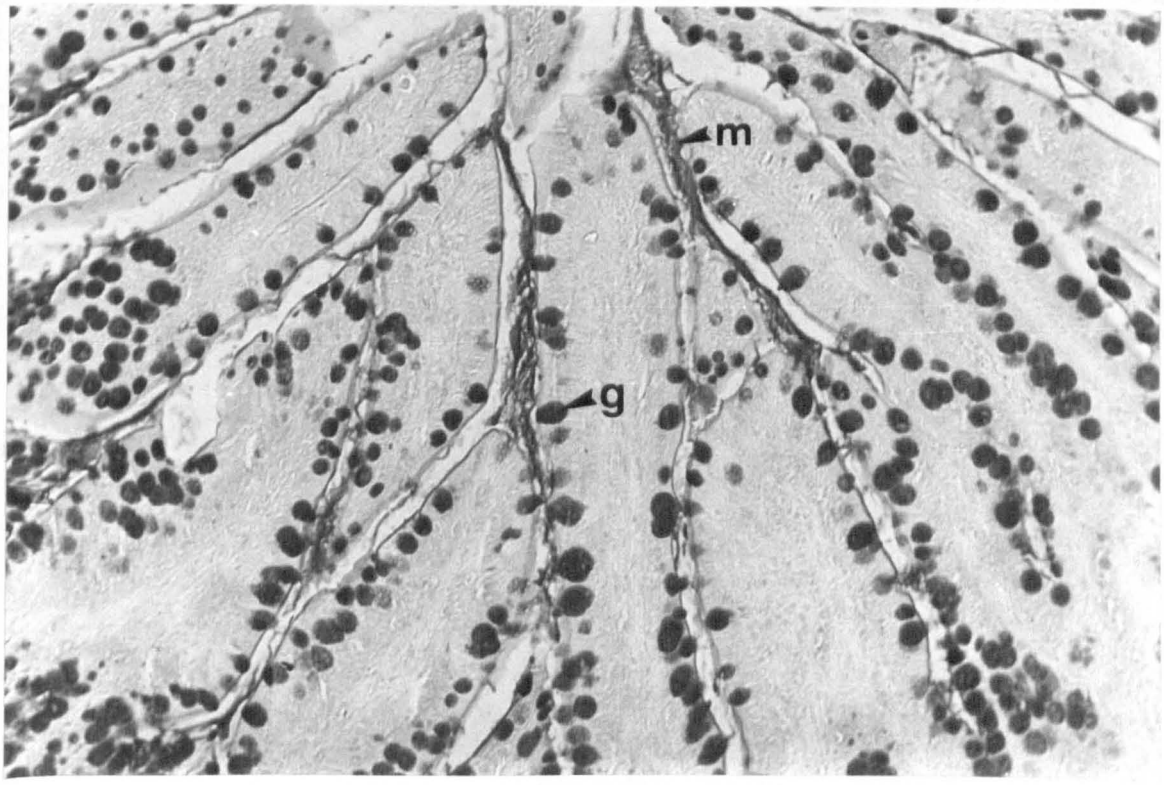


Figure 6.1. Number of goblet cells per villus for control (n = 6) and *S. mansoni*-infected mice (n = 6) in the ninth week of infection (except Φ where n = 7). All values are medians shown with interquartile ranges. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$)

Plate 6.1. Transverse section from the distal end of the small intestine from an uninfected, control mouse. **g** - goblet cell. —— 50 μm

Plate 6.2. Transverse section from the distal end of the small intestine from an *S. mansoni*-infected mouse (ninth week of infection).
g - goblet cell; **m** - layer of mucus. —— 50 μm


g


m
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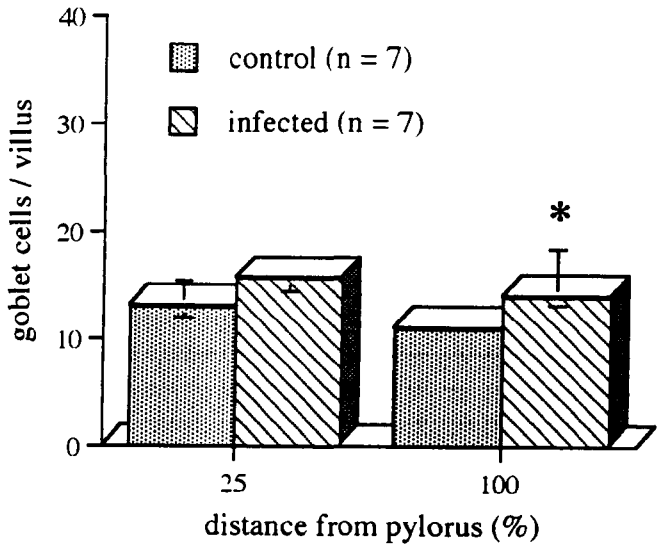

Figure 6.2. Number of goblet cells per villus for *S. mansoni*-infected at 6 weeks post infection and control mice. All values are medians, shown with interquartile ranges. (* $P < 0.005$)

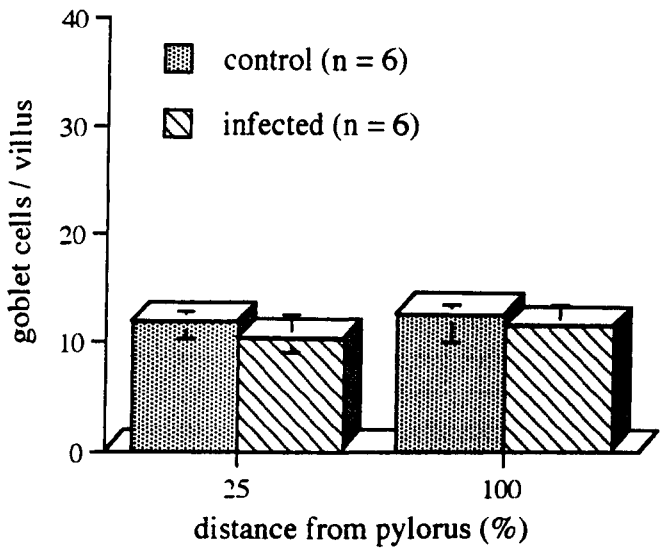

Figure 6.3. Number of goblet cells per villus for mice exposed to single-sex *S. mansoni* cercariae and uninfected control mice at 8 weeks post infection. All values are medians, shown with interquartile ranges.

Table 6.1. Goblet cell optical density for *S. mansoni*-infected mice at 16 weeks post infection (n = 7) and control mice (n = 7). (median values) (* $P < 0.05$)

Distance from pylorus (%)	Uninfected control mice	*S. mansoni*-infected mice
25	0.39	0.51
100	0.55	0.79 *

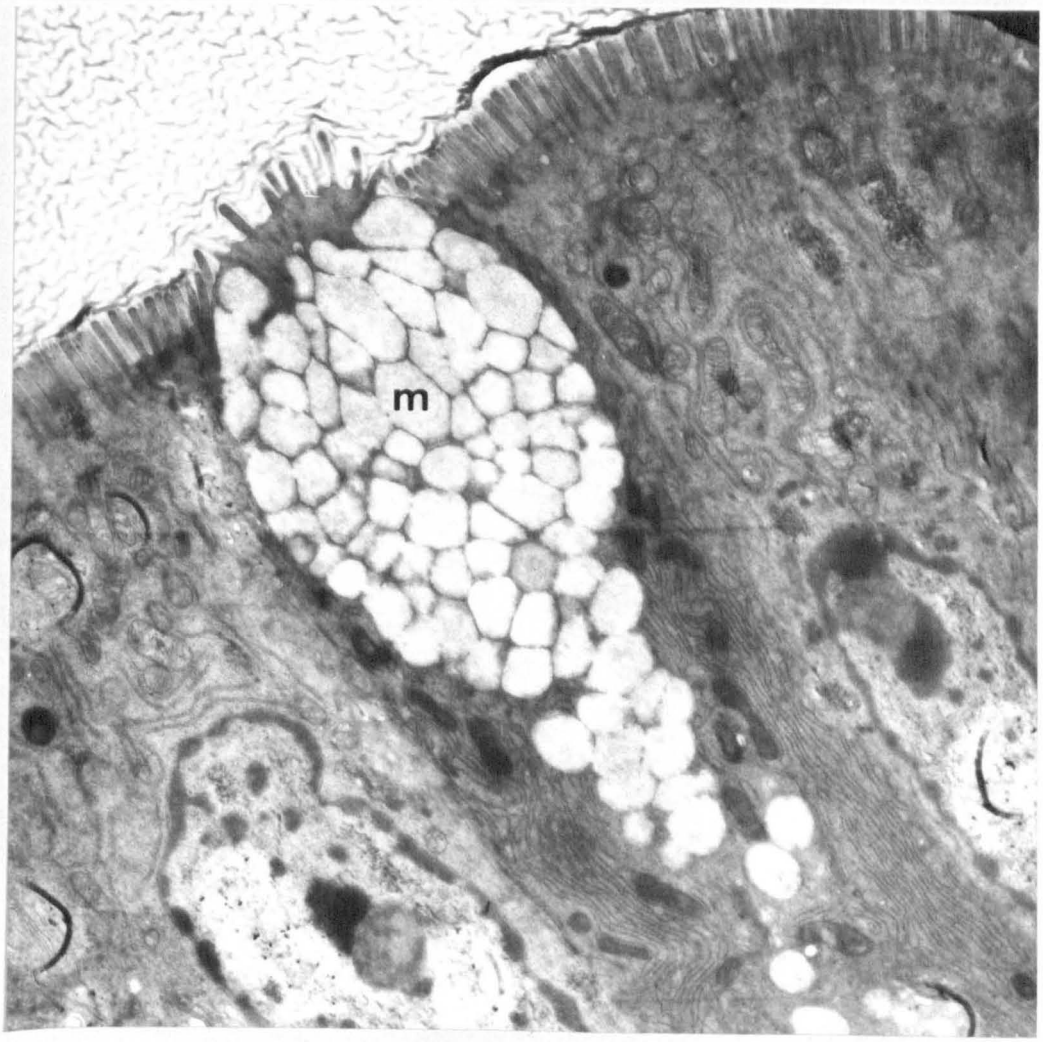

Plate 6.3. Transmission electron micrograph of a goblet cell from the small intestine of an uninfected, control mouse. The apical region of the cell is distended by many mucin granules (**m**). ——— 2μm

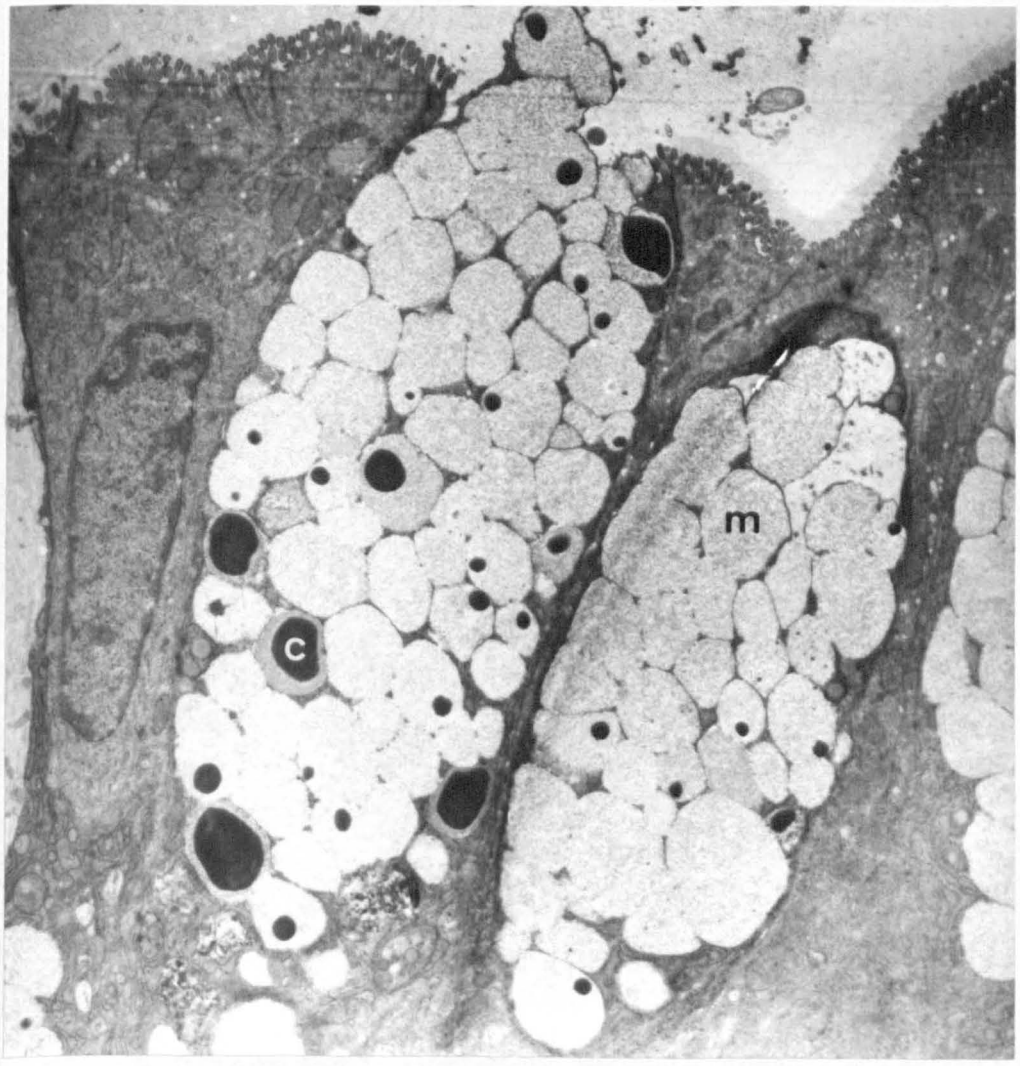

Plate 6.4. Transmission electron micrograph of the apical regions of goblet cells from an *S. mansoni*-infected mouse at nine weeks post infection. Some mucin granules (**m**) contain electron dense cores (**c**). ——— 2.5 μm

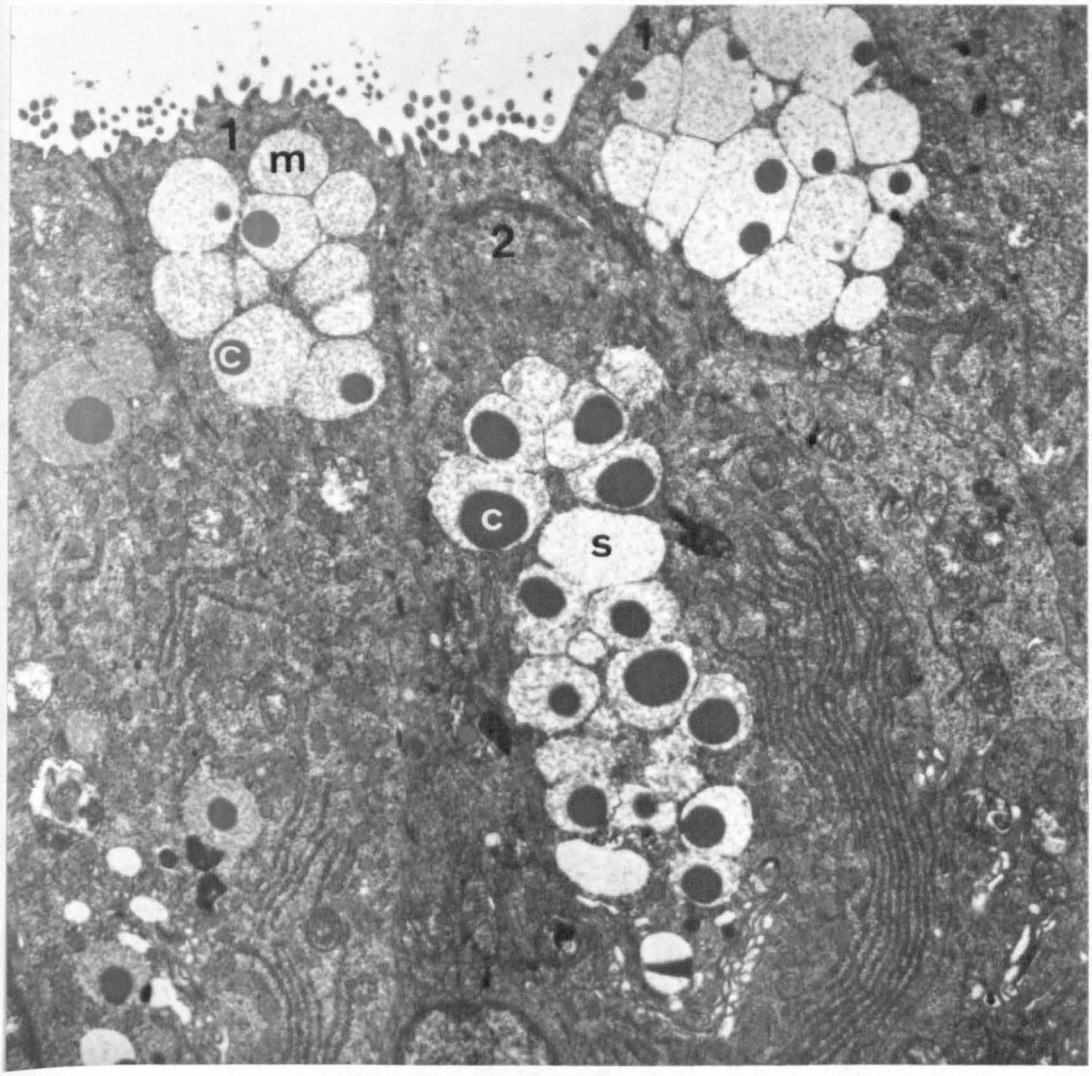

Plate 6.5. Transmission electron micrograph of immature goblet cells (**1**) and a Paneth cell (**2**) from the intestinal crypt of an *S. mansoni*-infected mouse in the ninth week of infection. Electron dense cores (**c**) are present in the mucin granules (**m**) of goblet cells and secretory granules (**s**) of the neighbouring Paneth cell. —— 2 μm

$P < 0.05$) (Table 6.1). In mice exposed to single-sex cercariae, no significant variation in goblet cell numbers was observed (25% region, Mann-Whitney, $W = 32.0$, $P > 0.05$; 100% region, Mann-Whitney, $W = 38.0$, $P > 0.05$) (Fig. 6.3).

The typical structure of a goblet cell from the proximal region of the small intestine of an uninfected mouse is shown in Plate 6.3. Goblet cells from infected mice were similar (Plate 6.4), except for the presence of condensed cores within some of the mucin granules. The mucin granules containing condensed cores were many and obvious in both mature goblet cells (Plate 6.4) and immature goblet cells in the intestinal crypts (Plate 6.5.) of infected mice. These dense cores varied in size, and ranged up to approximately 2 μm in diameter. In uninfected, control mice, these dense cores were observed rarely, and only in immature goblet cells in intestinal crypts.

The electron dense cores in goblet cells appeared very similar in structure to the electron dense cores of secretory granules in the neighbouring Paneth cell (Plate 6.5). The electron dense cores in Paneth cells were also in a similar size range, appearing to have a maximum diameter of approximately 1.2 μm. However, serial sections from both goblet cells and Paneth cells would be required to obtain true dimensional measurements of these electron dense cores.

6.3. Discussion

Goblet cell hyperplasia in the small bowel was observed to be a prominent feature of pathology during *S. mansoni* infection in mice. This phenomenon was apparent as early as six weeks in the course of infection, although goblet cell numbers were elevated only slightly at this time (1.27 times) when compared to cell numbers in the ninth week of infection (almost 3 times).

The correlation of the severity of goblet cell hyperplasia with increased distance from the pyloric sphincter appeared to reflect the qualitative assessment of greater granulomatous damage in posterior regions of the small intestine. Additional evidence suggestive of a link between granuloma formation and hyperplasia of mucus-producing

cells is the lower level of hyperplasia observed at six weeks post infection. The complete absence of goblet cell hyperplasia in animals exposed to single-sex schistosome infections also suggests a relationship between mucus production and egg-associated pathology.

The mechanisms responsible for goblet cell hyperplasia and mucus hypersecretion during helminth infection remain to be elucidated. Evidence suggests that mast cell products, or other inflammatory mediators, may influence mucus secretion. For example, mucosal mast cells are functionally active during the elimination of both *T. spiralis* and *N. brasiliensis* infections in rats (Woodbury *et al.*, 1984), and treatment of rats with anti-rat mast cell antiserum delays the expulsion of *N. brasiliensis* (Levy and Frondoza, 1983). Histamine induces goblet cell secretion of mucus in the rabbit colon (Neutra *et al.*, 1982). It is possible that histamine release from mast cells stimulates mucus secretion during *T. spiralis* and *N. brasiliensis* infections, although this mast cell product does not appear to induce mucus secretion in the small intestine of rabbits (Neutra *et al.*, 1982). A similar response may operate in the *S. mansoni*-infected mouse where an increase in the number mast cells is known to occur in the small intestine after the infection reaches patency (Kermanizadeh *et al.*, 1995).

Another potential mediator of mucus secretion in the parasitized host is immunoglobulin E (IgE). The intraduodenal antigen challenge of rats passively sensitised with antiserum rich in IgE antibodies appears to induce the anaphylactic release of mucus (Lake *et al.*, 1980). However, the interaction of IgE and mast cells to induce anaphylaxis may not be the only way by which this antibody might stimulate mucus secretion. The production of mucus in response to *N. brasiliensis* appears to be normal in mice that have few, if any, mucosal mast cells, but do produce antiworm IgE (Kojima *et al.*, 1980; Uber *et al.*, 1980). This observation has lead to speculation that IgE might bind directly to goblet cells to trigger mucus release. An elevation of IgE is one of the hallmarks of schistosome infection (Capron and Dessaint, 1992; Hagan, 1992).

More recently, platelet activating factor (PAF), a broad-spectrum mediator of inflammatory responses (Barnes *et al.*, 1988), has been proposed as a mucus secretagogue. PAF is implicated as a causative agent of the intestinal pathology observed during *N. brasiliensis*-infection in rats (Moqbel and MacDonald, 1990), and the hypersecretion of mucus is a feature of this infection (Miller, 1987). Furthermore, it has been suggested that PAF acts as a secondary mediator of tissue injury caused by TNF / lipopolysaccharide (Sun and Hsueh, 1988; 1991), and TNF is thought to be important in the granulomatous reaction to schistosome ova (Chensue *et al.*, 1989; Amiri *et al.*, 1992). Certainly, the granulomatous response appears crucial to the development of goblet cell hyperplasia, as mice with single-sex *S. mansoni* infections demonstrate no signs of increased mucus production.

A variety of other mediators have been proposed as mucus secretagogues (reviewed by Neutra and Forstner, 1987; Tse and Chadee; 1991). These include gastrointestinal peptides and hormones, and parasite-derived toxins. However, further research will be required to establish the mechanism of goblet cell hyperplasia in the *S. mansoni*-infected mouse.

The inclusion of electron dense bodies in many goblet cells of *S. mansoni*-infected mice proved to be an enigma. In the small intestine of the mouse, it appears that cells within the intestinal crypt may possess characteristics of more than one cellular phenotype, with both mucin granules and Paneth cell granules having been found within the same cell (Cheng and Leblond, 1974). It was suggested that these cells retained the ability to mature as either Paneth cells or goblet cells, but that the presence of more than one phenotype might also represent a breakdown in the cellular differentiation process within the crypt (Cheng and Leblond, 1974).

The electron dense cores in immature goblet cells within the intestinal crypts of *S. mansoni*-infected mice (Plate 6.5) may suggest that these cells have the potential to mature as either Paneth cells or goblet cells. Certainly, the cores are similar in appearance to the electron dense cores of secretory granules in Paneth cells of mice

(Satoh *et al.*, 1990; see also Plate 6.5). In addition, the secretory granules of the Paneth cells have a bipartite structure, with the dense core being surrounded by a halo of lower density. This peripheral halo of Paneth cells contains mucopolysaccharides (Spicer *et al.*, 1967).

However, these electron dense cores were also observed in mature goblet cells in infected mice (Plate 6.4), an observation that does not appear to have been made previously. This may be more indicative of a breakdown of normal cell maturation processes during goblet cell hyperplasia in the *S. mansoni*-infected host. Alternatively, the presence of increased numbers of cells containing these cores may suggest a protective role during infection. The similarities of the electron dense cores in mucin and Paneth cell granules, and the common origin of these cells from endodermal stem cells, may suggest a similar function. One of the major components of the Paneth cell secretory granule is lysozyme. This enzyme, found in the Paneth cells of both mice (Geyer, 1973; Satoh *et al.*, 1988) and humans (Geyer, 1973; Erlandsen *et al.*, 1974; Mathen *et al.*, 1987), is capable of cleaving the cell walls of certain bacteria. The bactericidal properties of lysozyme, if this enzyme is present in the dense core of goblet cells, might help prevent further insult to the already injured small intestine. It is recognised, however, that the inference of a common physiological function based on the morphological characteristics of these electron dense bodies is somewhat tenuous.

The increased production of mucus may, in itself, help to reduce microbial invasion by increasing the physical flushing of these organisms out of the gut, or by limiting the access of antigens to the surface of enterocytes. It is possible that mucus may also be involved as a non-specific mediator of interactive immunity between certain species of helminths. Mitchell (1979) used the term "interactive immunity" to describe a situation where one species of parasite elicits a host response that acts non-specifically to the detriment of a second, unrelated species of parasite. The enteric nematode *T. spiralis* and the cestodes *Hymenolepis diminuta* and *H. microstoma* provide examples of interactive immunity (Behnke *et al.*, 1977; Howard *et al.*, 1978).

Behnke *et al.* (1977) demonstrated that the rejection of *T. spiralis* infection in mice resulted in the premature loss of a concurrent *H. diminuta* infection, and it was suggested that this was attributable to the inflammation associated with the expulsion of the nematode. The cestode *H. microstoma* is also rejected if its intestinal phase coincides with intestinal inflammation induced by the expulsion of *T. spiralis*, but the tapeworm is not affected if its scolex has reached the bile duct (Howard *et al.*, 1978).

Recently, *S. mansoni* infection has been shown to have an antagonistic effect on superimposed infections of *H. diminuta* and *H. microstoma* in mice (Andreassen *et al.*, 1990). In patent *S. mansoni* infections, both *H. diminuta* and *H. microstoma* were rejected prematurely when compared to uninfected controls. In mice harbouring prepatent *S. mansoni* infections, the rejection of *H. diminuta* mirrored the response of uninfected controls. Thus the intestinal response to patent *S. mansoni* infection in mice appears to have the same affect upon superimposed infections of *H. diminuta* and *H. microstoma* as the intestinal response to *T. spiralis*. As goblet cell hyperplasia is observed during both these infections, it is possible that the production of increased amounts of mucus may have an inhibitory effect on these tapeworms.

An increase in staining density of goblet cells from *S. mansoni*-infected mice was observed at 16 weeks post infection. Although the intensities of histochemical stains are infrequently used for quantitative analyses, the simultaneous processing, cutting and staining of paired intestinal samples from infected and uninfected mice allowed the use of photometry to obtain these data. The observed increase in staining of mucopolysaccharide in infected mice may indicate a change in mucin composition.

Changes in the composition of mucin have a protective role during *N. brasiliensis* infection in rats. Koninkx *et al.* (1988) noted a significant increase in acidic mucins in the goblet cells of rats 15 days after infection with this nematode. This change in mucin synthesis coincided with the expulsion of *N. brasiliensis*, thereby indicating a protective role for the compositional alteration of mucus. It has recently been demonstrated that the changes in mucin structure during this infection appear to

be due, at least in part, to an immune-mediated alteration of the sugar residues of goblet cell mucins (Ishikawa *et al.* 1993). This alteration in mucin composition seems to be important not only in the expulsion of this parasite, but also in preventing its establishment after secondary exposure (Ishikawa *et al.*, 1994). However, a similarly protective role for changes in mucin composition during *S. mansoni* infection cannot be possible due to intravascular habitat of this worm.

Following acute ethanol injury of the stomach, a thick, gelatinous coat of mucus is observed to cover re-epithelializing mucosa (Morris *et al.*, 1981; Sellers *et al.*, 1987). Wallace and Wittle (1986) demonstrated that this layer of mucus is important to epithelial recovery by showing that the repair process was inhibited by mucolytic agents. Comparison of undamaged regions of mucosa with ethanol-damaged mucosa revealed that the mucus covering re-epithelializing tissue was more fluid than the normal, relatively rigid, adherent mucus layer, and that this altered mucus was resistant to shrinkage by fixatives such as ethanol (Lacy, 1985; Sellers *et al.*, 1987). Histological examination of the small intestines from *S. mansoni*-infected mice often revealed the presence of a thick layer of mucus covering villi; a similar layer of mucus on control mice was either absent or much thinner. The apparently thicker mucus coat on intestinal villi of infected mice, and the possible change in mucin structure that was suggested by photometry, may be analogous to the protective role of mucus in epithelial recovery that is observed in response to ethanol injury in the stomach (Wallace and Wittle, 1986).

A thicker layer of mucus, or a change in mucus composition, may upset normal patterns of nutrient uptake in the small intestine. A thin but continuous mucus gel layer acts as a diffusion barrier for nutrients, retarding the free diffusion of a variety of nutrients to the membrane hydrolytic enzymes (Smithson *et al.*, 1981; Proust *et al.*, 1984). A thickening of this layer may indicate an inhibitory effect on nutrient uptake. For example, Wein and Van Campen (1991) suggest that mucus appears to trap or bind iron in proportion to the amount of mucus that is secreted. A depleted mucus layer in

iron deficient rats accompanied an increase in iron absorption, and increased mucus levels observed in animals fed a high dietary level of iron were suggested to protect against excessive iron absorption (Wein and Van Campen, 1991). Quarterman *et al.* (1991) suggested the conflicting view that intestinal mucin delivers iron to enterocytes for absorption, as iron-deficient animals were found to have increased amounts of mucus on the intestinal surface. As mice infected with *S. mansoni* become anaemic (DeWitt and Warren, 1959; Mahmoud, 1982), an unimpeded uptake of iron in the small intestine would be particularly important during this infection. The potential link between mucus production and nutrient uptake in the small intestine of mice infected with *S. mansoni* warrants further investigation.

6.4. Conclusions

The most obvious benefit of goblet cell hyperplasia in the *S. mansoni*-infected mouse would seem to be an increase in intestinal lubrication; this would presumably ease the passage of foodstuffs through the inflamed intestine. Mucus may also play a part in the repair of epithelial cells damaged by the passage of schistosome eggs into the gut lumen. Any change in the structure and amount of mucus may affect the host's ability to protect against infection or its normal pattern of nutrient uptake. The beneficial or detrimental nature of these changes remains to be established.

The results from this study indicate that goblet cell hyperplasia may be a feature of human schistosomiasis mansoni. This would be consistent with the observation that intestinal schistosomiasis can cause diarrhoea with blood and mucus (Mohamed *et al.*, 1990). As granulomatous damage in the human small intestine appears to be focal, any hyperplasia of mucus-producing cells may also be focal in nature. It would be interesting to determine if goblet cell hyperplasia occurs in humans infected with *S. mansoni*. However, any changes in mucus synthesis and secretion are likely to prove much more difficult to observe in people due to the high probability of co-existing organisms in the small intestine, many of which might also exert an affect on mucus production in the gut.

6.5. Summary

1. Goblet cell hyperplasia in the small intestine of *S. mansoni*-infected mice was observed to be a prominent feature of intestinal pathology.

2. Hyperplasia of mucus-producing cells occurred as early as six weeks post infection, but was more pronounced later in the course of infection.

3. The presence of schistosome ova in the host small intestine appeared to be important in the induction of goblet cell hyperplasia, as no goblet cell proliferation was observed during single-sex worm infections.

4. A change in the optical density of goblet cells stained with Alcian Blue, combined with the frequent presence of a thicker layer of mucus on the villi of infected mice, was suggestive of a change in mucus structure.

5. Electron dense cores were observed in mucin secretory granules in both immature and mature goblet cells of infected mice.

Chapter 7

Protein malnutrition and intestinal pathology

7.1. Introduction

The small intestine appears capable of adapting to a wide variety of stimuli. For example, the lengths of villi may increase during lactation (Lichtenberger and Trier, 1979) and after partial resection of the bowel (Hanson *et al.*, 1977, 1977a; Williamson, 1979). There is some indication that mucosal hypertrophy is linked to enhanced iron absorption in pregnant rats fed on an iron deficient diet (Southon *et al.*, 1989). Smooth muscle hypertrophy occurs as a response to surgically induced stenosis in the small bowel (Gabella, 1979).

The presence of parasites in the host may affect the small intestine in similar ways. In rabbits infected with *Nematodirus spathiger*, villous hypertrophy is observed in the distal region of the small intestine at the same time as the lengths of villi in the jejunum are reduced (Hoste *et al.*, 1993). A similar response appears to occur in rabbits infected with *Trichostrongylus colubriformis* (Hoste *et al.*, 1988). Smooth muscle hypertrophy occurs in the small intestines of pigs infected with *Ascaris suum* (Stephenson *et al.*, 1980).

Teleological explanations for these responses appear obvious. The smooth muscle hypertrophy induced by stenosis is likely to be a response to the impeded flow of digesta through the small bowel. The increased muscle mass would allow greater forces of propulsion to maintain the flow of nutrients through the gut. In a similar way, *A. suum* would inhibit the flow of digesta and, again, hypertrophy of the smooth muscle would help to rectify this problem. The increased surface area of villi in pregnant rats fed on an iron deficient diet would presumably maximise the chance of absorption of this mineral. Likewise, damage caused by parasites in the anterior region of the gut may be, at least in part, compensated for by mucosal hypertrophy in more distal regions of the small intestine.

The essential nature of the small intestine in maintaining adequate nutrient supplies suggests that it would be advantageous for the gut to have the ability to respond to change in such ways. Driven by the assumption that resources would not be directed into the hypertrophy of smooth muscle, goblet cell hyperplasia or villous hypertrophy in the *S. mansoni*-infected mouse unless there was some benefit for the host, similar teleological explanations were offered for these aspects of intestinal pathology in Chapters 4 - 6. It was suggested that smooth muscle hypertrophy allowed the maintenance of mixing and propulsive forces in a bowel made more rigid by the granulomatous response to schistosome eggs. Villous hypertrophy was suggested to offset a reduction of the microvillus surface area. It was proposed that goblet cell hyperplasia might play a role in villous repair and increase the lubrication level of the inflamed small intestine.

As the energy cost of such processes would be relatively great, and parasitic disease and malnutrition commonly coexist (Crompton and Nesheim, 1982), it was of interest to establish if malnourished hosts could respond in similar ways. Varied interactions exist between infection and host nutrition (Scrimshaw *et al.*, 1968; Crompton, 1993). The relationships are generally classified as being either synergistic or, perhaps less commonly, antagonistic (Scrimshaw *et al.*, 1968; Beisel, 1982). In a synergistic relationship, malnutrition increases the susceptibility of a host to a pathogen and/or makes worse the consequences of infection and malnutrition. This results in a more serious condition for the host than would have been predicted by the occurrence of either disease or malnutrition in isolation. In an antagonistic relationship, the combined effects of infection and malnutrition are less than would be expected during infection alone.

It was the aim of the work described in this chapter to establish if a synergistic relationship existed between protein malnutrition and small intestine pathology in the *S. mansoni*-infected mouse. As changes in the small intestine were accompanied by a redistribution of body weight (Chapter 4), reduced food intake and possibly a change in

the efficiency of nutrient utilisation (Chapter 3), a further aim was to investigate these aspects of nutritional disturbance in protein-malnourished mice.

7.2. Results

7.2.1. Worm burden

The number of schistosomes recovered from infected mice fed on diets of varying protein content are shown in Table 7.1. The amount of protein in the diet was not found to have any significant effect on the number of worm pairs recovered from infected mice (Kruskal-Wallis ANOVA, $H = 4.32$, D.F. = 2, $P > 0.05$). However, the recovery of male worms from mice was significantly influenced by the concentration of protein in the diet (Kruskal-Wallis ANOVA, $H = 6.09$, D.F. = 2, $P < 0.05$). A multiple comparison test revealed that a significantly greater number of male worms ($P < 0.05$) were recovered from mice fed on the 2% protein diet for the last two weeks of the experimental period when compared to those fed on a 4% diet.

7.2.2. Growth patterns and food intake

The body weight of mice allocated to the various dietary and infection protocols did not differ significantly immediately prior to infection or sham infection (Kruskal-Wallis ANOVA, $H = 4.06$, D.F. = 3, $P > 0.05$). The growth patterns for these mice are shown Fig. 7.1. The protein content in the diet did not influence the growth of mice in the first week (Kruskal-Wallis ANOVA, $H = 4.29$, D.F. = 3, $P > 0.05$), second week (Kruskal-Wallis ANOVA, $H = 7.06$, D.F. = 3, $P > 0.05$), third week (Kruskal-Wallis ANOVA, $H = 6.94$, D.F. = 3, $P > 0.05$), fourth week (Kruskal-Wallis ANOVA, $H = 3.65$, D.F. = 3, $P > 0.05$), fifth week (Kruskal-Wallis ANOVA, $H = 3.57$, D.F. = 3, $P > 0.05$) or sixth week (Kruskal-Wallis ANOVA, $H = 5.99$, D.F. = 3, $P > 0.05$) of the experiment. After seven weeks, the body weight of mice was found to vary significantly (Kruskal-Wallis ANOVA, $H = 13.51$, D.F. = 5, $P < 0.05$), with

Table 7.1. Number of male worms and worm pairs recovered from mice fed on the 8, 4 and 2% protein diets. a : Significantly more male worms recovered from mice on the 2% diet than for mice on the 4% diet. ($P < 0.05$). All values are medians. For each experimental group n = 9.

	Male worms	Worm pairs
8% protein	13	6
4% protein	10	4
2% protein	24 [a]	11

Table 7.2. Percentage efficiency for the conversion of food eaten into body weight from the sixth week of infection onwards. a: efficiency significantly greater for control mice fed on the 8% protein diet than for infected mice fed on the 4% protein diet. For each experimental group n = 9. ($P < 0.05$) (Median values)

	Uninfected control mice	*S. mansoni*-infected mice
8% protein	2.49 [a]	1.37
4% protein	0.84	- 0.35

Table 7.3. Percentage efficiency for the conversion of protein eaten into body weight from the sixth week of infection onwards. a: efficiency significantly greater for control mice fed on the 8% protein diet than for infected mice fed on the 4% protein diet. For each experimental group n = 9. ($P < 0.05$) (Median values)

	Uninfected control mice	*S. mansoni*-infected mice
8% protein	30.98 [a]	17.32
4% protein	20.91	- 8.63

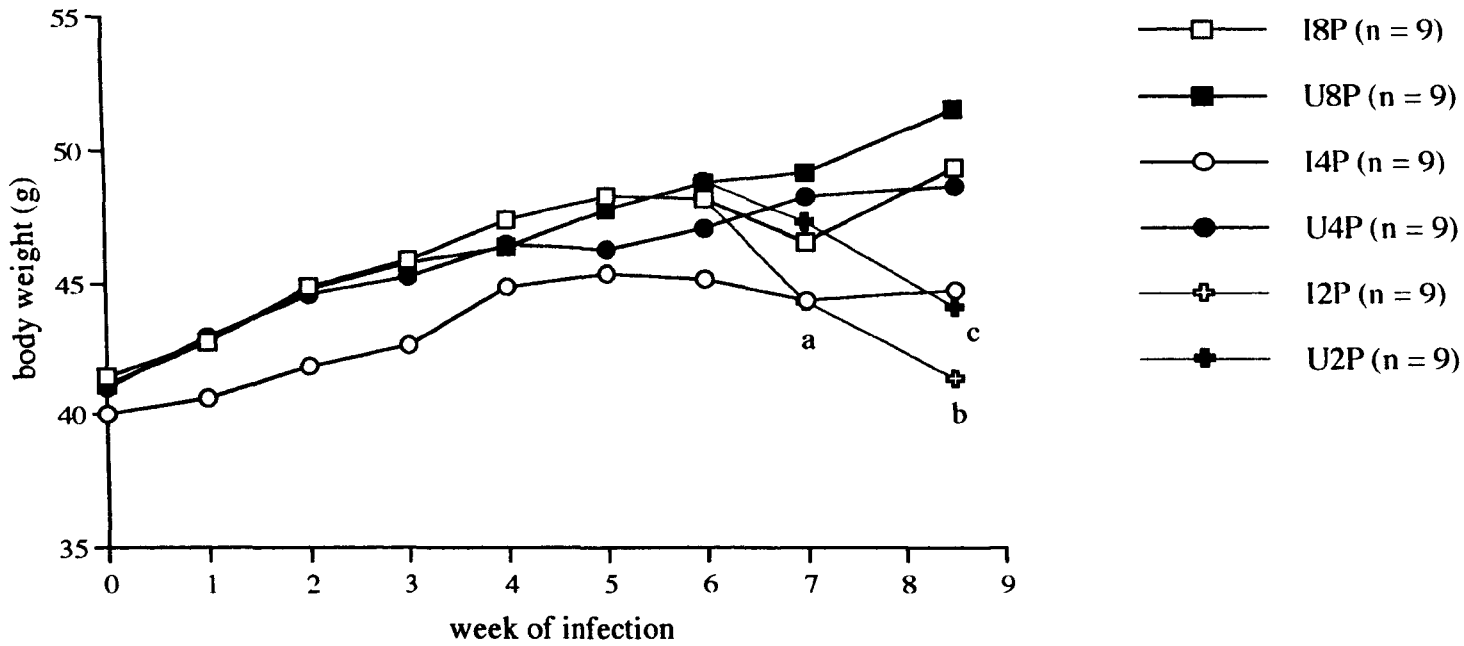

Figure 7.1. Median weekly weights (g) for *S. mansoni*-infected and control mice fed on 8, 4 and 2% protein diets. a: Infected mice on the 4% protein diet (I4P) were significantly lighter than control mice fed on the 8% diet (U8P). b: Infected mice fed on the 2% protein diet (I2P) were significantly lighter than mice fed on the 8% protein diet (I8P and U8P). c: Uninfected mice fed on the 2% diet (U2P) were significantly lighter than U8P. (multiple comparison tests $P < 0.05$). (Uninfected mice on the 4% diet - U4P)

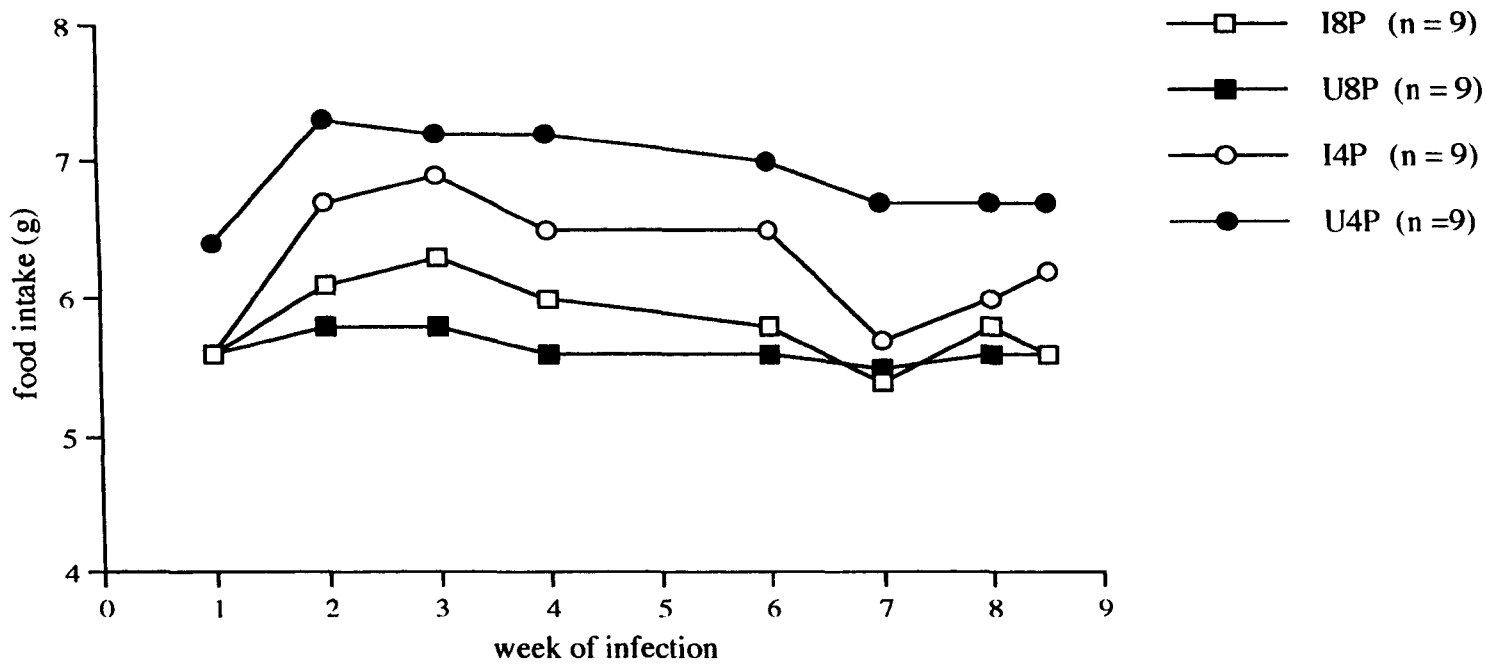

Figure 7.2. Median daily food intake (g) for *S. mansoni*-infected and control mice fed on 8 and 4% protein diets expressed on a weekly basis. Uninfected mice on the 4% protein diet (U4P) consumed significantly greater amounts of food than infected mice on the 4% protein diet (I4P), infected mice on the 8% protein diet (I8P) and uninfected mice on the 8% protein diet (U8P) except during week 6. Infected mice fed on the 4% protein diet consumed more food than I8P or U8P in weeks 2, 3, 4, and 6. Uninfected mice on the 8% protein diet ate less food than I8P during weeks 2 and 3. (multiple comparison tests $P < 0.05$)

Table 7.4. Carcass weights expressed as a percentage of body weight. All values are medians. a: carcasses of control mice on 8% diet significantly greater as a proportion of body weight than carcasses of infected mice ($P < 0.05$). b: carcasses of control mice on 4% diet significantly greater as a proportion of body weight than carcasses of infected mice ($P < 0.05$). For each experimental group n = 9.

	Uninfected control mice	*S. mansoni*-infected mice
8% protein	88.7 [a]	80.4
4% protein	86.5 [b]	79.4
2% protein	84.8	79.0

Table 7.5. Liver weights expressed as a percentage of body weight. All values are medians. a: livers of infected mice on 8% diet significantly greater as a proportion of body weight than livers of control mice ($P < 0.05$). b: livers of infected mice on 4% diet significantly greater as a proportion of body weight than livers of control mice on 2% diet ($P < 0.05$). c: livers of infected mice on 2% diet significantly greater as a proportion of body weight than livers of control mice on 2% diet ($P < 0.05$). For each experimental group n = 9.

	Uninfected control mice	*S. mansoni*-infected mice
8% protein	4.84	6.37 [a]
4% protein	4.01	5.43 [b]
2% protein	3.80	5.66 [c]

Table 7.6. Spleen weights expressed as a percentage of body weight. All values are medians. a: spleens of infected mice on 8% diet significantly greater as a proportion of body weight than spleens of control mice ($P < 0.05$). b: spleens of infected mice on 4% diet significantly greater as a proportion of body weight than spleens of control mice ($P < 0.05$). c: spleens of infected mice on 2% diet significantly greater as a proportion of body weight than spleens of control mice on 8% diet ($P < 0.05$). For each experimental group n = 9.

	Uninfected control mice	*S. mansoni*-infected mice
8% protein	0.24	0.79 [a]
4% protein	0.27	0.78 [b]
2% protein	0.28	0.66 [c]

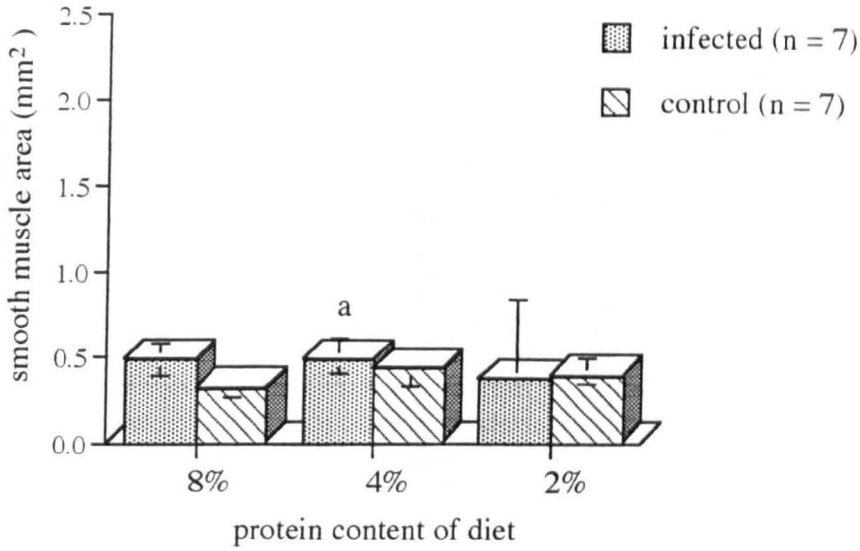

Figure 7.3. Smooth muscle area (mm^2) at a 25% distance from the pylorus for *S. mansoni*-infected and control mice fed on diets with varying protein content. (median values and interquartile ranges)
a: smooth muscle area for infected mice on 4% diet significantly larger than area for control mice fed on 8% diet. ($P < 0.05$)

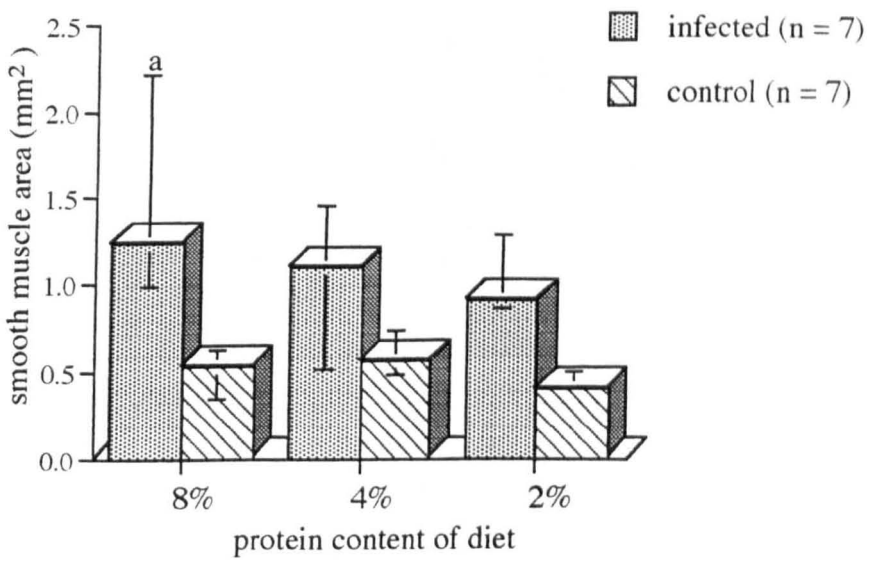

Figure 7.4. Smooth muscle area (mm^2) at a 100% distance from the pylorus for *S. mansoni*-infected and control mice fed on diets with varying protein content. (median values and interquartile ranges)
a: smooth muscle area for infected mice on 8% diet significantly larger than area for control mice fed on 8% diet. ($P < 0.05$)

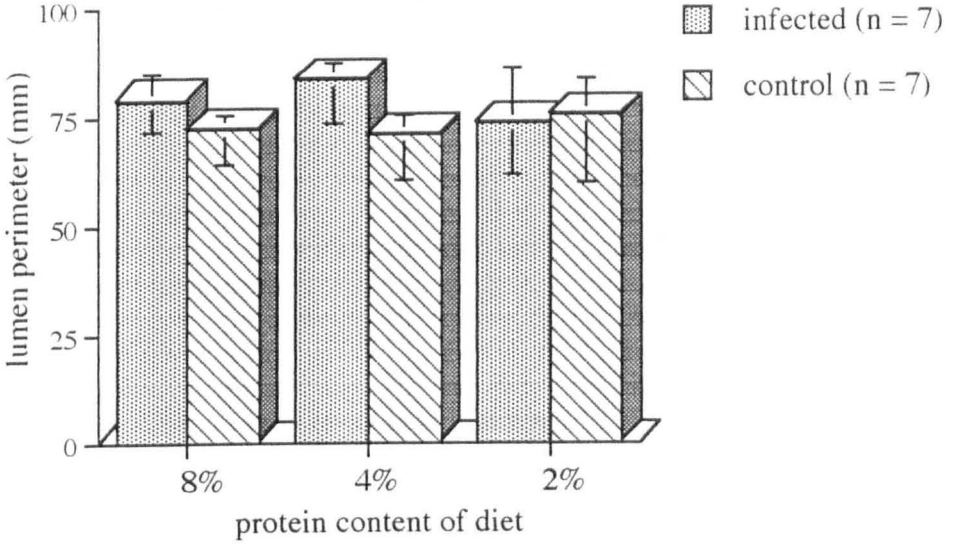

Figure 7.5. Lumen perimeter (mm) at a 25% distance from the pylorus for *S. mansoni*-infected and control mice fed on diets with varying protein content. (median values and interquartile ranges)

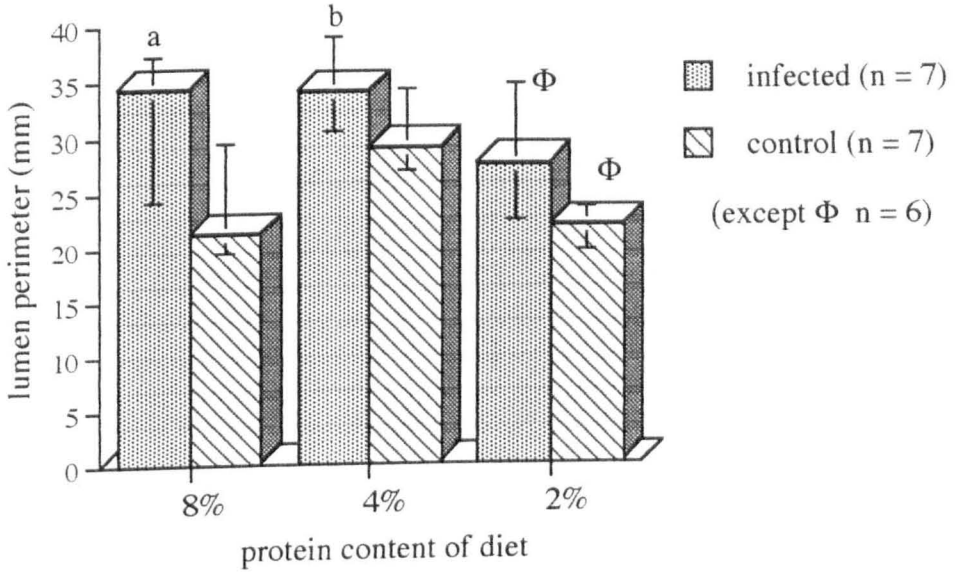

Figure 7.6. Lumen perimeter (mm) at a 100% distance from the pylorus for *S. mansoni*-infected and control mice fed on diets with varying protein content. (median values and interquartile ranges)
a: lumen perimeter for infected mice on 8% diet significantly larger than perimeter of control mice fed on 2% diet. ($P < 0.05$)
b: lumen perimeter for infected mice on 4% diet significantly larger than perimeter of control mice fed on 2% diet or 8% diet. ($P < 0.05$)

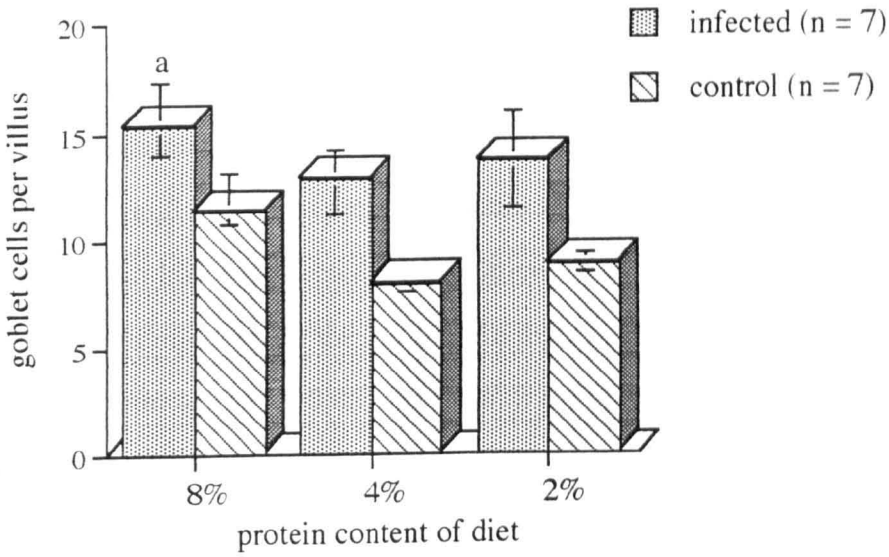

Figure 7.7. Number of goblet cells per villus at a 25% distance from the pylorus for *S. mansoni*-infected and control mice fed on diets with varying protein content. (median values and interquartile ranges)
a: numbers of goblet cells per villus for infected mice on 8% diet significantly greater than for control mice fed on the 4 or 2% diets. ($P < 0.05$)

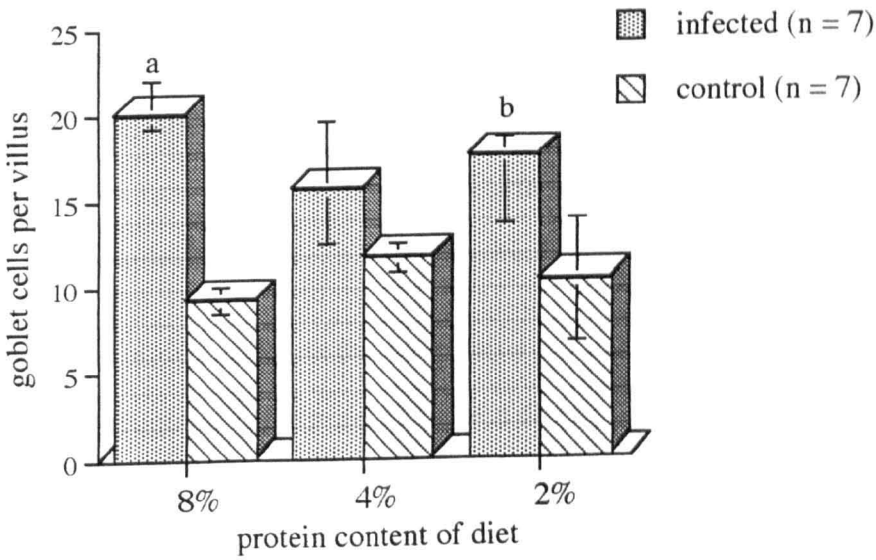

Figure 7.8. Number of goblet cells per villus at a 100% distance from the pylorus for *S. mansoni*-infected and control mice fed on diets with varying protein content. (median values and interquartile ranges)
a: numbers of goblet cells per villus for infected mice on 8% diet significantly greater than for control mice fed on 8, 4 or 2% diets. ($P < 0.05$)
b: numbers of goblet cells per villus for infected mice on 2% diet significantly greater than for control mice fed on 8% diets. ($P < 0.05$)

I4P[1] (*S. mansoni*-infected mice fed on the 4% protein diet) having a reduced body weight when compared to U8P (uninfected, control mice fed on the 8% protein diet) (multiple comparison test, $P < 0.05$). At the end of the experimental period significant variation in body weight was also recorded (Kruskal-Wallis ANOVA, $H = 23.10$, D.F. $= 5$, $P < 0.001$). Both U2P (uninfected, control mice fed on the 2% protein diet) and I2P (*S. mansoni*-infected mice fed on the 2% protein diet) were lighter in weight than U8P (multiple comparison test, $P < 0.05$), and I2P were also lighter than I8P (multiple comparison test, $P < 0.05$).

The daily food intake patterns of mice fed on 8% and 4% protein diets are shown in Fig. 7.2. Significant variation in the food intake was noted each week (week one: Kruskal-Wallis ANOVA, $H = 35.48$, D.F. $= 3$, $P < 0.001$; week two: Kruskal-Wallis ANOVA, $H = 103.13$, D.F. $= 3$, $P < 0.001$; week three: Kruskal-Wallis ANOVA, $H = 103.00$, D.F. $= 3$, $P < 0.001$; week four: Kruskal-Wallis ANOVA, $H = 104.57$, D.F. $= 3$, $P < 0.001$; week six: Kruskal-Wallis ANOVA, $H = 98.45$, D.F. $= 3$, $P > 0.001$; week seven: Kruskal-Wallis ANOVA, $H = 74.3$, D.F. $= 3$, $P < 0.001$; week eight: Kruskal-Wallis ANOVA, $H = 41.23$, D.F. $= 3$, $P < 0.001$; week nine: Kruskal-Wallis ANOVA, $H = 34.34$, D.F. $= 3$, $P < 0.001$). The daily food intake data was not included for week five as the mice accidentally received CRM mouse diet in addition to the formulated diet over a 24-hour period.

Uninfected mice feeding on the 4% protein diet consumed a significantly greater amount of food throughout the experiment except during week six (multiple comparison tests, $P < 0.05$). During weeks two, three, four and six, I4P also ate significantly more food than both I8P and U8P (multiple comparison tests, $P < 0.05$). Uninfected mice fed on the 8% protein diet consumed significantly less food than I8P during weeks two and three (multiple comparison tests, $P < 0.05$).

[1] For economy of style, the following abbreviations are in use: I8P - *S. mansoni*-infected mice fed on the 8% protein diet; U8P - uninfected control mice fed on the 8% protein diet; I4P - *S. mansoni*-infected mice fed on the 4% protein diet; U4P - uninfected control mice fed on the 4% protein diet; I2P - *S. mansoni*-infected mice fed on the 2% protein diet; U2P - uninfected control mice fed on the 2% protein diet.

The influences of dietary protein on the conversion efficiency of total food intake and total protein intake to body weight after the infections reached patency are shown in Tables 7.2 and 7.3. The percentage efficiency of conversion of food consumed into body weight was found to vary significantly (Kruskal-Wallis ANOVA, $H = 17.92$, D.F. = 3, $P < 0.001$), as was the percentage efficiency of protein consumed that was converted into body weight (Kruskal-Wallis ANOVA, $H = 12.93$, D.F. = 3, $P = 0.005$). In both cases, I4P were significantly less efficient in their use of available nutrients for growth than U8P (multiple comparison tests, $P < 0.05$).

7.2.3. Necroscopy

Because of the variation in terminal body weight, carcass weights and visceral organ weights were expressed as a percentage of body weight. Carcass weight, following removal of liver, spleen, small intestine and caecum, and following perfusion of the liver in both infected and uninfected mice, was found to vary significantly in its overall contribution to body weight between the various treatment groups (Table 7.4.; Kruskal-Wallis ANOVA, $H = 39.73$, D.F. = 5, $P < 0.001$). The carcass weights of U4P and U8P made a greater contribution to whole body weight than the carcass weights of any of the infected groups of mice (multiple comparison tests, $P < 0.05$).

Significant variation in liver weight expressed as a percentage of terminal body weight was also observed (Table 7.5.; Kruskal-Wallis ANOVA, $H = 38.00$, D.F. = 5, $P < 0.001$). The contribution of this organ to overall body weight was influenced by the infection status of the mice, with the liver weights of I8P contributing more than for any of the uninfected groups of mice, irrespective of the protein content of diet (multiple comparison tests, $P < 0.05$). The liver weight expressed as a percentage of body weight was also greater for both I4P and I2P when compared to U2P (multiple comparison tests, $P < 0.05$).

Infection status also appeared to be the major factor associated with the variation of spleen weight expressed as a percentage of body weight (Table 7.6.;

Kruskal-Wallis ANOVA, H = 40.40, D.F. = 5, $P < 0.001$). The spleens of I8P and I4P contributed more to body weight than in any of the uninfected groups of mice (multiple comparison tests, $P < 0.05$), and I2P spleen weights contributed more to body weight than the spleen weights of U8P (multiple comparison test, $P < 0.05$).

7.2.4. Histological observations

Schistosome infection was linked with significant variation in the area of smooth muscle in both the anterior region (Kruskal-Wallis ANOVA, H = 12.78, D.F. = 5, $P < 0.05$) and the posterior region (Kruskal-Wallis ANOVA, H = 22.60, D.F. = 5, $P < 0.001$) of the small intestine (Fig. 7.3. and 7.4.). In the 25% region of the small bowel, the smooth muscle area was slightly larger for I4P than for U8P (multiple comparison test, $P < 0.05$), whilst in the 100% region the muscle area was significantly larger for I8P when compared to U8P (multiple comparison test, $P < 0.05$).

The effects of protein consumption and infection on the lumen perimeter of the small intestine are shown in Fig. 7.5. and Fig. 7.6. No significant variation in this parameter was noted in the anterior region of the small intestine (Kruskal-Wallis ANOVA, H = 4.52, D.F. = 5, $P > 0.05$). However, in the posterior part of the small bowel, the lumen perimeter was significantly greater for I8P when compared to U2P, and for I4P when compared to U8P or U2P (Kruskal-Wallis ANOVA, H = 18.34, D.F. = 5, $P < 0.005$; multiple comparison tests, $P < 0.05$).

Goblet cell numbers were also influenced by the infection status of mice (Fig. 7.7. and Fig. 7.8.). In the anterior region of the small intestine significant variations in the numbers of this cell type were observed (Kruskal-Wallis ANOVA, H = 24.26, D.F. = 5, $P < 0.001$), with I8P having greater numbers than U4P or U2P (multiple comparison tests, $P < 0.05$). Goblet cell hyperplasia was also observed in the posterior region of the small bowel (Kruskal-Wallis ANOVA, H = 30.17, D.F. = 5, $P < 0.001$). More goblet cells per villus were present in I8P when compared to any of the groups of uninfected mice (multiple comparison test, $P < 0.05$). Infected mice fed on the 2%

protein diet also had more goblet cells per villus than U8P (multiple comparison test, $P < 0.05$).

7.3. Discussion

7.3.1. Statistical note

The data presented in this chapter were analysed using the Kruskal-Wallis one way analysis of variance (Kruskal-Wallis ANOVA). When Kruskal-Wallis ANOVA revealed significant variation between experimental groups, a multiple comparison test (Siegel and Castellan, 1988) was performed to establish which groups, if any, were significantly different from each other within 95% confidence limits. As with all statistical procedures, a lack of significant variation does not imply necessarily that a true difference between sample populations does not exist, but may instead reflect the sample variation and number of the samples collected for analysis. As the differences between two groups are computed with reference to the entire sample variation when using the Kruskal-Wallis ANOVA and multiple comparison test, this method of analysis is less sensitive than the Mann-Whitney test for comparison of two individual samples. This is stated to emphasise that a lack of statistically significant variation between two groups in this chapter does not necessarily negate the observations in previous chapters in which Mann-Whitney tests were applied routinely, and to explain why, on occasion, inferences are made from trends where no significant variation was observed.

7.3.2. Worm burden

Before interpreting the effects of malnutrition on the pathology caused during *S. mansoni* infection, it was necessary to quantify the worm burdens of infected mice and to establish how the intensity of infection might influence the severity of disease. As the parasite egg is the main aetiological agent of schistosomiasis, the number of worm pairs per mouse was considered to be the most important factor. No significant variation in the number of worm pairs was observed between mice fed on diets

containing different amounts of protein but having the same energy content. This is in accordance with the general observations of Knauft and Warren (1969). It was assumed, therefore, that any variation in the severity of pathology between the groups of mice fed on diets containing different quantities of protein could be attributed to the diet rather than the number of worm pairs.

The significantly greater number of male worms recovered from mice fed on the 2% protein diet when compared to the numbers recovered from mice fed on the 4% diet is difficult to explain. As recoveries of male worms from mice fed on the 4% and 8% diets are quite similar, and the mice on the 2% diet were only fed this reduced level of protein for the last two weeks of the experiment, it does not seem possible that malnutrition affected the number of schistosomes surviving to maturity. Knauft and Warren (1969) support the idea that protein malnutrition does not have any clear effect on the survival of worms. The increased number of male worms recovered from mice fed on the 2% diet may be due entirely to natural variation in host susceptibility, as all infected mice were exposed to cercariae from a single pool.

7.3.3. Growth patterns, food intake and necroscopy

The 8% protein diet was found to support adequately the growth of both uninfected and *S. mansoni*-infected mice, confirming the observations made in Chapter 3. Overt signs of malnutrition, as reflected by statistically lower mouse body weights, were noted for both infected and uninfected animals fed on the 2% protein diet. The combination of *S. mansoni* infection and the marginally deficient 4% protein diet also had an inhibitory effect on mouse growth.

The synergism between infection and malnutrition for I4P growth appeared to arise, at least in part, from a reduction of food intake after the *S. mansoni* infection reached patency. The clear cut reduction of food intake by *S. mansoni*-infected mice noted previously (Chapter 3) was, to an extent, confirmed by the more complicated results of the current investigation. Although no significant reduction in food

consumption was observed for I8P, the slight drop in food intake by these mice between weeks six and seven post infection did coincide temporally with the largest magnitude of reduced food intake that was noted previously (Chapter 3). The pattern of food consumption by I4P, however, appeared to mirror closely that of infected mice fed on the 8% protein diet in Chapter 3. When a direct comparison was made between U4P and I4P, the infected mice ate significantly less throughout the experimental period except for the sixth week of infection. As altered food intake was noted only in the first week of infection and after the infection reached patency in Chapter 3, this implies that a chronic protein malnutrition exacerbates the time scale for disturbance of normal feeding patterns during *S. mansoni* infection. That the reduction of food intake by I4P was made more severe after the infection reached patency is indicated by the observation that I4P consumed significantly more food than either U8P or I8P between the second and sixth week of infection, but not after this period.

Another notable observation made on the growth and food intake of these mice was the maintenance of growth rates by U4P when compared to both infected and uninfected mice on the 8% protein diet. The increased food consumption by U4P appears to account for this observation.

Because of the variable levels of food intake and growth between the four different groups of mice, and the differing intakes of energy and protein, the efficiencies of conversion of protein and nutrients consumed into body weight were used to investigate possible synergism more closely. Significant variation was recorded for both protein and nutrient conversion efficiencies between U8P and I4P. Overall, U8P were the most efficient group of mice, and when chronic malnutrition and infection occurred in isolation efficiencies appeared very similar. The lowest levels of efficiency in the conversion of protein and energy into body weight recorded for I4P demonstrated clearly that the double insult of infection and malnutrition interacted synergistically.

Although not statistically significant, efficiencies of protein and nutrient conversion to body weight were lower for infected mice when compared to their dietary

controls. Altered efficiencies in the conversion of nutrients to body weight would be expected if the same energy costly process of the redistribution of body weight during *S. mansoni* infection that was noted in Chapter 4 was also occurring in this study.

Carcass weights and organ weights were expressed in terms of percentage of body weight in the current investigation because of the significant variation of terminal body weights induced by protein malnutrition. This avoided any confusion of results that may have arisen from the scaling effects of body size and organ weights. The contribution of carcass weight to body mass was found to be lower in adequately nourished and marginally malnourished mice infected with *S. mansoni* when compared to their dietary controls. In the case of I8P, the reduction of carcass weight was accompanied by a significant increase in the contribution of spleen and liver weights to body mass. Increased spleen and liver weights were also noted for I4P when compared to U4P, but this was only significant for spleen weight. When I2P and U2P were compared, however, no significant changes in carcass weight or spleen weight were noted.

The smaller changes in body weight redistribution in severely protein malnourished mice infected with *S. mansoni* (I2P) appear to reflect the overwhelming effect of this dietary restriction. Any one of several factors may contribute to this lack of significant change in weight distribution. Firstly, as discussed in Chapter 3, the increase in visceral organ weights are all due largely to the host response to schistosome eggs. One aspect of the host response to this infection that is known to influenced by malnutrition is granuloma formation. Severe protein and calorie malnutrition in mice with schistosomiasis mansoni is associated with a diminished granulomatous response (Knauft and Warren, 1969; Akpom and Warren, 1975a). A diminished granulomatous response in this study could account for the apparently smaller degree of visceral organ pathology.

The suppressive effect of malnutrition on the host granulomatous response to eggs appears to result from an altered host response rather than an effect of dietary

deficiency on the antigenic nature of schistosome ova (Akpom and Warren, 1975a). It is generally agreed that the host response to these eggs is largely a T cell-mediated process (see Chen and Mott, 1988). Cell-mediated immunity is frequently impaired by undernutrition, with reductions in the number of circulating T lymphocytes, impaired maturation of T lymphocytes and decreased cutaneous hypersensitivity reactions among the commonly noted effects (reviewed by Chandra, 1983; 1984; Chandra *et al.*, 1987).

The cytokines co-ordinating T-cell responses may also be affected by malnutrition. For example, protein deficiency can reduce the cytokine-producing capacity of leukocytes (Kauffman *et al.*, 1986), with monocytes from malnourished children displaying a reduced interleukin-1 activity when exposed to endotoxin (Bhaskaram and Sivakumar, 1986). As cytokines play an important role in the control cell-mediated responses (Meager, 1990), it seems likely that malnourished mice with schistosomiasis demonstrate a reduced granulomatous response because of an impairment of both cell-mediated immunity and its co-ordination by cytokines.

A recent investigation of the affects of dietary restriction on murine cerebral malaria revealed that a small reduction in the amount of food made available to mice led to a considerable reduction in mouse mortality (Hunt *et al.*, 1993). Decreased mortality did not appear to be a consequence of reduced parasite replication, nor were there any large changes in the numbers of leukocytes in the major lymphoid organs. Instead, enhanced survival by mice with restricted food intake appeared to arise from an inhibition of TNF (tumour necrosis factor) production. Assuming that TNF is important in granuloma formation in mice with schistosomiasis, and that production of this cytokine is elevated during murine schistosomiasis as in the human disease (Chensue *et al.*, 1989; Zwingenberger *et al.*, 1990; Amiri *et al.*, 1991), an inhibition of TNF production in malnourished mice may, at least in part, be responsible for diminished granuloma size.

An inhibition of TNF production in severely protein malnourished mice provides a second hypothesis to explain the smaller effects of disease on body weight

distribution for I2P. As discussed in Chapter 4, TNF may be involved in proteolysis of skeletal muscle, with this process possibly providing some of the substrates for the increase in liver, spleen and small intestine size observed during murine schistosomiasis mansoni. If TNF production is reduced in protein malnourished mice, this might lessen the degree of skeletal muscle proteolysis, and explain the smaller change in carcass weight that are associated with severe malnutrition and infection in this study.

Beneficial effects of malnutrition have been observed during murine schistosomiasis mansoni (Knauft and Warren, 1969; Akpom and Warren, 1975). Mice fed on severely protein-deficient diets appear to harbour slightly smaller *S. mansoni*, and to have reduced numbers of eggs in their livers that is suggestive of a decreased egg output by adult worms (Knauft and Warren, 1969). This, again, should result in reduced manifestations of hepatosplenic disease, presenting a third mechanism by which only small changes in body weight distribution would occur in infected mice fed on the 2% protein diet.

In malnourished mice infected with *S. mansoni*, a reduction in the size of worms and their egg output, a diminished granulomatous response brought about by an impaired T-cell response or an altered pattern of cytokine release, and a reduction of TNF acting to induce skeletal muscle proteolysis would appear to be beneficial to the host. A reduction of hepatosplenomegaly accompanying a diminished granulomatous response in severely protein malnourished mice has been reported previously (Knauft and Warren, 1969). However, as the granulomatous response is generally considered to be protective against acute hepatotoxicity reactions in this disease (Byram and Von Lichtenberg, 1977; Doenhoff *et al.*, 1979; Amiri *et al.*, 1992), the benefit of reduced hepatosplenic disease must be somewhat debatable. Indeed, Knauft and Warren (1969) did observe necrosis of the liver parenchyma in mice fed on severely protein-restricted diets. In addition, haematocrit values for these mice were low and serum protein concentrations were very low. Knauft and Warren (1969) concluded that, ultimately, severe protein malnutrition was harmful for both the host and the schistosome.

Overall, as far as growth and nutrient utilisation are concerned, this investigation indicates that malnutrition and infection act synergistically when protein malnutrition is marginal. Severe restriction of dietary protein has a profound effect on both infected and control mice.

Some of the general effects of protein malnutrition on mouse growth during acute schistosomiasis mansoni have been examined previously (Knauft and Warren, 1969). Whilst the overall impression from the results of Knauft and Warren's study were similar to those of the current investigation, some differences were observed. For example, Knauft and Warren (1969) noted a fairly dramatic drop in the weight of infected mice fed on an 8% protein diet when compared to uninfected mice on the same diet. This is in contrast to the maintenance of I8P weights relative to U8P in this study. The disparity appears to relate to the use of smaller, and probably younger, mice by Knauft and Warren (1969), with uninfected mice on an 8% protein diet gaining approximately 70% of their starting weight in nine weeks. In the current study, a smaller 25% weight gain was observed in uninfected mice on the 8% diet. The greater requirements for protein during rapid growth appear to make the 8% diet used by Knauft and Warren (1969) more analogous to the 4% diet in this investigation.

Knauft and Warren (1969) also observed that uninfected mice on a 4% protein diet grew less than mice on the 8% diet. In the present study, the increased food consumption appeared to be the major factor in enabling U4P to maintain weight relative to animals with a higher protein intake. Therefore, the restriction of food intake to a predetermined energy requirement in Knauft and Warren's experiment appears to explain this conflict of data.

Overall, the results of this study and that of Knauft and Warren (1969) are in agreement with the fact that protein consumption and infection status can influence the growth of mice. The combined effects of *S. mansoni* infection and malnutrition are synergistic, and result in a reduction of host growth rate during acute infection. It should be noted, however, that a four week period of protein malnutrition had no

obvious synergistic effect on the growth of mice with chronic schistosomiasis mansoni (Akpom and Warren, 1975). This may relate to the short and transient nature of the malnutrition imposed by Akpom and Warren (1975), or to the down regulation of the host's immunological response to the schistosome egg in chronic disease (Smithers and Doenhoff, 1982).

Similar synergistic relationships between helminth infections and protein malnutrition are observed quite commonly in the laboratory setting. For example, *Trichuris muris* infection in mice is made more severe by protein deficiency, reducing the growth rate of mice and impairing their acquired immunity to this infection (Michael and Bundy, 1992). Similarly, immunity to *Heligmosomoides polygyrus* in the mouse is impaired by malnutrition (Slater and Keymer, 1988). Rats infected with *N. brasiliensis* and fed on a low protein diet were found to consume less food, gain less weight and to have an impaired immune response to this nematode when compared to either dietary controls or well-nourished, infected rats (Lunn *et al.*, 1986). Comparative observations have been made in animals of agricultural importance, including *Ascaris suum* in pigs (Forsum *et al.*, 1981) and *Haemonchus contortus* in lambs (Abbott *et al.*, 1986).

7.3.4. Intestinal pathology

In Chapters 4 - 6, smooth muscle hypertrophy, an increase in lumen perimeter and goblet cell hyperplasia in the small intestines of *S. mansoni*-infected mice were all observed to occur with greater severity in the posterior region of the bowel. The same overall pattern was also observed in this study.

Smooth muscle area in the proximal region of the bowel appeared to be affected only slightly by infection or nutritional status. The change was more dramatic in the distal region of the small intestine, with all infected mice demonstrating at least a two-fold increase of intestinal smooth muscle mass when compared to their dietary controls. That this was only significant for mice fed on the 8% protein diet probably reflects the statistical methods employed (see statistical note 7.3.1). Although not

significant, the smallest muscle mass for infected mice was observed for I2P. This would presumably reflect a smaller intestine size for mice with the lowest body weight. However, as the area occupied by granulomas were included in the measurements of muscle hypertrophy, this observation might also have been influenced by a diminished granulomatous response to schistosome ova similar to that noted in the liver and lungs of malnourished mice (Knauft and Warren, 1969; Akpom and Warren, 1975a).

In the proximal region of the small intestine, no significant change in lumen perimeter was observed, confirming the observations of Chapter 5. Villous surface area appeared to be influenced to a greater extent by infection status in the posterior region of the small intestine, with lumen perimeter being significantly greater for I4P when compared to U2P and U8P, and for I8P when compared to U2P. That no significant variation was recorded for infected mice on the 2% diet may again indicate a scaling effect of body size, which would have an influence on the outcome of the Kruskal Wallis ANOVA (see statistical note 7.3.1). Alternatively, it may relate to an affect of the acute period of severe malnutrition on the ability of the host to increase the villous surface area in response to this infection.

Significant increases in the numbers of goblet cells in the small intestines of infected mice were noted in both the anterior and posterior regions of the bowel. Infection status, rather than nutritional status, appeared to be the most important variable influencing goblet cell hyperplasia.

Overall, smooth muscle hypertrophy, an increase in intestinal surface area and goblet cell hyperplasia all appeared to occur to some extent in infected mice in the current study, regardless of the imposed protein restriction. In addition, there were no obvious signs of synergistic or antagonistic interactions between malnutrition and infection in relation to intestinal pathology.

This is in contrast to the intestinal pathology induced by *Nippostrongylus brasiliensis* infection in rats that appears to be exacerbated by protein malnutrition

(Martin, 1980). Intestinal damage that appeared to be localised in the jejunum of adequately nourished rats infected with this nematode was found to be more diffuse in rats on a low protein diet. Malformed villi, some of which were devoid of microvilli, were also observed. Whilst the intestines of adequately nourished mice returned to a normal structure by 20 days post infection, the villous architecture in malnourished animals was still deranged when the experiment was terminated at 24 days post infection (Martin, 1980).

It has been noted, however, that pigs infected with *Ascaris suum* and fed on a low protein diet have pathological changes in the small intestine similar to those in infected pigs fed on high protein diet. Stephenson *et al.* (1980) examined smooth muscle hypertrophy, villous structure and goblet cell hyperplasia in the small intestines of pigs infected with *A. suum* and fed on diets with either a high or low protein content. Smooth muscle area was increased in the intestines of pigs infected with *A. suum*, irrespective of the protein content of the diet. There was no significant variation in height of villi, but infected pigs had a smaller villus to crypt ratio. Again, this did not appear to be influenced by protein availability. Goblet cell hyperplasia was also observed in the intestines of infected pigs. However, the number of samples examined in relation to this parameter made it impossible to draw any conclusion about the influence of protein restriction on this hyperplasia. These results were therefore very similar to those of the current investigation, with intestinal pathology not appearing to be influenced greatly by a reduction of dietary protein.

The exacerbation of intestinal pathology by malnutrition may depend on the nutritional costs imposed by differing severities and types of infection. For example, *N. brasiliensis* infection in rats causes blood leakage into the small intestine (Martin, 1980), which must result in a loss of protein. This protein loss, in combination with the vast array of metabolic disturbances induced by this nematode in the rat which include anorexia, reduced growth and skeletal muscle atrophy (Robertson, 1989), may tip the balance towards a synergistic pathology in the small intestine when the host is subject

to this infection and protein malnutrition. In the current study, intestinal pathology does not appear to influenced greatly by nutritional status, suggesting that the protein cost of these responses during *S. mansoni* infection can be met adequately by the host, even if this results in a loss of body weight.

The redistribution of nutritional resources towards smooth muscle hypertrophy, an increase in villous surface area and goblet cell hyperplasia in the small intestine during periods of reduced protein intake may underline the importance of these responses in *S. mansoni*-infected mice. For example, there was some indication that villous surface area may have increased in U4P relative to U8P and U2P, although this was not significant. This observation would be consistent with the fact that the height of villi in the jejunum of rats deprived of protein for 45 days were of a comparable height to villi in the jejunum of well-nourished rats, even though the malnourished rats were less than one fifth of the size of adequately nourished animals (Hill *et al.*, 1968). In the malnourished animal, an increase in lumen perimeter would help to maximise the digestion and absorption of any available protein. In the *S. mansoni*-infected mouse subject to both malnutrition and pathological changes in the microvillus structure in the small intestine, the ability to increase the villous surface area may be even more important. That no similar trend was observed for mice fed on the 2% protein diet may be explained by the short time scale of this imposed malnutrition. It appears that the apparently adaptive response of maintaining a large jejunal surface area in protein-deprived rats takes longer than four weeks to develop (Hill *et al.*, 1968).

Smooth muscle hypertrophy may be essential to maintain an adequate flow of digesta through the bowel made more rigid by the presence of egg-induced granuloma formation. Mixing of digesta in the malnourished *S. mansoni*-infected host would also be important to maximise contact of nutrients with the luminal surface. If mucus is important for the repair of cells damaged by the passage of parasite ova through the mucosa, it would appear even more important in the malnourished host where the rate of intestinal epithelium replacement appears to be depressed (Deo and

Ramalingaswami, 1965; Hopper *et al.*, 1972). Overall, if the teleological explanations for these morphological changes in the small intestine are correct, they may prove even more vital for the survival of the malnourished host infected with *Schistosoma mansoni*.

7.4. Conclusions

Protein deficiency appeared to have little influence on the changes in intestinal structure that are associated with *S. mansoni* infection in the mouse. However, a synergistic interaction between this infection and malnutrition was evident for infected mice that underwent a marginal but chronic protein deficiency. This synergism appeared to be due to the combined effects of a reduction of food intake and a change in the efficiency with which energy and protein were assimilated as body weight. The reduction of the carcass weights of infected mice, accompanied by intestinal hypertrophy and hepatosplenomegaly, indicated that this change in efficiency seemed to arise, at least in part, from a redistribution of host resources. Severe protein malnutrition had a profound effect on both infected and control mice.

7.5. Summary

1. *Schistosoma mansoni* infection had a significant influence on the growth rate of mice fed on the 4% protein diet.

2. The 2% protein dietary regime caused a loss of weight in both infected and control mice.

3. Uninfected mice on the 4% protein diet maintained growth rates similar to adequately nourished mice. An increase in food intake appeared to account for this observation.

4. The food intake of *S. mansoni*-infected mice fed on the 4% protein diet was significantly reduced when compared to their dietary controls for most of the experimental period. This reduced food consumption appeared to be exacerbated after the infection reached patency.

5. The percentage efficiency of the conversion of protein and food consumed into body weight was significantly reduced in *S. mansoni*-infected mice fed on the 4% protein diet.

6. Carcass weights of infected mice fed on the 4% and 8% protein diets contributed less to overall body weight than carcass weights of dietary controls. Smaller changes were observed for mice fed on the 2% protein diet.

7. Smooth muscle hypertrophy in the small intestines of *S. mansoni*-infected mice did not appear to be influenced greatly by nutritional status. Similar observations were made for goblet cell hyperplasia and villous hypertrophy.

Chapter 8

General conclusions

Normal growth patterns in animals are generally considered to be adequate indicators of a healthy nutritional status. If the results presented in the preceding chapters are viewed from this perspective, then the maintenance of normal growth patterns in *Schistosoma mansoni*-infected mice suggests that nutritional status is little influenced by this parasite during the acute stages of infection. However, when other aspects of host pathology and nutritional physiology are considered, it is obvious that schistosomiasis mansoni has a nutritional cost for the laboratory mouse.

From the moment *S. mansoni* first infects its host, the cost of infection starts to grow. Although the schistosome must obtain its nutrients for growth and reproduction from the host, this energy demand is likely to be a relatively small toll of infection. Instead, the major nutritional drain for the host appears to arise from a diversion of resources into defence mechanisms and pathology associated with the eggs of *S. mansoni.* These responses to infection include granulomatous reactions to the parasite ova, a reduction of food intake, hypertrophy and hyperplasia of cells in the small intestine, and a redistribution of body weight away from skeletal mass towards some visceral organs.

Although the diversion of host resources into pathological responses to *S. mansoni* infection must be considered as a nutritional cost of disease, the overall effects on host mortality and morbidity should also be considered. For example, the role of the granulomatous response in preventing toxic reactions to ova (see Chapter 3) demonstrates the seemingly essential nature of this defence mechanism. While this is beneficial in lengthening the life expectancy of the infected host, the same response may result in the negative consequence of reduced host food intake during infection (Chapter 3), and, ultimately, can lead to the development of fatal pathology.

The costs and benefits of intestinal pathology during schistosomiasis mansoni are difficult to assess. It can be postulated that the hypertrophy of intestinal smooth muscle in infected mice helps to maintain normal motility patterns in an organ made more rigid by inflammatory reactions to schistosome ova (Chapter 4). The obvious cost of this response is the extra protein that is diverted to the gut. However, although an attempt was made to assess intestinal motility in *S. mansoni*-infected mice, it is not clear what affect muscle hypertrophy has on the movement of gut contents. It would be interesting to investigate further the functional effects of intestinal smooth muscle hypertrophy.

Likewise, the role of goblet cell hyperplasia during murine schistosomiasis mansoni is unclear (see Chapter 6). Increased mucus production may help to eliminate a variety of enteric helminths from the small intestine, but a similarly protective role cannot be attributed to this response during *S. mansoni* infection. Instead, it was suggested that goblet cell hyperplasia might allow for increased intestinal lubrication in the inflamed gut. A potential role for mucus in the repair of enterocytes damaged by schistosome ova was also suggested; this theory could be tested by examining the effects of mucolytic agents on epithelial cell recovery. Increased amounts of mucus in the intestines of infected mice might also be deleterious for the host by interfering with nutrient uptake or mucosal resistance to infection. It would be of value to investigate these hypotheses more fully.

The changes in intestinal structure observed during murine schistosomiasis also occur as a response to a variety of other enteric infections. These include *Nippostrongylus brasiliensis* and *Trichinella spiralis* infections in rodents, and *Ascaris suum* infection in pigs (reviewed by Castro, 1989; 1990; Moqbel and MacDonald, 1990). The trigger mechanisms responsible for these intestinal changes remain to be elucidated. The one common feature of these parasitic infections is the inflammatory response to the pathogen in the gut. It is also clear that cytokines are involved with the movement of cells to sites of inflammation (Klein, 1990).

The involvement of cytokines in inflammation lead to the hypotheses that PDGF (platelet-derived growth factor) might be important in the hypertrophy of intestinal smooth muscle (Chapter 4) and that PAF (platelet activating factor) might act as a trigger for goblet cell hyperplasia and mucus secretion (Chapter 5). The relationship between TNF (tumour necrosis factor) and reduced food intake was also investigated (Chapter 3). Although TNF was not detected in sera taken from *S. mansoni*-infected mice, this may relate to the difficulties of measuring TNF rather than a lack of production (Chapter 3). It is interesting to note that TNF can mediate the release of PDGF (Hajjar *et al.*, 1986), and that PAF may act as a secondary mediator for TNF (Sun and Hsueh, 1988, 1991).

Elevated production of TNF has been observed during human schistosomiasis mansoni (Zwingenberger *et al.*, 1990), and this cytokine also appears to be involved in the granulomatous response to schistosome eggs (Chensue *et al.*, 1989; Amiri *et al.*, 1992). TNF has also been proposed as the mediator of concomitant immunity during schistosomiasis (Hagan *et al.* 1993). In addition, circumstantial evidence suggests that TNF could be a common denominator linking much of the pathology observed during murine schistosomiasis. For these reasons, the discussion in this thesis has placed considerable emphasis on the involvement TNF during this disease, although production of this cytokine was not detected. While it is recognised that other factors may be responsible for the co-ordination of host responses to *S. mansoni* infection, the use of alternative bioassays or immunoassays to those employed in this study may help to resolve the issue of TNF production during murine schistosomiasis.

The information presented in this thesis suggests that, although there is a nutritional toll to murine schistosomiasis mansoni, the redistribution of host reserves into defence mechanisms appears to be important for the continued survival of the parasite-infected host. However, the delicate nature of this cost and benefit equilibrium was demonstrated by the synergism between protein malnutrition and infection. Perhaps of particular interest was the reduction in the efficiency of conversion of food eaten into

body weight in malnourished, infected mice. It was suggested in Chapter 3 that reduced efficiency might be offset by a reduction in the activity of infected mice. While this is possible for laboratory mice that have free access to food supplies, wild rodents must continue to forage for food. In a similar manner, the humans most likely to have schistosomiasis are often the agricultural workers who lack the financial resources to rest when ill. Maintaining activity levels may tip the balance of this disease towards weight loss and a deteriorating nutritional status. A variety of other environmental factors, such as concurrent infections, may also be synergistic with *S. mansoni* in relation to host nutrition.

It was also proposed that skeletal muscle might be the source of protein for the additional immunological and inflammatory agents produced in response to this infection (Chapter 4). If skeletal muscle wasting occurs in the infected host, this might explain, in part, the altered activity patterns that are observed in hamsters with patent *S. mansoni* infections (Kavaliers and Podesta, 1988). In relation to mouse activity during infection, an investigation of the size and aerobic capacity of a muscle such as the gastrocnemius may provide further insight into the influence of schistosomiasis mansoni on physical fitness.

Overall, this thesis suggests that there is a nutritional cost to *S. mansoni* infection in mice, and that the relationship between *S. mansoni* infection and nutritional status is dynamic. Whilst human infections are almost invariably much lower in intensity than those in mice, a nutritional toll for humans with this insidious disease must exist. A more complete appreciation of the magnitude of this toll remains to be established through further investigations of the interactions between infection and nutrition.

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