INVESTIGATION OF SHARED ANTIGENS BETWEEN *SCHISTOSOMA MANSONI* AND THE INTERMEDIATE HOST, *BIOMPHALARIA PFEIFFERI*

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ABSTRACT

Schistosomiasis is a major health problem especially in the developing countries within the tropics. The search for an effective vaccine has however been frustrated by two main contraints namely, the antigenic complexity of the parasite and the difficulty of producing adequate research material from the tiny parasite stages. These two problem would be overcome if an alternative source of immunising antigens could be found. The present work involved a search for such antigens from the snail vector of Schistosoma mansoni, Biomphalaria pfeifferi.

Antigen fractions from various parts of the vector snail were isolated using conventional immunochemical techniques. Similarly antigens were obtained from different stages of S. mansoni using homogenisation and centrifugation techinques. A battery of antisera of all the antigenic preparations were raised in the rabbits and antigenic cross-reactivity was investigated using gel diffusion technique. The potency of each antisera was ascertained by reacting it with its homologous antigen. The results obtained clearly indicate that both the digestive gland and the alimentary canal of the snail vector, possess antigens which cross-react with S. mansoni egg antigens. No such cross-reactivity was detected between the foot of B. pfeifferi and any of the stages of S. mansoni. Similarly, no shared

antigens were detected between the digestive gland of the non-vector snail *Bulinus africunus* and *S. mansoni*.

In order to establish their immunising potential and the degree of protection, the snail antigens were used to immunise groups of hamsters and the numbers of adults worms recovered from the immunised groups were compared with those from control groups. The results were statistically significant and indicated that the best protection was achieved using antigenic preparations from either digestive gland or the alimentary canal of the snail. The percentage worm reduction for the digestive gland and its two antigenic fractions were 28.5%, 65.0% and 52.4% respectively, while that of the alimentary canal and its two antigenic fractions were 45.6%, 52.6% and 56.6% respectively. The least worm reduction was obtained from the foot and its two antigenic fractions. These were 12.3%, 26.2% and 24.6% respectively.

The results therefore, of both antigenic cross-reactivity and immunisation experiments strongly suggest the presence of shared antigens between *Schistosoma mansoni* eggs and the intermediate host, *Biomphalaria pfeifferi*.

These results agree with those obtained by other workers. Lehman and Ruahini (1982) obtained 63.9% protection

against _S. mansoni_ when they immunised mice using crude extracts of the whole snail, _B. pfeifferi_. Similarly Carlos _et al_. (1985) found out that the digestive gland of _S. mansoni_'s Puerto Rican vector, _Biomphalaria grabrata_ shared 2.8% of its antigens with _S. mansoni_.

Suggestion are made for further research work in trying to develop a vaccine against schistosomiasis.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1 (a) General Introduction

Schistosomiasis is one of the major health hazards in the tropical countries where it affects 200 - 300 million people. Prolonged infections with schistosomiasis results in generalised weakness and incapacitation of the patient and may eventually lead to death. The disease is caused by the trematode worms of the genus *Schistosoma*. Although a number of species have been reported to infect humans, only three of these are of significant importance. These include *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*.

Schistosoma haematobium which causes urinary schistosomiasis occurs in Africa, Mauritius, Near East, Iraq, Portugal and India. *Schistosoma mansoni* which causes intestinal schistosomiasis occurs in Africa and South and Central America, while *S. japonicum*, the other causative agent for intestinal schistosomiasis is restricted to the Far East countries including the Phillipines, China, Thailand, Laos and Cambodia.

In Kenya, where it is estimated that at least 1.5 million people are infected with schistosomiasis, the two important species are *S. haematobium* and *S. mansoni*. *Schistosoma haematobium* is found in the coastal

districts of Kilifi, Kwale, and at the lower reaches of the river Tana, in Taveta, Machakos and Kitui districts and also in scattered foci in Muranga, Kiambu and Western Kenya. Schistosoma mansoni is mainly distributed in Machakos and Kitui districts. It is also found in Muranga, Kiambu, Kirinyaga and along the shores of Lake Victoria and Lake Jipe (Highton, 1974).

1 (b) Morphology and Life Cycle

The life cycle of the three human infecting species of Schistosoma is similar and may therefore be represented by a description of the life cycle of S. mansoni which was described first by Bilharz (1852).

The male is creamish in colour, broader than the female, bears a gynaecophoric canal and measures 6-12 mm long, with a diameter of 1.1 mm. The number of testes varies from 4 to 13 and these are anteriorly placed. The female is dark, slender and relatively larger than the male measuring 10-20 mm long by 0.6 mm wide. The uterus is placed in the anterior half of the female. The eggs of S. mansoni measure 140 µm by 61 µm and possess characteristic lateral spine. These are laid by the female adult worm in the mesenteries from where they must penetrate into the lumen of the intestine of the definitive host and are then passed out with faeces.

Eggs hatch into miracidia in fresh water under conditions of light and warmth. The miracidia then swim

actively by means of cilia until they encounter an appropiate snail, or die within 48 hours. Penetration into the vector is through the soft parts of the body and is accomplished with the help of secretions from the anteriorly placed penetration glands. Once inside the snail, the miracidia loses its ciliated epidermal layer and develops into a mother sporocyst which soon becomes filled with germ balls. Mother sporocysts bursts after 8 days releasing several germ balls. These migrates to the digestive glands where each develops into a thin-walled daughter sporocyst. A further process of asexual multiplication takes place and daughter sporocyst becomes filled with final larval stage, the cercariae. Emergence of cercariae from the snail occur 4 - 5 weeks after infection and is influenced by lihgt. One snail may emmit between 1,000 and 2,000 cercariae daily, though with time, this level may decrease. Cercariae normally measure 300 - 400 µm in length and have a biforked tail. Once out of the vector, cercariae may swim in water for 12 to 24 hours. When they come into contact with the wet skin of a definitive host, they penetrate between the hair follicles by means of anterior spines and the cytolytic secrection of the cephalic glands. The penetration process takes 5 - 15 minutes and cercariae lose their tails and become schistosomula. The schistosomula enter peripheral

lymphatic or venous vessels and are carried to the lungs where they may be observed 4-7 days after penetration. They then move from the lungs to the portal vessels where they grow into male and female adults. These then pair up and remain in copula with the female lying in the gynaecophoric canal of the male. In this state, the adult worm pairs migrate to the mesenteric veins where the female begins to lay eggs. Eggs of S. mansoni appear in stool 3 months after the cercariae penetration.

1 (c) Host Preference

The three Schistosoma species which are important in human infection differ in their host preference.

Schistosoma haematobium adult worms live in the veins of the urinary bladder of man, who is the only important definitive host. S. mansoni adult worms live in the lower mesenteric veins of man and more rarely in some rodents and baboons while S. japonicum adult worms are found in the upper mesenteries of the small intestines in man, cattle, water-buffalo, horses, dogs, cats, rodents and monkeys.

The parasites may also develop to maturity in a number of experimental animals. These are often referred to as permissive hosts. They include mice, hamsters, vervet monkeys and Rhesus monkeys.

Snails of various genera act as intermediate hosts for different species of schistosomes. The geographical distribution of the snails therefore influence the endemicity of the parasite. S. haematobium is transmitted by the species of the genus Bulinus where two species of bulinid snails are important in that respect. The africanus (subgenus Physopsis is an important vector particularly in Eastern and Southern Africa and in the southern parts of the Sahara. Then there is the truncatus group (subgenus Bulinus) which acts as an intermedite host in the near East and in some parts of West Africa. S. mansoni is transmitted by species of genus Biomphalaria. These snails are specific for S. mansoni and four groups are important vectors in Africa. These include (i) the pfeifferi group which includes some of the most efficient vectors. This group is found in streams, seepages and a variety of man-made waterbodies including water channels, dams and swimming pools. Isolated populations are found in the Sahara in Algeria (Ranson, 1953), South West Libya (Mandahl-Barth, 1958) and Chad. B. pffeiferi is also found in Arabia (Arfaa, 1972). The group is widespread south of an arbitrary line passing approximately through Asmara in Ethiopia (Brown, 1965) the Nile/Atbara confluence in Sudan (Malek, 1958; Williams and Hunter, 1968), Lake Chad and Lake Senegal. Recorded South West limits lie on the plateau of South Angola (Wright, 1963a) and in North West Transvaal (Van Eeden and

Combrick, 1966). It also occurs in a narrow zone extending down the coast of Natal to Port Saint Johns (Van Eeden et al., 1965). Its absence in most of South Africa highveld and South West is thought to be probably due to combined adverse effects of cool winter climate and low rainfall. The pfeifferi group is the most important intermediate host of S. mansoni in Africa. The other groups of Biomphalaria snails are (ii) the choanomphala group, species of which occur mainly in lakes and therefore form the main vector along the shores of Lake Victoria; (iii) the alexandria group which occurs sporodically in the North, East and South Africa and (iv) the sudanica group which occurs in swamps in both East and West Africa (Mandanl-Barth, 1958). The main intermediate hosts of S. japonicum are members of the genus Oncomelania. This is a small conically shaped snail measuring 3 - 8mm. The species involved in transmission are O. hupensis in China mainland, O. nosophara in South West China, O. formosana in Taiwan and O. quadrasi in the Phillipines (Cheng, 1971).

Both Bulinus and Biomphalaria are aquatic but Oncomelania is amphibious. The snail vectors of human schistosomiasis live in well aerated water with vegetation. The latter serves as food and supplies leaf surface for egg deposition. In addition snails feed on algae and absorb calcium for their shells. Although snails can survive at higher temperatures, they seem to

thrive best at an optimum temperature range of 22-23°C (Malek, 1963). Muddy irrigation canals are ideal for snail breeding as these encourage vegetation growth and mud also absorbs harmful chemicals. Snails are also found in slow-flowing water of ponds, dams, lakes and slow streams or rivers.

Most snail populations fall during the rainy seasons and increase in the drier and warmer months of the year. However, where water temperatures are more stable, some snail populations (e.g. O. quadrasi in Far East), show no seasonal trends. Although droughts and floods reduce the population, some infected snails do survive and carry infection from one wet season to another (Malek, 1963). Predators of snails include birds and other large snails like Marisa cornuarietus and Tarebia granifera (Webbe, 1969). Most snails are hermaphroditic. After mating, the embryo inside the egg develops into a juvenile snail still inside the egg. The eggs then hatch releasing young snails. The young snails feed on algae and other vegetable debris in the water.

1 (d) Pathogenicity

Pathology due to schistosomal infections has been the subject of major review by several workers (W.H.O., 1967; Edington and Gilles, 1976 and Warren, 1973) and only a brief discussion is given below. The discussion

may be divided into three parts in accordance with the three main stages of the disease. These include (a) the invasion stage (b) the maturation of the infection and (c) the chronic phase of the disease. The invasive stage includes the actual penetration of the definitive host by cerceriae and the following 2 to 7 days during which the cercariae migrate from the skin to the lungs. The phase is marked by a moderate to intense skin reaction at the site of penetration, depending on the individual's sensitivity to the parasite. Individuals living in an endemic area show little or no reaction to cercarial penetration while visitors develop marked skin rashes and urticaria. The severity of the skin reaction is in addition related to the number of invading cercariae and to the species of schistosome involved. In general terms, the reaction is strongest in _S. japonicum_ infections and least in _S. haematobium._ In heavy infections, the migrating schistosomula may cause severe inflammatory reactions in the lungs and the liver. These may be associated with coughing and pneumonia.

The acute phase starts two to eight weeks after infection. At this time, male and female worms arrive in the liver and egg deposition begins. The characteristic pathological manifestation in this stage, in moderate and heavy infections is an acute febrile reaction often referred to as the Katayama syndrome

(Clarke *et al.*, 1970). This is accompanied by eosinophilia lasting for several days or weeks. This is an allergic reaction to metabolic products of schistosomes and their eggs. There may be abdominal pains.

The maturation stage begins 4 to 8 weeks after exposure and is characterised by production and excretion of eggs. Eggs induce an inflammatory reaction in the walls of the colon and rectum, with formation of granulomas where giant cells and epitheloid cells are surrounded by loose fibrous tissue. These granulomas may coalesce or become calcified causing gross intestinal changes associated with malfunctioning of the digestive system. These are characterised by diarrhoea, vomiting and abdominal pains.

The chronic phase of schistosomiasis is marked by a reduction in egg extrusion and extensive fibrosis. Papillomas and inflammatory polyps often develop and in severe cases can lead to obstruction of the lumen of the colon. Eggs are repeatedly carried to the liver in the portal veins and may become lodged in the portal tract. Such eggs are taken in by multinucleate giant cells and surrounded by inflammatory cells. As the lesions heal, some degree of fibrosis is usually left. Hepatomegaly is common and may lead to hypertension in the portal blood vessels. The reaction to the eggs in the liver may eventually cause the periportal fibrotic reaction

known as 'Symmers clay pipe stem fibrosis.' The functioning of the liver, however, is not altered.

1 (e) Host-Parasite Relationship

Following the infection of a mammalian host by *S. mansoni*, the various components of the immune system are activated against the different stages of the worm.

That antibodies play a role in the host defence against the parasite, has been shown by several workers. Clegg and Smithers (1972) demonstrated that serum obtained from a monkey which had been hyperimmunised by exposure to cerceriae of *S. mansoni* contained an antibody which killed young schistosomula *in vitro*. This antibody was also reported in sera from rabbits, rats and mice infected with *S. mansoni* (Murell and Clay, 1972) and from humans infected with *S. mansoni* and *S. haematobium* (Smith and Webbe, 1974). It was Clegg and Smithers (1972) who identified this antibody as belonging to the lgG class.

Macrophages have been identified as a crucial component of the protective immune response in immunised mice (James *et al*., 1982a, b). Upon stimulation with schistosome antigen, T-cells from immunised mice produce lymphokines which activate normal peritoneal macrophages for non-specific as well as tumoricidal activity. Macrophages recovered from the site of specific antigen challenge in immunised mice are activated to kill 3 hour

schistosomula in vitro (James *et al.*, 1984). James, Skamene and Meltzer (1983) found out that inbred mouse strains carrying known macrophage defects could not be protected against challenge infection by either primary infection or immunisation with irradiated cerceriae.

Seven to 10 days old schistosomula , whether in lungs or liver are not susceptible to macrophage-mediated attack. However, 2.5 weeks old schistosomula are susceptible to activated macrophage-mediated cytotoxicity (Sher *et al.*, 1982). Recent ultra-structural studies carried out by McLaren and James (1985) showed that 2.5 weeks old schistosomula of *S. mansoni* which are killed by activated macrophages exhibit morphological disturbances similar to those observed in 3 hour parasites. The cellular disruption appears to be confined to the sub-epithelial regions only. The main targets for the macrophage mediated attack appear to be the muscle bundles beneath the surface and gut epithelia. This indicates that the toxic mediators responsible for the parasite death may diffuse into schistosomula across the interface.

Fearon and Wong (1983) showed that immune complexes of schistosome antigens activate the alternative pathway of complement thereby causing the production of anaphylotoxins which are chemotactic for macrophages. Macrophages are also found in large numbers in the inflammatory lesions surrounding dead worms and eggs

trapped in the livers of infected mice and rats (Knopf, 1979).

Infection with *S. mansoni* results in high blood eosinophilia in the host (WHO, 1974) and these have also been histologically demonstrated in the granulomas surrounding eggs in human (Hsu *et al.*, 1980). Eosinophils were also found in close association with eggs and teguments of worms at various stages of destruction in the skin and liver of experimentally infected mice and rats (Butterworth *et al.*, 1979). Furthermore there is evidence to suggest that eosinophils play an important role in production and regulation of inflammatory mediators which cause trapping and damaging of worms (Akenase, 1977).

Schistosome stages are able to survive in the definitive host despite the above mentioned host defense mechanisms. In order to achieve this survival, the various schistosome stages have adapted different evasive mechanisms.

Data derived from *in vitro* studies indicate that soon after penetrating the skin, schistosomula acquire several mechanisms for evading host immune responses (McLaren, 1984). Of these mechanisms, the reduction of surface antigenicity seems to play a major role. Two major hypothesis have been proposed to explain the reduction of surface antigenicity of schistosomula .

The first proposes that the parasite antigens persist on the surface but are hidden or masked from the host immune system by acquired host molecules (Smithers, Terry and Hockley, 1968). This hypothesis is based largely on circumstantial evidence which has inversely linked host antigen acquisition with decreasing parasite expression (Goldring *et al.*, 1977). The second hypothesis suggests that the parasite sheds off its surface antigen and therefore reduces anti-parasite antibody binding to older schistosomes. This suggestion is based on studies in which schistosomula cultured *in vitro* and in defined media which are free of host molecules spontaneously lose their ability to bind anti-schistosome antibodies (Dessaint *et al.*, 1981). This hypothesis was also recently supported by work done by Pearce, Basch and Sher (1986). These workers cultured schistosomula both *in vitro* and *in vivo* and showed, using indirect immunofluorescence assays that schistosomula recovered from mice at 24h and 5-10 days post infection bound low or insignificant amounts of a variety of anti-schistosome antibodies. These included antibodies from chronically-infected and radiation attenuated cercariae - vaccinated mice; vaccinated rabbits and rabbits hyper-immunised with non-living larval and adult schistosome antigen preparation. Parasites maturing *in vitro* lost their binding ability after 10 days post-transformation. That this reduction in antigenicity was due to shedding of surface antigens

was suggested by the observation that the reduced ability of these parasites to bind anti-parasite antibodies coincided closely with the loss of ^{125}I-labelled surface proteins (Pearce, Basch and Sher, 1986).

The adult worm seem to survive better and for very long periods in the definitive host. However, the reason for their survival has never been adequately explained. Damian (1964) postulated the phenomena of molecular mimicry to explain why the host does not recognize the worms as foreign. Kemp, Greene and Damian (1974) showed an antigenic determinant on the surface of adult worms of S. mansoni which cross-reacts with α_2 macroglobulin of mice. As this antigenic determinant is both in worms grown in rhesus monkey and in mice, it must be of parasitic origin. Dean (1974) showed the existence of shared antigens of host origin. These are surface glycolipids acquired by parasite and presumably mask the parasite antigens thereby rendering the worm less immunogenic. There are in addition secretion antigens released by the worm to the blood circulation (Kussel et al., 1975). These evoke the production of antibodies which react with antigens away from the intact worm. Such a mechanism would protect the adult worm from host immune attack. Yet another mechanism believed to play a role in the survival of the adult worm in the host is the discovery of host-like antigens on the surface of

the worm. Schistosomula have a few of these antigens and cerceriae have none (Clegg and Smithers, 1972).

These are probably but a few of the mechanisms used by schistosomes to evade the immune mechanism of the definitive host.

1(f) Schistosomiasis Control

Four main approaches have been principally applied in the attempt to control schistosomiasis. These have included (a) control of the snail vector (b) chemotherapy (c) improved sanitation and (d) prevention of water contact.

Although molluscicides have been widely used in the control of snails, this approach has had several limitations. Firstly, the chemicals are expensive and their application must be undertaken frequently. Some of the molluscicides (e.g. sodium chlorophenate) cause skin irritation and are therefore potentially dangerous to the handler. Others are absorbed in the soil causing persistence of chemicals in the environment (e.g. copper sulphate). Moreover, most of the molluscicides currently in use kill non-target beneficial molluscs. These drawbacks have limited the potential of this approach to control. Biological methods of control of snails have also been tried. This approach has been carried out using birds and large snails such as Marisa

carnuarietus and *Tarebia granifera* which feed on other snails. The method has been used in the Caribbean, apparently with some success in controlling *Biomphalaria glabrata* (Webbe, 1969). However the biological control faces a problem because of the overwhelming productivity rates of the snail populations.

The second approach to the control of schistosomiasis involves a proper disposal of fecal and urinary waste in order to avoid contamination of the environment with ova. Building of latrines would, if widely practised ensure that this is achieved. However, in practice, not every homestead in endemic areas has a latrine and in some cases, the local populations have cultural beliefs that are opposed to the practice.

Attempts to reduce human contact with infected water have not been successful. In order to discourage children from bathing in dams and ponds, alternative bathing places must be constructed. Such undertakings would be expensive and in most endemic areas the countries are already financially strained. Many a times the snail-infected streams or dams form the only water sources for the surrounding population. Provision of piped water to all the rural communities is prohibitively expensive. Similarly, it has not been possible in most endemic countries to completely stop infection of irrigation cannals with cercariae-carrying snails and these form some of the most serious infection

points.

Chemotherapy has been used extensively in the control of schistosomiasis. Antimonials were the first drugs to be used for treatment of schistosomes. Tarta emetic had been in use since 1918 but was replaced by the trivalent antimonials. However, the last decade has seen a shift from the antimonials to newer and safer drugs. The reason for the shift was mainly due to the severe side effects often associated with the administration of antimonials. These included skin ulceration at the point of injection, vomiting and abnormalities in liver function.

Another drug which was widely used between 1948 to the mid-sixties but now is abandoned due to its frequent and often severe side effects is Lucanthone hydrochloride. The side effects included nausea, vomiting, abdominal discomfort and occassionally diarrhoea. Niridazole has been used for the three important human schistosomes since 1964. However, its use is declining now, not only because of its side effects, but it has also been shown to be mutagenic in various test systems (Legator et al.,1975 and McCann et al., 1975) and carcinogenic in mice and hamsters (Bulayo et al.,1977 and International Agency for Research on Cancer, 1977).

Two recent drugs have given very encouraging cure rates

and fewer and less serious side effects. These drugs are Oxamniquine and Praziquantel. Oxamniquine has been used to treat acute, subacute, chronic and complicated cases of *S. mansoni* infection with uniformly good effects. Numerous trials in Brazil and the African continent have confirmed the drug's efficacy (Silva *et al.*, 1974; Clarke *et al.*, 1976; Katz *et al.*, 1976 and 1977 and Omer, 1978) and have shown that the therapeutic response varies with age and with geographic origin of the infection. In general, Oxamniquine is well torelated. The most frequent side-effects have been dizziness, drowsiness and headache. Vomiting and diarrhoea have also been reported, but their relationship to dosage is more tenous. Hallucinations and psychic excitement following oxamniquine are known (Katz *et al.*, 1976).

Praziquantel is a newly developed heterocyclic pyrozino-isoquinoline compound. It is effective against all the 3 important human schistosomes. For these three schistosomes, the cure rates are very high, usually above 70% (Wegner, 1979 and Biltricide Symposium, 1980). Virtually all trials to date have confirmed the absence of toxic effects of the drug on vital organs, systems and functions. Side-effects of treatment are generally mild and disappear within 24 hours. The most frequent symptoms reported are epigastric pain, diffuse abdominal discomfort, nausea, anorexia, diarrhoea, dizziness,

headache, pruritus and fever. So praziquantel is a drug of choice. It is well torelated, has no significant effect on liver, renal, haemapoetic or other functions and is highly effective against all schistosomes parasitising man. The only drawback is that it is expensive and so it cannot be made available to most of the local people in the endemic areas. So chemotherapy as a method of control faces the problem of undesirable and often fatal side effects. Even the drug of choice, praziquantel, bears the disadvantage of being expensive. The parasites can develop drug resistance, too.

1 (g) Immunoprophylaxis

1 (g) (i) Use of the Different Schistosome Stages

The search of a vaccine against schistosomiasis began as early as the 1930's when Ozawa (1930) reported that he had induced partial resistance against *Schistosoma japonicum* in dogs by injecting them with adult worm material. Since then, various workers have used a variety of preparations in attempt to induce significant protection. The most widely used materials have come from the egg stage. Kagan (1958) using eggs of *S. mansoni* to immunise mice was not able to induce resistance but found that instead the mice became more susceptible to the schistosome infection. Also Smithers (1962) did not stimulate any detectable degree of resistance to *S. mansoni* when he immunised rhesus monkeys with egg homogenate.

Ozawa (1930) used cercarial emulsion of S. japonicum to immunise dogs and observed that the animals were protected. However when Sadun and Lin (1959) used cercarial emulsion to immunise mice against S. japonicum, no protection was induced. Recently Ford et al. (1984) was able to obtain significant protection using live cercariae. He immunised Fischer rats by a single immunising dose of S. mansoni cerc riae and obtained 67-74% protection.

Vaccination with surface antigens obtained from newly transformed schistosomula and purified by affinity chromotagraphy has been shown to partially protect mice and rhesus monkey against challenge infection with S. mansoni (Smith and Clegg, 1985). However this process faces two major problems (i) only very low levels of immunity are achieved and (ii) the target molecules are most probably carbohydrate, making Recombinant DNA synthesis for these epitopes not possible using the conventional high yield procaryotic cloning systems. Auriault et al. (1985) was able to induce significant protection using schistosomulum-released products. Rats were immunised with either total schistosomulum released products or 25,000 to 30,000 MW molecules purified fractions. Significant protection against a challenge infection by Schistosoma mansoni was obtained. lgE and to a lesser extent lgG antibodies represented the major

humoral mediators of the cytotoxic reactions responsible for the killing of schistosomula.

Sadun and Lin (1959) obtained a significant worm reduction in mice which had received three injections of a crude, fat-free adult worm antigen preceeding exposure. In contrast to these positive findings, Vogel and Mining (1953) failed to induce any resistance against S. japonicum in two monkeys, one having received minced worms and the other dried pulverised worms.

Intradermal injection of non-living antigens of S. mansoni in combination with the bacterial adjuvant, Mycobacterium bovis strain bacillus Calmette-Guerin (BCG) induced partial resistance to challenge infection in mice (James, 1985). Hillyer et al. (1980) used secreted antigens as a vaccine. They implanted S. mansoni in a diffuse chamber in a mouse. This stimulated antibody formation against secreted antigens, but no protection against a challenge infection was conferred. Metabolic antigens of S. japonicum and S. mansoni were used by Sadun and Lin (1959) and Levin and Kagan (1960) respectively to induce resistance against homologous parasite in mice. The former authors obtained a reduced worm burden following challenge, while the latter noticed increased survival of host. Murell and Clay (1972) used a vaccine from metabolic products obtained by collecting antigens secreted by adult S. mansoni in a protein-free tissue culture medium. 40% fewer adult

worms were recovered as compared to the control. Ramalho-Pinto *et al.* (1976) used isolated adult surface membrane to immunise against *S. mansoni* but no protection was achieved.
More recently, Balloul *et al.* (1987) obtained significant protection using DNA. The complementary DNA sequence encoding a 28,000 MW antigen of *S. mansoni* was isolated and expressed in *Escherichia coli*. A fusion protein with MW 25,000 was produced. Injection of this protein induced a strongly cytotoxic humoral response in rats, hamsters and baboons, composed of both lgE and lgG antibody classes. Immunisation of rats and hamsters with the protein gave significant protection against a challenge with live cercariae.

Although stages of *S. mansoni* or their products do confer some protection, none has given complete protection. Also, they are in such minute quantities that to prepare a vaccine which can be used on a large scale, would be logistically improbable. Thus the use of fractionated homogenates of adult worms or stages of parasite may not be easily achievable. This calls for other alternative approaches to prophylaxis.

1 (g) (ii) Use of Immune Serum

Attempts to confer protection using hyperimmune serum has given some protection in some cases and failed to do so in others. The protective effect of passively

transferred immune serum in the mouse was observed by Mahmoud et al. (1975). However, Maddison et al. (1978a) showed using B cell-deficient mice that depletion of humoral immune competence did not affect the course of a primary infection with Schistosoma mansoni in this model.

Passive immune serum alone did not confer protection to recipient mice irrespective of the route of serum transfer or of cercarial challenge with Schistosoma mansoni (Maddison and Kagan, 1979). Nevertheless mice that received both sensitized cells and immune serum were protected against challenge infection by subcutaneous exposure. The immune serum could be transferred as late as 8 days after subcutaneous challenge, suggesting that the protection was afforded in part by a late parasite killing mechanism which functions after the schistosomula have migrated through the lungs.

1 (g) (iii) Use of Irradiated Stages of S. mansoni

Mice vaccinated by highly irradiated cercariae or schistosomula of Schistosoma mansoni develop resistance to subsequent challenge infection with normal cercariae (Bickle et al., 1979). Such resistance is first evidenced as early as 10 days after vaccination, reaches peak levels at 4-6 weeks and gradually wanes

(Minard et al., 1978). Since the attenuated larvae used for vaccination die during the first few weeks in the host, acquired resistance to schistosomiasis can be studied in this model without the accompanying complications of worm or egg induced pathology (Byram et al., 1983). That the resistance to infection induced by vaccination is indeed the result of a specific immune response is suggested by the failure of S. mansoni vaccinated mice to develop levels of protection against challenges with Schistosoma japonicum (Cheever et al., 1983). Furthermore, resistance to S. mansoni infection fails to develop in vaccinated mice deprived of B or T lymphocytes (Sher et al., 1982) and can be transferred to normal mice by parabiosis (Dean et al., 1981). Finally vaccination with irradiated cercariae has been shown to induce prolonged state of sensitization of host B and T lymphocytes (James et al., 1981). When an immunised animal is challenged with the homologous parasite, it is able to react immunologically against it (Correa-Oliveira et al., 1984).

These encouraging findings coupled with the difficult in inducing protective immunity using non-viable antigenic preparations, have stimulated interests in the potential of attenuated larvae as a vaccine against human and domestic animal schistosomiasis. However, this approach has been slowed down by several limitations. First, the larval stages of schistosomes have a short survival

period, yet they have to be produced in large numbers. Secondly, the safety in using these irradiated schistosomes as a vaccine, especially in human beings is still questionable.

1 (g) (iv) Shared Antigens

There is need for a different kind of vaccine for schistosomiasis. As discussed above, schistosome antigens have not given adequate protection and also they are not available in large enough quantities. The irradiated larvae of schistosomes have their limitation too, especially as they are required in such high numbers for effective immunisation. A vaccine should be safe, easy to produce and obtainable in adequate quantities. The alternative to using schistosome homogenate is to look for heterogenous antigens. Work done using such antigens from the snail vector have given encouraging results. The snail is larger compared to any of the schistosome stages, and is much easier to breed in large enough numbers.

Hillyer *et al.* (1977) showed that there were shared antigens between *S. mansoni* and the liver fluke, *Fasciola hepatica.* Mice and hamsters innoculated with extracts of *F. hepatica* developed antibodies which were reactive with extracts from adult worms of *Schistosoma mansoni.* Animals immunised with *F. hepatica* were challenged with *S. mansoni.* A control was set with

animals which had not been immunised but which were exposed to *S. mansoni*, too . It was found that the immunised animals had antibody titre eight times higher than the control.

Yoshino and Cheng (1978) reported that newly hatched miracidia of *Schistosoma mansoni* possess membrane-associated determinants antigenically similar to certain components of the haemolymph from the snail, *Biomphalaria glabrata.* Basch and Diconza (1976) using an immunofluorescent assay, found cross-reaction between snail antigens and schistosome sporocysts cultured in absence of snail-derived substances.

Jackson (1976) showed that *S. haematobium* cercariae developing in snail haemolymph shared antigens with their molluscan host. Antisera to *Bulinus africanus* snail was raised in rabbits by innoculating them with crushed, lyophilised, non-infected whole snail material suspended in distilled water and dispersed in equal volume of complete Freund's adjuvant. Fifteen freshly prepared cercariae were incubated in rabbit antisera to snail and fifteen in normal rabbit serum. Both antisera had been fluorescein-labelled. The cercariae incubated with fluorescein-labelled rabbit anti-snail serum fluoresced strongly, indicating that snail antigens were present on their surfaces. Similarly immunologic cross reactivity between snail and adult schistosome

determinants was demonstrated by Kemp *et al.* (1974). Later Deelder *et al.* (1975) found cross-reaction between certain snail antigens and anti-schistosome serum from human patients.

Tsuji *et al.* (1978) using rabbits immunised with extracts of either adults or eggs of a Yamanashi (Japanese) strain of *S. japonicum*, showed by immunoelectrophoresis that such anti-schistosome serum reacted with extracts of *Oncomelania hupensis nosophara* (Japanese host snail) giving seven precipitin lines. Using the same system these authors observed that the anti-schistosome serum gave five precipitin lines with *O.h. quadrasi* (vector in Phillipines) and two lines with *O.h. formasana* (the vector in Taiwan). Corresponding infection rates of Yamanashi strain of *S. japonicum* in these strains were 92%, 30% and 9% respectively. Antigens from snails of other non-hosts generally gave either one line (e.g. *Bulinus*) or none (e.g. *Lymnae*, *Biomphalaria*, *Pila* and *Semisalcospira*). These results indicate that the more suitable the molluscan host, the greater the antigenic similarity, with the adult trematode.

Lehman and Ruaini (1982) prepared antigens from 3 species of uninfected snails, *Biomphalaria pfeifferi*, host of *S. mansoni*; *Bulinus africanus*, host of *Schistosoma haematobium* and *Lymnea natalensis*, host of *Fasciola gigantica*, all from the same locality. The antigens were injected intraperitoneally into mice. The

mice were later challenged with cercariae of S. mansoni and protection was assayed six weeks later. One control group was given adjuvant alone while another group was untreated. Mice immunised with B. pfeifferi and adjuvant showed a reduction of 63.9%; those immunised with B. africanus gave 42.6% and those with L. natalensis 14.6%. Adjuvant alone gave 20.6% and B. pfeifferi alone 13.7% reduction. Thus a crude extract of the molluscan host either alone or with adjuvant did confer some protection. There is not much work done using materials from Biomphalaria pfeifferi to immunise against S. mansoni. However, the results obtained by Lehman and Ruaini (1982) show that there might be a possibility of obtaining a vaccine from the snail vector.

1 (h) Objectives

1. To separate the antigens of the snail into different components.

2. To carry out analytical studies to find out whether there are shared antigens between the snail vector and the different developmental stages of *S. mansoni.*

3. To use the antigenic components to immunise hamsters.

4. To compare results obtained from analytical studies (2) with those from immunisation of hamsters (3).

CHAPTER 2 MATERIALS AND METHODS

2(a) MATERIALS

2(a)(i) Parasites

A strain of *S. mansoni* obtained in the field from Machakos district was used for all the work involving preparation of antigens. Naturally infected snails from the field were collected and screened for *S. mansoni* infection. Snails were put in the dark for 2 days and then put under strong light so that those with a patent infection would shed cercariae. Only snails with a single infection of *S. mansoni* were maintained in the laboratory. The strain of *S. mansoni* used for challenging immunised hamsters came from laboratory infected snails maintained at the schistosomiasis laboratory in the Kenya Medical Research Institute (KEMRI).

2(a)(ii) Definitive Host

Golden hamsters originally obtained from the International Laboratories for Research in Animal Diseases (ILRAD) and then bred in the Zoology Departmental animal house were used as definitive host for most of the work. Additional hamsters were obtained from the International Centre of Insect Physiology and Ecology (I.C.I.P.E.) animal house.

2(a)(iii) Intermediate Host

The snail vector *Biomphalaria pfeifferi* was bred in the laboratory from a strain originally obtained from Machakos district.

2(a)(iv) Rabbits

Mature New Zealand white rabbits were obtained from the International Centre of Insect Physiology and Ecology (I.C.I.P.E.), the Department of Biochemistry and from the local farmers.

2(b) **METHODS**

2(b)(i) Preparation of Schistosome Antigens

Preparation of Cercariae Antigen

Naturally infected snails were collected from the field in Machakos district. These were screened and confirmed to be infected with *S. mansoni* by careful cercarial examination. Cercariae obtained from such snails were pooled together, concentrated by slow centrifugation (100g) and stored at -20 ^{0}C until a total of 405,650 was obtained. These were then sonicated for 9 minutes in a cold bath using Sonifier Cell Disruptor, Model W 185. The homogenate was centrifuged at 5,000g for 10 minutes at 0 ^{0}C and the supernatant was stored at -20 ^{0}C.

Preparation of Adult Worm and Egg Antigens

Golden hamsters were exposed to *S. mansoni* cercariae using the ring method and perfused after 7 weeks as described by Smithers and Terry (1965). The

livers from these animals were preserved. Adult worms recovered from the hamsters were pooled together and stored at -20 °C. A total of 4,316 worms were collected. These were hand homogenised and the homogenate was centrifuged at 5,000g for 10 minutes at 0 °C. The supernatant was stored at -20 °C. The livers from the perfused hamsters were homogenised at low speed for 25 seconds. Physiological saline was added to the homogenates which were then passed through a series of sieves of decreasing mesh size (405 µm; 180 µm; 106 µm) and then collected in a 45 µm sieve. The eggs thus obtained were then washed twice in physiological saline and stored at -20 °C. About 93,600 eggs in total were obtained. These were sonicated in a cold bath for 9 minutes, using Sonifier Cell Disruptor, Model W 185. The homogenate was contifuged at 5,000g for 10 minutes at 0 °C and the supernatant was stored at -20 °C.

2 (b)(ii) Preparation of Snail Antigens

Six hundred Laboratory bred *Biomphalaria pfeifferi* snails were dissected in physiological saline. Three different parts of the snail: the digestive gland, the foot and the gut were separated, homogenised using a hand homogeniser and the homogenate centrifuged at 5,000g for 10 minutes at 0 °C. The supernatant was stored at -20 °C.

The above procedure was repeated for *Bulinus africanus*, though it was only the digestive gland which was used.

2 (b)(iii) Protein Estimation

The concentration of schistosome antigens and snail antigens was determined using a modification of Lowry's technique for protein measurement with Forlin reagent (Lowry et al., 1951). 25 mls of solution B(2% $NaCO_3$ and 0.2% Potassium sodium tartrate mixed in a ratio of 1:1) was mixed with 5 mls of 0.5% copper sulphate to make solution C. Dilution of standard bovine serum albumin and of the snail and schistosome antigens were made and 500 µl of each was incubated in solution C for 10 minutes at room temperature. Physiological saline was used as a blank control. After this duration, phenol reagent was added into each of the tubes and the solutions were then incubated in the dark for 30 minutes. Optical densities were determined at 750nm and a curve of optical density against concentration of standard protein was plotted. Optical densities of the antigens were read off the standard curve and their concentrations were estimated. Table (a) shows the final concentration of the various antigen preparation.

ANTIGEN	CONCENTRATION mg/ml
S. mansoni	
Egg homogenate	0.39
Cercaria homogenate	0.56
Adult worm homogenate	50.77
B. pfeifferi	
Digestive gland homogenate	29.30
Foot homogenate	12.70
Gut homogenate	11.80

Table (a) Concentrations of the snail and *S. mansoni* antigen preparations.

2(b)(iv) Column Fractionation of Snail Antigen

Phosphate buffered saline (PBS), pH 7.4 was used to swell sephadex G200 and also to elute all the antigens. A glass column of 1cm diameter and 15 cm length was prepared using the swollen sephadex G200 and connected to a LKB fraction collector which was used to fractionate the antigens. Neat digestive gland antigen was added to the column. The peaks of the different proteins were collected and stored at -70 ^{o}C. The same procedure was repeated for the foot and gut antigens.

The seperation profiles for the different fractions of the 3 antigens are shown in figures (i), (ii) and (iii). The fractions collected were concentrated by lyophilization. The lyophilized antigens were later reconstituted with distilled water and then stored at - 20 ^{0}C. Two peaks were obtained for each of the antigens as shown by the elution profiles.

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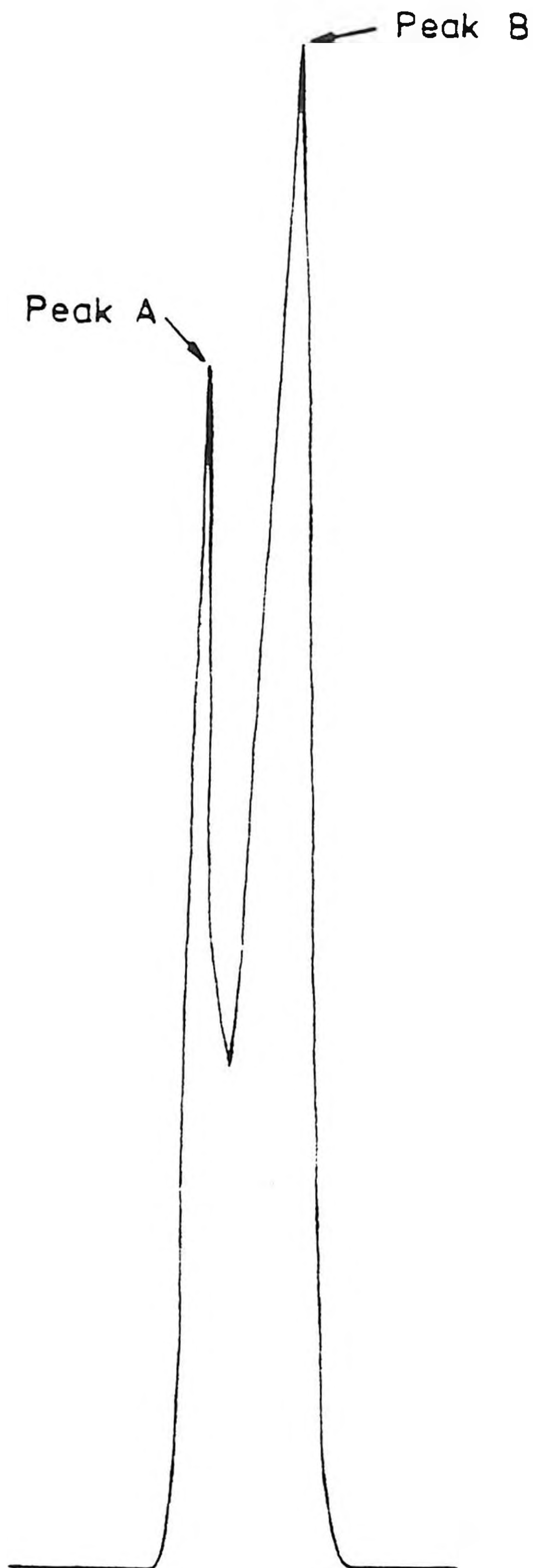

Figure (i) Elution profile of *B. pfeifferi* neat digestive gland antigen.

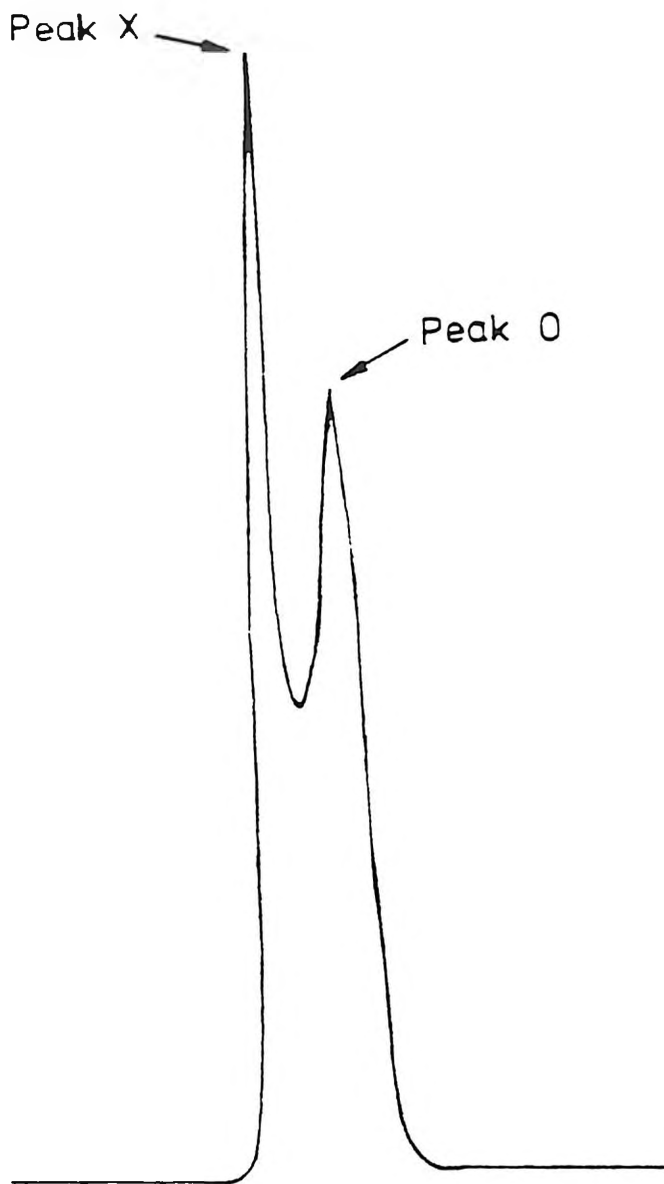

Figure (ii) Elution profile of B. pfeifferi neat foot antigen.

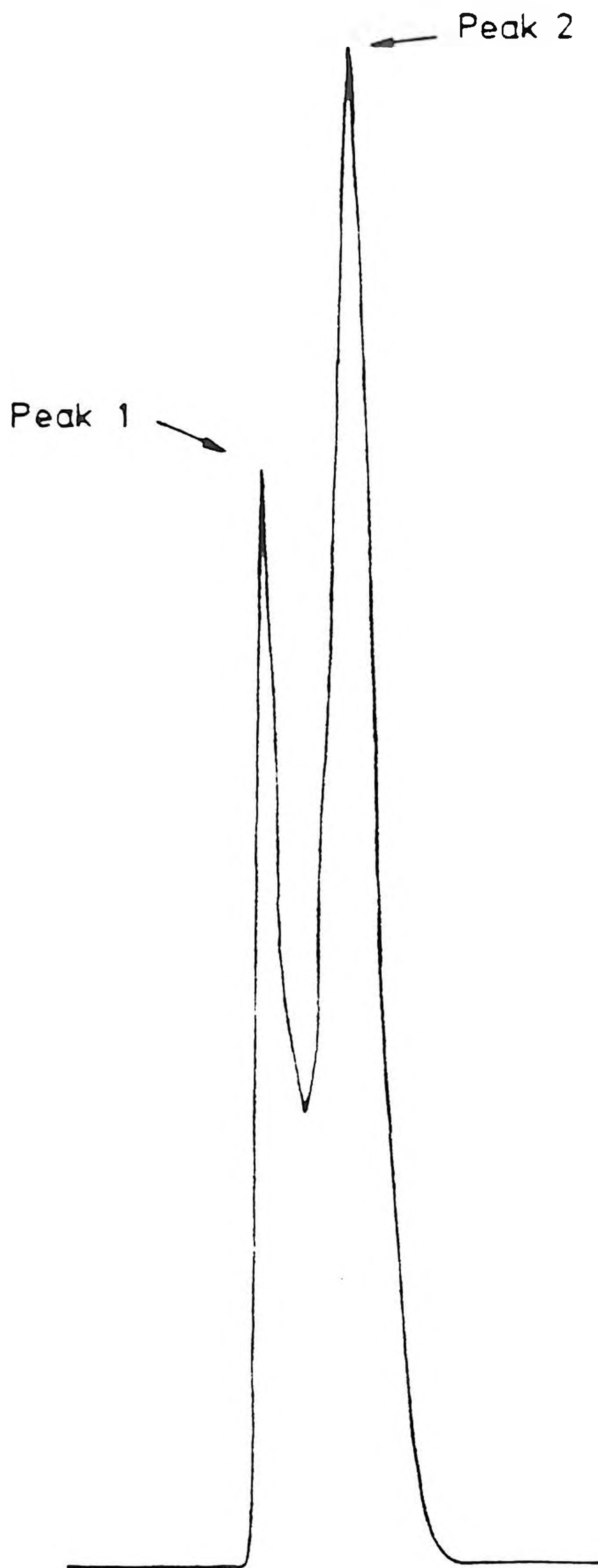

Figure (iii) Elution profile of B. pfeifferi neat gut antigen.

2(b)(v) Preparation of Antisera

Antisera were prepared against antigens from eggs, cerceriae and adult worms of *S. mansoni* and against digestive gland, foot and gut antigens of *B. pfeifferi.* For each of the antigens, two New Zealand white rabbits (2(a)(iv) were used. 0.5 mls of either neat or diluted antigens (in physiological saline) was emulsified with equal voume of Incomplete Freund's adjuvant. Each rabbit was given 6 injections of the mixture, 4 subcutaneously at the back and 2 intramusculary at the hind legs. The rabbits were boosted weekly. After the second boosting, the rabbits' sera were tested using simple gel diffusion as described by Ouchterlony (1949). Once a strong precipitin line was obtained, the rabbit was bled out 4 days after the last boost. The serum collected was stored at -20 $^{\circ}$ C. The following six antisera were obtained:-

Rabbit anti - *S. mansoni* egg serum

Rabbit anti - *S. mansoni* cercaria serum

Rabbit anti - *S. mansoni* adult worm serum

Rabbit anti - *B. pfeifferi* digestive gland serum

Rabbit anti - *B. pfeifferi* foot serum

Rabbit anti - *B. pfeifferi* gut serum.

2 (b)(vi) Simple Gel Diffusion (Ouchterlony, 1949)

Barbitone buffered agarose gel (1.1% agarose in 0.05M barbitone buffer pH 8.6 containing 2% Polyethylene glycol) was used in all experiments. Snail and schistosome antigens were reacted overnight at room temperature and in a moist chamber with the antisera prepared in the rabbits. The plates were then rinsed in physiological saline and then in distilled water before they were photographed. The gels were then dried naturally and stained using coomassie blue. The results are represented by plates 1-15 in the next Chapter.

The above procedure was repeated for *Bulinus africanus* digestive gland antigen and the antisera prepared in the rabbits.

2(b)(vii) Immunisation of Hamsters

Fifty five golden hamsters were divided into eleven equal groups. These were carefully marked with picric acid to avoid any mixing up of groups. Nine groups were immunised with either neat or fractions of the snail antigens. Two control groups included one non-immunised and one injected with adjuvant alone. 50 μl of each antigen was diluted in 150 μl of physiological saline

and emulsified with an equal amount of Incomplete Freund`s adjuvant. The mixture was given in a single intramuscular injection in one of the hind limbs for each of the hamsters in the 9 experimental groups. Each hamster in the adjuvant control group was given 200 µl of physiological saline in equal amounts of Incomplete Freund's adjuvant. The hamsters were immunised three times at weekly intervals and exposed to S. mansoni cercariae one week after the final immunisation. The method of exposure is described below.

2(b)(viii) Exposure of Hamsters to S. mansoni Cercariae

The hamsters were exposed to S. mansoni cercariae using the ring method described by Smithers and Terry (1965). The hair on the abdominal skin was clipped by an electric shaver. The hamsters were anaesthetised by an intraperitoneal injection of 1ml of anaesthesia (10% sodium pentabarbitone in 10% ethanol) per 100g of body weight. The shaved abdominal skin was swabbed with damp cotton wool before rings were placed on the shaved area. A suspension containing 135 cerceriae was put in rings and the hamsters left for 40 minutes to allow the cerceriae to penetrate through the skin. The hamsters were perfused for adult worms six weeks post exposure.

2(b)(ix) Recovery of *S. mansoni* Adult Worms

Six weeks after exposure, the hamsters were perfused following the method described by Smithers and Terry (1965). They were anaesthetised using a mixture of sodium pentabarbitone and sodium citrate. The hamsters were then dissected to expose the abdominal and thoracic cavities and the hepatic portal system was perfused using citrated saline (1.5 % sodium citrate in physiological saline). The worms from each hamster were collected in a petri dish with citrated saline. Perfusion was stopped only when the mesenteries looked clean. The liver was removed and pressed between two glass slabs and any trapped worms were counted. Both male and female worms were counted and the numbers for each groups were pooled together. Percentage worm reduction was calculated as :-

$$\frac{\text{No of worms recovered from control} - \text{No of worms recovered per group}}{\text{No of worms recovered from control}} \times 100$$

CHAPTER 3 RESULTS

3(a) Gel Diffusion

All antigens formed precipitin lines when reacted against their homologous antisera. This is shown by Plates 1 and 2. Also precipitin lines were formed between all antigens prepared from one organism and antisera raised against the same organism as shown by Plates 3 and 4.

Precipitin lines were formed between some of the antigens of the snail and some of the antisera prepared against S. mansoni stages. Neat digestive gland antigen and its two fractions A and B and neat gut antigen and its two fractions 1 and 2 all cross-reacted with Rabbit anti-S. mansoni egg serum as represented by Plates 5 to 9. Fraction 1 of the gut also reacted with cercaria and adult worm antisera (Plates 10 and 11). However, there were no precipitin lines formed between the neat foot antigen and its fractions and all the antisera raised against S. mansoni stages.

The antigens of S. mansoni cross-reacted with some of the antisera prepared against B. pfeifferi, too. S. mansoni egg and adult worm antigens formed precipitin lines with both the antisera prepared against B. pfeifferi digestive gland and gut (Plates 12 - 14).

Cercariae antigen cross-reacted with antisera raised against digestive gland only (Plate 15).

Bulinus africanus digestive gland antigen only formed a precipitin line with antiserum raised against *B. pfeifferi* digestive gland.

The results of all the reactions are shown in Table (b).

ANTIGENS	ANTISERA					
	Biomphalaria pfeifferi			Schistosoma mansoni		
	Digestive gland	Foot	Gut	Adult Worm	Cercaria	Egg
Biomphalaria pfeifferi						
Digestive gland and Fractions A and B	+	+	+	−	−	+
Foot and Fractions X and O	+	+	+	−	−	−
Gut and Fraction 2	+	+	+	−	−	+
Fraction 1 of Gut	+	+	+	+	+	+
Bulinus africanus digestive gland	+	−	−	−	−	−
Schistosoma mansoni						
Adult worm	+	−	+	+	+	+
Cercaria	+	−	−	+	+	+
Egg	+	−	+	+	+	+

Table (b) Simple gel diffusion results obtained by reacting B. pfeifferi and S. mansoni antigens with the various antisera.

KEY +: precence of precipitin line(s)
−: absence of precipitin line(s)

Plate 1 Simple gel diffusion showing precipitin lines between _B. pfeifferi_ digestive gland and its homologous rabbit antiserum.

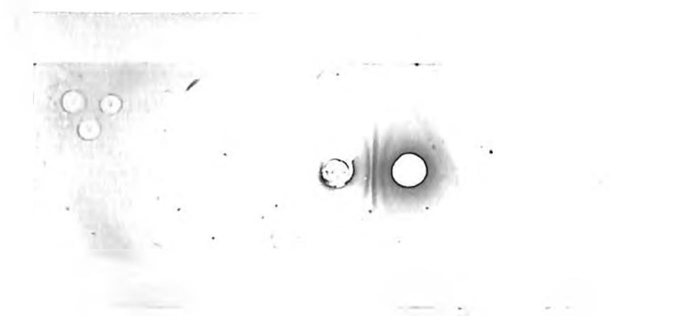

Plate 2 Simple gel diffusion showing precipitin lines between _S. mansoni_ adult worm homogenate and its homologous rabbit antiserum.

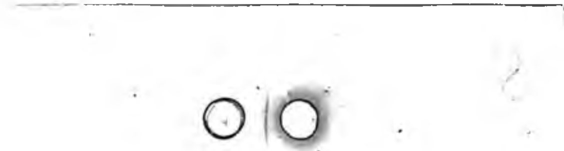

Plate 3 Simple gel diffusion showing precipitin lines between a fraction of *B. pfeifferi* foot homogenate and antiserum raised against the snail digestive gland.

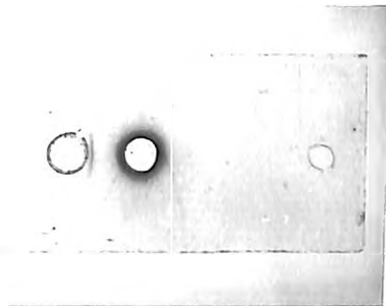

Plate 4 Simple gel diffusion showing precipitin lines between *S. mansoni* cercariae homogenate and antiserum raised against *S. mansoni* eggs.

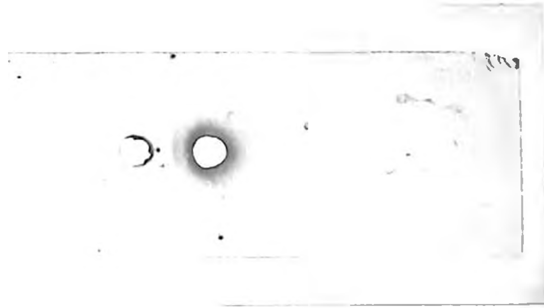

Plate 5 Simple gel diffusion showing precipitin line between neat *B. pfeifferi* digestive gland homogenate and antiserum raised against *S. mansoni* eggs.

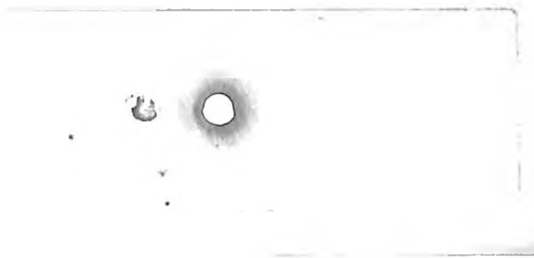

Plate 6 Simple gel diffusion showing precipitin line between a fraction of *B. pfeifferi* digestive gland and antiserum raised against *S. mansoni* eggs.

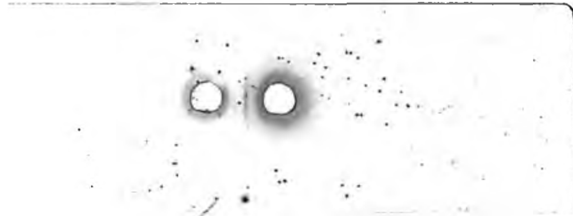

Plate 7 Simple gel diffusion showing precipitin line formed between neat *B. pfeifferi* gut homogenate and antiserum raised against *S. mansoni* eggs.

Plate 8 Simple gel diffusion showing precipitin line formed between a fraction of *B. pfeifferi* gut and antiserum raised against *S. mansoni* eggs.

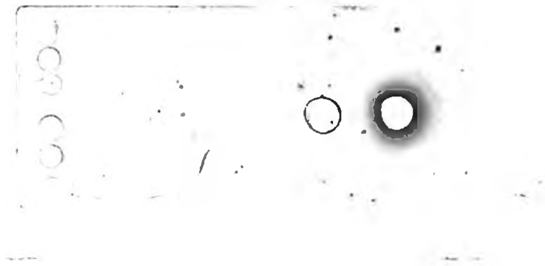

Plate 9 Simple gel diffusion showing precipitin line between a fraction of B. pfeifferi gut and antiserum raised against S. mansoni eggs.

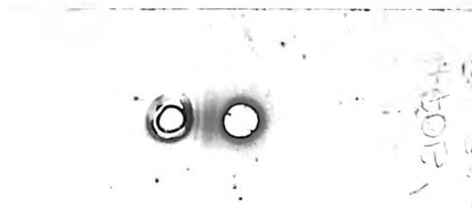

Plate 10 Simple gel diffusion showing precipitin lines between a fraction of B. pfeifferi gut and antiserum raised against S. mansoni cercariae.

Plate 11 Simple gel diffusion showing precipitin line between a fraction of *B. pfeifferi* gut and antiserum raised against *S. mansoni* adult worm.

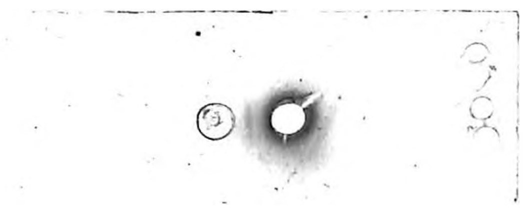

Plate 12 Simple gel diffusion showing precipitin line between *S. mansoni* egg homogenate and antiserum raised against *B. pfeifferi* digestive gland.

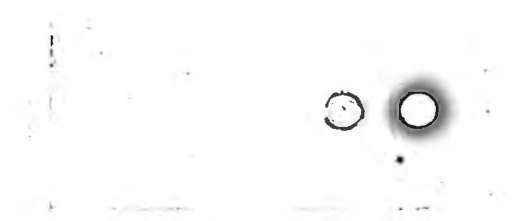

Plate 13 Simple gel diffusion showing precipitin line between <u>S. mansoni</u> adult worm homogenate and antiserum raised against <u>B. pfeifferi</u> digestive gland.

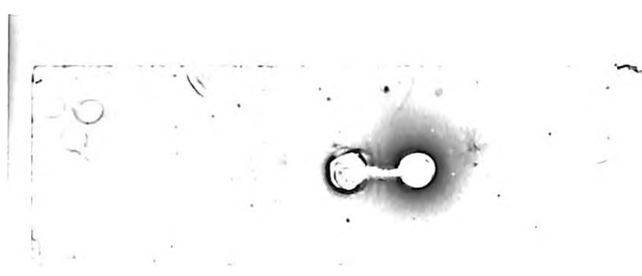

Plate 14 Simple gel diffusion showing precipitin lines formed between <u>S. mansoni</u> adult worm homogenate and antiserum raised against <u>B. pfeifferi</u> gut.

Plate 15 Simple gel diffusion showing precipitin line between *S. mansoni* cercariae and antiserum raised against *B. pfeifferi* digestive gland.

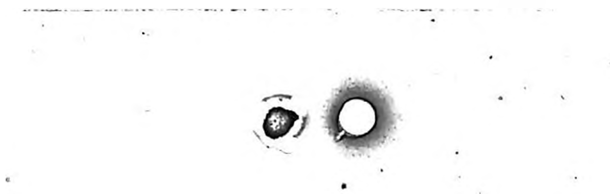

Plate 16 Control.

3(b) Worm Recovery

Nine groups of Golden hamsters were given a series of 3 injections of either neat or fraction of snail antigens emulsified in Incomplete Freund's adjuvant before each hamster was exposed to 135 cercariae of S. mansoni. The tenth group was given 2 injections of adjuvant alone and another control group received no immunisation. The results of worm recovery for each of the 11 groups is given in Table (c).

The numbers of female and male worms recovered do not seem to differ much. So the immunisation had the same effect on both sexes. The means of the different treatment do differ and this difference is empasized when the treatments are compared to the controls. The percentage worm reduction was calculated using the formula given in 2 (b) (ix). The worm reduction between adjuvant and unimmunised control was 2.6% which is minimal. However, all the antigens both neat and fractions showed a worm reduction of more than 10% as compared to the unimmunised control. The foot and its two fractions, fraction O and fraction X gave the least worm burden reduction, having 12.3%; 24.6% and 26.2% respectively. Neat digestive gland had 28.5%; neat gut 45.6%; fraction B (digestive gland) 52.4%; fraction I (gut) 52.8% and fraction 2 (gut) 56.6% . The highest reduction was obtained with fraction A (digestive gland)

having 65.0%. These results show that there was a reduction of worm burden in hamsters which were treated with snail antigens. The best results were given by hamsters immunised with neat gland antigen and neat gut antigen and their fractions.

In order to confirm the above results, it was neccesary to analyze the results using the analysis of variance approach which takes into consideration the variation within group as well as within treatments. This analysis is presented in Tables (d) and (e) below. The calculated F-ratio is 4.4. This is greater than the tabulated F-ratio at p(0.01) which is 4.1. This shows there is a statistically significant difference between the treatments, hence immunising the hamsters affected the number of worms recovered.

The fractions of both the digestive gland and gut antigens gave a higher protection than their crude mixtures. A possible explanation could be that the fractions share some complementarity in their epitopes. These epitopes would interact with each other in the crude mixture leaving just a few sites for the immune components to bind to. This would in turn lead to a lower antibody level. When the antigens are separated, each would express more binding sites than previously and hence more components of the immune system would bind leading to a higher titre of the antiserum.

TREATMENT	NO. OF HAMSTERS	NO. OF WORMS RECOVERED			MEAN	S.D.	PERCENTAGE WORM REDUCTION
		MALES	FEMALES	TOTAL			
DIGESTIVE GLAND	5	113	108	221	44.2	12.9	28.5%
FRACTION A	5	57	51	108	21.6	10.3	65.0%
FRACTION B	5	78	69	147	29.4	7.8	52.4%
FOOT	5	134	137	271	54.2	28.6	12.3%
FRACTION X	5	114	114	228	45.6	9.4	26.2%
FRACTION O	5	113	120	233	46.6	6.3	24.6%
GUT	5	86	79	165	33.0	13.9	45.6%
FRACTION 1	5	75	71	146	29.2	7.0	52.8%
FRACTION 2	5	67	67	134	26.8	4.3	56.6%
ADJUVANT	5	141	160	301	60.2	16.8	2.6%
CONTROL	5	156	153	309	61.8	24.7	0.0%

Table (c) Worm recovery for groups of hamsters infected with 135 cercariae of S. mansoni per animal. Group 1-9 were immunised with various snail antigens. Group 10 and 11 are controls.

<u>ANALYSIS OF VARIANCE</u>

REPLICATE	NEAT DIGESTIVE GLAND	FRACTION		NEAT FOOT	FRACTION		NEAT GUT	FRACTION		ADJUVANT	CONTROL	TOTAL
		A	B		X	O		1	2			
1	51	24	33	101	52	46	45	34	22	82	65	
2	34	33	40	46	41	57	33	20	33	71	29	
3	63	5	26	47	32	45	13	24	26	47	94	
4	41	25	19	23	56	40	47	31	24	41	48	
5	32	21	29	54	47	45	27	37	29	60	73	
TOTAL	221	108	147	271	228	233	165	146	134	301	309	2263

Table (a) Number of worms recovered from each hamster and the Group totals.

	DEGREE OF FREEDOM (d.f.)	CORRECTION SUM OF SQUARES	MEAN SQUARES VARIANCE	F-RATIO
BETWEEN GROUP TREATMENT	10	9769.2	976.9	4.4
WITHIN GROUP	44	9751.6	221.6	
TOTAL	54	19520.8		

TABLE (e) The working of the F-ratio

CHAPTER 4 DISCUSSION

Much work has been done in search of a vaccine for schistosomiasis. The different schistosome stages: eggs (Kagan, 1958); cercariae (Ford *et al.*, 1984); schistosomulum-released products (Auriault *et al.*, 1985); adult worm (Ozawa, 1930) and DNA (Balloul *et al.*, 1987) have been used as vaccines but with minimal success. This is mainly due to the difficulty of producing adequate materials from these parasite stages.

Lehman and Ruaini (1982) showed that the e may be shared antigens between *Schistosoma mansoni* and its snail vector *Biomphalaria pfeifferi.* They used crude extracts of the snail and obtained a 63.9% protection in mice. If it can be confirmed that there are shared antigens between *S. mansoni* and its snail vector, then the snail, which is bigger and easier to breed would be a better source of antigens for both vaccination as well as immunodiagnosis.

In this study, soluble neat antigens were prepared from the digestive gland, foot and gut of *Biomphalaria pfeifferi* and were separated into their fractions by gel filtration. Soluble antigens from egg, cercariae and adult worms of *S. mansoni* were prepared by centrifuging the homogenate. Antisera for the neat snail proteins and the schistosome stages were prepared by injecting rabbits with the respective proteins.

Results from gel diffusion showed that the antisera prepared against the snail and schistosome antigens were potent as they reacted with homologous antigens and also antigens from the same organism. Neat digestive gland antigen and its two fractions A and B and neat gut antigen and its two fractions 1 and 2 all formed precipitin lines with antiserum of S. mansoni egg. This suggests that there are shared antigens between the snail digestive gland and gut and S. mansoni egg. This was further affirmed by the fact that S. mansoni egg and adult worm antigens formed precipitin lines with antisera against B. pfeifferi digestive gland and gut. Also cercariae antigen formed a precipitin line with antiserum against digestive gland. Egg antigens have the highest specificity for schistosomes and have been used in most of the immunodiagnostic work for schistosomiasis. Cross-reactivity between either the digestive gland and gut antigens and S. mansoni egg antiserum or S. mansoni egg antigen and antisera of the snail digestive gland and gut, therefore strongly supports evidence of shared antigens between S. mansoni and B. pfeifferi. No precipitin line was formed between the foot antigens and its two fractions X and O and any of the schistosome-stages' antisera. Also no precipitin lines were formed between S. mansoni egg, cercariae and adult worm antigens and the foot antiserum. Therefore no shared antigens between the snail foot and S. mansoni stages were detected.

Bulinus africanus digestive gland antigen did not form a precipitin line with antisera raised against schistosome stages. This shows that no antigens were detected between _Bulinus africanus_ and _S. mansoni_. _B. africanus_ digestive gland antigen only cross-reacted with antisera raised against the digestive gland of _B. pfeifferi_ suggesting that _B. africanus_ might not share antigens specific to _B. pfeifferi_ cells but rather other functional proteins like enzymes. As the digestive glands from these two snail species perform the same function, they can produce the same enzyme which is common to all snails. The enzyme would be responsible for the cross-reactivity between the two.

The results on antigenic cross-reactivity are in agreememt with those obtained from the immunisation experiment. The lowest reduction of worm burden was given by immunising hamsters with foot antigen and its two fractions O and X. These are 12.3%, 24.6% and 26.2% respectively. Neat gut antigen and its two fractions 1 and 2 gave a reduction of 45.6%, 52.8% and 56.6% respectively. Neat digestive gland and its two antigens A and B gave the following reduction: 28.5%, 52.4% and 65.0% respectively. Adjuvant alone gave only 2.6% protection. The results showed that hamsters were protected when they were immunised with neat digestive gland and gut antigens and their fractions. Analysis of variance confirmed that the results were statistically significant at p(0.01).

Therefore the worm reduction is due to immunisation.

The above results show strong evidence of shared antigens between S. mansoni and its intermediate host B. pfeifferi. However, it might be argued that the shared antigens between the schistosome worm and the gut antigens was due to non-specific immunity because there are materials in the gut foreign to the snail e.g. food material and bacteria. Bout et al.,1977 was able to give 80-100% protection against S. mansoni by injecting C57B1 inbred mice which had a 14 day infection, with bacillus Calmette- Guerin (BCG). So if there were any bacteria in the gut, they would have possibly contributed to the total antigenic components attributed to the gut and hence provide some protection. However, unlike the gut, the digestive gland is free from contamination with non-snail material. So, the antigenic cross-reactivity and the protection afforded by the digestive gland can only be attributed to its proteins alone, but not to non-specific immunity. This then strongly supports the evidence of shared antigens between the snail and S. mansoni.

The possibility of shared antigens between the snail vector and S. mansoni parasite is supported by several other workers. Basch and Diconza (1976), using an immunofluorescent assay, found cross-reaction between snail antigens and schistosome sporocysts cultured in

absence of snail derived substances. Jackson (1976) showed that schistosome cercariae in haemolymph shared antigens with their molluscan hosts. Certain B. grabrata antigens were shown to cross-react with S. mansoni antiserum from human patients (Deelder et al., 1975). Tsuji et al., (1978) using sera from rabbits immunised with extracts of adults or eggs of S. japonicum to react with extracts obtained from different snail species, was able to show by immunoelectrophoresis that the more suitable a molluscan host is, the greater the antigenic similarlity with the adult trematode. One of the antigens shared between S. mansoni and its Puerto Rican vector, B. glabrata, was shown to react with sera of mice and humans infected with S. mansoni (Kemp et al., 1983). Carlos et al., (1985) reacted anti-schistosome serum against antigens derived from Biomphalaria grablata digestive gland. They found that 2.8% of the antigens were shared by the vector and the parasite. Lehman and Ruaini (1982) obtained 63.9% worm reduction when they used crude extracts of B. pfeifferi to immunise mice against S. mansoni. Therefore the results obtained from analytical procedure are not isolated.

More work should be done using materials from the snail vector. As the highest protection was obtained from fraction A of the digestive gland, and also as there is little chance of contaminating the digestive gland with

material from outside the snail, it might of value to concentrate on purified materials from the digestive gland. Other models closer to man especially primates can be used for immunisation experiments. If protection is obtained in these models, one can be sure they are getting closer to developing a vaccine which can be used in man and hence protect him from the miseries of schistosomiasis.

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