ABSTRACT.

Studies on the development of resistance in experimental murine schistosomiasis

by

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Experiments were performed in mice on resistance to challenge with Schistosoma mansoni and S. mattheei cercariae following previous bisexual, single sex or irradiated infections. Resistance to reinfection was demonstrated following bisexual infection with S. mansoni using outbred T.O. mice and it appeared that such resistance was not stimulated by cercarial transformation products. T.O. mice from three different suppliers exhibited markedly different levels of resistance and mortality. Bisexual infection with S. mattheei resulted in resistance to both homologous challenge and heterologous challenge with S. mansoni. Infections with S. mansoni worms of one sex only, stimulated significantly lower resistance than light bisexual infections. Injection of eggs alone failed to stimulate resistance, suggesting that both worms and eggs may be required.

Studies on irradiation of S. mansoni and S. mattheei showed that the minimum radiation doses required to produce sterile infections were 2.3 kr and 2.7 kr respectively, due predominantly to sterilization of the female worms. Radiation-induced death of 2.3 kr-irradiated parasites occurred mainly in the liver, but higher doses resulted in death earlier in the migration pathway, with parasites exposed to 40 kr dying at the site of infection.

Varying levels of resistance followed percutaneous or intramuscular infection with larvae irradiated with 2.3-160 kr. This resistance was demonstrated by significantly reduced worm and egg burdens and by longer survival of the vaccinated mice. Maximal resistance was demonstrated earlier following vaccination with highly irradiated parasites (20-40 kr) than with 2.3 kr-irradiated parasites, although comparable levels were eventually reached. The resistance was not transient, being demonstrated 17 weeks post-infection. Neither unirradiated male parasites alone nor dead irradiated parasites could confer resistance suggesting that, in the absence of eggs, death of the parasites within the host is necessary for the induction of resistance. Resistance was not increased by varying the number of irradiated larvae, the number of vaccinations, the route of vaccination or by the simultaneous administration of B.C.G. Irradiated S. mattheei infections conferred only weak resistance to homologous challenge and vaccination with irradiated S. mansoni or S. mattheei failed to confer significant resistance to heterologous challenge.

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CHAPTER 1. INTRODUCTION.

1.1 The existing measures for controlling schistosomiasis, including chemotherapy, molluscicide and improved water management, have not prevented the spread of disease (Terry, 1973). Therefore, there is considerable interest in attempting to develop vaccination procedures. This thesis is concerned with the development of resistance to *Schistosoma mansoni* and *Schistosoma mattheei* in the mouse, with particular emphasis on vaccination with radiation-attenuated larvae. The studies were aimed at developing optimal vaccination procedures that would consistently result in a high degree of resistance. It was hoped that such studies carried out with the human parasite would (a) help establish the feasibility of developing a highly effective live vaccine for testing in primates and, hopefully, subsequently lead to vaccination procedures against the human disease, and (b) result in the development of a non-pathogenic method of inducing resistance for studying mechanisms of resistance in mice. Induction of resistance in the absence of mature worms and the eggs they produce is potentially useful for immunological studies on resistance in that the host is exposed to a more restricted number of antigens. Also the severe pathology associated with schistosome eggs and the consequent host mortality are avoided. More specifically, for example, the effect of rigorous T-cell deprivation on the development of resistance has proved rather difficult to study as deprived, infected mice die soon after patency, suffering liquefatic necrosis of liver tissue surrounding trapped eggs. The studies with the sheep and cattle parasite, *S. mattheei*, were performed in parallel with experiments carried out by Dr. M.G. Taylor at Winches Farm Field Station aimed at developing a live vaccine against ovine and bovine schistosomiasis. Apart from the economic benefits that such vaccines may produce

in developing animal rearing areas of Africa, it was hoped that development of the cattle and sheep vaccine in a natural host/parasite system would help elucidate the feasibility and methodology of developing a vaccine against the human disease.

Previous attempts to vaccinate experimental animals with irradiated cercariae have proved encouraging particularly in the S. japonicum/rhesus monkey (Macaca mulatta) system. Nevertheless, interest in the development of an irradiated vaccine against S. mansoni in the rhesus monkey waned in the 1960's with the demonstration that a large number of irradiated cercariae had to be given in order to stimulate resistance. Rodents have also been used as experimental hosts for investigation of irradiated vaccines but there is a lack of consistency between the published results of different workers. However, in view of the failure to induce resistance consistently in any host with non-living preparations of schistosomes, it was considered that a re-evaluation of the resistance induced by irradiated infections was worthwhile. The mouse was chosen as the experimental host largely because of its cheapness and convenience. Its possible relevance as a model for schistosomiasis is considered in Section 1.2.3.

This thesis is presented in 8 chapters. This, the first chapter, comprises an extensive review of the literature concerned with the development of resistance by man and experimental animals against schistosome infections with particular reference to S. mansoni. The materials and methods employed are described in Chapter 2. Chapter 3 contains a group of experiments concerned with the induction of resistance using primary bisexual infections with unattenuated parasites of

S. mansoni and S. mattheei. These were initially undertaken as a baseline for the studies using attenuated infections and include studies of the effect on resistance of the size and route of administration of the primary infection and of the strain of murine host. Chapter 4 is concerned with resistance following infection with worms of one sex. Such studies, initiated on the widely held assumption that the adult worm alone is responsible for induction of resistance, were aimed at developing a relatively non-pathogenic and antigenically less complex procedure for induction of resistance in the mouse for the purposes of mechanistic studies. Chapter 5 deals with the migration and development of gamma-irradiated schistosome larvae in the mouse and the pathological consequences of such infections. These studies provide information on the longevity and site of death of larvae exposed to doses of radiation in the range 0 - 40 kr, which have been studied for their resistance-inducing potential. Chapters 6 and 7 contain the studies on the parameters involved in the induction of resistance by vaccination with irradiated S. mansoni and S. mattheei respectively. Chapter 8 comprises a summary of the results obtained and conclusions drawn.

1.2 Literature Review - Evidence for the development of resistance and for the involvement of the immune system in resistance to S. mansoni and S. mattheei.

It is not proposed to review the whole subject of 'immunity' in schistosomiasis as this has been done elsewhere (Stirewalt, 1963; Smithers and Terry, 1969a and 1976; Smithers, 1976). Throughout this thesis care has been taken to avoid use of the term 'immunity' as a synonym for 'resistance' because the immune system has not, in the

majority of instances been implicated as the mediator of resistance. Attempts to establish the key factors governing resistance are plagued by the fact that a wide variety of experimental hosts and schistosome species have been studied and findings in one system are not necessarily applicable to others. Thus the following literature review has been largely restricted to studies of S. mansoni in man, subhuman primates and the mouse and of S. mattheei in sheep. Evidence that man develops a degree of resistance under natural conditions would encourage attempts to develop prophylactic vaccines although an absence of such evidence would not preclude the possibility of artificially inducing resistance and 'improving on nature'. A knowledge of the pattern of infection in man also provides a useful framework in which to analyse the possible relevance to the human of results observed in experimental animals.

Research workers have inevitably turned to the use of experimental animals for studying resistance to schistosomes. Of the subhuman primates, the rhesus monkey in particular has been extensively used. This Asian monkey had initially been used in studies on S. japonicum (Vogel and Minning, 1953) and was relatively easily available in the 1960's for the studies using S. mansoni. The rhesus was found to develop marked resistance to reinfection and many of the currently held ideas have been derived from such studies. However, the rhesus is not typical of subhuman primates and different results have been obtained using the African Cercopithecus monkeys and also baboons (Papio spp.) and chimpanzees (Pan satyrus). Of the rodents, both rats and mice have found favour at various times over the years. The studies using mice, the experimental host used throughout the experiments reported herein, are extensively reviewed. The rat behaves very differ-

ently from the mouse in its response both to infection and reinfection with S. mansoni, showing a high degree of natural resistance, failing to develop persistant chronic infection and showing only transient resistance to reinfection. The literature on the rat is thus not discussed here but is introduced where considered relevant.

1.2.1 S. mansoni - Man.

Several authors have reviewed the evidence that man develops resistance to reinfection with schistosomes and their conclusions vary: "Undoubtedly man (and animals) acquire immunity to schistosomes" (Smithers and Terry, 1969a); "Clinical data from human infections, however, give almost irrefutable evidence of man's acquired immunity" (Stirewalt, 1963); "This concept (that resistance to reinfection with schistosomesis common and must be the factor controlling the prevalence and intensity of infection), unsupported by hard data and explainable by other factors, has had a stultifying influence on research" (Warren, 1973). Direct or fortuitous experimentation in man, based on known exposure to schistosome cercariae with the subsequent demonstration of the presence or absence of infection assessed by faecal egg output, has naturally been limited and in fact no such data exists for S. mansoni infections.

The circumstantial evidence provided by epidemiological studies can be interpreted as supporting the concept of acquisition of resistance to reinfection by man. In the majority of endemic areas studied, both the point (age) prevalence of infection and the intensity of infection (eggs/gm. faeces) increase to a peak, usually in the

second decade of life, and thereafter decline. Such a pattern is particularly typical of areas endemic for S. haematobium, and mathematical analyses strongly indicate that acquired resistance is playing a fundamental role (McCullough and Bradley, 1973; Bradley and McCullough, 1973; Wilkins, 1977). With S. mansoni, which follows a similar trend, the decline is not always so pronounced but Omer et al. (1976), reporting on the pattern in the Gezira region of the Sudan, found that the prevalence rates rose steeply to a peak of about 80% between the ages of 10-20 years, and subsequently declined; this fall was more dramatic in females (to 20% by 45 years) than in males (to 40% by 45 years). Egg output dropped concurrently from around 600 to 300 eggs/gm. of faeces. In a study by Siongok et al. (1976) in Kenya, prevalence rates of almost 100% were reached by 20 years, subsequently declining to 70% by 40-50 years. Females showed peak egg intensities of 1,000 eggs/gm. of faeces at 10-14 years, dropping to 300 eggs/gm. of faeces at 20-24 years. Males showed peak intensities of 1,000 eggs/gm. of faeces at 20-24 years, which dropped to 200 eggs/gm. of faeces at 25-29 years. Similarly, Kloetzel (1963) and Lehman et al. (1976), working in Brazil, showed that after the peak there was little sign of a decline in prevalence, while egg output dropped dramatically. Ongom and Bradley (1972), however, described a focus in West Nile, Uganda, in which peak prevalence approched 100% in the early teens with intensities of 1,000-1,500 eggs/mg. of faeces and, although apparently similar in terms of prevalence and intensity to the population studied by Siongok et al. (1976), the high egg output continued throughout life in males and only dropped to about half peak levels in females. Such a plateau or decline of prevalence and intensity can be explained by assuming that in endemic areas infected people gradually develop an acquired resistance to reinfection and, as

established worms die, the egg output falls. Warren et al. (1974) estimated the mean life span of the Yemani strain of S. mansoni in man to be between 5-10 years.

Other possible reasons for the falling egg counts in older people should also be considered, for example, decreased output of eggs by older worms or a hindered passage of eggs through the gut wall due to fibrosis in older patients. However, Cheever (1968a), as a result of post-mortem studies in Brazil, noted that the number of eggs/gm. of faeces/worm pair did not change significantly with the age of the host. It has also been suggested that older people are less susceptible to schistosomiasis than children but when Kleotzel and Da Silva (1967) studied a group of adult Brazilian males who moved from a non-endemic to an endemic area of S. mansoni they found that prevalence and intensity of infection rose to a peak after 15-18 years of residence in the area and then fell in a pattern similar to that observed amongst children brought up in an endemic area. Age-related differences in patterns of exposure to infected water might also be important but unfortunately, in most of the above mentioned epidemiological surveys water contact studies were not performed although Jordan (1972), working in St. Lucia, has reported in one study that 85% of water contact occurred in people younger than 20 who are those passing most of the eggs.

Although age-related differences in water contact might seem to provide an appealingly simple explanation of observed prevalence patterns, some evidence based upon comparisons of areas with high and low intensities of infection lends support to the theory of acquired resistance. Lehman et al. (1976) observed that, in one study area associated

with generally low egg counts, there was a slower rising age specific prevalence rate than in other areas of higher intensity, and that the fall in intensity was most dramatic in these latter areas, even though the age-related pattern of water contact in both would presumably have been comparable. Jordan (1972) compared epidemiological data from 41 settlements in St. Lucia grouped according to prevalence 1-25%, 26-50%, 51-75% or 76-100%. In areas with prevalence rates below 50%, the intensity of infection in people both below and above 20 years was similar, while, where the prevalence level was 50-100%, egg output was considerably higher in the young age group and was double the adult figure in areas where prevalence exceeded 76%. Clarke (1966) has provided similar data from Rhodesia. If it is assumed that the age related patterns of water contact in the various areas studied were broadly similar the above findings indicate that an acquired resistance is developed, and is more effective in areas of high prevalence where the intensities of infection in the younger age groups are greater. However, Ongom and Bradley's (1976) data indicate that this is not always the case. To explain this situation it has been postulated that such resistance as might develop can be overcome by continuous heavy exposure.

Studies on the incidence and rate of increase of intensity of infection following chemotherapeutic cure of patients could, theoretically, throw some light on the question of resistance. Kloetzel (1967b) reported on the pattern of infection following treatment of children in a hyperendemic region of Brazil. Prevalence (100% before treatment) had returned to 55% after 6 months and 83% after 4 years, at which time the egg excretion was one third of the pretreatment level. Assuming chemotherapy was reasonably effective, it appears that reinfection did

occur fairly rapidly indicating that the treated children displayed little if any resistance. However, the treatment was given to children under the age of ten and thus before the postulated resistance had begun to operate. Cook et al. (1974) reported that 2 years following treatment of St. Lucian patients with hycanthone, evidence of reinfection was seen in 45% of patients in an area of high endemicity and in 5% in an area of lower endemicity. Interestingly, reinfections were more common in those under 15 years than in those above and intensity of infection at this time was 2-3 times greater in the age group 0-9 years than in older patients, though in all age segments the intensity was very low. While the data could be interpreted as indicating that resistance was more evident in the patients over 15 years than in the younger ones, the author again observed that it was also explicable by the greater water contact of the younger group. The paucity of information on this subject leaves equivocal the questions as to whether resistance exists and persists in the absence of an active infection.

Considered as a whole, the epidemiological data can best be explained by postulating that, after a period of 10-20 years during which worm load increases, man can develop a partial resistance to reinfection, the degree of which is greatly influenced by the pattern of transmission. Bradley and McCullough (1973) proposed such a interpretation for their epidemiological data on human S. haematobium infections, and observed that it was consistent with the concept of concomitant immunity, described by Smithers and Terry (1969b) as a consequence of their studies of S. mansoni in the rhesus monkey (see section 1.2.2). The term concomitant immunity (Gershon et al., 1967) as applied to schistosomiasis described a situation in which the host is resistant

to reinfection but cannot at the same time rid itself of an established population.

Sera from humans infected with S. mansoni have raised levels of IgG and IgE, though there is some disparity between the results with IgM (Hillyer, 1969; Antunes et al., 1971; Dessaint et al., 1975). It is believed that much of this immunoglobulin is non-specific and this has been demonstrated for IgE (Dessaint et al., 1975). It is well documented, however, that at least some is schistosome specific and can be detected by numerous serological tests employing a variety of antigens (cercarial, adult worm or egg extracts) (for reviews of serodiagnosis - Kagan and Pellegrino, 1961; Sadun, 1967; Sadun, 1976). Specific antibodies belonging to the classes IgG, IgM and IgE have been detected (Hillyer, 1969; Dessaint et al., 1975). Whether any of the detectable antibodies are involved in resistance remains uncertain, however, there is general agreement that serological results do not correlate with worm burden and antibody levels detected serologically do not appear to bear any mechanistic relationship to the postulated pattern of resistance (Sadun, 1967).

The time course of development of immediate hypersensitivity, an assay for reaginic antibody activity, does correlate with the putative pattern of development of resistance. The degree of reactivity to cercarial or worm antigen is greatest in adults and lowest in children. Amongst adults the duration of infection bears a positive relationship to reactivity and amongst children there is a correlation between responsiveness and intensity of infection (Kagan and Pellegrino, 1961; Kloetzel and Da Silva, 1967; Warren et al., 1973a; McKay et al.,

1973). Dessaint et al. (1975), however, found no correlation between specific IgE levels and the clinical course of infection.

The attempted transfers of resistance by injection of relatively large quantities of serologically reactive gamma globulin from infected adults into either infected young adults (Warren et al., 1972) or parasitologically negative children (Cook et al., 1972) failed to significantly alter the course of infection in the former group or influence incidence and intensity in the latter group. Thus, to date, there is no evidence that antibody alone can mediate resistance in man.

Infected humans develop delayed skin sensitivity to intradermal injections of adult or cercarial antigens (Wolfson et al., 1969). On the basis of histological appearance and the time course of its appearance, Moriearty and Lewert (1974a) concluded that this delayed skin sensitivity (assessed at 24 or 48 hours after antigen injection) is a manifestation of cellular (delayed) hypersensitivity. Support for this conclusion comes from the demonstration by Wolfson et al. (1972) of inhibition, in the presence of adult worm antigen, of migration of peripheral blood leucocytes from subjects with positive delayed skin sensitivity. Warren et al. (1973) reported that 66% of infected St. Lucian adults developed a delayed hypersensitivity intradermal response compared with 31% of children in the age groups 5-9 years and considered this a consequence of the children having had less intense exposure to sensitizing antigens. Moriearty and Lewert (1974b) considered that the frequency of delayed skin reactivity was related to the frequency of past exposure to schistosome infection and tentatively concluded - ".. delayed hypersensitivity appears to be a response

which might be associated with resistance". Warren et al. (1975) failed to transfer delayed skin hypersensitivity to children by injection of transfer factor prepared from patients with chronic infections and so their failure to protect the children means little.

More recently, the effect of 'immune' serum and cells from S. mansoni-infected humans on schistosomula has been studied in vitro. Smith and Webbe (1974), using the culture system with which Clegg and Smithers (1972) detected antibody in the serum of infected rhesus monkeys that was lethal for schistosomula in vitro (see section 1.2.2), also demonstrated that, in the presence of complement, sera from infected humans killed a significantly higher percentage of schistosomula than did control sera. Capron et al. (1973) reported a similar lethal factor in sera from infected humans. The relevance of this lethal activity to resistance in man is unknown but analogous lethal antibody in the rhesus monkey and the rat appear not to be involved in resistance.

Butterworth et al. (1974) showed that a factor in inactivated sera from infected patients damaged schistosomula only in the presence of normal human peripheral blood leucocytes as assessed by release of ^{51}Cr from the labelled schistosomula. The antibody activity was associated with an IgG fraction and maximal cytotoxic effect was associated with an eosinophil-rich polymorphonuclear leucocyte fraction. Separation by differential centrifugation and treatment of the cells with specific antisera showed that the eosinophil was indeed the major cytotoxic cell (Butterworth et al., 1975) a finding further supported by studies using eosinophil-enriched cell populations (Butterworth et al. (1977). In this connection it should be noted that Mahmoud et al.

(1975a) reported having ablated the resistance of mice to reinfection by treatment with specific anti-eosinophil serum. Sher et al. (1977a), however, reported that there was no correlation between activity or titre of serum in this eosinophil-mediated ^{51}Cr release test and the duration of known infection in man.

Thus the evidence that man develops resistance to reinfection and that any such resistance involves the immune system remains largely circumstantial.

1.2.2 Subhuman primates.

The rhesus monkey has been used extensively as an experimental host for the study of development of acquired resistance to S. mansoni and much of our knowledge concerning resistance has arisen from such studies. The basic pattern of a primary infection has been described by numerous authors (reviewed by Smithers and Terry, 1969a). Following a heavy primary infection, faecal egg output increases from around week 6 to peak at 8-20 weeks and subsequently drops dramatically to a low level that can be maintained for over a year and may fall to zero. Cheever and Powers (1972) have shown that this drop reflects a spontaneous cure. Between weeks 12-27 post infection, 2/3 of the established worms died in monkeys infected with 600 cercariae. However, monkeys infected with 100 cercariae did not show this spontaneous cure and the faecal egg output continued to rise throughout the study. The absence of a dramatic self cure in rhesus monkeys infected with relatively low numbers of cercariae (below 200) is a general finding (Smithers and Terry, 1965a; Sadun et al., 1966; Ritchie et al., 1966).

Before discussing the factors involved in the development of resistance by the rhesus, a brief consideration will be given as to exactly what has been meant by resistance and how it is manifest. Throughout the studies described below there has been a lack of consistency in the criteria used to assess the course of a challenge infection. It is apparent that clinical protection, egg output and worm burden are not necessarily comparable. Smithers and Terry (1967) demonstrated that the absence of a rise in faecal egg output following challenge of preinfected monkeys does not necessarily mean that the monkeys have resisted reinfection. Perfusion of 2 monkeys given a primary infection of 100 cercariae and challenged 16 weeks later with 2,000 cercariae showed that 9-10 weeks after challenge (while the faecal egg output of both was comparable and significantly lower than that of the challenge control monkeys) their worm burdens represented 5% and 35% of the challenge dose compared with 48% for the challenge controls. Thus one of the monkeys had obviously only partially resisted reinfection though the faecal egg output of both was comparable and significantly lower than that of the challenge controls. The worms of the partially resistant animal were only half the size of those from the controls and their fecundity was evidently severely diminished. Foster and Broomfield (1971) have noted a similar stunting and reduction in fecundity of worms in resistant monkeys. Lichtenberg and Ritchie (1961) in discussing the data of Naimark et al. (1960) concluded that the solid resistance to challenge in terms of faecal egg output that was apparent in the previously infected monkeys was manifest in three phases. Firstly, fewer worms reached the portal system, some schistosomula being retained during migration, mainly in the lungs; secondly, those that did reach the liver remained immature and thirdly, all were eventually eliminated.

A reduction in fecundity of challenge worms has also been noted in resistant animals belonging to other species of primates (see next section). In some cases (Smithers and Terry, 1967) clinical protection has been observed in experimental monkeys passing large numbers of faecal eggs normally associated with severe illness. A contributing factor to this type of protection may have been a reduction in granuloma size caused by modulation of the immune reaction to the eggs trapped in the tissues which has been shown to occur in chronic infections in both mice and rhesus monkeys (Andrade and Warren, 1964; Cheever and Powers, 1969).

Several workers have reported that following heavy primary infections (500 - 2000 cercariae) rhesus monkeys can develop solid resistance to a challenge as indicated by an absence of rise in faecal egg output post-challenge. (Naimark et al., 1960; Smithers and Terry, 1965a; Maddison et al., 1971) N.B. The papers by Ritchie et al. (1966) and McMullen et al. (1967) contain much of the same data as was presented by Naimark et al. (1960). Smithers and Terry (1967) reported that rhesus monkeys infected with 100 cercariae and challenged 17 weeks later with 1800 cercariae showed a negligable increase in faecal egg output post-challenge, while the egg output of challenge-control monkeys rose dramatically and the animals died. Monkeys infected with 25 or 50 cercariae were clinically protected but did show a rise in faecal egg output following challenge. When the interval between primary infection (with 200 or 1000 cercariae) and challenge was shortened only some of the monkeys showed evidence of resistance (Smithers and Terry, 1965a). The above studies show that the rhesus monkey can become solidly resistant following a single, fairly heavy primary exposure provided the interval between primary infection and challenge is sufficiently

long. Furthermore, it appears that the resistance can be manifest in monkeys in which the primary infection has not been resolved (self-cured).

Warren (1973) has pointed out that in an endemic area man is generally repeatedly exposed to low cercarial densities. Foster and Broomfield (1971), in an attempt to more closely simulate such a pattern of exposure, infected a rhesus monkey with 25 cercariae every 14 days on a total of 19 occasions. The egg output rose continuously throughout the observation period up to day 319 post initial infection and thereafter showed signs of declining. At necropsy shortly afterwards the worm recovery represented 49% of the applied cercariae indicating that resistance to reinfection had not occurred. Similarly, Naimark et al. (1960) exposed a group of rhesus monkeys to 25-50 cercariae on 26 occasions at 35 day intervals. Egg output reached a maximum level after about 200 days, remained high up to 500 days and subsequently, declined, in some cases precipitously. Thereafter a low level output was maintained despite continued challenge. It appears, therefore, that when exposure to cercariae resembles that of man, the pattern of infection tends to be similar also, with a slow gradual increase in worm burden and faecal egg output preceding the development of resistance.

With reference to the development of resistance in schistosomiasis, Newsome (1956) concluded - "There is little evidence, but much presumption, that a condition of premunition as described by Sergent et al. (1925) is developed'. In their studies of 1965a and 1967, Smithers and Terry challenged a series of monkeys whose faecal egg output from the

primary infection had fallen to, or persisted at, a low level. In the resistant monkeys, egg output did not increase post challenge but the previous low level of egg production either continued or fell to zero. They concluded (Smithers and Terry, 1969b) - "Clearly, whatever the nature of the immune response that prevents the schistosomula of the challenge from maturing, this response does not at the same time necessarily destroy established adult worms or prevent them from producing eggs". They introduced the term "concomitant immunity" to describe this situation, avoiding use of the term premunition which would imply that persistence of the primary infection worms was essential to the perpetuation of the resistant state. The papers by Ritchie et al. (1966) and McMullen et al. (1967) reported solid resistance in a number of rhesus monkeys which had been exposed to cercariae on several occasions over a long period of time and in which the faecal egg output had fallen to zero. In a number of instances no adult or immature worms attributable to a prior infection were recovered at perfusion post challenge. Furthermore, Vogel (1962) demonstrated that chemotherapeutically cured rhesus monkeys were able to strongly resist challenge given from $7\frac{1}{2}$ to 34 months after treatment. Thus, the rhesus monkey can develop a sterile 'immunity' under certain circumstances.

In all of the above experiments in which resistance has been demonstrated, the monkeys have been exposed to penetrating cercariae, migrating schistosomula, adult worms and eggs: The role of each of these various stages in stimulating resistance will now be considered. Injection of eggs subcutaneously, intravenously or directly into the mesenteric veins of rhesus monkeys has not been shown to stimulate any resistance (Smithers, 1962; Smithers and Terry, 1967). Single-sex

infections with S. mansoni do not result in egg production and so can be used to study the role of eggs in the induction of resistance. In two experiments reported by Smithers (1962) four monkeys were infected with 2000 - 2500 male or female cercariae, challenged at 23 weeks with 100 cercariae and perfused 8 weeks later. In the first experiment substantial resistance was demonstrated in two monkeys infected with female cercariae. The equal sex ratio among the worms recovered at perfusion indicates that the primary unisexual infection had not persisted. In the second experiment, in which the worm recovery from the challenge control monkey was unexpectedly low, there was no evidence of resistance in the monkey pre-infected with male cercariae and evidence of partial resistance in the monkey pre-infected with female cercariae. Hsü (1969) reported that monkeys infected with large numbers of cercariae of one sex (4,500 - 17,000) and challenged approximately 600 days after the first exposure were highly resistance in terms of faecal egg output and worm burden.

To see whether the penetrating and migrating stages of the parasite contributed to the stimulation of resistance, Smithers and Terry (1967) transferred adult worms directly into the mesenteric veins of rhesus monkeys and challenged them 10 weeks after transfer. On the basis of faecal egg output following challenge and/or worm burden at perfusion, none of the monkeys were completely resistant to challenge while others were almost completely susceptible. It is apparent that the above transfer of worms did not result in as consistant protection as had been observed with whole infections. Possibly the fact that the monkeys were only exposed to the adult worms for 10 weeks (an attempt to stimulate the degree of exposure to the adult phase that a monkey

infected for 16 weeks would have received) may account for this difference. Consistent with this is the demonstration by Smithers (1968) that two monkeys that had received transplanted worms were highly resistant to a challenge given 14-16 weeks after transfer. Transfer of worms that had been cut transversly in half and which produced very few eggs stimulated variable resistance, one monkey being susceptible, another highly, and a third partially resistant to reinfection. The most convincing evidence that the adult worm alone can stimulate resistance to reinfection is provided by the demonstration by Smithers (1968) that monkeys which had received 160 transplanted male or female worms were highly resistance in terms of faecal egg output to a challenge given 14-16 weeks post transfer. Such monkeys had been exposed to neither migrating stages or eggs. On the basis of the above findings Smithers and Terry (1967) concluded that in a normal infection it is the adult worm that provides the major stimulus to resistance to S. mansoni in the rhesus monkey. Assuming that this resistance was immunologically mediated, Smithers (1968) stated - "Thus the antigens which are targets for the host immunological attack on the invading schistosomula, must also be present on the adult worm". Shared antigens between adult worms and larval stages have indeed been demonstrated by immunoelectrophoresis (Capron et al., 1965; Sadun et al., 1965). To explain how it was possible for the worms from a primary infection to persist at a time when the challenge infection was being destroyed by an immunological attack which had been stimulated by adult worm antigens it was postulated, with some support from in vitro studies, that antigens either of parasite or host origin that were known to be shared between host and parasite serve to mask the adult from the immunological attack that it had instigated (see review by Smithers and Terry, 1976).

It follows from the above that the cercariae or early schistosomula express the antigens against which the immune response is directed and thus should be able to stimulate resistance in the absence of adult worms. Pertinent to this question are the attempts which have been made to vaccinate experimental animals by exposing them to irradiated cercariae that fail to develop into mature adults. Smithers (1962) demonstrated marked partial protection of monkeys infected on 2 occasions with a total of 13,000 cercariae irradiated with 2.0-3.0 kr and challenged 14 weeks later. Such low doses of radiation, particularly 2.0 kr allowed survival of a proportion of stunted and largely sterile worms. Sadun et al. (1964) immunized monkeys with 5 weekly doses of 5,000 irradiated cercariae and challenged them 30 days later. Markedly better resistance (83% fewer worms than controls) was produced by cercariae irradiated with 2.5 kr than with either 4 or 10 kr (26% and 42% respectively, fewer worms than controls). These results indicated that the small proportion of stunted worms that survive irradiation may be largely responsible for the stimulation of resistance. However, Hsü et al. (1969) have reported resistance in monkeys immunized with cercariae exposed to 24 and 48 kr that do not survive past the schistosomular stage, perishing mainly in the dermal tissue (Lichtenberg and Sadun,1963; Hsü et al., 1963a). Such monkeys, immunized on 3-5 occasions with a total of 25-40,000 cercariae and challenged 271-801 days after the first immunization, were found to harbour a mean of 76% fewer worms than the challenge controls. Thus resistance can be induced in the rhesus monkey by exposure to the schistosomular stage alone

Rhesus monkeys infected with S. mansoni have raised levels of immunoglobulin much of which appears not to be schistosome specific

(Freeman et al., 1970). Schistosome specific antibodies, including reaginic antibodies can be detected in monkeys infected with normal and irradiated cercariae and in monkeys injected with eggs or extracts of cercariae and adult worms, but in no case does the presence or level of detectable antibody seem to correlate with resistance (Smithers, 1962; Jackowski et al., 1963; Sadun et al., 1964 and Maddison et al., 1971). As in infected humans (Smith and Webbe, 1974), the serum of infected rhesus monkeys can damage schistosomula in culture (Clegg and Smithers, 1972). A lethal effect caused by antibodies in the IgG class is dependent upon the presence of heat labile factors in fresh normal serum, probably complement. A growth inhibiting effect, probably caused by a different IgG antibody is independent of such factors. Following a small infection (175 cercariae) no marked lethal effect could be detected in 3 out of 4 monkeys 16 weeks later at a time when the rhesus regularly demonstrates a high level of resistance to challenge (Smithers and Terry, 1969b), though a marked growth inhibiting effect was present at this time. Murrell and Clay (1972), who also demonstrated cytotoxic antibodies in vitro, have questioned their role in resistance to schistosomes. All attempts to directly establish a role for antibody alone in mediating resistance, by the passive transfer of serum from infected monkeys, have failed to confer any protection (Meisenhelder et al., 1960; Ogilvie et al., 1966; Maddison et al., 1976; Clegg and Smithers, 1976). In this latter study the transferred serum contained high titres of lethal antibody.

Delayed skin hypersensitivity (D.H.), indicative of the presence of specifically sensitized cells, has been demonstrated in infected monkeys (Maddison et al., 1973). Maddison et al. (1976) were able

to transfer D.H. to monkeys by injection of transfer factor from infected donors. Recipients of hyperimmune serum plus 'immune' transfer factor were partially, though significantly, protected from a challenge infection. However, hyperimmune serum plus 'normal' transfer factor, which did not result in skin test conversion, resulted in comparable protection. Thus, under these circumstances, development of D.H. seemed to be incidental to resistance. Possible mechanisms whereby normal transfer factor could stimulate cellular components in the recipient are discussed by Maddison et al. (1976). This latter paper is fundamental as it provides the only evidence of immune system involvement in resistance to reinfection in the rhesus monkey. The results suggest an intimate interaction between humoral and cellular immune mechanisms. In an earlier study (Maddison et al., 1971) immunosuppressive treatments aimed at suppressing the humoral or the cellular components of the immune response failed to affect the expression of resistance to reinfection.

With reference to the relevance of their rhesus monkey/*S. mansoni* studies to human schistosomiasis, Smithers and Terry (1969b) stated: "Undoubtedly, the rhesus is more adept at becoming resistant than is man. It seems to us, however, a priori unlikely that in two primates, both of which support the development of the parasite, there are two distinct mechanisms of immunity against the same parasite. They may differ in degree, but hardly, we believe, in kind!" A brief review of the findings in other primate systems serves to throw some light on the extent to which the rhesus monkey can be considered representative of sub-human primates in general.

Cheever and Duvall (1974) have reported that following exposure to 600 cercariae, the grivet monkey (Cercopithecus aethiops) supports a prolonged infection and shows little tendency to self cure. Such infected monkeys were repeatedly challenged with about 1000 cercariae over 30 months. During the course of these challenge exposures, the faecal egg output increased at first but eventually stabilized. Perfusion results gave evidence of the development of partial resistance as indicated by a lower percentage recovery of challenge worms (19%) compared with the recovery following a single infection (47%) and by a reduced fecundity in terms of tissue eggs/or faecal eggs/worm pair recovered. The results of perfusion following challenge of monkeys 23 and 28 months after a 600 cercariae primary infection indicated that a lower degree of partial resistance had developed (35% recovery of the challenge cercariae). The closely related green monkey (C. sabaeus) shows a similar prolonged egg output following primary exposure (Meisenhelder and Thompson, 1963; Ritchie et al., 1967). Ritchie et al. (1967) could detect little evidence of resistance in monkeys challenged 12 or 60 months after primary infection with 100-200 cercariae. Monkeys exposed repeatedly to 20 cercariae over 32 months showed a gradual increase in faecal egg output and very little evidence of having developed any resistance. It is possible that the apparent differences in the response of the green and grivet monkey merely reflect differences in experimental protocol. While it does appear that the grivet monkey can develop a degree of concomitant immunity, both this and the green monkey are very poor at developing resistance as compared with the rhesus monkey.

Sadun et al. (1966) concluded that, in terms of the persistance of infection, the pattern of egg production and tissue egg distribution, the baboon (Papio spp.) appears to follow a course closely related to that of man. Following a primary infection eggs appear in the faeces at 4-5 weeks, increase in number over the next 2-3 months and thereafter remain relatively constant (Sadun et al., 1966; Taylor et al., 1973a). Such relatively constant egg output may continue for at least 2-3 years (Damian et al., 1976). While this appears to be the general pattern of infection following a single primary infection, an early peak in egg output (from week 6-12) followed by a sharp decline to about 1/3 peak values has been reported in a number of baboons infected with 2,000 cercariae (Damian et al., 1976). Sturrock et al. (1976) reported apparent cessation of egg production in one baboon 11 weeks after infection with 500 cercariae. Complete self cure has only been reported once in a baboon whose faecal egg output dropped to zero between 12-17 weeks post infection with 15,000 cercariae and it was subsequently found that the worms had died (Taylor et al., 1973a). Taylor et al. (1973b) reported that the baboon demonstrates partial resistance to reinfection when challenged 18 weeks after a primary infection with 500 cercariae. There was no rise in faecal egg output post challenge in 2 out of 4 such baboons, though egg excretion due presumably to the primary infection persisted, indicating the operation of concomitant immunity. Results at perfusion showed that the reduction in worm burden (48%) was paralleled by a reduction in tissue egg count (49%) indicating that there was no effect on the fecudity of the challenge worms that did establish themselves.

The paper by Damian et al. (1976) presents data on reinfection studies in baboons. Unfortunately, the experimental designs are very varied and are only recorded for 9 out of 17 experimental animals. Amongst these selected examples primary exposure varied from 200-2000 and re-exposure on one or two occasions with 1000 or 2000 cercariae was given from 4-32 months later. The faecal egg output data presented for individual baboons indicated that 2 out of 9 showed no rise in faecal egg output following re-exposure. When taken as a group the 17 'repeatedly exposed' baboons showed no evidence of resistance in terms of percentage worm burden or egg count/worm pair (both faecal and tissue) compared with baboons given a single exposure. Damian et al. (1974) exposed a group of baboons, at 3 month intervals to small numbers of cercariae (100-50) totalling 700 given over 36 months. Faecal egg output did not continue to rise throughout this time period and declined in a number of the baboons. 40 months after the first exposure, half the group were challenged with 1000 cercariae. There followed no apparent rise in faecal egg output and the percentage recovery of worms at perfusion was significantly less in baboons that had been challenged (29%) than in those that had not (46%). Thus repeated low level exposure over a long period appeared to have conferred a degree of resistance to reinfection. Mean daily faecal egg output/worm pair during the month preceding necropsy and the tissue egg count/worm pair were significantly lower in these trickle infected animals than in those singly or 'repeatedly' infected which were reported in the above paper (Damian et al, 1976). This finding was interpreted as indicating the operation of an immune process resulting in inhibition of egg output. However, there was some indication of a reduction in fecundity with duration of infection in singly and 'repeatedly' infected baboons, and when the values

for tissue eggs/worm pair are compared for single or repeated infections of more comparable duration to the 'trickle' infections there is no significant difference.

In a study by Sturrock et al. (1976) baboons given 5, monthly, exposures to 200 cercariae showed no evidence of having developed resistance in terms of worm burden in that they harboured more worms than baboons given a single exposure to 1000 cercariae and only 22% fewer worms than the cumulative total calculated from baboons infected as controls for each of the 5 exposures. The values for tissue eggs/worm pair and mean daily faecal egg output/worm pair were significantly lower in 3 out of the 6 repeatedly exposed baboons than in the single infection controls. This was tentatively interpreted as indicating the operation of an immunologically mediated depression of egg production.

Damian et al. (1972) reported an attempt to induce resistance in baboons following transfer of worms directly into the mesenteric system of baboons as Smithers and Terry (1967) had done in the rhesus. Transfer of 80 worm pairs directly into the portal system did not confer any significant protection against challenge with 2000 cercariae 2-3 months after transfer. However, as resistance has not been demonstrated at such an early time post infection this result contributed little to an understanding of the conditions necessary for stimulation of resistance in baboons though it does further indicate the difference between the baboon and the rhesus in development of resistance.

Two attempts to induce protection of baboons by infection with radiation attenuated organisms have been reported by Taylor et al.

(1976a). Baboons exposed on weeks 0, 4 and 8 to 5000 cercariae irradiated at 6.0 kr were not significantly protected against a challenge with 500 cercariae on week 15. Another group were injected intramuscularly with a total of 31,415 schistosomula irradiated at 2.1 and 2.4 kr over a period of 13 weeks. Such infections resulted in a mean worm burden of 420 stunted and largely sterile worms. Similarly vaccinated animals which were challenged with 35000 cercariae 8 weeks after the last injection showed no significant resistance either in terms of egg output or worm burden.

While there are some inconsistencies in the published data concerning the circumstances under which the baboon may develop resistance to reinfection, it is evident that resistance is invariably weak and frequently absent.

Of all the primates studied, the chimpanzee (Pan satyrus) is phylogenetically closest to man and Sadun et al. (1966 and 1970) have shown that the course of disease and the num ber and location of worms in this animal resembles that in the human. In the latter study faecal egg output rose following a single infection with 1000 or 2000 cercariae and remained relatively constant during the 3 years of study. Repeated monthly exposures of chimps to 100 or 250 cercariae resulted in a gradual increase in faecal egg output during the first 24 months followed by a more or less steady output. At perfusion such animals were shown to have a higher percentage worm burden than singly exposed chimps and the tissue egg load/worm pair was comparable. There was thus no evidence of resistance either in terms of diminished worm burden from succeeding exposures or in terms of a suppression of fecundity.

The unavoidable conclusion from the above is that primates in general, unlike the rhesus monkey, do not readily develop resistance to reinfection with S. mansoni and seemingly the more similar their pattern of infection is to that in man, the weaker is their response. Thus any prophylactic vaccine will have to do better than merely mimic the resistance induced by a whole infection. It is considered that further experimentation in primates, which are becoming prohibitively expensive, would not be justified until experiments in the more convenient rodent models give some indication of possible fruitful approaches.

1.2.3 Mice.

S. mansoni infections in the mouse develop to maturity and, provided the cercarial dose is not excessive, proceed to a chronic phase (Stirewalt et al, 1951). Lightly infected mice, harbouring 1-3 worm pairs show little tendency to spontaneous cure, such burdens being maintained at least up to 84 weeks post infection (Cheever, 1965; 1969). Worm burdens in heavily or massively (100-500 cercariae) infected mice show little evidence of declining up to week 16 (Stirewalt et al., 1951); the lower percentage worm burdens recorded thereafter may be largely explained by the earlier death of the more heavily infected mice. However, Kloetzel (1967b) and Kuntz and Malakatis (1955), the latter who reported active infection 119 weeks post infection, have reported death of worms in prolonged infections.

Egg output per worm pair appears to be constant during the course of prolonged infections such that a steady state ultimately obtains with the number of eggs/worm pair in the tissues (liver/or intestines) remaining relatively constant from 6-12 months after infection, reflecting a balance between egg production and destruction of eggs in the tissues (Perlowagora-Szumlewicz, 1964a; Cheever, 1965; Kloetzel, 1967b; Cheever, 1969). Cheever (1969) reported that this steady state was also reflected in the faecal output/worm pair which peaked 100-200 days post infection and remained at this level up to 300 days. Moore et al. (1949) similarly reported steady egg output from 80 - 270 days. However, Kloetzel (1967b) reported that faecal egg output/worm pair declined rapidly from a peak 60 days post infection. Cheever (1968a) reported that a steady state also exists in human infections. Furthermore, the number of eggs/worm pair in the tissues and the tissue distribution of eggs between gut and liver were broadly comparable for man and mouse. Warren and De Witt (1958) and De Witt and Warren (1959) have demonstrated that infected mice develop manifestations of chronic disease characterised by liver and spleen enlargement, portal hypertension and oesophageal varices, a syndrome closely resembling hepatosplenic schistosomiasis in the human.

Thus in terms of persistence of infection, pattern of egg production and disease manifestation, the mouse broadly resembles man. However, in terms of infection the two are markedly different. The lightest possible infection in a mouse (1 worm pair) represents an intensity of the order of 50 worm pairs/kg. body weight, whereas the heaviest infestations encountered in man at autopsy by Cheever (1968a) were approximately 5 worm pairs/kg. Accepting this quantitative difference, the mouse has proved to be a useful and convenient host in which to study the development of resistance to reinfection.

Table A, presented as Appendix 2, records most of the experimental data in the literature concerned with actively acquired resistance to S. mansoni in the mouse following previous infection with the homologous parasite. Certain of the data have been recalculated to accommodate them in this table. Resistance is assessed by exposing the mice to an initial (primary) infection, subsequently re-exposing them to a challenge infection (superinfection) and recovering the adult worms by perfusion. A control group given the primary infection alone allows an estimate to be made of the number of challenge-derived parasites maturing in the superinfected mice and a comparison with the worm burden in a control group given the challenge alone enables the degree of resistance to be estimated. Studies not conforming to this design, Thompson (1954), Lurie and De Meillon (1957), Perlowagora-Szumlewicz (1964a), Sher et al. (1974) are not included in tabular form but are discussed where relevant. It is difficult to draw sound conclusions from the tabulated data concerning the parameters of the primary infection necessary to stimulate significant resistance to reinfection; different mouse strains have been used by the various authors and in no instance is their experimental protocol the same. That the mouse can develop partial resistance is demonstrated in 39 of 52 recorded experimental groups, where a statistically significant reduction of the challenge infection was observed.

The operation of resistance in the mouse is confirmed by the demonstration of increased survival time following lethal challenge of previously infected mice. Olivier and Schneidermann (1953) performed a series of experiments to test whether a light initial infection with S. mansoni would protect mice against the effects of a challenge exposure

known to be lethal to normal mice. Mice were challenged with 360-583 cercariae 13-16 weeks following light bisexual infection. The mean survival time, after challenge, of the preinfected group was not significantly different from that of the challenge controls, though the two groups differed in their survival pattern, deaths in the preinfected group occurring over a much longer period than in the challenge control group. Levine and Kagan (1960) recaluclated this data, excluding deaths prior to the seventh week post-challenge which they considered due to the initial infection. Using this data, the mean survival time of the preinfected group is significantly longer than that of the controls. Referring again to Table A, the operation of concomitant immunity (Smithers and Terry, 1969b) is clearly demonstrated in the studies of Olivier and Schneidermann (1953) in which the challenge infection was distinguished by size from the primary infection. No obvious correlation exists between the mean number of worms persisting from the primary infection and the degree of resistance, but it is apparent that relatively few worms (3-5) can stimulate significant protection, as shown by Olivier and Schneidermann (1953), Sher et al. (1974) and Mahmoud et al. (1975a). Resistance, as assessed by recovery of schistosomula from the lungs (Olivier, 1952; Clegg, 1965) 4-6 days after challenge of mice given a primary infection 12 weeks previously, appeared to be independent of the level of primary exposure (Sher et al., 1974). No direct relationship exists between the degree of protection and the size of the challenge dose. In fact, as shown by Hunter et al. (1962), a relatively constant proportion of the challenge dose fails to mature irrespective of the number of challenging cercariae.

How long does resistance take to develop? Stirewalt (1953) was able to demonstrate that the resistance observed when mice were challenged one hour or one day after an initial infection was due to local inhibition of penetration of the challenge cercariae, which were applied at the same site as those of the primary infection. Hunter et al. (1967a) reported significant resistance 6 weeks after a primary exposure while Hunter et al. (1962) and Stirewalt (1953) demonstrated resistance when mice were challenged eight weeks after a primary infection but not when challenge was only four weeks after infection. Similarly, Sher et al. (1974) were unable to demonstrate resistance at three weeks but found strong resistance by twelve weeks. Using the lung recovery assay they reported that the degree of resistance increased from about 35-45% at 7 weeks to 50-70% at 15 weeks post primary infection. The results of Hunter et al. (1962) indicate that resistance is maximal by eight weeks and is not markedly affected by prolonging the immunizing interval. Ritchie et al. (1963), however, reported that mice challenged 24 weeks after primary infection were significantly more resistant than mice challenged at 16 weeks. With regard to the question as to how long resistance takes to develop in the mouse, it should be emphasized that the influence of the size of the primary infection has been largely overlooked. Resistance may be demonstrable earlier if the initial worm load is greater. However, the experimental designs recorded in Table A are so varied that no such correlation can be made. Lurie and De Meillon (1957), Thompson (1954) and Frick et al. (1965) have shown that mice can develop resistance during the course of a trickle infection in so far as the percentage worm burdens following trickle infections were lower than those following a single exposure. However, the data of Hunter et al. (1962) show that repeated primary infections do not stimulate any greater resistance to challenge than a single infection.

For the sake of completeness, it should be recorded that Thompson (1954) failed to demonstrate resistance in mice challenged 12 weeks after a primary infection with 50 or 100 cercariae in so far as the percentage worm recovery 3-4 weeks post challenge (25.8%) was comparable with that observed after a single exposure (20.8%). Similarly, Perlowagora-Szumlewicz (1964a) failed to demonstrate resistance in mice challenged 8 weeks after primary exposure to 100 cercariae. Such failures to demonstrate resistance under similar experimental conditions to those successfully employed by other workers may be due to mouse strain differences as are reported in Chapter 3 of this thesis. The existence of such strain differences means that experiments without a control group showing that the particular mouse strain involved does develop resistance may be worthless (e.g. the experiments discussed below on resistance following chemotherapy).

Although there is evidence from the studies in Rhesus monkeys that single sex infections can stimulate resistance to reinfection (i.e. that eggs are not necessary for the induction of resistance), experiments in the mouse model have not clearly defined the stage(s) of infection responsible for stimulation of resistance. As noted above, resistance has only been demonstrated at a time when the worms from the primary infection have reached maturity and oviposition has commenced. Such findings prompted Lichtenberg et al. (1963) to study the role of the egg in induction of resistance. Mice were injected intravenously with 1,800 S. mansoni eggs and challenged 40 days later with 200 cercariae. Worm recoveries 50 days post challenge, from these and from control mice, were not significantly different. Moore et al. (1963) failed to demonstrate any resistance in mice given 10 subcutaneous injections of 400-1,000 eggs

and challenged from 2-42 days after the last injection. Although these administrations of eggs in no way simulate egg deposition in infected mice and accepting that resistance was not studied in mice harbouring a whole infection, these results indicate that the egg alone cannot be responsible for resistance.

That the adult worm alone can stimulate resistance has not been convincingly demonstrated either. Olivier and Schneidermann (1953) reported marginally statistically significant, low grade, partial resistance in mice harbouring an all male worm population. Protection was considerably weaker than that reported for bisexual infections, though direct comparisons were not performed. Sher et al. (1974), employing the "lung chop" technique, have demonstrated that mice harbouring an all male worm infection yield 54% fewer schistosomula than uninfected controls, a resistance comparable with that observed with a numerically comparable bisexual infection. However, comparable levels of resistance took longer to develop following single sex infection (12 weeks) than bisexual infection (7 weeks). These results were obtained by the lung chop assay and corroborative data on worm perfusions were not presented. Both Thompson (1954) and Perlowagora-Szumlewicz (1966) failed to demonstrate resistance after heavy primary infection with 100-500 cercariae of one sex. However, as reported above, both also failed to demonstrate resistance in bisexually infected mice. Evidence that single sex infections do not promote protection was presented by Olivier and Schneidermann (1953). Mice initially infected with only male cercariae did not show a prolonged survival time post challenge compared with challenge controls, whereas mice initially infected with a bisexual infection did.

Thus, in the opinion of the author, it has not been clearly demonstrated which stage(s) of an active infection contribute to the development of acquired resistance in the mouse. Whether the resistance that has been demonstrated in mice harbouring an active infection depends upon the persistence of such active infection is a fundamental question and has been studied by several workers who have studied resistance following chemotherapeutic treatment of infected mice. Cheever et al. (1965) detected no resistance in mice challenged 2.5 - 5 weeks after almost complete cure. However, it was not demonstrated that untreated mice demonstrated resistance and thus cannot be concluded that resistance was abrogated. Striebel and Sarasin (1975) failed to demonstrate resistance in mice that had been treated 8 weeks following a primary percutaneous infection through they also failed to test the resistance of untreated mice. Partial resistance was reported following drug treatment of mice when the primary and challenging infections were given by the subcutaneous route. The experiment reported by Gold and Lengy (1975) did include an infected/untreated control group. However, mice in this group and in the infected/treated group showed no evidence of resistance in terms of worm burden, highlighting the danger of interpreting negative results in the absence of a positive control. The only evidence that the course of a challenge infection is influenced by previous exposure and treatment comes from the work of Campbell (1963) who demonstrated that the mean survival time of mice, challenged following chemotherapeutic cure of a single or repeated previous exposure(s), was significantly longer than that of challenge controls. The primary infection(s), totalling from 500 to 400 cercariae were drug terminated from 7-28 days post infection and thus mature egg producing infections were not established. Prolonged survival does not, of course, mean that

the challenge infection has been partially eliminated, it may merely have been delayed or its pathological consequences ameliorated.

Resistance has been demonstrated in mice infected with radiation attenuated cercariae. Table B, presented as Appendix 3, summarizes the data presented in the literature on such studies. Statistical analysis was only possible for 24 experimental groups in 19 of which a statistically significant reduction of the challenge infection was demonstrated. Both Villella et al. (1961) and Radke and Sadun (1963) reported greatest resistance with cercariae exposed to the low levels of x-radiation (950-3,000 rads) which allowed survival of a small proportion of the cercariae as stunted worms. Murrell et al. (1975) also reported marked resistance in mice exposed to ultra violet-irradiated cercariae, a small proportion of which survived to become stunted adults. However, Radke and Sadun (1963) were able to induce resistance with cercariae irradiated with 5 kr. and Erickson and Caldwell (1965) reported greater protection with cercariae irradiated with 8 or 10 kr. than with 6 or 4 kr. As cercariae exposed to doses of around 4 kr. and above fail to develop to the adult stage (Villella et al., 1961; Radke and Sadun, 1963; Erickson and Caldwell, 1965), these results indicate that the adult worm is not essential for the stimulation of resistance in the mouse and that resistance induced by irradiated infections does not depend upon the persistence of infection. A more detailed consideration of the above literature is presented in Chapter 5, but in conclusion here it should be observed that resistance following infection with irradiated organisms is not entirely predictable even under similar experimental protocol, and that there is disagreement in the literature as to the best irradiation dose with which to attenuate the infection

to produce maximum resistance. However, in view of the variable and generally limited success of attempts to induce resistance with metabolic or somatic extracts of cercariae or worms (Watts, 1949; Thompson, 1954; Levine and Kagan, 1960; Ritchie et al., 1962; Da Silva and Ferri, 1968; Murrell and Clay, 1972; Murrell et al., 1975; Minard et al., 1977), and with the possible exception of single sex infections, infection with radiation attenuated organisms is the only way of conferring protection without giving the mouse a pathogenic whole infection. [Non-specific resistance has been demonstrated in mice injected with living BCG (Fauve and Dodin, 1976; Bout et al, 1977), and talc (Fauve and Dodin, 1976).]

Mice infected with bisexual, single sex or irradiated infections develop schistosome specific antibodies against cercarial, adult worm and egg antigens, detectable by a variety of serological techniques (Stirewalt and Evans, 1955; Radke and Sadun, 1963; Jaimes and Lichtenberg, 1965; Sadun et al., 1965; Bruijning, 1965; Hillyer and Frick, 1967; Kien Truong et al., 1970; Ambroise-Thomas and Andrews, 1976 and Katz and Colley, 1976a) but antibody levels appear to be unrelated to the degree of resistance.

However, despite early unsuccessful attempts with passive transfer (Stirewalt and Evans, 1953) and paraboisis (Hunter et al., 1967), more recent reports indicate that approximately half of the resistance demonstrable in chronically infected donors can be transferred to recipients by passive serum transfer (Sher et al., 1975; Mahmoud et al, 1975a; Hillyer et al., 1975). Sher et al. (1977b) reported that successful transfer was associated with an IgG fraction. Perlowagora-Szumlewicz (1964b) reported transfer of protection from donors infected with irradiated

cercariae, though the donors themselves were not resistant. Mahmoud et al, (1975a) were able to abrogate the resistance demonstrable in chronically infected mice by depletion of their eosinophil population with anti-eosinophil serum, a procedure which also abrogated the resistance transferable with serum and it was concluded that resistance is mediated by specific antibody acting in concert with eosinophils. Lichtenberg et al. (1976) have described a dramatic increase in the number and percentage of eosinophils associated with microabcess-like foci which appeared in the skin at the time of challenge of previously infected mice as compared with controls. However, it was not established that such mice were resistant.

It has been clearly demonstrated that the schistosome egg granuloma is a manifestation of delayed cell mediated hypersensitivity (see Warren 1976) which can be ablated by rigorous T cell deprivation (Buchanan et al., 1973). However, a role for C.M.I. in resistance to reinfection has not been established although Katz and Colley (1976b) have reported a delayed cell-mediated response to an intradermal injection with a soluble cercarial antigen preparation. Hunter et al. (1967b) failed to demonstrate transfer of resistance in paraboised animals or by injection of spleen, lymph node or peritoneal exudate cells from infected mice. Similarly Sher et al. (1975) failed to transfer resistance with spleen cells from chronically infected donors and also failed to affect the resistance of such donors by ALS depletion of T cells at the time of challenge. Indirect evidence for the involvement of specifically sensitized cells comes from the work of Colley et al. (1972) who demonstrated that while passive transfer of serum from infected mice resulted in an intense polymorphonuclear infiltrate in

the skin of recipients 5 hours post challenge, transfer of lymph node and spleen cells resulted in a delayed hypersensitivity type mononuclear cell infiltrate which was maximal by 30 hours at which time many schistosomula were observed to have undergone degeneration. Unfortunately it appears that no attempt was made to determine the worm burden in these recipient animals.

1.2.4 S. mattheei

S. mattheei is a common parasite of domestic livestock in Southern and Central regions of Africa (Hussein, 1973) and is responsible for outbreaks of clinical and fatal schistosomiasis characterised by dramatic loss in condition, weakness, severe anaemia and diarrhoea (Lawrence, 1968). Lawrence (1974) reported that following infection of sheep with 3,000 cercariae, eggs first appeared in the faeces 7 weeks later and the mean egg counts rose slowly to peak 20 weeks post infection, thereafter showing a gradual decline. The worm burdens did not show a parallel decline indicating a decline in fecundity of worms or a restricted passage of eggs into the faeces. Preston and Webbe (1974) reported that sheep exposed to 500 cercariae showed no evidence of resistance, either in terms of worm or egg burdens, to a challenge with 500 cercariae 14 weeks after the primary infection although the female worms from superinfected sheep were significantly shorter than from the controls. Dargie et al. (1977) reported that sheep challenged with 10,000 cercariae 63 weeks after a primary exposure to 10,000 cercariae harboured 20% fewer worms than controls given the challenge alone. A more pronounced effect on the fecundity of the worms was

observed, there being a 31% reduction in the number of eggs/worm pair in the superinfected animals compared with the controls. Possibly as a consequence of this lower egg production the clinico-pathological changes post challenge were slower to develop and much milder in the superinfected sheep. Despite the low levels of resistance stimulated by whole infections, Taylor et al. (1976b) have reported strong partial resistance in sheep vaccinated with radiation-attenuated infections. Sheep vaccinated on four occasions with 10,000 cercariae or artificially-transformed 'schistosomula' irradiated with 3 or 6 kr. exhibited marked resistance to challenge (56-80% reduction in worm burden).

Heterologous protection against S. mattheei by infection with S. mansoni has also been studied. Taylor et al. (1976b) infected sheep on four occasions at monthly intervals with 10,000 cercariae of S. mansoni and challenged them 4 weeks after the last exposure with 5,000 cercariae of S. mattheei. There was no evidence of protection against this challenge either in terms of worm or egg burdens. However, Preston et al. (1972) reported that sheep exposed to 20,000 S. mansoni cercariae and re-exposed to 10,000 S. mansoni cercariae one year later showed partial resistance to a challenge with S. mattheei administered 13 weeks later. S. mattheei worm burdens were reduced by a mean of only 17% compared with controls but tissue egg counts showed reductions of 41-52%.

It has been demonstrated that cattle also develop resistance to reinfection with S. mattheei following either homologous (Lawrence, 1973) or heterologous (S. mansoni) (Hussein et al., 1970) primary exposure, but no vaccination studies using irradiated parasites have yet

been reported. Superinfection and vaccination studies with S. mattheei in the mouse have not previously been reported.

CHAPTER 2. MATERIALS AND METHODS.

2.1 Maintenance of the parasites and their snail hosts.

2.1.1 Schistosomes used.

S. mansoni (Puerto Rican strain).

Eggs of this strain were isolated from a patient and sent to London in 1964. Since then it has been maintained in albino *Biomphalaria glabrata* from Puerto Rico, and white mice or hamsters.

S. mattheei (South African strain).

A Nelspruit strain has been maintained in *Bulinus globosus* from Nelspruit and hamsters at the School since 1965.

2.1.2 Maintenance of snails.

Snails were kept in 10 litre glass tanks containing water dechlorinated by passage through an activated charcoal/glass wool filter and allowed to stand in plastic dustbins containing guppies (*Lebistes reticulatus*) - hence 'guppy water'. Each tank contained a maximum of 40 snails. Gravel and weed were not introduced but *Daphnia* were routinely added to restrict excessive algal growth. The water was continuously aerated by pumps and attached air stones. Snails were fed on dried lettuce. Uneaten lettuce and faeces fouling the tanks were routinely removed by suction using a peristaltic pump, and the tanks topped up with fresh water. The aquaria were thermostatically maintained at 24-26^{o}C and illumination provided by 'warm white' fluorescent tubes controlled to give a 12 hr day from 8 a.m. to 8 p.m.

2.1.3 Maintenance of schistosomes.

Live schistosome eggs were extracted from mice and hamster livers by macerating the tissue, straining it through a wire mesh filter (mesh 36/inch) into a urine glass and repeatedly sedimenting the precipitate with 0.85% saline until the supernatant became clear. About 15 mins. was allowed for each sedimentation. A final washing with ice-cooled distilled water was performed to remove traces of saline. After the final washing, the sediment was poured into petri dishes and placed under a strong light. Miracidia hatched within a few minutes and were caught using a drawn out Pasteur pipette under the low power of a dissecting microscope. This procedure also constituted the hatching test for the presence of viable eggs. Snails were individually exposed to miracidia (10 for S. mansoni, 5-6 for S. mattheei) in the wells of haemagglutination trays. The wells were topped up with water, covered with a sheet of glass and left under aquarium conditions for 4-5 hours (S. mattheei) or overnight (S. mansoni). Such snails were individually screened for infection 28-38 days later by placing them in 3" x 1" glass tubes containing 3-5 ml. of guppy water and exposing these to light. Any shedding snails were removed to the infected tanks. Snails which proved to be negative after 2 test screenings were killed.

Infected snails required for shedding of cercariae were kept in the dark in tanks containing perspex linings. Each tank contained two such liners which had holes drilled in the bottom such that when they were lifted the water drained away and the snails were retained within. This facilitated rapid removal. The snails were transferred to glass beakers containing clean guppy water and placed in a 30°C water bath under strong illumination.

The cercarial suspension shed from infected snails was separated from the snails and the majority of snail faeces by straining through a plastic tea strainer. An estimate of the cercarial concentration was made by counting the cercariae in five samples of 0.1 or 0.2 mls. removed from the well agitated suspension with an automatic pipette (Finpipette, Eppendorf). The cercariae were stained with Lugol's iodine and counted under a dissecting microscope.

2.2 Experimental techniques (Schistosomes).

2.2.1 Production of cercariae of one sex.

Snails were individually infected with S. mansoni as above but with single miracidia. Of 332 snails thus infected at various times a total of 43 (13%) subsequently shed cercariae. Individual mice were exposed to the cercariae from a single infected snail and perfused 4-5 weeks later to establish the sex of cercariae. Although the proportion of snails shedding male or female cercariae varied from batch to batch, an exactly balanced sex ratio was found overall.

2.2.2 Irradiation of cercariae.

Suspensions of cercariae were concentrated using a Millipore filter apparatus (pore size 8 µm). Cercariae introduced into the upper part of this apparatus migrate to the surface under the influence of an artificial light. Contaminating matter such as snail faeces was readily removed using a pasteur pipette and excess water removed by gentle suction through the filter. Cercariae were concentrated to about 1000/ml. and the suspension poured into clean Sterilin universal tubes. The tubes of cercariae were transported in a thermos glask to the National Insitute for Medical Research, Mill Hill, where they were irradiated using an A.E.C. Gammabeam 650, ^{60}Co source. The complete contents of the tube were within a 95% range of accuracy of the course. The dose rate of radiation employed was either around 2 kr./min. (for doses of 0-10 kr.) or 13 kr./min. for higher doses. The abbreviation kr. is used for kilorad (1,000 rads) throughout.

2.2.3 Transformation of cercariae into injectable 'schistosomula-like larvae'.

Collection in vitro through isolated skin.

The technique described by Stirewalt and Uy (1969) and Clegg and Smithers (1972) in which cercariae were allowed to penetrate excised mouse abdominal skin which had been shaved and scraped to remove the subcutaneous fat and blood vessels was employed. The membrane was held in a tube of 15 mm. internal diameter to which approximately 10,000 - 15,000 cercariae were applied. After 3 hours, schistosomula had collected as a pellet at the tip of the lower portion of the tube which contained Earle's medium with lactoalbumin hydrolysate (Gibco-Bio Cult). The excess medium was removed with a pipette, the pellet agitated to resuspend the larvae and aliquots removed for counting. For brevity such larvae one referred to as 'skin-somules'.

Mechanical disruption and incubation.

The procedure described by James and Taylor (1976) which incorporated that of Colley and Wikel (1974) was used. The cercarial suspension was concentrated to a minimal volume in a Millipore concentration apparatus and 50 mls. of Earles medium added. The suspension was again concentrated and the procedure repeated once. The final suspension in about 5 mls. of medium was transferred to a Sterilin universal tube. Separation of cercarial heads from tails was effected by 14 passages (both ways) of this suspension through a 21g needle attached to a 10 ml. syringe. The volume was then increased to 25 mls. with medium containing 100 units/ml. of penicillin and 100 mg/ml. of streptomycin and then incubated for 40 minutes at 30°C. After incubation the tube was

gently shaken and allowed to stand for 5 minutes to sediment the organisms. The supernatant containing actively swimming tails was removed down to about 1 ml. The organisms were examined and if contaminated with many cercarial tails, the resuspension and sedimentation was repeated. For brevity these larvae one referred to as 'somules'.

2.3 Mammalian host used.

Male T.O.("Tyler's Original") albino mice aged 6-8 weeks, weighing 20-25 gms. were used for most of the experiments. This strain was originally sent to the National Institute for Medical Research, Mill Hill, from the Tyler Institute, Copenhagen, in 1953. It has subsequently been kept by several animal suppliers, from three of which mice were obtained: Animal Suppliers Ltd. (Woolmer Green, Herts.), Bantin and Kingman (Aldbrough) and A. Tuck and Sons Ltd., (Battlesbridge, Essex). Over the years, stocks have been interchanged between these breeders and thus a direct lineage to the original stock cannot be traced.

Two inbred syngeneic strains of CBA mice were used: CBA/Lac and CBA/H-T6T6. Both of these are H-2^K and neither rejects skin grafts from each other (E. Leuchars - personal communication). These mice were either bred on site, or obtained from the Chester Beatty Research Institute, London, or from Bantin and Kingman, Aldbrough. Both males and females were used. Doenhoff (personal communication) has found no significant differences between males and females or between mice from different animal suppliers with regard to the development of resistance to reinfection with S. mansoni. Furthermore, no inconsistancies

attributable to mice from different suppliers were apparent in the studies contained herein. Thus the mice are merely referred to as CBA throughout.

2.4 Experimental techniques (mice).

2.4.1 Infection of mice.

Percutaneous infection.

Infection of mice with cercariae by the percutaneous route was essentially as described by Smithers and Terry (1965b). Mice were anaesthetised by intraperitoneal injection of Nembutal (sodium pentobarbitone 60 mg/ml.) diluted 1 in 10 with saline. T.O. mice were given 7 mg. of Nembutal/100 gm. body weight and CBA mice 6 mg/100 gm. having proved to be more susceptible to the anaesthetic. In primary exposures the hair was shaved from the abdomen and the mice laid on their backs in a rack. The skin was moistened with guppy water and a nickel plated ring placed in position and secured with a strip of sellotape. The rings measured 1.3 cms. internal diameter, 2 cms. in height and held 1.2 mls. of fluid.

The required volume of cercarial suspension was administered with an automatic pipette through a hole cut in the sellotape covering the rings. After 20 mins., the suspension was removed and checked to ensure that the vast majority of cercariae had penetrated. Any subsequent infections, e.g. challenge exposures, were given on a different site, invariably on the flank.

Infection of mice with 'schistosomula'.

Estimates of the concentration of larvae were made by counting five 20 µl. aliquots. The concentration was then adjusted such that the required number of organisms were contained in 0.1 ml. This required volume was drawn from an agitated suspension into a 1 ml. syringe fitted with a 25g x $\frac{5}{8}$" needle. The syringe was held vertically downwards until injection was effected so that the larvae would settle just above the needle and not stick to the sides of the syringe. Intramuscular injections were given into the hamstring muscle in the hind leg. Intradermal injection was performed, 0.05 mls. at a time, into the skin on the shaved dorsal surface. Subcutaneous injection was beneath the loose skin at the back of the neck.

2.4.2. Perfusion of mice.

Worms were recovered from the liver and mesenteries of infected mice in a similar manner to that described by Smithers and Terry (1965b). Mice were killed by an intraperitoneal injection of 0.2 - 0.5 mls. of heparinized Nembutal (25 units/ml). The abdominal and thoracic cavities were opened and the mouse attached by spring clips to a vertical board of Perspex. The liver was allowed to hang into a filter funnel leading into a plastic Universal bottle (Sterilin) (See plate 1). With the intestines held out of the way, the hepatic portal vein was cut. The perfusion fluid was heparinized citrated saline (0.85% sodium chloride, 1.5% sodium citrate, 2 units/ml. heparin); 0.1% methiolate was used to prevent fungal growth. This fluid was injected into the left ventricle with a 50 ml. syringe and 18g needle. During this delivery, the guts and liver were gently massaged with the fingers, perfusion being continued until the gut veins and liver were free of blood. The needle was withdrawn and the guts and liver washed down to remove any attached worms.

Plate 1. The perfusion technique.

The funnel was removed and checked for adhering worms and the tube allowed to stand for about 5 mins. during which the worms settled, any remaining on the meniscus being pushed under with a needle. After this time, all but 1-2 mls. of the perfusate was carefully removed by means of a pasteur pipette attached to a suction pump. The erythrocytes were lysed with a few drops of 2.5% aqueous saponin, the tube gently shaken and the fluid plus worms poured into a squared plastic petri dish (Sterilin) or, where small worms were present, into a 1 ml. Sedgewick-Rafter chamber for counting. The tube was washed out with a small volume of perfusion fluid and then checked for the presence of any remaining worms under the dissecting microscope.

Collection of worms and perfusate in this manner was first introduced, to replace collection on a gauze, when small irradiated parasites were collected. It was subsequently adopted for routine perfusion of adult worms, proving both quick and efficient. The use of a syringe allows more sensitive control over the flow of perfusion fluid than could be achieved with a peristaltic pump. Worms flushed out of the liver with perfusion fluid passed directly over the liver into the funnel and did not become trapped in folds of mesentery as was found to happen when the guts were allowed to just hang down. Squashing of the liver between glass plates and searching through the mesenteries to check for retained worms was found to be necessary only when it was apparent that the organs had not been fully perfused as occasionally happened if a clot formed.

2.4.3. Recovery of schistosomula from the lungs of infected mice. ("lung chop").

The lung recovery assay was adapted from the procedures described by Clegg (1965) and Sher et al. (1974). The mice were killed with Nembutal containing 25 units/ml of heparin and the body cavity opened to expose the heart and lungs. The lungs were perfused of blood by cutting open the left ventricle of the heart and injecting 10 mls. of Hanks balanced salt solution (HBSS) containing 100 units/ml of penicillin and 100 mg/ml of streptomycin into the right ventricle. Perfusion was much improved by cutting open the left ventricle and subsequent addition of haemolytic gelatin Gey's as described by Sher et al. (1974) was found to be unnecessary.

The lungs were removed, washed in HBSS, finely chopped with scissors, and incubated in 10 mls. of HBSS with penicillin and streptomycin in sterile universal tubes in a 37°C water bath for 3 hours. The contents of each bottle was then filtered through a steel gauze (36 mesh/inch) rinsed twice, and the filtrate centrifuged for 3 mins. at 1,000 r.p.m. All but 1 ml. of the supernatant was removed by suction and the shaken centrifugate transferred to a Sedgewick-Rafter chamber for counting on an inverted microscope at x40 manification. The tube was washed out and the washings added to the chamber.

2.4.4. Recovery of worms from the portal system and lungs of the same animals.

This was essentially a direct tie up of the above two techniques. Worms from the portal system were perfused first, using HBSS, gentle pressure being applied to the syringe so that the lungs did not inflate. The lung recovery was then performed exactly as above.

2.4.5 Tissue egg counts.

The tissues for digestion were stored at -20°C until required. The gut contents were removed by slitting the gut with scissors and gently scraping away the contents with a spatula. Whole guts or liver were digested in 20 mls. of 5% KOH solution at 37°C for 16 hours in plastic Universal bottles (Cheever, 1968b). Generally, 50 or 100 µl. aliquots were placed on microscope slides, covered with a coverslip and examined at 32x magnification. When extremely low egg densities were encountered, volumes of up to 1 ml. were counted in a Sedgewick-Rafter chamber or the whole universal spun for 5 mins. at 1,000 r.p.m., all but 1 ml. of the fluid removed and the remaining 1 ml. was counted in a Sedgewick-Rafter chamber. Three such counts were performed, the total volume measured using a measuring cylinder and the values for eggs/organ calculated.

2.4.6. Histological techniques.

All tissue specimens collected for histology were fixed in 10% formol saline, dehydrated and embedded in paraffin wax. Sections were cut at 4-6 microns and stained routinely with haemotoxylin and eosin.

2.5 Experimental Design and Statistical Analysis.

In the studies on the development of resistance, mice were given an initial infection with either unirradiated cercariae (called the 'Primary (1^{o})' infection) or with irradiated cercariae (called the 'Vaccination'). Mice which only received these initial infections are referred to as '1^{o} controls' or 'Vaccine controls'. Other groups, initially infected in exactly the same way were subsequently challenged with relatively large numbers of cercariae. These are referred to as '1^{o} + Chall.' or 'Vacc. + Chall.' groups. Age and sex matched mice were also challenged at this time and called 'Chall. Controls.' In order to establish the mean number of challenge-derived worms that had established in the initially infected and challenged mice it was necessary to calculate:

mean number of worms in (1^{o} + Chall.) or (Vacc. + Chall.) -
mean number of worms in (1^{o} Control) or (Vacc. Control).

This calculation was considered valid as the standard deviation of the means for initially infected controls were invariably considerably lower than for the initially infected and challenged groups (Sher et al., 1974).

Resistance to challenge of initially infected mice was calculated from the mean worm burdens of the respective groups thus:

$$100 \times \frac{\bar{x}\ \text{Chall. Control} - \left[\bar{x}\ (1^{o} + \text{Chall.}) \text{ or } (\text{Vacc.} + \text{Chall}) - \bar{x}(1^{o}\ \text{Control}) \text{ or } (\text{Vacc. Control})\right]}{\bar{x}\ \text{Chall. Control}}$$

Egg count data was handled in exactly the same fashion.

Statistics were performed with the aid of a programmable HP-25 electronic calculator (Hewlett-Packard, Cupertino, California, U.S.A.). The Student 't' test was used to determine the significance of both worm and egg count data which in the vast majority of experiments followed a normal distribution. As the standard deviations of the worm counts from initially infected control groups were considerably smaller than those from the corresponding initially infected and challenged mice, these latter standard deviations were used together with the calculated means for challenge derived worm burdens in the analysis of significance. P values of <0.05 were taken as significant.

CHAPTER 3. OBSERVATIONS ON THE INDUCTION OF RESISTANCE IN THE MOUSE USING BISEXUAL INFECTIONS WITH UNATTENUATED PARASITES.

3.1 Introduction.

As a baseline for the studies on resistance following infection with radiation attenuated parasites (Chapters 6 and 7), it was considered advisable to establish that resistance could be developed following unattenuated bisexual infection using the particular host/parasite strains employed in these studies (viz. the T.O. and C B A strains of mice and the laboratory-passaged, Puerto Rican strain of S. mansoni and the Nelspruit strain of S. mattheei). This was particularly important in view of the reported failures of certain workers to demonstrate resistance to reinfection in the mouse (Thompson, 1954; Perlowagora-Szumlewicz, 1964a; Gold and Lengy, 1975). Studies performed in collaboration with Dr. M.J. Doenhoff on the development of resistance to reinfection with S. mansoni in the C B A mouse are included as Appendix 1 in the form of the pre-print of a paper to be published in the Journal of Helminthology. The experiments presented in this chapter were performed using the T.O. mouse strain.

The experiment to demonstrate resistance to reinfection with S. mansoni (section 3.2) involved an investigation of the influence of the size of the primary exposure on the degree of resistance stimulated. Also included in this experiment was a comparison of the efficacy of percutaneously applied cercariae and intramuscularly injected skin transformed somules or mechanically transformed somules in inducing resistance.

The subsequent finding that the T.O. 'strains' maintained by different animal suppliers respond differently with regard to the development of resistance against S. mansoni is presented in section 3.3.

In section 3.4 resistance to reinfection with S. mattheei was investigated as this had not previously been demonstrated. It was also of interest to determine whether heterologous infection with S. mattheei could induce a higher level of resistance to S. mansoni than homologous primary infection.

3.2 Homologous resistance following S. mansoni infection.

It has been reported that mice infected with 50-75 S. mansoni cercariae can partially resist a homologous challenge infection given 6-8 weeks later as assessed by recovery of adult worms at perfusion 6-8½ weeks post challenge (Stirewalt, 1953; Hunter et al., 1962; Hunter et al., 1967a). It has not been reported that a minimum number of worms are required in order to stimulate resistance and Sher et al. (1974) using the lung recovery assay found no correlation between the degree of resistance 12 weeks after a primary exposure and the primary worm burden. In experiment 1 primary infections with 20-80 larvae were investigated and resistance assessed at portal perfusion 5 weeks post challenge.

Colley and Wikel (1974) described the preparation of S. mansoni somules by mechanical disruption of cercarial heads from their tails followed by incubation in medium. Up to 24 hrs. of incubation such larvae still possessed stainable acetabular gland products. The surface tegument of similar mechanically transformed somules remains largely characteristically cercarial up to 30 mins. incubation (Brink et al., 1977), whereas schistosomula prepared by allowing penetration of isolated rat or mouse skin followed by incubation for 3 hrs. would have largely evacuated their gland contents (Stirewalt and Kruidenier, 1961) and developed a heptalaminate membrane (Hockley and McLaren, 1973). The possibility that products secreted during the in vivo transformation

of cercariae into schistosomula (Stirewalt, 1974) played a role in the induction of resistance to reinfection in the mouse was investigated below by establishing primary infections by intramuscular injection of the above two types of artificially prepared somules.

3.2.1 Experiment 1.

Groups of T.O. (A.S.L.) mice were infected with 20, 40, 60 or 80 S. mansoni larvae as shown in Table 1. 8 weeks later half of the mice in each group together with 10 uninfected controls were challenged with 100 cercariae each. The remainder of the infected mice served as controls for the primary infection. All the mice were perfused 5 weeks later.

The worm recoveries are shown in Table 1. Primary exposures to 20-80 cercariae resulted in mean worm burdens of 6.4 ± 3.0 - 18.9 ± 6.0. Administration of 80 larvae as percutaneously applied cercariae, intramuscularly injected somules or skin somules resulted in primary worm burdens of 18.9 ± 6.0, 13.0 ± 4.3 and 18.4 ± 7.4 respectively, between which there were no significant differences. In all of the superinfected groups, the calculated challenge-derived worm burden was significantly lower than that in the challenge control group. There were no significant differences between the challenge derived worm burdens in any of the superinfected groups.

These results showed that the T.O. (A.S.L.) mice could develop resistance to reinfection assessed by challenge 8 weeks after primary exposure and subsequent recovery of adult worms. The degree of resistance was independant of the size of the primary worm burden (in the range 6.4 - 18.9 worms) and also comparable whether the infection had been administered as percutaneously applied cercariae or intramuscularly injected somules that either possessed or had largely evacuated their acetabular gland contents.

TABLE 1. Experiment 1.

Worm recoveries from mice infected with 20-80 cercariae or somules of S. mansoni, challenged with 100 cercariae 8 weeks later, and perfused 5 weeks post challenge.

Group	Number of mice per group	Number of larvae in primary infection	Mean worm recovery (S.D.[3]) ♂	♀	⚥	Mean worm recovery attributable to challenge	Percent Reduction	Significance (P.)
1. 1° Control	9	20	3.7(1.9)	2.7(1.3)	6.4(3.0)			
2. 1° + Chall.	10	20	11.3(3.6)	10.1(3.5)	21.4(6.7)	15.0	51	<0.001
3. 1° Control	10	40	7.9(2.8)	5.8(2.8)	13.7(4.0)			
4. 1° + Chall.	10	40	16.1(5.7)	12.9(4.0)	29.0(5.1)	15.3	50	<0.001
5. 1° Control	8	60	10.7(3.6)	6.8(2.6)	17.5(4.8)			
6. 1° + Chall.	9	60	17.3(5.8)	12.9(5.0)	30.2(9.7)	12.7	59	<0.001
7. 1° Control	8	80	10.6(3.2)	8.3(3.1)	18.9(6.0)			
8. 1° + Chall	8	80	16.1(5.4)	13.7(3.4)	29.8(8.6)	10.9	65	<0.001
9. 1° Control	10	80[1]	7.3(2.4)	5.7(2.2)	13.0(4.3)			
10. 1° + Chall	11	80[1]	13.3(6.8)	9.3(3.8)	22.6(9.5)	9.6	69	<0.001
11. 1° Control	8	80[2]	11.0(5.4)	7.4(3.7)	18.4(7.4)			
12. 1° + Chall	9	80[2]	20.5(5.8)	15.4(4.7)	33.9(8.8)	15.5	50	<0.01
13. Chall. Co.	9	-	17.3(6.2)	13.6(3.9)	30.9(8.4)	30.9		

Notes - 1. I.M. - injected somules.
2. I.M. - injected skin-somules.
3. S.D. - Standard deviation of the mean.

3.3 Comparison of mouse 'strains'.

Major inconsistencies were noticed when T.O. mice from different suppliers were used in experiments like that discussed above. It was thus decided to compare mice from the 3 suppliers that had been used (Animal Supplies Ltd. (A.S.L.), Bantin and Kingman (B.K.), and Tuck). Differences in susceptibility to S. mansoni infection have been reported between mouse strains (Stirewalt et al., 1965). Marked differences have also been reported in the ability of 2 strains of hamster to resist reinfection (Smith and Clegg, 1976).

3.3.1 Experiment 2.

Mice from each of the 3 suppliers were infected with 35 cercariae and challenged 12 weeks later with 200 cercariae. The animals were perfused 24 days later. Perfusion at this time allows distinction of the primary from the challenge infection worms, thus obviating the need for controls for the primary worm burden. Early perfusion has been employed to assay resistance in the mouse by several workers (Kagan, 1952; Olivier and Schneiderman, 1953; Sadun and Lin, 1959) and more recently by Doenhoff et al. (in preparation - see appendix 1). This appendix, comprising collaborative work on resistance to reinfection in the C B A mouse describes the rationale for the design of experiments 2 and 3.

The worm count data is presented in Table 2. The mean numbers of primary infection worms in the 3 superinfected groups were not significantly different. Similarly, the means of the number of worms in the 3 challenge control groups were not significantly different, and therefore statistical comparison of the challenge derived worm burdens in the superinfected groups was valid. Significant resistance was apparent

TABLE 2. Experiment 2.

Worm recoveries from 3 'strains' of T.O. mice infected with 35 cercariae of S. mansoni, challenged 12 weeks later with 200 cercariae and perfused 24 days post challenge.

Mouse strain	Group	Number of mice per group infected / perfused	Mean worm recovery (S.D.) Primary ♂	Primary ♀	Primary ⚥	Challenge[1]	Percent Reduction	Significance (P.)
A.S.L.	1° + Chall	30/14	8.1(3.6)	5.6(3.0)	13.7(5.6)	21.9(17.4)	58	<0.001
	Chall. Co.	15/15				51.6(17.0)		
B.K.	1° + Chall.	30/17	8.2(4.3)	5.0(2.1)	13.2(5.8)	33.9(11.9)	37	<0.001
	Chall. Co.	21/20				52.9(16.0)		
Tuck	1° + Chall.	15/15	7.2(2.6)	5.9(2.3)	13.1(4.5)	51.5(23.8)	-12	>0.4
	Chall. Co.	15/15				47.9(10.9)		

Note 1. - The sex of the 24-day old challenge-derived worms could not be distinguished.

in the A.S.L. and B.K. 'strains' but there was no evidence of resistance in the Tuck 'strain'. Furthermore there were significantly fewer juvenile worms in the A.S.L. mice than in either the B.K. mice ($t=2.27$, $P<0.05$) or the Tuck mice ($t=3.8$, $P<0.001$) and significantly fewer in the B.K. mice than in the Tuck mice ($t=2.76$, $P<0.01$). Marked differences in the number of mice of each 'strain' surviving to perfusion were also noted. Although the primary worm burdens in all 3 'strains' were virtually identical, deaths up to the time of perfusion were: 0/15, Tuck; 13/30, B.K.; 16/30, A.S.L.

The above findings indicated marked differences in the response of the three strains. The A.S.L. mice developed marked resistance to reinfection and the Tuck 'strain' none, with the B.K. 'strain' intermediate.

3.3.2 Experiment 3.

This experiment was designed to confirm the above conclusions. The experimental design was identical to that described above except for the fact that challenge was performed 9 weeks after infection and the mice were perfused 21 days post challenge. It had been intended to allow a longer interval between challenge and perfusion (5 weeks) and for this purpose groups of controls for the primary infection alone were included and the mice were challenged three weeks earlier than in experiment 2. However, mice started dying at a faster rate than would have been expected from the mortality rate observed in experiment 2 and thus the perfusion was brought forward.

The worm recoveries are presented in Table 3. There were no significant differences between the primary worm burdens in any of the groups though there were significantly fewer worms in the B.K. challenge

TABLE 3. Experiment 3.

Worm recoveries from 3 'strains' of T.O. mice infected with 35 cercariae of S. mansoni, challenged 9 weeks later with 200 cercariae and perfused 21 days post challenge.

Mouse strain	Group	Number of mice per group infected / perfused	Mean worm recovery (S.D.) Primary ♂	Primary ♀	Primary ⚥	Challenge	Percent Reduction	Significance (P.)
A.S.L.	1° + Chall.	22/17	8.3(3.3)	7.2(2.6)	15.4(4.6)	19.1(12.5)	66	<0.001
	1° Co	18/14	7.3(4.3)	6.8(3.3)	14.2(6.1)			
	Chall. Co.	10/10				55.5(12.6)		
B.K.	1° + Chall.	22/15	8.7(2.6)	6.5(2.9)	15.3(4.3)	24.2(13.0)	50	<0.001
	1° Co.	18/8	8.4(2.1)	6.0(2.2)	14.4(3.5)			
	Chall. Co.	10/10				48.0(15.7)		
Tuck	1° + Chall.	20/20	7.0(2.8)	7.1(2.8)	14.1(4.6)	50.0(23.7)	26	<0.05
	1° Co.	20/17	6.8(2.5)	7.9(3.2)	14.7(5.0)			
	Chall. Co.	13/13				67.9(19.6)		

controls than in the Tuck controls ($P < 0.01$) thus precluding a direct statistical comparison. Although all of the strains showed significant resistance (A.S.L., $P < 0.001$; B.K., $P < 0.001$; Tuck, $P < 0.05$), it is evident that this was most marked in the A.S.L. mice and least in the Tuck mice, with B.K. mice again intermediate. Differences in mortality of the three strains were again observed (3/40, Tuck; 17/40, B.K., 9/40, A.S.L.).

The B.K. strain of T.O. mice is monitored regularly by the Genetic Monitoring System run by Dr. M.F.W. Festing of the Laboratory Animal Centre, Carshalton. Festing (1972) was able to distinguish the various inbred strains of mice by measuring their mandibles and has applied this parameter as a sensitive phenotypic indicator of genetic drift in mouse colonies. He kindly examined mice from the 3 'strains' used in experiment 3, and reported that while the A.S.L. and B.K. 'strains' were indistinguishable, the Tuck mice were obviously different. This finding shows that genetic drift had occurred during the maintenance of the Tuck strain, a drift which seemingly resulted in a reduced ability to develop resistance to reinfection with S. mansoni.

3.4 Homologous and heterologous resistance following S. mattheei infection.

In this section comparative observations were made on the ability of T.O. mice to develop resistance to reinfection with S. mattheei and to develop 'heterologous' resistance to S. mansoni after prior infection with S. mattheei. The ability of mice to develop resistance to reinfection with S. mattheei has not previously been studied but it is known that previous infection of mice with S. mattheei confers partial

'heterologous' resistance against challenge with S. mansoni (Amin and Nelson, 1969). Such heterologous resistance has been demonstrated in several host/schistosome systems (reviewed by Eveland, 1969) and it has been suggested that, in mice, such resistance may be more marked than in homologous systems (Sadun et al., 1961; Nelson et al., 1968). As a preliminary to testing the protective effect of heterologous irradiated infections with S. mattheei and S. mansoni, a repeat of the above work by Amin and Nelson (1969) was incorporated into the experiment described below.

3.4.1 Experiment 4.

62 T.O. (A.S.L.) mice were exposed to 30 S. mattheei cercariae and separated into 3 groups. 9 weeks later, mice in one group were challenged with 100 S. mattheei cercariae, mice in another group with 100 S. mansoni cercariae and mice in the third group were left unchallenged serving as controls for the primary infection. Owing to a technical failure almost half of the mice in the second of these groups had to be removed from the experiment. Appropriate challenge controls were included at this time. 7 weeks later all of the mice were perfused. The adult worms recovered from the mixed S. mattheei and S. mansoni infections were stained with aceto-alum carmine for species identification.

The worm recoveries are presented in Table 4. Significant partial resistance was demonstrated in both homologous and heterologous infections. The degree of heterologous protection against S. mansoni (69%) is consistent with that demonstrated under virtually identical experimental conditions by Amin and Nelson (1969). In their study, however, they demonstrated a reciprocal effect on the S. mattheei

TABLE 4. Experiment 4.

Worm recoveries from mice infected with 30 cercariae of S. mattheei, challenged 9 weeks later with 100 S. matheei or S. mansoni cercariae, and perfused 7 weeks post-challenge.

Group	Number of mice per group infected / Perfused	Mean worm recovery (S.D.) S. mattheei ♂	S. mattheei ♀	S. mattheei ⚥	S. mansoni ♂	S. mansoni ♀	S. mansoni ⚥	Percent Reduction	Significance (P.)
1. 1° Control	21/17	7.5(2.7)	6.1(2.5)	13.6(3.5)					
2. 1° + Chall. (S.matt.)	21/15	10.6(5.2)	7.5(2.6)	18.1(7.1)				82	<0.001
3. 1° + Chall. (S.mans.)	11/8	6.6(2.9)	5.8(1.9)	12.4(4.7)	3.8(1.3)	3.9(2.2)	7.6(3.2)	69	<0.001
4. Chall. Co. (S.matt.)	14/14	16.9(4.5)	8.1(4.6)	25.0(6.7)					
5. Chall. Co. (S. mans.)	8/8				12.4(5.2)	12.9(6.0)	25.3(8.0)		

primary infection in that there were 43% fewer S. mattheei worms in the superinfected mice than in the primary infection controls. No such effect was evident in the present experiment, the mean numbers of S. mattheei worms in the primary infection controls being 13.6 ± 3.5 and in the primary infection plus S. mansoni challenge, 12.4 ± 4.7.

3.5 Discussion.

Several experimental host species have been shown to develop resistance to reinfection with a variety of schistosome species (reviewed by Stirewalt, 1953; Smithers and Terry, 1969a). With specific reference to the mouse/S. mansoni system, Table A in Appendix 2 records that statistically significant resistance against homologous challenge has been demonstrated in 39 out of 54 experiments, and there is general agreement that resistance can be developed by 6-8 weeks following a primary exposure. It was demonstrated above that the T.O. (A.S.L.) mouse can manifest partial resistance to reinfection 8 weeks after a primary infection. The degree of partial resistance was, however, unaffected by the size of the primary worm burden which covered a range of 6-19 worms. A minimum threshold in the numbers of primary infection worms required to stimulate significant protection was, therefore, not apparent. Whilst it is possible that primary worm burdens of less than 6 worms would have failed to stimulate resistance, both Sher et al. (1974) and Mahmoud et al. (1975) have reported high partial resistance in mice harbouring primary bisexual infections of 4-5 worms. In these two studies, however, the mice were challenged 12-15 or 32 weeks after primary exposure. The only indication that the size of the primary worm burden can influence the development of resistance in the mouse comes from the work of Amin and Nelson (1969) who

reported significant resistance to heterologous challenge with S. mansoni in mice harbouring 3 S. mattheei worm pairs but not in mice harbouring 1-2 worm pairs. In this latter study mice were challanged 9 weeks after primary exposure. The lack of correlation of primary worm burden with the degree of resistance observed in the present experiment is consistent with the findings of Sher et al. (1974) and of Olivier and Schneidermann (1953) who also studied S. mansoni and with those of Sadun and Lin (1959) studying S. japonicum. However, in both the rat (Knopf et al., 1977) and the rhesus monkey (Smithers and Terry, 1967a) it has been shown that the size of the primary worm burden can influence the degree of resistance.

It has been shown that the induction of resistance in mice is not dependent upon penetration of skin by the cercariae of the primary infection (Frick et al., 1965; Murrell et al., 1975; Mahmoud et al., 1975a) as in these studies cercariae were injected either intraperitoneally or subcutaneously. The results of experiment 1 show that primary infections established by the intramuscular route are also effective. The comparable resistance of mice infected with cercariae, transformed somules that still possessed a trilaminate cercarial membrane and acetabular gland contents or skin transformed somules that had developed a heptalaminate membrane and largely evacuated their acetabular gland contents indicates that superinfection resistance in the mouse is not attributable to a response associated with the transformation of cercariae into schistosomula. This is supported by the failure to protect mice by immunization with cercarial secretion products although such immunization induced a variety of antibody responses (Minard et al., 1977).

Marked differences were observed between the T.O. mice obtained from 3 different animal breeders. Whilst all three 'strains' were equally susceptible to infection, the Tuck 'strain' mice were least susceptible to the lethal consequences of infection in both of the experiments performed. The A.S.L. 'strain' developed the highest degree of resistance to reinfection and the Tuck 'strain' the least, with the B.K. 'strain' intermediate. Although Smith and Clegg (1976) reported differences with regard to the development of resistance to reinfection with S. mansoni in two strains of hamster, a direct comparison of 6 different inbred strains of mice by Sher et al. (1974) revealed no obvious differences. However, the failure by certain workers to demonstrate resistance, notably Thompson (1954), Perlowagora-Szumlewecz (1964a) and Gold and Lengy (1975) may be explicable by postulating that they were using mice that, in the course of the inevitable inbreeding that would occur during the maintenance of a colony of outbred mice, had developed an 'unresponsiveness' similar to that evidenced in the Tuck 'strain'. It is perhaps relevant that in the three studies cited above, as in the present study, the mice used were Swiss albino mice. Whilst it is conceivable that differing environmental conditions obtaining during the rearing of the different 'strains' of mice used in the present study could in some way have resulted in phenotypic differences affecting the response to schistosomes, this seems an unlikely explanation particularly in view of the evidence for genetically controlled differences reported by Dr. M.F.W. Festing.

There are several reports in the literature of genetically determined host differences in susceptibility and development of

resistance to parasitic infections. Many of these are cited in a paper by Wakelin (1975) in which he was able to identify responder and non-responder mice, with regard to the immune expulsion of the nematode Trichuris muris, amongst populations of the random bred Schofield strain of mouse. It has been suggested that such responsiveness/unresponsiveness to parasitic infections may be associated with the presence/absence of such specific immune response (I.R.) genes directly controlling immune responses as have been reported by McDevitt and Benaceraff, 1969.

It is premature to hypothesize further about the 'strain' differences reported herein. However, if the results could be reproduced with animals bred on site under the same conditions and the differences shown to be genetically controlled a number of interesting experiments could be performed. Of interest would be comparative analysis of general immune responses to non-schistosome antigens and particularly of schistosome specific responses possibly involved in the development of resistance and/or the pathology associated with infection.

Dargie et al. (1977) have shown that sheep can develop partial resistance to reinfection with S. mattheei, but a greater degree of resistance has been produced by vaccination with radiation-attenuated infections (Taylor et al., 1976b). Thus the above demonstration that mice develop a strong partial resistance to reinfection with S. mattheei indicated that the mouse might prove a useful 'model' in extending the development of a live attenuated vaccine against S.

mattheei (see Chapter 7). The demonstration of heterologous protection afforded by S. mattheei against S. mansoni confirmed the results of Amin and Nelson (1969). However, the degree of protection afforded was comparable with that produced by homologous superinfection in experiment 1, indicating that, in terms of resistance to reinfection, there is no advantage in using heterologous rather than homologous infections to produce resistance to reinfection in the mouse, although there are obvious advantages in other models where the heterologous immunizing infection fails to develop and is thus non-pathogenic e.g. S. rodhaini and S. bovis in the baboon (Taylor et al., 1973).

CHAPTER 4. STUDIES ON THE DEVELOPMENT OF RESISTANCE FOLLOWING SINGLE SEX INFECTION.

4.1 Introduction.

As a consequence of their studies of S. mansoni in rhesus monkeys, Smithers and Terry (1969b) concluded that it is the adult worms, as distinct from the migrating stages or the eggs, that are responsible for the stimulation of resistance to reinfection. Particularly relevant to this conclusion was the demonstration that rhesus monkeys develop marked resistance following infection with worms of one sex (Smithers, 1962; Hsü, 1969) and also following direct transfer of worms of one sex into the mesenteric veins of uninfected recipients (Smithers, 1968). If resistance to reinfection in the mouse is also stimulated solely by the adult worms, then mice infected with worms of one sex would provide the ideal system in which to study the putative immunological basis of resistance. Such mice would be exposed to fewer antigens than if they harboured egg producing populations of both male and female worms and consequently the immune responses elicited would be more restricted and yet include those responsible for the mediation of resistance. Furthermore, the problem of host mortality consequent upon egg deposition would be eliminated. The development of resistance against S. mansoni by mice harbouring single sex infections has not been extensively studied. Olivier and Schneidermann (1953) reported low grade (25-28%) protection 16 weeks after infection of mice with male worms. This level of resistance was considerably weaker than that reported by these authors in mice with bisexual primary infections although direct comparisons were not made. Sher et al. (1974) assaying resistance by the lung chop technique reported comparable protection (50-60%) in mice with male or mixed primary infections of 12 weeks

duration. The experiments described here investigate whether single sex infections stimulate comparable resistance to that demonstrated in the acute stage of bisexual infections in mice (Doenhoff et al. - see Appendix 1).

4.2 Comparisons of resistance following male, female or mixed infections of 8 weeks duration.

4.2.1 Experiment 5.

20 CBA mice were infected with 50 male cercariae, 20 with 50 female cercariae and 10 with 30 cercariae of mixed sexes. Half of the mice infected with cercariae of one sex and all of those infected with mixed cercariae were challenged 53 days later with 200 cercariae each, together with 10 challenge controls. The mice were perfused 25 days post challenge. The unchallenged single sex infected mice served as vaccine controls for the primary infection as it was uncertain whether the unmatched males or females would be readily distinguishable from the 25 day old challenge derived worms.

The worm recoveries are presented in Table 5. It transpired that the primary infection worms of both sexes could be distinguished from the juvenile, challenge derived worms. No significant differences were recorded between the mean numbers of single sex male or female primary infection worms in the primary infection controls and the super-infected groups. Thus the challenge infection had not affected the primary worm burdens. The mice harbouring a bisexual infection showed marked resistance to challenge (66%), the difference between the challenge derived worm burdens in these and the challenge controls being significant ($P < 0.001$). The group of mice harbouring a mean of 12.0

Table 5. Experiment 5.

Worm recoveries from mice infected with male, female or male and female cercariae, challenged 8 weeks later with 200 cercariae and perfused 25 days post challenge

Group	Number of mice per group	Number of cercariae in primary infection (sex)	Mean worm recovery (S.D.) Primary ♂	Primary ♀	Primary ♂+♀	Challenge	Percent reduction	Significance (P)
1. 1° Control	10	50(♂)	14.9(7.1)	-	14.9(7.1)	-	24	<0.05
2. 1° + Chall.	10	50(♂)	12.0(4.3)	-	12.0(4.3)	49.3(14.1)		
3. 1° Control	10	50(♀)	-	10.7(7.1)	10.7(7.1)	-	15	>0.1
4. 1° + Chall.	9	50(♀)	-	11.7(4.8)	11.7(4.8)	55.4(16.8)		
5. 1° + Chall.	8	30(♂+♀)	3.4(1.4)	4.4(2.1)	7.8(2.8)	21.8(13.8)	66	<0.001
6. Chall. Co.	10	-	-	-	-	65.0(13.5)		

male worms showed marginally significant resistance (24%, P <0.05). The mice infected with female cercariae were not significantly resistant. The mean number of challenge worms in the previously bisexually infected mice was significantly lower (P <0.001) than the mean numbers in the mice infected with male or female worms, the mean recoveries from which were not significantly different (P = 0.4).

Experiment 6 was essentially a repeat of the above.

4.2.2. Experiment 6.

The design, outlined in Table 6, is virtually identical to that of experiment 5, except that controls for the primary infection were dispensed with and perfusion was performed at 24 days post challenge.

The results are presented in Table 6. The mean primary worm burdens were virtually identical in all three groups (♂-11.6 ± 4.6, ♀-11.0 ± 7.9, ♂+♀-11.9 ± 5.1). However, whereas the mice harbouring a patent bisexual infection were significantly resistance to challenge (75%, P <0.001), no significant resistance was apparent in the mice harbouring an all male infection (20%, P >0.05) and low grade, though significant resistance was apparent in the mice preinfected with female worms (24%, P <0.01). The mean number of challenge derived organisms in the bisexually infected mice was significantly lower than that from the mice infected with male worms (P <0.001) or female worms (P <0.001), the difference between these latter two means not being significantly different (P >0.6).

The results of the two above experiments indicate that the resistance apparent 8 weeks after primary infection with relatively small numbers of cercariae is dependant upon the presence of a mature

Table 6. Experiment 6.

Worm recoveries from mice infected with male, female or male and female cercariae, challenged 8 weeks later with 200 cercariae and perfused 24 days post challenge

Group	Number of mice per group	Number of cercariae in primary infection (sex)	Mean worm recovery (S.D.) Primary ♂	Primary ♀	Primary ♂+♀	Challenge	Percent reduction	Significance (P)
1. 1° + Chall.	10	50(♂)	11.6(4.6)	-	11.6(4.6)	35.7(11.0)	20	>0.05
2. 1° + Chall.	10	50(♀)	-	11.0(7.9)	11.0(7.9)	34.1(6.4)	24	<0.01
3. 1° + Chall.	10	17(♂) + 17(♀)	5.8(3.3)	6.1(2.3)	11.9(5.1)	11.3(13.1)	75	<0.001
4. Chall. Co.	9	-	-	-	-	44.8(8.2)	-	-

infection. The possibility that large numbers of worms of one sex could stimulate comparable resistance is investigated below.

4.2.3 Experiment 7.

Groups of CBA mice were infected with 50, 150 or 300 male or 18 male plus 18 female cercariae. 8 weeks later these mice, together with a group of challenge controls, were challenged with 200 cercariae each. All the mice were perfused 21 days post challenge.

The results are presented in Table 7. Mice in the group harbouring a mean of 20.4 $\pm$ 9.5 male worms were not significantly resistant (7%, $P > 0.4$). Marginally significant resistance was apparent in the group harbouring a mean of 81.7 $\pm$ 9.2 male worms (19%, $P < 0.05$) and a higher degree of resistance was apparent in the group harbouring a mean of 138.7 $\pm$ 52.7 male worms (34%, $P < 0.01$). However, the difference between the challenge derived worm burdens in these three groups were not significant (see Table 7). Maximum resistance was apparent in the bisexually infected group (73%, $P < 0.001$) and the mean number of challenge derived worms in this group was significantly lower than that in any of the three single-sex groups (see Table 7).

4.3 Discussion.

By infection of rhesus monkeys with worms of one sex it has been clearly demonstrated that the adult worm alone can stimulate resistance to reinfection with both S. mansoni and S. japonicum in this host (Vogel and Minning, 1953; Vogel, 1962; Smithers, 1962; Hsü, 1969). In all of these studies large numbers (1,300 - 17,000) of cercariae were employed in the primary infection. However, Smithers (1968)

Table 7. Experiment 7.

Worm recoveries from mice infected with varying numbers of male cercariae or with male and female cercariae, challenged 8 weeks later with 200 cercariae and perfused 21 days post challenge.

Group	Number of mice per group	Number of cercariae in primary infection (sex)	Mean worm recovery (S.D.) Primary ♂	Primary ♀	Primary ♂+♀	Challenge	Percent reduction	Significance (P)
1. 1° + Chall.	10	50 ♂	20.4(9.5)	-	20.4(9.5)	63.4(18.9)	7	>0.4
2. 1° + Chall.	10	150 ♂	81.7(9.2)	-	81.7(9.2)	55.5(14.0)	19	<0.05
3. 1° + Chall.	6	300 ♂	138.7(52.7)		138.7(52.7)	45.0(22.6)	34	<0.01
4. 1° + Chall.	9	18 ♂ + 18 ♀	8.1(1.5)	8.0(3.2)	16.1(3.5)	18.2(11.6)	73	<0.001
5. Chall. Co.	10	-	-	-	-	68.2(7.4)		

't'-test comparison of mean numbers of challenge derived worms

Group		Group	
1 vs. 2	>0.3	4 vs. 1	<0.001
1 vs. 3	>0.1	4 vs. 2	<0.001
2 vs. 3	>0.2	4 vs. 3	<0.01

demonstrated that transfer of 160 male or female worms directly into the mesenteric veins of rhesus monkeys conferred comparable high grade protection against challenge 14-15 weeks later as did 80 worm pairs. Almost complete resistance can be demonstrated in rhesus monkeys infected with 100 cercariae (resulting in about 40 adult worms) (Smithers and Terry, 1965a), and although it has not been demonstrated that an equal number of cercariae of one sex alone can stimulate such protection it is generally concluded that the eggs are not necessary for the stimulation of resistance in this host. It has been tacitly assumed that the same principles apply to the development of resistance in other hosts.

The development of marked resistance by mice to reinfection with several schistosome species broadly coincides with the onset of egg laying by the primary infection (S. mansoni - Stirewalt, 1953; Hunter et al., 1962; Sher et al.,1974: S. japonicum - Sadun and Lin, 1959: S. douthitti - Kagan, 1952: heterologous protection of S. mattheei against S. mansoni - Amin and Nelson, 1969). This correlation provides circumstantial evidence that eggs or at least reproductively active worms may be involved in the stimulation of resistance in the mouse. Such a correlation does not apply to all host/schistosome models. In the hamster some resistance is demonstrable 4 weeks after primary infection and by 6 weeks it is maximal (Smith et al., 1976). The same authors have also shown that resistance to reinfection with S. haematobium in the hamster develops to a maximal level by 6 weeks following primary exposure, i.e. before patency of S. haematobium in this host. Resistance to reinfection with S. mansoni in the rat reaches a maximal level 4-6 weeks after primary exposure (Smithers and Terry, 1965c; Maddison et al., 1970; Perez et al., 1974). Smithers and Terry (1965a) demonstrated partial resistance in 2 of 3 rhesus monkeys challenged 4 weeks after primary infection with S. mansoni,

although consistant high grade resistance was not demonstrable until 16 weeks.

An obvious way of analysing the requirement for a mature egg producing primary infection in order to stimulate resistance is to study single sex infections. Kagan (1952) was unable to demonstrate resistance against S. douthitti in mice infected for up to 211 days with male worms. However, resistance was developed by 61-63 days following exposure to female worms and by 35-37 days following bisexual infection. As the female S. douthitti worms lay eggs parthenogenetically in the absence of a male infection, these results were taken as indicating that the egg was involved in resistance. Olivier and Schneidermann (1953) reported low grade (25-28%) protection 16 weeks after infection of mice with male S. mansoni cercariae. This level of resistance was considerably weaker than that reported by these authors in mice with bisexual primary infection, although direct controlled comparisons were not made. Sher et al. (1974) reported comparable protection in mice with male or bisexual primary infections but observed that equivalent levels of resistance took longer to develop in the mice with single sex infections. A similar finding was reported by Lin et al. (1954) in so far as single sex infections of 3-8 months duration in mice resulted in comparable protection against homologous challenge with S. japonicum as a bisexual infection of 6 weeks duration. The results of experiments 5 and 6 reported here indicate that the resistance apparent 8 weeks after primary infection was dependant upon the presence of mature actively reproducing worms and/or influenced by the presence of eggs. It seemed possible, at this point, that the worms in single sex infections were not metabolizing and turning over their surface membrane as rapidly as the actively reproducing worms in bisexual infections and thus not providing sufficient stimulus to the

hosts' defense mechanisms. Such a theory would have been consistent with the slower development of resistance in mice harbouring single sex infections (Sher et al., 1974). Female worms in single sex infections certainly do not reach full size or maturity (Erasmus, 1973) but males develop normally in terms of size and sexual development. Experiment 7 showed some indication of greater resistance as the primary male worm burden was increased from a mean of 20.4 to 81.7 to 138.7. However, the degree of resistance in mice harbouring a mean of 138.7 male worms was less than half and significantly lower than that in mice harbouring a mean of 16.1 male and female worms. This indicated the involvement of eggs or some factor associated with reproduction in the induction of resistance in the mouse at least during the acute stage of infection.

Injection of eggs either subcutaneously or intravenously has failed to stimulate resistance in both rhesus monkeys and mice (Smithers, 1962; Smithers and Terry, 1967; Moore et al., 1963; Lichtenberg et al., 1963). Thus it appears that the possible involvement of eggs in resistance is not due to their possession of antigens responsible for stimulation of a protective immune response. Another possibility to be considered is that the eggs produce a more non-specific effect on migrating schistosomula as a consequence of the inflammatory granulomatous responses associated with eggs trapped in the liver or the lungs through which the schistosomula have to migrate. It has been reported that inflammation in the lungs induced by intravenous injection of killed bacteria can markedly reduce the worm burden in hamsters when stimulated at a time when schistosomula are migrating through the lungs (Smith et al., 1975). In this connexion there would have been a number of eggs in the lungs at the time of challenge of the bisexually infected mice in the present experiments

(Doenhoff - personal communication). However, in the experiment of Lichtenberg et al. (1963) eggs were injected into the tail vein and the mice challenged at a time of pronounced granulomatous inflammation around eggs in the lungs. Similarly, as part of an experiment not presented here in full, 10 CBA mice were injected intravenously via the tail vein with 1,000 S. mansoni eggs on two occasions at an interval of one week and challenged 3 days later with 200 cercariae together with a group of previously untreated mice. The worm recoveries 21 days post challenge were not significantly different (59.9 $\pm$ 11.6 - egg injected, 51.8 $\pm$ 12.5 - controls). With regard to the involvement of eggs in the liver, in the above experiments performed by Smithers and Terry (1967), eggs were injected directly into the mesenteric veins of rhesus monkeys and presumably lodged in the livers. There is thus convincing evidence that the egg alone cannot stimulate resistance.

The final possibility to be considered is that eggs and worms act synergistically. Mahmoud et al. (1975a) have presented evidence that the eosinophil, possibly acting in concert with specific antibody, is involved in the effector arm of immunity in the mouse. Blood and bone marrow eosinophil counts rise around the time of patency of S. mansoni infections and injection of eggs intravenously or subcutaneously into mice results in a rapid peripheral blood eosinophilia (Mahmoud et al., 1975b). It is possible, therefore, that the egg is involved in stimulating eosinophils, which, acting in concert with specific antibody stimulated by adult worm antigens, comprise the effector arm in killing schistosomula. Investigations along this line would involve comparisons of the resistance of mice, harbouring worms of one sex, which had or had not been injected with eggs intravenously or subcutaneously. While such hypotheses are demanded by the results obtained herein, more basic work on the stimulation of resistance by

single sex infections in mice seems the first priority. Marked resistance following single sex infection in the mouse has only been demonstrated by Sher et al. (1974) using the lung recovery assay of resistance. Such success and the relative failure to induce resistance reported herein should be investigated by perfusion of adult worms 6-8 weeks post challenge. The results of Sher et al. (1974) are in fact not inconsistent with those of the experiments cited here, as they reported that marked resistance following single sex infection did not develop until 12-13 weeks. Therefore, it would be interesting to test the resistance of mice with longer-standing single sex infections than were studied in the experiments described here. It may be that eggs merely function quantitatively in accelerating the appearance of resistance. If it is confirmed that mice do ultimately become resistant following infection with worms of one sex, then such would provide a useful simplified model in which to study the mechanism of resistance.

CHAPTER 5. THE EFFECTS OF GAMMA RADIATION ON THE DEVELOPMENT OF S. MANSONI AND S. MATTHEEI INFECTIONS.

5.1 Introduction.

Gamma- and x-radiations are electromagnetic ionising radiations of short wave length which have essentially similar effects on biological material (Smith et al., 1974). Exposure of the infective stages of a variety of helminth parasites to such radiation has been shown to result in reduced infection rates and inhibition of growth, development and reproductive capacity. The literature on this subject has recently been fully reviewed by Oothuman (1976).

Exposure of the cercariae of a number of schistosome species to doses of gamma- or x-radiation in excess of 3-4 kr. has been shown to prevent the developing worms surviving to the adult stage. Slightly lower doses of between 2-3 kr., however, result in the normal migration and subsequent persistence of a small proportion of radiation damaged, morphologically abnormal and often sterile adults:- S. mansoni - Villella et al. (1961), Smithers (1962), Perlowagora-Szumlewicz and Olivier (1963), Radke and Sadun (1963), Perlowagora-Szumlewicz (1964a and 1966), Erickson and Caldwell (1965), Villella and Weinbren (1965), Oliveira et al, (1971): S. japonicum - Hsü et al. (1963a): S. haematobium - Dr. C. James (pers. comm.): S. incognitum - Tewari and Biswas (1972): S. mattheei - Taylor (1975).

The irradiated parasites which fail to reach the adult stage and location in the host perish at some stage during their migration and histological studies have shown that as the dose of radiation is increased so this death occurs earlier in the migration. Thus Hsü et al. (1963a)

showed that most S. japonicum cercariae exposed to 24-48 kr. die, as schistosomula, in the skin of percutaneously infected mice or rhesus monkeys and Lichtenberg and Sadun (1963) showed the skin to be the site of death of virtually all S. mansoni larvae exposed to 50 kr. The nature of the host response and histopathological consequences of the death of irradiated parasites in the various host organs were also investigated in these earlier studies.

The aim of the present investigation was to confirm and extend the observations on the effects of gamma radiation on S. mansoni and include some additional observations on S. mattheei. Interest was initially centred upon accurately defining the dose of radiation that would result in a persistent infection of sexually sterile adults. This dose is referred to as the 'minimum sterilizing dose' (M.S.D.) and was established for use in the studies on stimulation of resistance because it has been demonstrated that the adult worm can be the major stimulus to the development of resistance (Smithers and Terry, 1969a) and because the reported attempts to induce resistance in experimental animals had generally indicated that maximal resistance was stimulated by such minimally irradiated persistent infections (Villella et al., 1961; Radke and Sadun, 1963; Smithers, 1962; Hsü et al., 1963b; Sadun et al., 1964). The differential effects of this M.S.D. on the survival and fecundity of male and female worms was studied and the persistence of such infections followed up to 200 days post infection. The chapter also includes studies on the migration and site of death of S. mansoni worms exposed to a range of radiation doses up to 40 kr. With the exception of a study by Erickson (1965) of the migration of larvae exposed to 8 kr., knowledge of the site of death of parasites exposed to a range of radiation doses has been based on histological studies which at best are only semi-quantitative. In the present study

histological observations were combined with recovery of parasites from the lungs and liver. T.O. (A.S.L. or B.K.) mice were used in all of the experiments in this chapter.

5.2 The minimum sterilizing dose (M.S.D.) for S. mansoni infections.

5.2.1 Experiment 8.

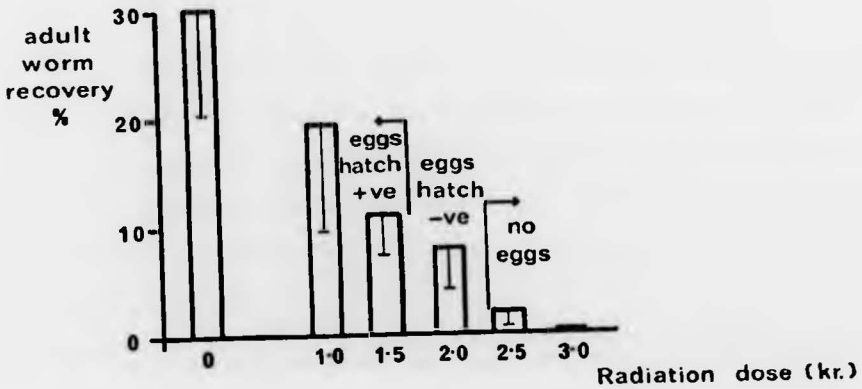
This was essentially a repeat of earlier published work, aimed at broadly defining the M.S.D. for infections arising from percutaneously applied cercariae. 54 mice were separated into 6 groups. Mice in the first group each received 125 unirradiated cercariae. Mice in each of the remaining groups received the same number of cercariae exposed to one of 5 different radiation doses from 1.0 - 3.0 kr. The mice were perfused 8 weeks later, the worms counted and the presence of eggs was deduced by microscopic examination of squashes of liver tissue. The hatching test was used to determine the viability of any eggs present.

The results are shown in Figure 1a. The mean numbers of worms recovered from mice exposed to cercariae irradiated with 1.5, 2.0 or 2.5 kr. were statistically significantly lower than the mean from mice exposed to unirradiated cercariae. All of the mice in the 0 - 2.0 kr. groups were found to harbour worms while 2 of the 9 (22%) in the 2.5 kr. group were parasite free as were all of the mice in the 3.0 kr. group.

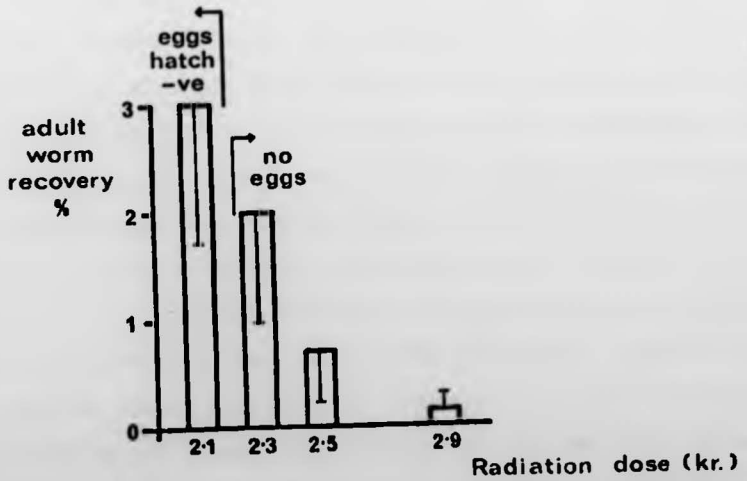
Viable eggs were found in the livers of mice infected with cercariae irradiated with 0 - 1.5 kr. Eggs were seen in liver squashes from mice exposed to cercariae irradiated with 2.0 kr. but these proved to be non-viable in the hatching tests. No eggs were seen in the livers of mice exposed to cercariae irradiated with 2.5 kr. and the hatching

FIGURE 1 Effect of radiation on recovery of adult S.mansoni.

a) infection with 125 cercariae, perfusion 8 weeks



b) i.m. injection with 520 larvae, perfusion 12 weeks



tests proved negative. Thus it was confirmed that the M.S.D. for infections of 125 cercariae lay between 2.0 - 2.5 kr.

5.2.2 Experiment 9.

This experiment was designed to define more accurately the M.S.D. for infections produced by intramuscularly injected somules. The mice in each of 4 groups (6 mice/group) were injected intramuscularly with 520 somules irradiated with 2.1, 2.3, 2.5 or 2.9 kr. The mice were perfused 12 weeks later, the worms counted and the livers checked for eggs.

The results are shown in Figure 1b. Eggs, which proved to be non-viable in the hatching test, were seen in the liver squashes of 4 of the 6 mice injected with somules irradiated with 2.1 kr. For mice injected with somules irradiated with 2.3 - 2.9 kr., no eggs were seen in any liver squashes and all of the hatching tests proved negative. Fragments of dead worms and associated lesions were occasionally seen in squashes of liver from mice in all of the groups. Worms were recovered from all of the mice infected with somules irradiated with 2.1 and 2.3 kr. These worms displayed a range of morphological abnormalities, some appearing almost normal and others extremely stunted. Of the mice infected with schistosomula irradiated with 2.5 and 2.9 kr., 1 out of 6 and 3 out of 6 respectively were parasite free and all the worms recovered were extremely stunted. Thus the range of radiation doses from which to choose an M.S.D. for infections comprising about 500 somules is extremely narrow, eggs being present in the livers of mice infected with 2.1 kr. somules and 1 out of 6 mice in-

fected with 2.5 kr. somules being parasite free. Consequently, 2.3 kr. was taken as the M.S.D. for use in many of the studies described herein, though subsequent studies have shown that a few eggs can be produced by a small proportion of the worms which develop from larvae exposed to 2.3 kr. (See Sections 5.3 and 5.6).

Staining of the 2.3 kr. worms with carmine showed that while none of the females possessed visible eggs or ovaries, 43% of the males had some visible testes. It thus appeared that 2.3 kr. resulted in sterility in the females but not obviously in the males.

5.3 Differential effect of the M.S.D. on male and female worms.

5.3.1 Experiment 10.

This apparently differential effect of 2.3 kr. on the development of the reproductive capacity of male and female worms was investigated by percutaneously infecting mice with either male or female cercariae irradiated with 2.3 kr. (R ♂ or R ♀) together with unirradiated cercariae of the opposite sex (N ♂ or N ♀). The experimental design is shown in Table 8. The mice were perfused 8 weeks after infection, the worms recovered, stained with carmine and examined for evidence of ovaries or testes, and the liver and intestines digested for egg counts.

The results are shown in Table 8. The mean number of male worms recovered from mice in group B (R ♂ + N ♀) was not significantly different from the mean recovered from mice in group D (R ♂ + R ♀). Similarly, the recovery of female worms from mice in group C (N ♂ + R ♀) was not significantly different from that from mice in group D (R ♂ + R ♀). Thus the number of 2.3 kr. male or female worms surviving was similar

Notes on Experimental design of Experiment 10.

1. Group A. Each mouse given 50 normal male cercariae + 50 normal female cercariae (N ♂ +N ♀).

   Group B. " " " 1,200 irradiated (2.3 kr.) male cercariae + 50 normal female cercariae (R ♂+ N ♀).

   Group C. " " " 50 normal male cercariae + 1,200 irradiated (2.3 kr.) female cercariae (N ♂+ R ♀).

   Group D. " " " 1,200 irradiated (2.3 kr.) male cercariae +1,200 irradiated (2.3 kr.) female cercariae (R ♂+ R ♀).

(3 mice/group).

2. Worms of indeterminate sex have been excluded (10.7 $\pm$ 5.5).

TABLE 8. Experiment 10.

TABLE 8. Experiment 10.

Worm and egg recoveries from mice infected with irradiated (2.3 kr.) and/or unirradiated cercariae of S. mansoni

	Group A[1]	Group B	Group C	Group D[2]
Mean number of male worms with visible testes (± S.D.)	23 ± 10	18 ± 5	17 ± 3	11 ± 2
Mean number of male worms without visible testes	0	11 ± 6	0	13 ± 3
Mean number of female worms with visible ovary	23 ± 5	25 ± 4	5 ± 2	2 ± 2
Mean number of female worms without visible ovary	0	0	92 ± 16	81 ± 8
Mean number of eggs/liver	34,623 ± 11,649	28,083 ± 8,138	6,835 ± 2,924	196 ± 88
Mean number of eggs/gut	57,614 ± 34,130	35,330 ± 13,928	4,417 ± 3,270	28 ± 38
Mean total eggs/worm pair	4,333 ± 1,030	2,436 ± 481	648 ± 317	9 ± 5
Mean percent eggs in liver	41 ± 9	45 ± 3	65 ± 16	91 ± 10

whether their partners were irradiated with 2.3 kr. or unirradiated. The recoveries of 2.3 kr. male worms in groups B (R ♂+ N ♀) and D (R ♂+ R ♀) represented respectively 2.4% and 2.0% survival of the number of male cercariae applied while the recoveries from group C (N ♂+ R ♀) and group D (R ♂+ R ♀) of 2.3 kr. female worms represented respectively 8.1% and 7.0% survival of the female cercariae applied. Thus in terms of survival, female larvae were less susceptible to the effects of 2.3 kr. than were male cercariae. Dilations of the caeca of male worms similar to those described by Perlowagora-Szumlewicz (1964a) and Villella and Weinbren (1965) were observed in 48% of the 2.3 kr. worms.

The tissue egg count data has been presented as the number of eggs/worm pair. The number of worm pairs per animal represents the maximum number of potential worm pairs, this being limited by the number of worms of the less numerous sex. The mean total number of eggs/worm pair recovered from mice in group B (R ♂+ N ♀), 2,436 $\pm$ 481, was significantly ($P < 0.05$) lower than from mice in group A (N ♂+ N ♀), 4,333 $\pm$ 1,030. Staining of the male worms from group B showed that a mean of 11 out of 29 (38%) lacked any visible testes. Some of them appeared far too stunted to pair properly and others were of indeterminate sex. If the mean number of eggs/worm pair is calculated with reference to the number of male worms possessing visible testes, the value is 3,493 $\pm$ 504 which is not significantly different from the value for group A (N ♂+ N ♀). Thus it seems likely that the figure of 2,436 $\pm$ 481 eggs/worm pair is not a reflection of the reproductive capacity of all the persisting 2.3 kr. male worms but rather a reflection of the range of reproductive capacity.

The egg count/worm pair value for mice in group C (N ♂ + R♀), 648 ± 317, although significantly lower ($P < 0.01$) than the value for group A (N♂ + N♀), 4,333 ± 1,030, does show that at least some of the females developing from cercariae exposed to 2.3 kr. did have the capacity to produce eggs. However, the fact that the egg count/worm pair in group C (N♂ + R♀) was significantly lower ($P < 0.02$) than that in group B (R ♂ + N ♀) clearly shows that 2.3 kr. has a greater effect on the reproductive capacity of females than of males. It must be pointed out that the figure of 648 ± 317 eggs/worm pair is unrepresentative of the average reproductive capacity of a random sample of 2.3 kr. female worms. In group C the irradiated females outnumbered the normal males by a mean ratio of almost 6:1. If it is assumed that the normal males would have paired preferentially with the most normal of the irradiated females, the figure of 648 ± 317 may not be representative of the fertility of the large excess of unpaired females. Staining of the 2.3 kr. females revealed that a mean of only 5 out of 97 possessed a visible ovary. These females with visible ovaries were the largest and most normal looking of the females and most were found in copula at perfusion.

A mean of only 9 ± 5 eggs were found in the tissues of mice in group D (R ♂ + R♀). The majority of these were merely malformed egg shells lacking any miracidial remnants.

Major differences were noted in the tissue distribution of eggs in the four groups. In the mice in group A, 41% of the eggs were in the liver as compared with 45% in group B, 65% in group C and 91% in group D.

5.4 Persistence of S. mansoni worms exposed to the M.S.D.

5.4.1 Experiment 11.

In this study, mice infected with organisms exposed to 2.3 kr. were perfused at intervals up to 200 days post infection to determine the length of time 'sterile' worms would survive and how the infected mice would tolerate such an infection. Mice were infected by intramuscular injection of 500 somules. 32 mice received unirradiated somules and 42 mice received somules irradiated with 2.3 kr. 4 mice from each group were perfused 5, 12, 14, 18, 24, 32 and 46 days post infection and a further 4 mice from the 2.3 kr. group on each of days 63, 90 and 200. The recoveries are expressed graphically in figure 2. The lengths of the irradiated worms recovered on days 63, 90 and 200 were measured in a graduated counting chamber. The percentage recovery of worms from the unirradiated infection increased rapidly from day 5 to plateau at day 24. This plateau, representing approximately 33% recovery of the number of larvae injected, was maintained up to day 46, the remaining 4 mice having died by day 63. Up to day 14 there was no significant difference between recoveries from the unirradiated and 2.3 kr. infections. The 25% recovery of worms from the 2.3 kr. infection on day 14 represented 77% of the 33% plateau recovery of unirradiated worms. Between days 14 and 18 there was a significant drop in the number of 2.3 kr. worms recovered such that at day 18 the mean recoveries from the unirradiated and 2.3 kr. infections were significantly different ($P < 0.001$). From day 18 to day 46 there was a gradual decline in recovery of irradiated worms and from day 46 to day 200 recoveries remained remarkably constant at between 2 - 3%. Worms were found in all of the mice infected with irradiated organisms (range 7 - 25 worms from days 46 - 200).

FIGURE 2 Effect of 2·3 kr. on survival of S.mansoni

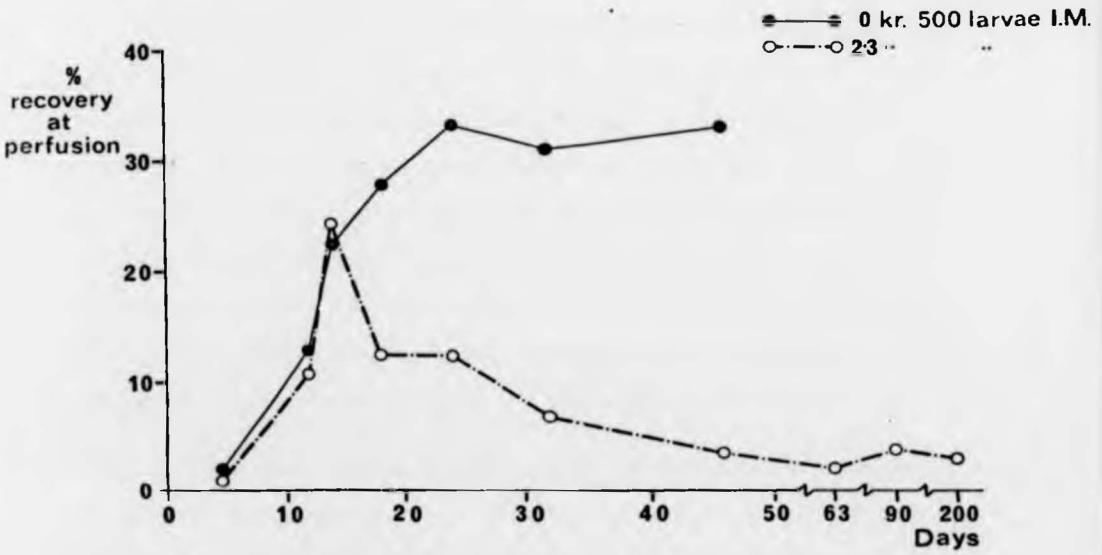
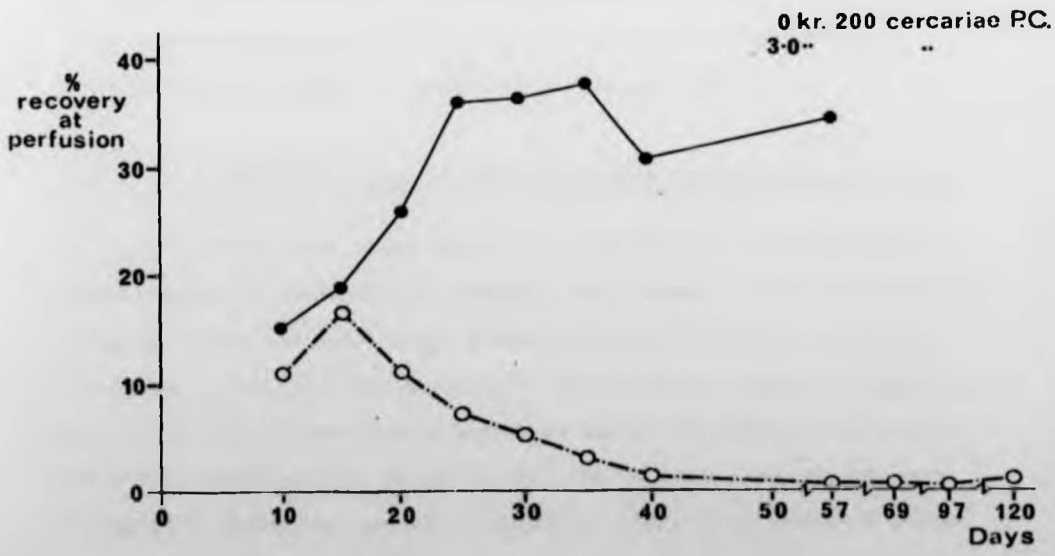


FIGURE 5 Effect of 3·0 kr. on survival of S.mattheei



Of the 42 mice initially infected with larvae irradiated with 2.3 kr., one died on day 46. No eggs could be seen in the liver and the cause of death of this mouse was unknown. Two mice were killed because of superficial abscesses secondary to injuries incurred in fighting. Red patches, presumably sites of haemorrhage were seen on the surface of the lungs of mice killed on days 14 and 18 and occasional white spots were seen on the livers of mice killed on days 14 - 46. Microscopic examination of squashes of such pieces of liver tissue frequently revealed disintegrating worms or patches of pigment. Histological sections of such lesions are presented in section 5.6.

The mean lengths of the sterile worms recovered on days 63, 90 and 200 were respectively :- ♂ -4.9 ± 2.0, ♀ -4.4 ± 1.2; ♂ -5.5 ± 1.8, ♀ -4.6 ± 1.9; ♂ 5.3 ± 1.4, ♀ -4.6 ± 1.6. The mean lengths of unirradiated S. mansoni adults recovered from the challenge controls in Experiment 18 (Chapter 6) were ♂ -8.6 ± 1.8 and ♀ -13.3 ± 1.0 (20 worms of each sex counted). Thus, the sterile worms were considerably smaller than unirradiated worms. Moreover, as mentioned in Section 5.3 they displayed a range of morphological abnormalities.

5.5 Survival and site of death of larvae exposed to 2.3 - 40 kr.

It has been shown above that, as the dose of radiation to which larvae are exposed is increased, the number of worms recoverable from the liver and mesenteries 8 weeks post infection correspondingly decreases. Thus the vast majority of schistosomula exposed to doses of 2.3 kr. and above must perish somewhere during the course of migration from the infection site to the liver. The following experiments were designed to study the pattern of migration and site of death of larvae

exposed to radiation doses in the range 2.3 - 40 kr. used to attenuate infections in the studies of the development of resistance to reinfection described in Chapter 6.

5.5.1 Experiment 12.

Mice in each of four groups (40 mice/group) were injected intramuscularly with the following numbers of irradiated somules:- 495 at 0 kr, 406 at 2.3 kr, 494 at 4 kr and 448 at 10 kr. The intramuscular route was chosen as it has proved to be a quick and efficient method of infection (James & Taylor, 1976). The results of a preliminary experiment had shown that schistosomula could be recovered from the lungs of mice infected by intramuscular injection of 10 kr somules in comparable numbers with those recovered from mice infected with unirradiated larvae. On days 3, 4, 5, 6, 8, 12, 16 and 26 after injection, "lung chops" and liver perfusions were performed on 5 mice from each of the groups.

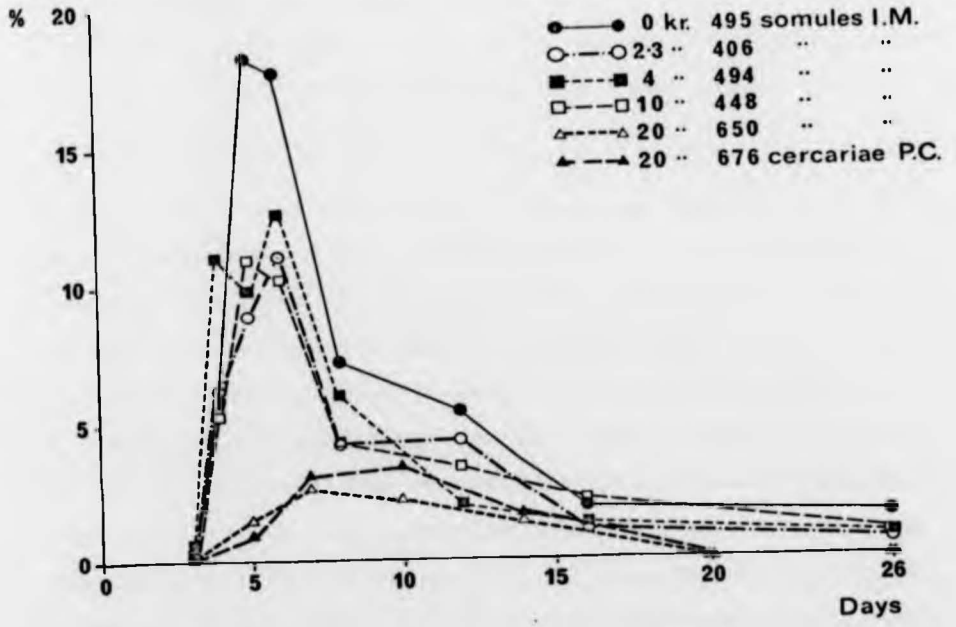
The results from this study are for comparative purposes combined with those of a further study in which mice were infected either percutaneously (P.C.) or intramuscularly (I.M.) with larvae exposed to 20 kr or 40 kr. It was unknown whether larvae exposed to such high doses would be capable of leaving the site of infection and reaching the lungs or whether their capacity to do so was affected by the two different routes of administration, both of which have been used in vaccination studies (Chapter 6). Mice in each of four groups (35 mice/group) received the following numbers of larvae:- 676 (P.C.) cercariae or 650 (I.M.) somules irradiated with 20 kr. or 616 (P.C.) cercariae or 635 (I.M.) somules irradiated with 40 kr. After the

20 mins. allowed for penetration the water in which the cercariae had been suspended was removed from the rings of 10 mice from each of the two cercarial groups and searched for cercariae following the addition of a few drops of Lugol's iodine. Samples from the 20 kr group contained a mean of 4.4 $\pm$ 3.3 cercariae, and those from the 40 kr group, a mean of 6.6 $\pm$ 4.9 cercariae. Though no comparisons were made with the pentration of unirradiated cercariae it is apparent that penetration of cercariae is not significantly affected by exposure to 20 kr or 40 kr. It is assumed that lower doses would similarly not markedly affect penetration. On days 3, 5, 7, 10, 14, 20 and 26 after infection "lung chops" and liver perfusions were performed on 5 mice from each of the groups.

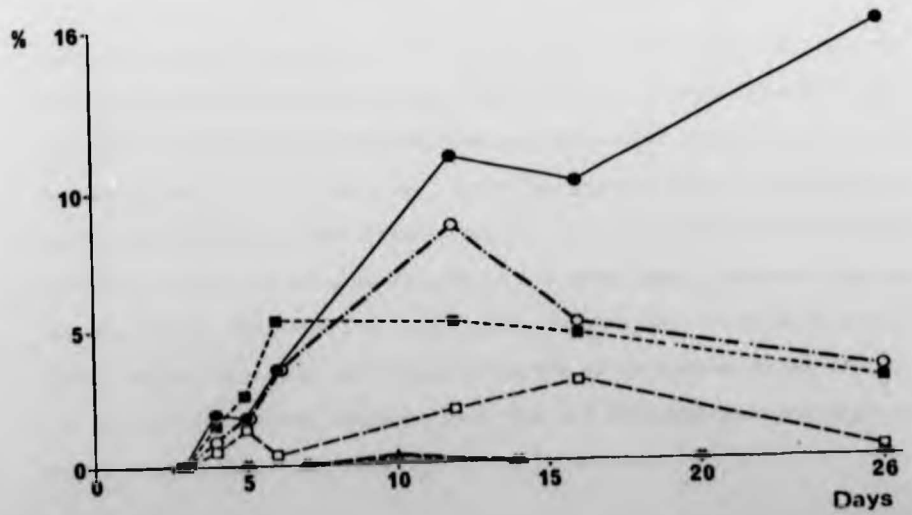
The percentage recoveries of schistosomula from the lungs in these two experiments are shown in figure 3a. The patterns of recovery from mice infected with unirradiated larvae and larvae exposed to 2.3 - 10 kr are very similar. Peak recoveries from all of these groups occurred on days 5 and 6. There was no significant difference between the recoveries from these groups for any time point except for a marginally significant difference between the 0 kr and the 2.3 kr infections on day 5 ($P < 0.05$) and between the 0 kr infection and the 2.3 kr and 10 kr infections on day 6 ($P < 0.05$). A marked drop in recoveries of lung form schistosomula occurred between days 6 and 8 and thereafter there was a gradual decline. A small proportion (0.6 - 1.4%) of typical lung-form organisms (Clegg, 1965) was recovered from each of these groups on day 26. With both percutaneous and intramuscular routes of infection the pattern of recovery of organisms exposed to 20 kr was markedly different from that observed above. The most striking difference was the absence in the 20 kr infection of a definite peak between days 5 - 6, percentage recovery increasing gradually until day 7.

FIGURE 3 Effect of radiation on migration of schistosomula

a % recovery from lungs



b % recovery from portal system



Similar recoveries were obtained on day 10 after which the numbers fell, declining to zero by day 20. Only one schistosomulum was recovered from the lungs of the mice infected with larvae exposed to 40 kr. This was from the percutaneous group on day 10.

The pattern of recovery of worms from the liver and mesenteries is shown in figure 3b. Recoveries from the mice infected with unirradiated somules showed a gradual increase throughout the study period. No worms were recovered at any time from mice infected with cercariae or somules exposed to 40 kr and a mean recovery of only 1.3 $\pm$ 1.1 worms was recorded for mice exposed to larvae irradiated with 20 kr (on day 10). Worms from the mice infected with larvae irradiated with 0 - 10 kr were first detected on day 4. On day 6 and thereafter recoveries from the mice infected with 10 kr larvae were significantly lower than those from mice infected with unirradiated larvae. Maximum recovery (2.9%) occurred on day 16 falling by day 26 to 0.3%. Recoveries from the 4 kr infection rose to a peak at day 6 (5.3%), and thereafter were significantly lower than those from the unirradiated infection, gradually falling to 2.9% by day 26. Recoveries from the 2.3 kr infection were not significantly different from those from the unirradiated infection during the first 12 days, but were significantly lower thereafter. There was a marked drop in recoveries from the 2.3 kr infection between days 12 and 16. A similar marked drop in recovery of 2.3 kr worms was observed between day 14 - 18 in experiment 11 described in section 5.4. At no time was there a significant difference between the recoveries from the 2.3 and 4.0 kr infections. Both were significantly higher than the recoveries from the 10 kr infection on days 6 and 26. On day 12 the recovery from the 2.3 kr infection was significantly higher than that from the 10 kr infection.

While this above study provides information concerning the pattern of arrival and survival in the liver and lungs of irradiated parasites it provides only circumstantial evidence as to their site of death. The following studies of the histopathological consequences of infection with irradiated organisms provide additional information.

5.5.2 Experiment 13.

30 mice were each injected intramuscularly with 500 somules irradiated with 2.3 kr. This infection was performed at the same time and with the same batch of somules as were used in experiment 11, section 5.4 At intervals from 5 - 230 days after infection, 2 or 3 mice were killed by cervical dislocation and the lungs and livers rapidly removed and fixed in 10% formol saline. Serial wax sections of these tissues were cut at 6μ and stained with haematoxylin and eosin.

At day 5, a few intact schistosomula devoid of any host tissue reaction were found in lung capillaries, arterioles and occasionally within alveoli. Small, scattered haemorrhages were seen but could not be directly related to the presence of schistosomula. A single degenerated parasite was seen embedded in a tiny nodule consisting of mononuclear cells. On day 12, several intact schistosomula without reaction were present though there was a mild infiltration of mononuclear cells around some of the blood vessels. A few cellular nodules consisting of mononuclears and eosinophils were seen in close association with blood vessels or haemorrhagic foci in which degenerated schistosomula could be found. By day 18, the frequency and magnitude of such reactions

were more intensified and there were haemorrhages, diffuse interstitial mononuclear cell infiltration and multiple, comparatively large nodules around disintegrated schistosomula. The latter lesions were characteristically distributed near the edges and in the interstitium of the lung. Some of them revealed giant cells and fibroplasia while others, probably newly formed, consisted predominantly of mononuclears and eosinophils (see Plate 2). In addition, there was an intense focal perivascular infiltration of eosinophils and neutrophils. Some such vessels showed subintimal eosinophilic infiltration and swelling of their lining endothelial cells. By day 24 relatively few of the focal lesions could be seen but some of these near the borders of the lung had coallesced substituting the alveolar parenchyma. The perivascular infiltrate now comprised predominantly mononuclears. At day 32, no schistosomula associated with discrete lesions could be seen. However, there was a heavy perivascular infiltration involving most parts of the lung. Signs of fibrosis were observed at the edges of the lung. At day 46, the lungs presented a fairly normal appearance except for a single nodule containing a few giant cells and polymorphs. In the centre of this nodule was a degenerated parasite containing a large amount of black pigment. This parasite was obviously not a lung-form schistosomulum and it must be assumed that it either developed into a feeding worm ectopically in the lung or had travelled to the lungs from the portal system. This lesion appeared to be becoming organized by fibrous tissue. Some perivascular mononuclear cell reaction was still apparent though much less pronouned than at day 32 and was largely confined to minor branches of the blood vessels. Focal pigmentation occurred. At day 64, there was a similar degree of lymphoid reaction around blood vessels and pigmentation of the alveolar macrophages. A mild degree of peribronchial lymphoid hyperplasia was evident and this persisted though with reduced intensity at day 90. Slight mononuclear cell aggregation around some of the pulmonary bronchioles and blood vessels

could be seen at day 140. However, there was a relatively large inflammatory focus around a dead parasite at the lung edge in one series of the sections. This lesion was organised and inside it a few emphysematous alveoli, bronchial remnants and vessels were still discernible. At day 230, there was a fairly marked infiltration of lymphycytes and polymorphs around bronchi and arterioles in one part of the section. The alveolar parenchyma in this vicinity showed areas of collapse and emphysema with some accumulation of lymphocytes, polymorphs, macrophages and nuclear fragments. Though no parasite remains were seen this lesion may have represented further resolution of a similar lesion to that described at day 140. Diffuse changes were minimal, with only very few pholymorphs and other inflammatory cells in the interstititial tissue.

In liver sections, no schistosomula were found at day 5 and there were no pathological lesions in the organ. At day 12, a few intact schistosomula free of surrounding reaction were seen in small portal venules and their numbers increased by day 14, when there was also a slight intra-lobular infiltration of mononuclear cells. At day 18, many schistosomula were seen and although some of them were free of local inflammatory reaction, the majority occurred in vessels surrounded by monoculear cells and eosinophils (see Plate 3). In addition, severe portal phlebitis with a dense accumulation of mononuclears and eosinophils was found in association with dead schistosomula inpacting portal veins (see Plate 4). At these sites, inflammatory cells extending from the perivascular zone surrounded degenerated and necrotic hepatocytes in their immediate vicinity. In addition, circumscribed foci of coagulative necrosis resembling infarcts were seen in the

Legends to plates.

The sections represented in plates 2-6 were from mice infected with 500 S. mansoni somules irradiated with 2.3 kr. The sections were stained with haematoxylin and eosin.

Plate 2. Section of lung 18 days post infection (x230), showing a cellular nodule comprising mononuclear cells and eosinophils surrounding a degenerating schistosomulum.

Plate 3. Section of liver 18 days post infection (x330) showing a worm in a portal venule eliciting an infiltration comprising mononuclears and eosinophils.

Plate 2.

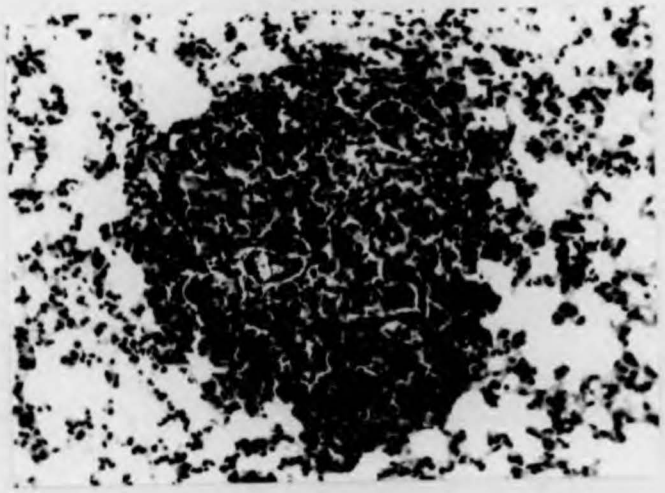


Plate 3.

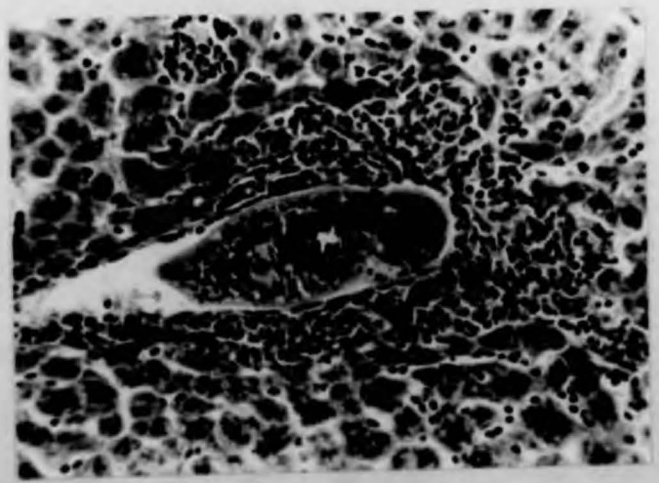


Plate 4. Section of liver 18 days post infection (x150) showing a degenerating worm surrounded by a cellular infiltrate of mononuclear cells, eosinophils and giant cells.

Plate 5. Section of liver 24 days post infection (x100) showing a circumscribed focus of coagulative necrosis containing degenerate, necrotic hepatocytes and surrounded by inflammatory cells.

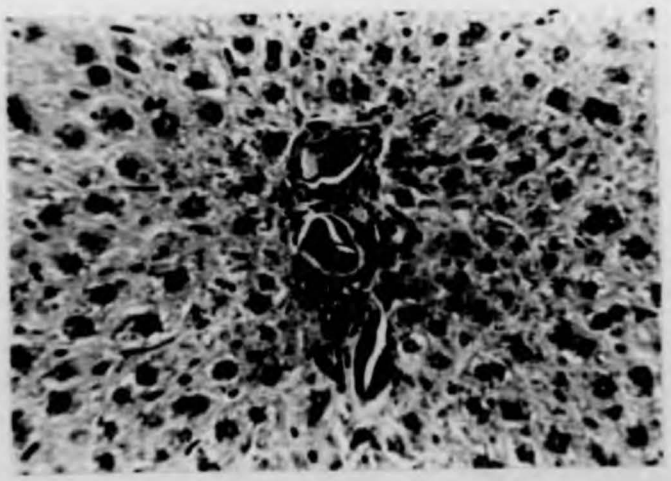
Plate 4.

Plate 5.

parenchyma (see Plate 5). In the centres of these necrotic masses, serial sections revealed disintegrated parasites, pigment and dead neutrophils. These three types of lesion, represented in plates 3, 4 and 5 probably represent differing stages in the evolution of lesions associated with intravascular death of the parasite. At day 24, such lesions were more pronounced. There was severe inflammation of the portal veins due to dead worms and there was comparatively more wide-spread infiltration of mononuclear cells and eosinophils in between the surrounding parenchymal cells. Several intra-parenchymal nodules containing schistosomular remnants, inflammatory cells and necrotic liver cells were seen, the hepatocytes peripheral to these nodules were also affected and showed vacuolization and pyknosis. At day 32, however, there was evidence of a starting resolution of the lesions with fewer inflammatory nodules and necrotic foci. However, a patchy residual infiltration of mononuclear cells and eosinophils was still present. At day 46 the liver was essentially normal apart from swelling and proliferation of the Kuppfer cells. A single very small granuloma around a calcified egg was seen. It comprised epithelioid cells, fibroblasts and a peripheral ring of round cells. At day 64 a single area of phlebitis was seen with infiltration of mononuclear cells, polymorphs, eosinophils and macrophages perivascularly. The reaction extended along the length of the portal vessel but the lesion could not be associated with parasite remnants. There were, again, small collections of mononuclear cells intralobularly with some pigment accumulation within the Kuppfer cells. At day 140 a few eggs and shells were seen inside small portal vessels. They were engulfed by giant cells, often impregnated with pigment and incited little or no mononuclear cell reaction (see Plate 6). There was a mild focal perivascular lymphocytic infiltration around portal vessels. One portal branch, containing an intact worm was, however, surrounded by a rela-

Plate 6. Section of liver 140 days post infection (x330) showing abnormal S. mansoni eggs inside a small portal venule. The eggs, lacking miracidia and impregnated with pigment, have elicited minimal cellular reaction.

Plate 6.



tively marked cellular reaction comprising a mixture of lymphocytes and eosinophils. Other intact worms were seen in other vessels eliciting no surrounding reaction. At 230 days, the sections showed more accentuated but essentially similar changes to those described at 140 days. That is, dead calcified eggs (? eggshells) in small venules, often enclosed by giant cells, flukes in the larger vessels evoking a mild or moderate, predominantly lymphocytic, perivascular infiltration and marked accumulation of pigment.

5.5.3. A further study was performed to assess the relative involvment of the lungs and liver as sites of the focal pathology consequent upon the death of schistosomula irradiated with 4 or 10 kr.

Experiment 14.

Mice were infected as in experiment 13 but with 500 somules irradiated with 4 or 10 kr. Two mice for each radiation dose were killed on days 5, 13, 23 and 33 post infection and the livers and lungs serially sectioned.

The histopathological changes observed were identical in kind to those described for the 2.3 kr infection. At day 5, a few intact schistosomula, largely devoid of cellular reaction were seen in the lung sections from mice in both groups. However, by day 13 numerous cellular nodules were seen, surrounding degenerate schistosomula. By day 23 and 33 such lesions had become successively less numerous. Focal host cellular reactions to parasites in the liver were first seen in the 13 day sections, at which time they were comparable in number in both groups. Very few such lesions were seen at 23 and 33 days in the 10 kr group but in the 4 kr group the numbers were increased somewhat

at day 23 and comparable numbers were again seen on day 33.

5.6 The M.S.D. for S. mattheei infections.

In the next three experiments comparative observations were made on the effects of low levels of radiation on S. mattheei infections. Taylor (1975) showed that when cercariae of S. mattheei were exposed to 6.0 kr no worms survived to maturity in mice. Exposure to 3.0 kr resulted in about 2% survival, while exposure to 1.5 kr resulted in comparable recoveries to the unirradiated infection.

5.6.1 Experiment 15.

In order to establish an M.S.D. for S. mattheei infections mice were each infected with 125 cercariae exposed to a range of radiation doses from 0 - 3.0 kr. The mice (9-10/group) were perfused 9 weeks after infection and the livers checked for eggs by examination of squashes of liver tissue. The viability of any eggs present was assessed in hatching tests. The results are shown in figure 4a. Exposure of cercariae to radiation doses up to 1.5 kr had no significant effect upon adult worm recovery and viable eggs were present in all mice examined. There was a significant reduction in the number of worms recovered from mice infected with cercariae irradiated with 2.0-3.0 kr. Eggs were found in 6 of the 10 mice exposed to cercariae irradiated with 2.0 kr but they proved non-viable in the hatching tests. No eggs were seen in the liver squashes of mice infected with cercariae irradiated with 2.5 or 3.0 kr and the hatching tests proved negative. On the basis of this result 2.5 kr was taken at the M.S.D. for S.matheei infections comprising 125 cercariae. However, 3.0 kr which resulted

in a 12% recovery in the above experiment was used to attenuate S. mattheei infections in studies of the development of resistance to reinfection in mice (see Chapter 7). 3.0 kr was also used in vaccination studies in sheep (Taylor et al., 1976).

5.6.2 Experiment 16.

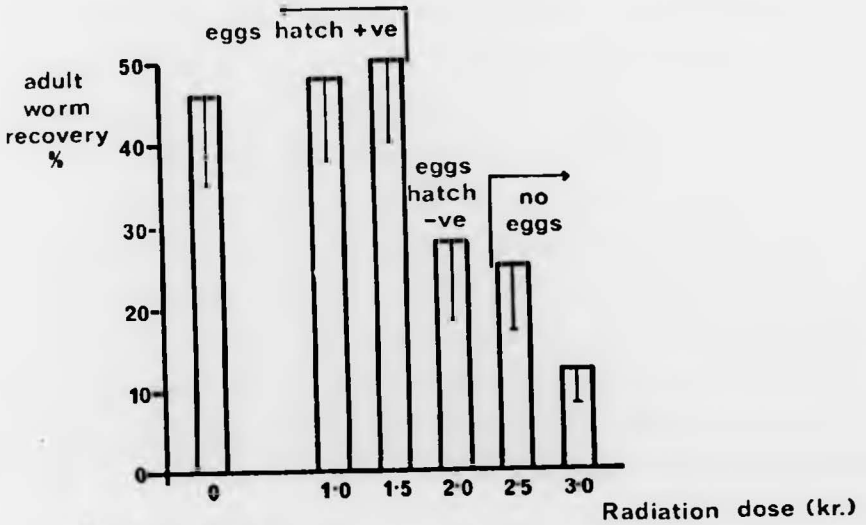
This study was designed to follow the survival of worms exposed to 3.0 kr. Mice were infected percutaneously with 200 cercariae either unirradiated or irradiated with 3.0 kr. At various times after infection 8-10 mice from both groups were perfused and the worms counted. The results are shown in figure 5 (page 104). Percentage recoveries from the unirradiated infections increased to a plateau at 20-25 days representing a 30-35% recovery. Recoveries from the 3.0 kr. infections showed a parallel increase up to day 15, when there was a 17% recovery, and thereafter declined to around 0.5%, a level that persisted up to 120 days. No deaths attributable to the irradiated infection occurred during this study and no eggs were detected in squash examinations of liver tissue. This is not surprising since, of the mice killed on days 45-120, 30% were parasite free and the mean recovery was only one worm. Thus these results indicate that infection of mice with 200 cercariae irradiated with 3.0 kr was not an effective schedule for establishing a "sterile" worm population, although 3.0 kr has been successfully used to establish sterile worm populations (see Chapter 7).

5.6.3 Experiment 17.

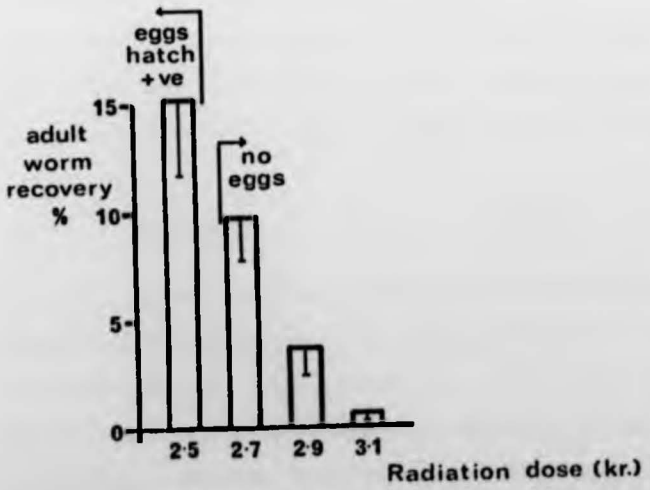
As the M.S.D. established in this experiment was to be used in immunization experiments in sheep (Taylor et al., 1976; Bickle et al.

FIGURE 4 Effect of radiation on recovery of S.mattheei

a) infection with 125 cercariae, perfusion 9 weeks



b) infection with 740-995 somules, perfusion 10 weeks



Appendix 4), relatively large numbers of somules were used and were administered by intramuscular injection. Mice in each of 5 groups (5 mice/group) received the following numbers of somules:- 970 irradiated with 2.3 kr, 810 irradiated with 2.5 kr, 740 irradiated with 2.7 kr, 795 irradiated with 2.9 kr or 995 irradiated with 3.1 kr. The mice were perfused 10 weeks later, the worms counted and the livers checked for eggs. The results are shown in figure 4b. All of the mice exposed to schistosomula irradiated with 2.3 kr had died with typical murine hepatosplenomegaly. The worm recovery from the mice infected with 2.5 kr somules was 15.4%. Eggs were seen in liver squashes of all 5 of the mice and although the vast majority were black and apparently non-viable, miracidia appeared in the hatching tests proving the viability of some. No eggs were seen in the liver squashes of mice infected with somules irradiated with 2.7 - 3.1 kr and no miracidia were seen in the hatching tests. All of the mice were found to harbour parasites. Staining of the worms exposed to 2.7 kr, which was taken as the M.S.D., revealed that although none of the females possessed a visible ovary, 51% of the males had visible testes. However, more females survived the irradiation (♂ = 19%, ♀ = 68%, worms of indeterminate sex = 13%).

5.7 Discussion.

As was reported by Radke and Sadun (1963) and Erickson and Caldwell (1965) exposure of *S. mansoni* cercariae to increasing doses of radiation from 0 - 3 kr resulted in a virtually linear decrease in the number of parasites surviving to perfusion 8 weeks post infection. *S. mattheei* cercariae behaved somewhat differently. Doses up to 1.5 kr had no significant effect on the percentage recovery of adult worms, a near linear decrease in recovery occurring between 1.5 - 3.0 kr. Further differences were observed in the susceptibility of these two parasites to particular radiation doses, e.g. exposure of mice to 125

cercariae irradiated with 3 kr resulted in a 12.5% recovery of S. mattheei worms whereas no S. mansoni worms survived this radiation dose.

The minimum dose of radiation (M.S.D.) that prevented egg production by S. mansoni infections was found to be between 2.0 - 2.5 kr, thus agreeing with the results of previous authors cited in the introduction to this chapter. When mice were injected with 500 somules, it was found that 1 out of 6 mice infected with 2.5 kr organisms was parasite free, while eggs were present in mice infected with 2.1 kr organisms. Thus 2.3 kr was taken as the M.S.D. for infections of around 500 larvae and this dose was subsequently used in vaccination experiments (Chapter 6). Stunted, largely sterile worm infections were consistently produced with this radiation dose resulting in percentage recoveries in the range 1.0 - 4.4%. The results of experiment 11 showed that despite the fact that a high proportion of the irradiated parasites reaching the liver died between 14-46 days post infection, thereafter the percentage survival remained relatively constant at between 2-3% until the termination of the experiment 200 days post infection. The finding of eggs in experiments 10 and 13 show that 2.3 kr is not an absolute sterilizing dose for S. mansoni infections. As mentioned above, the choice of 2.3 kr represents a compromise between the need to avoid the pathology caused by eggs and the need to consistently produce stunted infections with a manageable number of infective larvae. The random nature of radiation damage and the general variation in biological vigour inevitably results in some parasites suffering more damage to vital organs than others. This is evidenced by the fact that only a small proportion of parasites survive at all and amongst those that do there is a considerable range of morphological abnormalities.

Thus it is not surprising that eggs should occasionally be encountered in infections with 2.3 kr larvae particularly when vast numbers of cercariae or somules are used for infection such as the 2,400/mouse in experiment 10.

However, a number of facts indicate that the 2.3 kr infections invariably produce only egg shells. Even with the 2.1 kr infections in experiment 9, no miracidia were seen in the hatching tests, although eggs were readily seen in liver squashes. Minimal host cell response was observed around the few eggs seen in the livers of mice infected with 2.3 kr larvae in experiment 13 and the eggs themselves lacked any visible miracidial remnants (see plate 6). On an ultrastructural level, Dr. John Shaw (personal communication) has shown that about 5% of the females in a mixed infection exposed to 2.3 kr possessed vitelline cells which were nevertheless rarely organised into a well developed vitelline gland. Of those that did possess a vitelline gland and thus were capable of forming and discharging egg shell material, only a few possessed an ultrastructurally normal ovary. Thus more females had the potential, in morphological terms, to produce only egg shells than to produce ova.

Staining and microscopic examination of the 2.3 kr worms from experiment 9 revealed that while none of the females possessed a visible ovary, 43% of the males possessed testes. This indication that the sterility of the 2.3 kr infections was due to sterility of the females was investigated by infecting mice with irradiated worms of one sex and unirradiated worms of the opposite sex, (experiment 10). In terms of the number of worms surviving to perfusion, females were less

susceptible to the effects of 2.3 kr than males. This predominance of females over males has been observed in virtually all of the infections with 2.3 kr worms (see Chapter 6). Similarly Perlowagora-Szumlewicz (1964a) reported that in infections with 2.0 kr cercariae, female worms consistently outnumbered males. Greater susceptibility of male worms than females to the effects of ionizing radiation has been reported with other helminth species: Oesophagostomum radiatum (Riek and Sadun, 1960), Dictyocaulus filaria (Jovanovic et al., 1961), Ancylostoma caninum (Miller, 1964).

The egg recovery data from experiment 10 clearly shows that the sterility of the 2.3 kr infections is due predominantly to sterility in the females, the irradiated males being almost as fertile as unirradiated males when paired with normal females. However, such irradiated males did not constitute as competent partners for the irradiated females as did normal males this being reflected in an overall lower fecundity and a shift to the liver in the distribution of eggs. Thus the percentage of total eggs found in the livers of mice infected with irradiated females and normal males was 65% as compared with 91% in the mice infected with irradiated females and males. This finding may well be partly explained by the observation of Oliveira et al. (1971) that in mice infected with cercariae irradiated with 2.5 kr, 91% of the worms were found in the liver as compared with only 15% in an unirradiated infection, this inability of the irradiated worms to occupy a normal position in the host possibly reflecting the fact that they were markedly smaller than normal worms. Particularly characteristic abnormalities most commonly amongst the male worms were the haematin filled dilatations of the caecae, also noted by

Perlowagora-Szumlewicz (1964a). Villella and Weinbren (1965) similarly reported that radiation in the range 2.0 - 2.5 kr resulted in a decrease in body length, parenchymal vacuolation and ventricular swelling.

The above observations on the fecundity of 2.3 kr-irradiated worms have important practical implications for the theoretical use of 'sterile' worm infections as a means of stimulating resistance. Thus, although the 2.3 kr infections produce very few eggs which themselves elicit a minimal host reaction, a proportion of both the males and the females could constitute fertile partners for any invading normal worms in the event of resistance being only partial. Perlowagora-Szumlewicz (1964c and 1966) who performed a similar study with infections irradiated with 2.0 kr also observed that the irradiated worms could reproduce successfully in the presence of unirradiated partners.

The M.S.D. established for S. mattheei somules injected intramuscularly was 2.7 kr. This confirmed the earlier observations that S. mansoni with an M.S.D. of 2.3 kr was more susceptible to the effects of radiation than S. mattheei. Despite the very low recovery of 3.0 kr parasites in experiment 16, infections with S. mattheei larvae exposed to 3.0 kr successfully produced sterile worm infections in three experiments, reported in Chapter 7, giving recoveries of 3.4, 2.7, 2.9, 3.1 and 6.1%

Interpretation of the data on the migration and site of death of irradiated S. mansoni schistosomula depends upon understanding the route of migration of unirradiated infections. Clegg and Smithers (1968) showed that about 30% of unirradiated cercariae which enter the

skin of mice die there shortly afterwards. As only 30-40% of the infecting larvae establish themselves as adult flukes a further 30-40% must die somewhere on route from the skin to the liver and mesenteries. Miller (1976) proposed a systemic route for the migration of S. mansoni in mice. Thus, schistosomula reach the lungs largely via the vascular system, although a small proportion enter the lymphatics. Following arrival in the lungs, the majority migrate through the capillary bed to enter the systemic circulation and are thus passively transported to the various organs of the body. Miller postulated that approximately 10% of the larvae would reach the liver and mesenteries at this time, the remainder migrating through the capillary beds of the various other organs of the body, eventually to return to the lungs and so commence a further systemic circuit. Histological sections reveal schistosomula in virtually every organ of the body including the brain (M. Nilsson, personal communication) thus supporting this proposed systemic route.

The negligable recovery of schistosomla from the lungs of mice infected with percutaneously applied cercariae or intramuscularly injected somules irradiated with 40 kr indicates that the larvae failed to reach and/or penetrate blood vessels in the skin or muscle and thus presumably perished at these sites of infection. Similarly, the low recovery of schistosomula from the lungs of mice infected with 20 kr larvae indicates that a large proportion must have stayed near the site of infection. The extremely low recoveries from the liver indicate that most of the 20 kr parasites that did reach the lungs died there, although a very small proportion could have died in other organs of the body during the systemic migration. The fact that no parasites were recovered from either the lungs or the liver 20 days post infection indicates that the 20 kr infection had died out by this time although the possibility that parasites may have persisted in the skin, muscle

or other organs of the body cannot be excluded. Hsü et al. (1963a) reached similar conclusions with regard to infections with S. japonicum cercariae exposed to 24 and 48 kr. Their histological sections of lung tissue revealed minimal reaction in the 24 kr infection while in the 48 kr infection no schistosomula or their remnants were detected. With neither radiation dose were schistosomula seen in liver sections. However, in the skin, lesions associated with dying larvae were most pronounced. In the 24 kr infections, degenerate schistosomula were seen in the skin up to 21 days post infection showing that, although unable to penetrate blood vessels, the larvae can survive for a relatively long period of time extravascularly. Despite the fact that such high doses evidently prevent the vast majority of parasites leaving the site of infection, Lichtenberg and Sadun (1963) reported finding a degenerate schistosomulum in the lungs of a mouse 50 days after infection with 200 cercariae irradiated with 50 kr. Furthermore, a single granuloma containing the remains of a metazoan parasite, presumably a schistosomulum, was seen in the liver sections from a similarly infected mouse in this study.

Unlike the 20 kr infection, lung recoveries from the 2.3, 4.0 and 10.0 kr infections peaked at the same time as those from the unirradiated infection and although the peak recoveries were consistently lower amongst the irradiated infections, the differences were not consistently significant. While it is possible that significant differences would have been recorded had the sample size been increased, the results do indicate that exposure of larvae to 2.3 - 10 kr does not result in marked radiation-induced mortality at the site of infection or in a delay in arrival in the lungs. Lichtenberg and Sadun (1963), however, reported that granulomas associated with dead parasites

were more common in the skin of mice infected with S. mansoni cercariae irradiated with 5 kr than in unirradiated infections. Hsü et al. (1963a) reported that exposure of S. japonicum cercariae to 6 kr did not result in an increase in deterioration of parasites in the skin or significantly alter the rate of migration, while exposure to 12 kr resulted in a slight retardation but not increased mortality. Subsequent to the peak, the lung recoveries showed a similar pattern of decline in both the irradiated and unirradiated infections giving no indication that the irradiated parasites suffered either delay or enhanced mortality in the lungs.

However, exposure to 2.3, 4 and 10 kr resulted in an altered recovery from the liver. In the unirradiated infection, the numbers of worms recovered by perfusion increased steadily from days 4 - 26. Recoveries from the 2.3 kr infections showed a parallel rise until day 12 and thereafter fell. Similarly, in experiment 11, the recoveries of 2.3 kr and unirradiated worms were virtually identical up to day 14 but by day 18 a sharp drop in recovery of 2.3 kr worms had occurred. This peak on day 14 represented a 25% recovery of irradiated parasites as compared with the 33% plateau recovery of unirradiated worms. The histopathological studies on 2.3 kr infections show that the focal lesions around dying schistosomula in the lungs, first apparent on day 12 were most numerous on day 18 becoming successively less frequent on days 24 and 32. The pronounced pathology on day 18 in the lungs together with the somewhat reduced peak recovery from the liver indicates that exposure to 2.3 kr caused a proportion of the parasites to succumb in the lungs between days 12 - 24. Furthermore, the relatively marked

drop in perfusion recoveries that occurred around day 14 - day 18 indicates a considerable mortality at around the same time amongst the worms that had reached the liver. The histological sections of liver tissue revealed that the focal lesions around dying parasites first became apparent on day 18, were most pronounced and numerous on day 24 and had declined in number and undergone considerable resolution by day 32. Lichtenberg and Sadun (1963) studying the fate of 2.5 kr worms did not look at sections of tissue taken between days 14 - 28 post infection and thus although their report of sporadic granulomas in the lungs and liver at these two times is consistent with what was found in the present study, they would have missed the time of maximum death and resultant pathology. Hsü et al. (1963a) concluded that S. japonicum parasites exposed to 1.7 or 3.0 kr underwent a relatively normal migration to the liver where the majority died between days 15 - 21.

Subsequent to the death of most of the 2.3 kr worms during the first 2 - 4 weeks of infection sporadic focal lesions were found in both the lung and the liver up to 230 days post infection. The history of the parasites responsible for the defined lesions in the lungs on days 46, 120 and 230 is uncertain. They could have been delayed in the lung and developed ectopically or moved somehow from the liver to the lung. Certainly the parasite seen in the lesion on day 46 was considerably more advanced than a lung-form schistosomulum. Although a small proportion of the 2.3 kr worms can persist for long periods of time in the portal system, sporadic degeneration will occur with resultant pathology as was observed on days 64 and 140.

The percentage recoveries, from the liver, of parasites exposed to 4 kr paralleled those from the unirradiated and 2.3 kr infections up to day 6 and thereafter showed a gradual decline. Recoveries from the 10 kr infection remained low throughout with maximum recovery on day 16. Thus as the dose of radiation is increased from 2.3 to 4.0 to 10.0 kr so fewer parasites reach, or at least are recoverable from, the liver. With both 4 and 10 kr infections focal lesions associated with dying parasites in the lungs were most frequent 13 days post infection, the numbers having declined successively by days 23 and 33. Thus of the parasites that fail to reach the liver a proportion die in the lungs. The very low recovery of 10 kr parasites from the lungs and liver 26 days post infection together with the fact that the numbers of lesions associated with degenerate parasites in both of these organs had declined by this time shows that the vast majority had perished during this first 3 - 4 weeks of infection. The parasites that escaped from the site of infection presumably died in the lungs and in the various other organs of the body during their systemic migration such that only a relatively small proportion reached and died in the liver. Larvae exposed to the lower doses 4 and 2.3 kr had an increased mean survival time this being reflected in more parasites reaching the liver and, with a dose of 2.3 kr, surviving there for long periods. The conclusions concerning the site of death of S. mansoni parasites exposed to 0 - 10 kr are intermediate between the conclusions of Lichtenberg and Sadun (1963) and those of Erickson and Caldwell (1965). Lichtenberg and Sadun (1963) concluded that the majority of schistosomula exposed to 5 kr died in the lungs whereas Erickson and Caldwell (1965) concluded that the majority of schistosomula irradiated with 8 kr died in the

liver. The former study was based entirely upon histological observations and perhaps the most critical period between 14 - 28 days post infection was not studied. Erickson and Caldwell (1965) recovered parasites from the lungs and liver and reported a peak recovery of 8.0 kr worms from the liver on day 14, representing about 30% of the number of worms ultimately recoverable from mice infected with unirradiated cercariae. By comparison, in the present study, the peak recoveries of 4.0 and 10.0 kr worms from the liver represented respectively 33% and 18% of the final recovery of unirradiated worms. Thus the 8.0 kr parasites studied by Erickson and Caldwell behaved similarly, with regard to their ability to reach the liver, to the 4 kr parasites in the present study. Hsü et al. (1963a) concluded, on the basis of histological evidence, that with an exposure of 12 kr, the skin, lungs and liver played nearly equal roles as organs of destruction of S. japonicum schistosomula.

The nature and resolution of lesions associated with dying schistosomula in the lungs and the liver were essentially as described by the above authors. The close association of the dying and degenerating parasites with the surrounding inflammatory cells of the host would seem to provide an ideal site for recognition of parasite antigens, both metabolic and somatic. This idea that the schistosomular granuloma may be important as a site of generation of an immune response is further developed in Chapter 6. Although such lesions show complete resolution the clinical significance of the transient pathology remains unknown. Furthermore, the consequences of irradiated parasites dying in other organs of the body during their systemic migration have not been investigated.

CHAPTER 6. STUDIES ON THE DEVELOPMENT OF RESISTANCE FOLLOWING EXPOSURE TO RADIATION-ATTENUATED S. MANSONI INFECTIONS.

6.1 Introduction.

Resistance following infection with radiation-attenuated cercariae has been studied in a variety of host/schistosome systems (S. mansoni: Mouse - Villella et al (1961), Radke and Sadun (1963), Perlowagora-Szumlewicz and Olivier (1963), Perlowagora-Szumlewicz (1964a, 1964c, 1966), Erickson and Caldwell (1965), Murrell et al. (1975); Rat - Smithers and Terry (1965c); Rhesus monkey - Smithers (1962), Sadun et al. (1964), Hsü et al. (1969); Baboon - Taylor et al. (1976a). S. japonicum: Mouse - Hsü et al.(1965a); Rhesus monkey - Hsü et al. (1962, 1963b, 1965b, 1969, 1975); Chimpanzee - Hsü (1970). S. incognitum: Mouse - Tewari and Biswas (1972). S. mattheei: Sheep - Taylor et al (1976b).

The studies described here were designed to confirm and extend the results of previous investigations in order to provide a more complete analysis of the parameters involved in the induction of resistance against S. mansoni in the mouse. An efficient and reproducible vaccinating procedure would provide a useful system for studying the mechanism of resistance thus avoiding the need to use pathogenic whole infections. Furthermore, it was hoped that these studies would help establish the feasibility of developing a live vaccine against schistosomiasis.

6.2 Effect of radiation dose.

In the majority of reported studies mice were vaccinated with cercariae exposed to radiation doses of up to 10 kr and resistance assessed by challenge of the mice 6-10 weeks later. Within this range of radiation doses the most successful protection was achieved with the low doses which allowed survival of a small proportion of stunted parasites (Villella et al, 1961; Radke and Sadun, 1963). Therefore, the initial experiments reported here were designed using an essentially similar protocol to that employed by these latter two groups.

6.2.1 Experiment 18.

Groups of T.O.(A.S.L.) mice were infected with 200 cercariae irradiated with 3.0, 6.0 or 10.0 kr, challenged 8 weeks later, together with challenge controls, with 100 cercariae and perfused 8 weeks post challenge. A group of 5 mice infected with 3.0 kr cercariae were unchallenged serving as vaccine controls.

The worm recoveries are presented in Table 9. No worms were recovered from the vaccine controls. The mean worm recoveries from all vaccinated groups were significantly lower than from the challenge controls. There was no significant difference between the mean recoveries from mice vaccinated with 3.0 and 6.0 kr cercariae but both were significantly lower than the mean recovery from mice vaccinated with 10.0 kr cercariae ($P < 0.01$ and $P < 0.02$ respectively).

TABLE 9. Experiment 18.

Worm recoveries from mice vaccinated with 200 irradiated cercariae, challenged 8 weeks later with 100 cercariae and perfused 8 weeks post challenge

Group	Number of mice per group	Radiation dose (Kr)	Mean worm recovery (S.D.) ♂	♀	Σ	Percent Reduction	Significance (P.)
1. Vacc.+ Chall.	17	3.0	4.7(3.8)	6.5(5.6)	11.2(8.9)	59	<0.001
2. Vacc.+ Chall.	19	6.0	5.2(2.9)	8.1(4.4)	13.3(6.9)	52	<0.001
3. Vacc.+ Chall.	22	10.0	7.3(3.6)	12.7(6.1)	20.0(8.8)	27	<0.05
4. Ch. Co.	10	-	10.9(2.5)	16.6(5.9)	27.5(8.1)	-	

6.2.2 Experiment 19.

This experiment was essentially similar to the previous one but included a group vaccinated with cercariae exposed to 2.3 kr, the dose selected in chapter 5 for producing a persistent sterile worm infection.

T.O.(A.S.L.) mice in each of three groups were infected with 260 cercariae irradiated with 2.3, 3.0 or 6.0 kr. Two subgroups from those infected with 2.3 and 3.0 kr cercariae were not challenged serving as vaccine controls. The remainder of the mice, together with a group of challenge controls, were challenged with 100 cercariae 8 weeks after vaccination. All the mice, including the vaccine controls were perfused 8 weeks post challenge.

The worm recoveries are shown in Table 10. The vaccine controls for the 2.3 kr infection harboured a mean worm burden of 10.6 $\pm$ 4.8 worms. A few malformed eggs were seen in the liver squash preparations from one of the eight mice but no miracidia were seen in the hatching test. The mean worm recovery from the 3.0 kr infections was 1.1 $\pm$ 0.9. 2 of the 6 mice were parasite free and all of the worms in the remaining mice were extremely stunted. There were no significant differences between the challenge-derived worm recoveries (corrected for worms persisting from the vaccination procedures) from any of the vaccinated groups but all 3 were significantly lower than the mean worm recovery from the challenge controls.

TABLE 10. Experiment 19.

Worm recoveries from mice vaccinated with 260 irradiated cercariae, challenged 8 weeks later with 100 cercariae and perfused 8 weeks post challenge.

Group	Number of mice per group	Radiation dose (kr)	Mean worm recovery (S.D.) ♂	♀	⚥	Mean worm recovery [1] from challenge (S.D.)	Percent reduction	Significance (P)
1. Vacc. Co.	8	2.3	3.8(2.8)	6.8(2.7)	10.6(4.8)			
2. Vacc.+ Chall.	15	2.3	8.9(4.9)	12.0(4.0)	20.9(8.3)	10.3(8.3)	51	<0.01
3. Vacc. Co.	6	3.0			1.1(0.9)[2]			
4. Vacc.+ Chall.	14	3.0	6.3(2.5)	7.7(2.6)	14.0(4.5)	12.9(4.5)	38	<0.02
5. Vacc.+ Chall.	14	6.0	5.9(1.7)	6.2(2.6)	12.1(3.9)	12.1(3.9)	42	<0.01
6. Chall.Co.	11	-	9.7(4.9)	11.2(4.9)	20.9(9.6)	20.9(9.6)		

Notes - 1. Calculated where necessary by subtraction of the mean number of worms in the vaccine controls from the mean number in the vaccinated and challenged group.

2. All of the worms recovered from these animals were very stunted and the majority of indeterminate sex.

6.2.3 Experiment 20.

This experiment was designed to test whether C B A mice could be similarly partially protected. In addition to the radiation doses 2.3 and 6.0 kr that had been successful used above, a dose of 30 kr was also included as such high doses had been successfully used by Hsü et al.(1969) in rhesus monkeys. Vaccination by injection of 500 irradiated somules was chosen as a result of the findings in experiment 26, section 6.5, that such vaccination could be as effective as percutaneous infection with cercariae. Challenge was delayed until 12 weeks post vaccination because it was thought possible that the persistent survivors of the 2.3 kr would by that time have boosted the resistance.

Groups of C B A mice were vaccinated by intramuscular injection of 500 somules irradiated with 2.3, 6.0 or 30 kr. The mice were challenged 12 weeks later with 100 cercariae and perfused 8 weeks post challenge. Appropriate vaccine- and challenge-controls were included.

The worm recoveries are presented in Table 11. The vaccine controls for the 2.3 kr infection harboured a mean of 5.0 $\pm$ 3.1 sterile worms; no eggs were seen in liver squash preparations from any of the mice. The challenge-derived worm recoveries from all 3 vaccinated groups were significantly lower than from the challenge controls. The greatest degree of resistance was observed in the group vaccinated with

TABLE 11. Experiment 20.

Worm recoveries from C B A mice vaccinated with 500 somules irradiated with 2.3-30 kr, challenged 12 weeks later with 100 cercariae and perfused 8 weeks post challenge.

Group	Number of mice per group	Radiation dose (kr)	Mean worm recovery (S.D.) ♂	♀	Σ	Mean worm recovery from challenge (S.D.)	Percent reduction	Significance (P.)
1. Vacc Co.	8	2.3	1.9(1.0)	3.1(2.0)	5.0(3.1)			
2. Vacc.+ Chall.	13	2.3	15.8(4.6)	8.4(3.4)	24.2(5.6)	19.2(5.6)	37	<0.001
3. Vacc.+ Chall.	12	6.0	14.8(6.1)	6.3(3.7)	21.1(8.3)	21.1(8.3)	31	<0.01
4. Vacc.+ Chall.	14	30.0	12.0(4.4)	4.9(3.3)	16.9(7.2)	16.9(7.2)	45	<0.001
5. Chall. Co.	14	-	20.9(4.1)	9.6(2.7)	30.5(6.8)	30.5(6.8)		

30 kr somules although there were no significant differences between the worm recoveries from any of the vaccinated groups.

6.2.4 In view of the success achieved by vaccination with somules irradiated with 30 kr, a further experiment was set up to investigate the protective effect of somules exposed to even higher doses of radiation (20-160 kr). A group of mice given a primary infection with unirradiated parasites was included for comparison.

Experiment 21.

Groups of male C B A mice were intramuscularly injected with 500 somules irradiated with 20, 40, 80 or 160 kr. Mice in another group were injected with 35 unirradiated somules. The larvae exposed to all of the radiation doses appeared normally active at the time of injection. All the mice were challenged with 200 cercariae 10 weeks post vaccination and perfused 21 days post-challenge (see Appendix 1).

The mean worm recoveries are presented in Table 12. The mean challenge-derived worm recoveries from all 5 of the experimental groups were significantly lower than from the challenge controls. The highest degree of resistance was observed in the mice infected with 35 unirradiated somules. The mean challenge-derived worm recovery from these mice, although significantly lower than that from the groups vaccinated with 40 and 160 kr somules ($P < 0.05$ and $P < 0.02$ respectively), was not significantly different from that from the groups vaccinated with 20 and 80 kr somules. The only significant difference between the worm recoveries from the vaccinated groups was between those infected with 20 kr and 160 kr somules ($P < 0.05$).

TABLE 12. Experiment 21.

Worm recoveries from mice given a primary (1^{o}) infection of 35 cercariae or vaccinated intramuscularly with 500 somules irradiated with 20 - 160 kr, challenged 10 weeks later with 200 cercariae and perfused 21 days post challenge

Group	Number of mice per group	Radiation dose (Kr)	Mean worm recovery from challenge	Percent reduction	Significance (P.)
1. 1^{o} + Chall	7	0	16.7(11.2) [1]	70	< 0.001
2. Vacc.+ Chall.	12	20	22.1(12.3)	60	< 0.001
3. Vacc.+ Chall.	10	40	26.3(6.7)	52	< 0.001
4. Vacc.+ Chall.	10	80	26.8(9.7)	51	< 0.001
5. Vacc.+ Chall.	10	160	37.8(18.9)	31	< 0.05
6. Chall. Co.	-	-	54.8(13.5)		

Note. 1. Mean recovery of primary infection worms in group 1 was ♂-4.4 ± 1.0, ♀-4.3 ± 1.7, Total 8.7 ± (1.7).

6.2.5. The results of the above 4 experiments indicate that the degree of resistance apparent upon challenge of mice 8-12 weeks post vaccination is comparable whether the vaccinating cercariae or somules are exposed to a low dose of radiation (2.3 kr) which allows persistance of a small proportion of parasites as sterile adults or to higher doses (20 kr +) which appear to prevent the vast majority of parasites from leaving the site of infection (see Chapter 5). Furthermore, the level of such resistance can be almost as high as that induced by a primary infection with unirradiated parasites.

6.3 Time of development and persistence of resistance in relation to radiation dose.

In the majority of experiments reported in the literature and in all of those experiments described in section 6.2 the efficacy of vaccination of mice with S. mansoni cercariae or somules irradiated with varying doses of gamma- or x-rays has been assessed by challenge at one time point, usually 6-12 weeks post vaccination. The experiments in this section investigate the relationship between the times of development and persistence of resistance and the dose of attenuating radiation.

6.3.1 The first experiment in this section is concerned with the time of development and persistence of resistance following vaccination with somules irradiated with 40 kr.

Experiment 22.

Five groups of C B A mice were vaccinated by intramuscular injection of 500 somules irradiated with 40 kr. At intervals of 1, 2, 4, 8 or 16 weeks post vaccination, the mice in one of these groups were challenged with 200 cercariae. Appropriate challenge controls were included and the mice were perfused 6 weeks post challenge.

The worm recoveries are presented in Table 13. The mean worm recovery from mice challenged one week post vaccination was not significantly different from that of the controls but the mean worm recoveries from vaccinated mice challenged thereafter were significantly lower than from their respective challenge controls. The level of resistance of mice challenged 2, 4, 8 and 16 weeks post vaccination remained reasonably constant, viz. 39, 26, 28 and 31%. In summary, therefore, the resistance to challenge induced by vaccination with 40 kr somules first becomes apparent between 1 and 2 weeks post vaccination and persists at a relatively constant level at least until 16 weeks post vaccination.

6.3.2 The next 2 experiments were designed to compare the time of onset and persistence of resistance induced by somules exposed to 40 kr and lower doses of radiation.

Experiment 23.

Groups of C B A mice were vaccinated by intramuscular injection of somules irradiated with 2.3, 10, 20 or 40 kr. Half of the

TABLE 13. Experiment 22.

Worm recoveries from mice vaccinated with 500 somules irradiated with 40 kr, challenged 1-16 weeks later with 200 cercariae and perfused 6 weeks post challenge

Group	Number of mice per group	Interval to Challenge (weeks)	Mean worm recovery (S.D.) ♂	♀	Σ	Percent reduction	Significance (P.)
1. Vacc.+ Chall.	9	1	41.4(6.7)	42.1(10.1)	83.6(15.4)	-3	>0.7
2. Chall. Co.	9	1	43.6(9.6)	37.4(8.7)	81.0(14.8)		
3. Vacc.+ Chall.	10	2	23.3(5.7)	24.2(7.2)	47.5(11.4)	39	<0.001
4. Chall. Co.	10	2	39.3(8.7)	38.5(6.1)	77.8(11.6)		
5. Vacc.+ Chall.	10	4	37.8(6.9)	28.6(7.3)	66.4(12.6)	26	<0.001
6. Chall. Co.	10	4	48.3(6.1)	41.1(7.0)	89.4(11.1)		
7. Vacc.+ Chall.	10	8	32.2(10.2)	11.2(4.8)	43.4(13.6)	28	<0.01
8. Chall. Co.	10	8	42.9(8.7)	17.3(3.7)	60.2(10.8)		
9. Vacc.+ Chall.	10	16	30.0(7.1)	24.7(7.5)	54.7(13.7)	31	<0.001
10. Chall. Co.	10	16	45.2(7.6)	33.9(5.0)	79.1(7.5)		

mice in each group were challenged 23 days later with 200 cercariae and the other half similarly challenged 56 days later. Appropriate challenge controls were included at each time. The mice were perfused 6 weeks post challenge.

The worm recoveries are presented in Table 14. For the 23 day challenge the mean worm recoveries from mice vaccinated with 10, 20 and 40 kr somules were significantly lower than from the challenge controls but the mean recovery from the 2.3 kr group was not significantly different. The greatest degree of resistance occurred in the 20 kr group (41%) although the mean worm burden in this group was not significantly different from the mean worm burdens in either the 10 kr or 2.3 kr groups and only marginally significantly different ($P = 0.05$) from that in the 40 kr group. (The influence of the unusually high standard deviation of the mean worm burden from the 2.3 kr group (group 2) on the result of the 't' test should, however, be noted).

By 8 weeks post vaccination this pattern had altered. The mean worm burdens in the 2.3, 20 and 40 kr groups were significantly lower than in the controls and also than in the 10 kr group, the mean recovery from which was not significantly different from the controls. As in experiment 22, the degree of resistance manifest at 8 weeks by the 40 kr group was essentially similar to that apparent at the earlier time of 23 days. Similarly, the degree of resistance in the 20 kr groups had remained essentially the same. However, the level of resistance of the 2.3 kr group had more than doubled while that of the 10 kr group had more than halved.

TABLE 14. Experiment 23.

Worm recoveries from mice vaccinated with 500 somules irradiated with 2.3-40 kr, challenged either 23 or 56 days later with 200 cercariae and perfused 5 weeks post challenge.

	Group	Number of mice per group	Radiation dose (Kr)	Mean worm recovery (S.D.) ♂	♀	⚥	Percent reduction	Significance (P.)
23 day challenge	1. Vacc. Co.	10	2.3	2.0(1.3)	2.3(1.9)	4.3(2.0)		
	2. Vacc.+ Chall.	10	2.3	43.7(15.8)	30.4(10.8)	74.1(25.0)	21	>0.05
	3. Vacc.+ Chall.	10	10.0	33.4(12.0)	25.4(8.3)	58.7(17.3)	34	<0.001
	4. Vacc.+ Chall.	10	20	30.5(11.7)	21.7(7.2)	52.2(17.8)	41	<0.001
	5. Vacc.+ Chall.	10	40	38.0(9.1)	28.4(4.6)	66.4(11.8)	25	<0.01
	6. Chall. Co.	10	-	49.3(9.2)	39.2(8.2)	88.5(15.4)		
56 day challenge	7. Vacc. Co.	9	2.3	1.3(1.1)	2.4(1.0)	3.8(1.7)		
	8. Vacc.+ Chall.	10	2.3	23.9(7.9)	21.7(8.1)	45.6(14.7)	47	<0.001
	9. Vacc.+ Chall.	9	10.0	36.6(10.8)	30.4(7.2)	67.0(17.3)	15	>0.1
	10. Vacc.+ Chall.	10	20	23.3(10.4)	17.3(5.6)	40.6(15.0)	49	<0.001
	11. Vacc.+ Chall.	10	40	33.0(8.8)	28.8(7.8)	61.8(14.()	22	<0.02
	12. Chall. Co.	9	-	44.1(5.3)	34.9(10.6)	79.0(14.3)		

Thus it appeared that somules exposed to increasing doses of radiation up to 20 kr produced increasing degrees of resistance early post vaccination. By 8 weeks, however, while the resistance induced by 20 or 40 kr somules had remained constant, that induced by 2.3 kr somules, which produced a sterile worm infection, had increased.

6.3.3 It was of obvious interest to see what would happen to the relative levels of resistance induced by organisms exposed to different radiation doses if challenge were delayed even longer. Thus a comparison was made between the resistance apparent 6 weeks post vaccination and that apparent at 17 weeks. For comparative purposes a group of mice given a primary infection with 35 unirradiated cercariae was included for the 6 week challenge.

Experiment 24.

Groups of C B A mice were vaccinated by percutaneous exposure to 500 cercariae irradiated with 2.3, 10, 20 or 40 kr. Mice in a further group were given a primary exposure to 35 unirradiated cercariae. This latter group together with half of the mice in each of the vaccinated groups were challenged 6 weeks post vaccination. The remainder of the mice were challenged 17 weeks post vaccination. Appropriate challenge controls were included at each time. Mice were perfused 21 days post challenge.

The worm recoveries are presented in Table 15. The mean challenge-derived worm burdens in all of the vaccinated groups were significantly lower than in the challenge controls at both 6 and 17 weeks.

TABLE 15. Experiment 24.

Worm recoveries from mice given a primary exposure to 35 unirradiated cercariae or vaccinated with 500 cercariae irradiated with 2.3-40 kr, challenged 6 or 17 weeks later with 200 cercariae and perfused 21 days post challenge

	Group	Number of mice per group	Radiation dose (Kr)	Mean adult worm recovery (S.D.) ♂	♀	Σ	Mean worm recovery from challenge	Percent reduction	Significance (P.)
6 week challenge	1. 1° + Chall.	9	0	6.4(2.3)	5.0(1.5)	11.4(3.7)	8.1(6.9)	75	<0.001
	2. Vacc.+ Chall.	10	2.3	2.8(1.8)	4.9(2.5)	7.7(3.6)	17.6(8.5)	46	<0.001
	3. Vacc.+ Chall.	8	10.0				14.4(6.2)	56	<0.001
	4. Vacc.+ Chall.	10	20				11.5(3.6)	65	<0.001
	5. Vacc.+ Chall.	10	40				19.9(4.8)	39	<0.001
	6. Ch. Co.	10	-				32.4(5.9)		
17 week challenge	7. Vacc.+ Chall.	10	2.3	2.6(2.1)	3.1(1.8)	5.7(3.2)	20.5(5.2)	65	<0.001
	8. Vacc.+ Chall.	10	10.0				40.1(17.4)	32	<0.02
	9. Vacc.+ Chall.	10	20				32.9(9.2)	45	<0.001
	10. Vacc.+ Chall.	10	40				40.1(13.1)	32	<0.01
	11. Ch. Co.	10	-				59.3(13.2)		

At 6 weeks maximum resistance was observed in the group infected with unirradiated cercariae. Mean worm recoveries from the groups vaccinated with cercariae irradiated with 2.3, 10 and 20 kr were not significantly different, but the recovery from the 40 kr group was significantly higher than from either the 10 kr group ($P = 0.05$) or the 20 kr group ($P < 0.001$). Whilst this pattern is essentially similar to that observed following the 8 week challenge in experiment 6 with regard to the 2.3, 20 and 40 kr vaccinations, the results for the 10 kr vaccination are somewhat different. The reasons for this discrepancy are not apparent.

By 17 weeks the degree of resistance in the mice vaccinated with 2.3 kr cercariae had increased while that in the 10, 20 and 40 kr groups had declined such that the mean worm recovery from the 2.3 kr group was significantly lower than from the mice vaccinated with 10 kr ($P < 0.01$), 20 kr ($P < 0.01$) or 40 kr ($P < 0.001$) cercariae.

6.3.4 Without laying too much emphasis on the statistical differences observed above, certain clear trends emerge from the above 3 experiments. The resistance following vaccination with irradiated somules first becomes apparent between 1 and 2 weeks. The resistance apparent 3 weeks following vaccination is greatest in mice vaccinated with 20 kr somules, and shows a fall off as the radiation dose is decreased to 2.3 kr or increased to 40 kr. The level of resistance induced by 40kr somules remains relatively constant between 2 and 16 weeks. Similarly the resistance induced by 20 kr cercariae is comparable at 3 and 8 weeks but appears to drop a little between 6 and 17 weeks. In experiment 25, section 6.4, there was a slight decline in

the level of resistance induced by 20 kr cercariae between 3 weeks and 8 weeks. The results with 10 kr vaccination are somewhat conflicting but both experiments 23 and 24 indicate a decline of resistance with time. In contrast, the resistance following vaccination with 2.3 kr somules increases with time such that by 8 weeks it is comparable with that induced by 20 kr organisms and by 17 weeks somewhat higher.

6.4 Comparison of the resistance induced by large numbers of irradiated cercariae, large numbers of unirradiated cercariae of one sex and small numbers of cercariae of both sexes.

The increase in resistance with increasing radiation dose apparent at 3 weeks, described above, indicated that the radiation treatment of the larvae was in some way fundamental to the development of resistance at this time. The increase of resistance with time in mice vaccinated with 2.3 kr cercariae or somules could be attributed to a concomitant type immunity (Smithers and Terry, 1968) stimulated by the small proportion of surviving sterile worms. This interpretation is, however, inconsistent with the fact that infection with worms of one sex fails to stimulate significant resistance to challenge 8 weeks post primary infection (see Chapter 4). Relevant to both of these points are the results of the following experiment in which a comparison was made between the resistance apparent 3 weeks and 8 weeks following infection with irradiated cercariae, unirradiated male cercariae or unirradiated cercariae of both sexes.

Experiment 25.

Groups of CBA mice were infected percutaneously with 35 unirradiated cercariae of mixed sex, 300 unirradiated male cercariae or 500 of mixed sex irradiated with 20 kr. Three weeks later half of the mice in each of these groups were challenged with 200 cercariae, the remaining half being similarly challenged 8 weeks after infection. Appropriate challenge controls were included at each of these times. All mice were perfused 21 days post challenge.

The worm recoveries are presented in Table 16. The mice infected with irradiated cercariae showed a marked resistance to challenge 3 weeks post vaccination (80% fewer worms than the controls, $P < 0.001$), while those given a primary bisexual infection were only marginally resistant (21%, $P < 0.05$) and those infected with unirradiated male cercariae were not significantly resistant (13%, $P > 0.1$). By 8 weeks, the mice harbouring the primary bisexual infection had developed a high degree of resistance to challenge (73%, $P < 0.001$). The reason for the much lower primary worm burden in the group challenged 8 weeks after infection compared with that challenged at 3 weeks is unknown. Resistance in the group infected with irradiated cercariae had dropped from that evidenced 3 weeks post vaccination, although remained highly significant (58%, $P < 0.001$). As in experiment 3, Chapter 4, the mice infected with 300 male cercariae showed no evidence of resistance against the 8 weeks challenge.

TABLE 16. Experiment 25.

Worm recoveries from mice infected with 35 cercariae of mixed sexes, 300 male cercariae or 500 cercariae irradiated with 20 kr, challenged 3 or 8 weeks later with 200 cercariae and perfused 21 days post challenge.

	Nature of initial infection	Number of mice per group	Mean adult worm recovery(S.D.) ♂	♀	♂+♀	Mean worm recovery from challenge	Percent reduction	Significance (P.)
3 week challenge	35 (♂+♀)	10	7.8(1.4)	9.0(2.5)	16.8(3.1)	73.0(17.0)	21	<0.05
	300 (♂)	8	109.5(51.2)	-	109.5(51.2)	79.6(16.8)	13	>0.1
	500 (20kr)	9	-	-	-	18.6(8.0)	80	<0.001
	Ch. Co.	10				91.9(18.3)		
8 week challenge	35 (♂+♀)	8	4.0(1.8)	3.3(2.0)	7.3(3.3)	11.6(10.7)	73	<0.001
	300 (♂)	9	122.4(26.3)	-	122.4(26.3)	40.1(13.7)	7	>0.5
	500 (20kr)	10	-	-	-	17.9(6.6)	58	<0.001
	Ch. Co.	10				43.0(8.6)		

Thus there are evidently fundamental differences between the time course of development of resistance following the three types of infection described. Resistance following exposure to small numbers of cercariae of both sexes develops slowly, increasing markedly between 3 weeks and 8 weeks. Resistance following exposure to large numbers of cercariae irradiated with 20 kr, however, develops rapidly, being highly significant just 3 weeks after infection and appearing to decline a little between 3 weeks and 8 weeks. Most interesting, in view of the success with irradiated cercariae is the demonstration that 300 unirradiated male cercariae which produce a massive worm burden fail to stimulate significant resistance to challenge given either 3 weeks or 8 weeks post primary infection.

6.5 Effect of size of vaccinating infection.

Villella et al. (1961), Radke and Sadun (1963) and Erickson and Caldwell (1965) reported that increasing the number of irradiated cercariae to which mice were exposed did not result in an increasing level of resistance. This apparent lack of dose dependancy was investigated in two experiments, one involving 2.3 kr cercariae and the other 40 kr somules.

6.5.1 In this study tissue egg burdens were assessed in order to test whether the observed protection in terms of worm recoveries was reflected in reductions of tissue egg load. In addition to the investigation of dose dependancy, this experiment also included the first attempt to induce resistance with intramuscularly injected somules or

skin-somules. The influence of the size of challenge dose on the degree of resistance was also investigated.

Experiment 26.

Cercariae or schistosomula irradiated with 2.3 kr were used for vaccination. Groups of T.O.(A.S.L.) mice were infected with 500, 300 or 200 cercariae or injected intramsucularly with 210 somules or skin-somules, as shown in Table 17. Vaccine controls were included for the mice vaccinated with cercariae, but insufficient somules or skin-somules were obtained for the establishment of vaccine controls for the groups vaccinated with such larvae. 8 weeks post vaccination the mice were challenged with 100 cercariae except for one group of mice from those vaccinated with 200 cercariae, which was challenged with 300 cercariae. Appropriate challenge controls were included at this time. 60 days post challenge all of the mice were perfused and the livers and guts of the mice vaccinated with 500, 300 and 200 irradiated cercariae and of the challenge controls were removed and their tissue egg loads estimated following digestion.

The mean worm and egg burdens are presented in Tables 17 and 18 respectively. The vaccine control mice infected with 500, 300 and 200 cercariae harboured mean worm burdens of 9.3 $\pm$ 4.6, 4.4 $\pm$ 1.8 and 3.5 $\pm$ 2.1 respectively. None of the mice were parasite free. Eggs were seen in the liver of one of the mice infected with 500 cercariae but these proved non-viable in the hatching test. All of the vaccination treatments produced significant protection in terms of worm burden

TABLE 17. Experiment 26.

Worm recoveries from mice vaccinated with varying numbers of cercariae or somules irradiated with 2.3 kr, challenged 8 weeks later with 100 or 300 cercariae and perfused 60 days post challenge.

Group	Number of mice per group	Number of irradiated larvae	Number of challenge cercariae	Mean worm recovery (S.D.) ♂	♀	⚥	Mean worm recovery from challenge (S.D.)	Percent reduction	Significance (P.)
1. Vacc. Co.	7	500	-	3.1(2.1)	6.1(3.4)	9.3(4.6)			
2. Vacc.+ Chall.	14	500	100	14.2(5.1)	14.0(5.1)	28.2(9.9)	18.9(9.9)	55	<0.001
3. Vacc. Co.	6	300	-	1.2(0.9)	3.2(1.7)	4.4(1.8)			
4. Vacc.+ Chall.	16	300	100	11.6(4.2)	11.5(4.4)	23.1(8.2)	18.7(8.2)	55	<0.001
5. Vacc. Co.	6	200	-	1.5(0.9)	2.0(1.4)	3.5(2.1)			
6. Vacc.+ Chall.	13	200	100	13.9(4.0)	14.6(4.9)	28.5(8.8)	25.0(8.8)	40	<0.001
7. Vacc.+ Chall.	8	200	300	25.0(5.5)	31.2(13.7)	56.2(16.4)	56.2(16.4)	41	<0.001
8. Vacc.+ Chall.	8	210[1]	100	10.5(4.0)	11.3(3.4)	21.8(6.2)	21.8(6.2)	48	<0.001
9. Vacc.+ Chall.	8	210[2]	100	10.0(3.7)	10.0(5.1)	20.0(8.6)	20.0(8.6)	52	<0.001
10. Ch. Co.	9	-	100	19.8(6.1)	22.0(5.8)	41.8(11.6)	41.8(11.6)	-	
11. Ch. Co.	6	-	300	43.2(6.5)	51.3(9.2)	94.5(15.8)	94.5(15.8)	-	

Notes - 1. skin-somules I.M.
2. somules I.M.

TABLE 18. Experiment 26.

Mean tissue egg recoveries

Group	Mean number of eggs per animal (S.D.) Liver	Gut	Total	Percent reduction	Significance (P.)
2 [1]	15,802(6,185)	16,518(6.766)	31,320(11,482)	61	<0.001
4	18,077(7,917)	17,653(6,528)	35,730(13,261)	55	<0.001
6	23,386(7,681)	28,759(8,103)	52,145(14,266)	35	<0.01
10	33,222(12,621)	47,000(16,320)	80,222(28,566)	-	

Note- 1. Refer to table 17.

and no significant differences were recorded between the mean challenge derived worm burdens from any of the vaccinated groups. The mean tissue egg loads of mice vaccinated with 500 and 300 cercariae were not significantly different but both were significantly lower than that of the mice vaccinated with 200 cercariae ($P < 0.001$, $P < 0.01$ respectively). The reductions in tissue egg load, 61, 66 and 35% respectively in the groups vaccinated with 500, 300 and 200 cercariae correlate reasonably well with the corresponding reductions in total worm burden, i.e. 55, 55 and 40% respectively. However, the number of worm pairs is the important measure of egg production. By subtraction of the mean numbers of male and female worms in the vaccine controls from the mean numbers of males and females respectively in the vaccinated and challenged animals, it is possible to estimate the mean number of challenge derived worm pairs. Thus the mean number of challenge derived worm pairs in the groups vaccinated with 500, 300 and 200 cercariae were calculated as 7.9, 8.3 and 12.4 which in fact represent the mean number of female worms (the less predominant sex). In relation to the mean number of worm pairs in the challenge controls, 19.8, these figures represent reductions of 60, 58 and 37% respectively, which correlate very well with the reductions in egg load. This close correlation validates the practice of subtracting the mean worm burden in the vaccine controls from that in the vaccinated and challenged mice in order to calculate the percentage resistance to challenge. This apparent dose dependancy of resistance assessed by egg burden resulted from an imbalance in the male/female worm ratio in the vaccinated animals compared with the controls, i.e. it appeared that resistance was more effective against females than males. Such an imbalance is

not characteristic of the results obtained in the other experiments reported here and thus this apparent dose dependancy based on egg burden is considered an artifact.

6.5.2 Experiment 27.

Groups of C B A mice were vaccinated by intramuscular injection of 50, 100, 200, 500, 1000 or 2000 somules irradiated with 40 kr. Eight weeks later these mice, together with a group of challenge controls, were challenged with 200 cercariae. The mice were perfused 6 weeks post challenge.

The worm recoveries are presented in Table 19. The mean worm recoveries from all of the vaccinated groups were significantly lower than from the controls. The mean worm recovery from the mice vaccinated with 50 somules was significantly higher than the recoveries from all of the other vaccinated groups except for the groups vaccinated with 200 somules. There were no other significant differences between any of the groups.

6.5.3 The results of these two experiments fail to show any consistant dose dependancy of resistance following vaccination with cercariae or somules irradiated with 2.3 or 40 kr. Although the degree of resistance in the latter experiment was somewhat more consistant with 500-2000 somules than with 50-200, 100 somules stimulated comparable resistance to that induced by 2000 somules. A minimum threshold in the number of cercariae required to stimulate resistance was not detected although, with the exception of the group vaccinated with 200 somules, all of the

TABLE 19. Experiment 27.

Worm recoveries from mice vaccinated with 50-2000 somules irradiated with 40 kr, challenged with 200 cercariae 8 weeks later and perfused 6 weeks post challenge.

Group	Number of mice per group	Number of irradiated somules per mouse	Mean worm recovery (S.D.) ♂	♀	♂+♀	Percent reduction	Significance (P.)
1. Vacc.+ Chall.	10	50	41.2(10.0)	30.8(7.5)	72.0(15.8)	24	<0.01
2. Vacc.+ Chall.	10	100	28.0(6.8)	25.7(6.3)	53.7(12.7)	45	<0.001
3. Vacc.+ Chall.	10	200	37.4(13.7)	30.6(7.9)	68.0(20.9)	29	<0.01
4. Vacc.+ Chall.	10	500	30.8(10.5)	22.8(6.1)	53.6(13.5)	46	<0.001
5. Vacc.+ Chall.	9	1000	29.7(7.3)	27.7(8.3)	57.3(13.3)	40	<0.001
6. Vacc.+ Chall.	10	2000	29.9(9.8)	26.7(8.7)	56.6(16.5)	40	<0.001
7. Ch. Co.	9	-	54.6(8.2)	40.4(9.4)	95.0(8.4)		

groups vaccinated with 100-2000 somules harboured significantly fewer worms than the groups vaccinated with 50.

6.6 Resistance induced by multiple compared with single vaccination.

There is very little evidence that the resistance induced in primates or rodents by vaccination with irradiated parasites can be enhanced by giving multiple vaccination. Nevertheless, comparative studies on resistance induced by single and multiple vaccinations with cercariae exposed to a range of radiation doses have not been performed.

Experiment 28.

Cercariae were exposed to 2.1, 6.0 or 30 kr. (The dose used to establish a 'sterile' worm population in this experiment was 2.1 kr as the M.S.D. for S. mansoni (see Chapter 3) had not been established at the time.) Groups of T.O.(B.K.) mice were infected with cercariae exposed to one of these doses according to the following regimen. At the first vaccination, the first group received 600 cercariae, the second 300 and the third 200. 4 and 8 weeks later, the third group received a further 200 cercariae. 6 weeks after the first vaccination the second group received a second exposure to 300 cercariae. Owing to a technical failure, the group which should have received 3 vaccinations with 200 cercariae irradiated with 6.0 kr was removed from the experiment. From each of the three groups vaccinated with 2.1 kr cercariae a subgroup was established, which was unchallenged, serving as a vaccine control group. The remainder of the mice were challenged 12

weeks after the first vaccination with 100 cercariae as also were a group of challenge controls. All of the mice were perfused 8 weeks post challenge.

The mean worm recoveries are presented in Table 20. The vaccine control mice vaccinated on 1, 2 or 3 occasions with cercariae irradiated with 2.1 kr harboured comparable worm burdens (26.8 $\pm$ 7.1, 27.8 $\pm$ 8.0, 25.0 $\pm$ 9.7 respectively), indicating that the first and second vaccinations had not stimulated resistance against the irradiated parasites from subsequent exposures. Although the livers of these vaccinated mice were not routinely examined for the presence of eggs, granulomas were observed on macroscopic examination of the liver surfaces of a number of the mice showing that 2.1 kr had not produced a sterile worm infection. There were no significant differences between the challenge derived worm recoveries from mice vaccinated on 1, 2 or 3 occasions with 2.1 kr parasites although only the groups vaccinated on 1 or 2 occasions harboured worm burdens significantly different from the controls. The groups vaccinated on 1 or 2 occasions with 6.0 kr cercariae were significantly resistant and, although the resistance stimulated by one vaccination was very low, the mean worm burden was not significantly different from that in the group vaccinated on two occasions. All three groups vaccinated with cercariae irradiated with 30 kr were significantly resistant to challenge and there were no significant differences between the mean worm burdens following single and multiple vaccinations. The degrees of resistance stimulated by 30 kr cercariae were consistently higher than with either 2.1 or 6.0 kr.

TABLE 20. Experiment 28.

Worm recoveries from mice vaccinated with cercariae irradiated with 2.1, 6.0 or 30 kr on 1-3 occasions with a total of 600 cercariae, challenged with 100 cercariae 12 weeks after the first vaccination and perfused 8 weeks later.

Group	Number of mice per group	Radiation dose (kr)	Vaccination schedule	Mean worm recovery (S.D.) ♂	♀	⚥	Mean worm recovery from challenge(S.D.)	Percent reduction	Significance
1. Vacc. Co.	8	2.1	1 x 600 (week 0)	7.8(2.3)	19.0(5.9)	26.8(7.1)			
2. Vacc.+ Chall.	13	2.1	1 x 600 (week 0)	28.2(7.7)	24.7(8.4)	52.9(14.7)	26.1(14.7)	32	<0.01
3. Vacc. Co.	8	2.1	2 x 300 (weeks 0 and 6)	10.3(2.5)	17.5(5.8)	27.8(8.0)			
4. Vacc.+ Chall.	12	2.1	2 x 300 (weeks 0 and 6)	28.9(8.9)	24.7(7.8)	53.6(14.3)	25.8(14.3)	33	<0.01
5. Vacc. Co.	7	2.1	3 x 200 (weeks 0, 4 and 8)	10.4(2.8)	14.6(7.5)	25.0(9.7)			
6. Vacc.+ Chall.	14	2.1	3 x 200 (weeks 0,4 and 8)	28.9(9.5)	27.0(7.8)	55.9(15.6)	30.9(15.6)	20	>0.1
7. Vacc.+ Chall.	10	6.0	1 x 600 (week 0)	22.7(5.7)	8.3(3.7)	31.0(8.1)	31.0(8.1)	19	<0.05
8. Vacc.+ Chall.	16	6.0	2 x 300 (weeks 0 and 6)	19.5(8.1)	4.6(3.3)	24.1(9.8)	24.1(9.8)	37	<0.001
9. Vacc.+ Chall.	17	30.0	1 x 600 (week 0)	16.1(6.6)	4.3(2.7)	20.4(8.1)	20.4(8.1)	47	<0.001
10. Vacc.+ Chall.	13	30.0	2 x 300 (weeks 0 and 6)	13.9(7.8)	4.6(3.5)	18.4(10.2)	18.4(10.2)	52	<0.001
11. Vacc.+ Chall.	15	30.0	3 x 200 (weeks 0,4 and 8)	17.7(7.0)	4.5(1.9)	22.2(8.2)	22.2(8.2)	42	<0.001
12. Chall. Co.	16			28.1(7.5)	10.4(5.2)	38.5(9.0)	38.5(9.0)		

These results show that mice vaccinated over an 8 week period, on 1, 2 or 3 occasions with the same total number of irradiated cercariae (600), exhibit comparable degrees of resistance. This applies to vaccination with cercariae exposed to 2.1, 6.0 or 30 kr.

6.7 Effect of route of administration of irradiated somules.

Experiment 29.

Groups of CBA mice were vaccinated with 500 somules irradiated with 40 kr administered by injection indradermally, intramuscularly, subcutaneously or intravenously. Eight weeks later these mice, together with a group of challenge controls, were challenged with 200 cercariae. The mice were perfused 6 weeks post challenge.

The worm recoveries are presented in Table 21. The mean worm recoveries from the groups vaccinated by intradermal, intramuscular or intravenous injection were significantly lower than from the controls. The mean recovery from the subcutaneously injected group was not significantly different from that in the controls and was significantly higher than the recoveries from the three other vaccinated groups which were themselves not significantly different.

Thus, administration of the irradiated parasites by intradermal or intravenous injection does not enhance the resistance that can be stimulated by intramuscular injection, while subcutaneously injected parasites appear unable to stimulate significant resistance.

TABLE 21. Experiment 29.

Worm recoveries from mice vaccinated with 500 somules irradiated with 40 kr, administered by a variety of routes. Mice were challenged 8 weeks later with 200 cercariae and perfused 6 weeks post challenge.

Group	Number of mice per group	Route of vaccination	Mean worm recovery (S.D.) ♂	♀	Σ	Percent reduction	Significance (P.)
1.	10	I.D.	36.6(18.0)	23.9(12.9)	60.5(30.1)	35	<0.02
2.	9	I.M.	38.8(7.6)	30.8(10.9)	69.6(16.8)	25	<0.01
3.	12	S.C.	51.3(8.5)	42.0(9.4)	93.3(13.5)	-1	>0.9
4.	8	I.V.	38.6(5.1)	33.6(6.6)	72.2(11.1)	22	<0.01
5. Chall.Co.	10	-	51.8(8.7)	41.0(5.4)	92.8(10.7)		

6.8 Effect of administration of irradiated parasites together with adjuvants.

The results presented so far in this chapter show that although resistance can be consistently stimulated by injection of highly irradiated (40 kr) parasites, which remain at the site of their injection (see Chapter 5), the level of such resistance is generally low. A demonstration that such low levels of resistance could be enhanced by administration of the vaccine together with an adjuvant that boosted the level of resistance, would obviously be an advance towards establishing the feasibility of developing a live vaccine against schistosomiasis. The following experiment was established to investigate the use of Bordatella pertussis, Corynebacterium parvum and B.C.G. as adjuvants in this system. Unfortunately, when the somules were examined after the injections had been performed it was found that those combined with B. pertussis and C. parvum were dead, possibly killed by the thiomersil used as a preservative in these two preparations. The results obtained are interesting, nevertheless, and are presented below.

Experiment 30.

Groups of C B A mice were injected with 500 somules irradiated with 40 kr. The somules were injected in 0.1 ml of Earle's medium alone or in saline containing 1×10^9 organisms of B. pertussis (B. pertussis vaccine - Lister Institute), 1×10^7 organisms of B.C.G. (B.C.G. vaccine B.P., percutaneous - Glaxo Laboratories Ltd.) or 0.7 mg dry wt. C. parvum (Coparvax - Wellcome). The mice were challenged 8 weeks later with 200 cercariae, together with a group of challenge controls. The mice were perfused 6 weeks post challenge.

TABLE 22. Experiment 30.

Worm recoveries from mice vaccinated with 500 somules irradiated with 40 kr administered together with adjuvant. Mice were challenged 8 weeks later with 200 cercariae and perfused 6 weeks post challenge

Group	Number of mice per group	Adjuvant	Mean worm recovery (S.D.) ♂	♀	⚥	Percent reduction	Significance
1	10	-	13.3(5.2)	12.5(4.6)	25.8(9.1)	41	<0.001
2	9	B.pertussis	25.0(3.2)	20.6(3.7)	45.6(4.1)	-4	>0.4
3	10	B.C.G.	16.9(6.0)	16.2(6.1)	33.1(10.6)	25	<0.002
4	10	C.parvum	22.3(6.7)	20.2(5.6)	42.5(10.3)	3	>0.7
5. Chall. Co.	10		22.9(5.3)	21.0(5.7)	43.9(7.2)		

The results are shown in Table 22. The mean worm recoveries from the groups of mice vaccinated with irradiated somules alone and irradiated somules plus B.C.G. were significantly lower than from the challenge controls. Mean worm recoveries from the groups given somules plus B. pertussis or C. parvum were not significantly different from the controls and both were significantly higher than the recoveries from the mice vaccinated with irradiated parasites alone ($P < 0.001$ and $P < 0.01$ respectively). Mean recoveries from the B.C.G. groups were significantly lower than from the B. pertussis group ($P < 0.01$) but not significantly different from the C. parvum group.

Thus B.C.G. given together with irradiated somules by intramuscular injection did not enhance resistance. Injection of the irradiated somules together with B. pertussis or C. parvum failed to stimulate resistance. Whilst it is possible that this was due to a suppressive effect on the development of an immune response it seems more likely, in view of the general failure to stimulate resistance with dead schistosome material, that it was due to the fact that the somules were dead on injection.

6.9 Effects of vaccination with highly irradiated parasites in terms of worm burden, egg burden and mortality due to a challenge infection.

The following experiment was designed to test whether the resistance conferred by vaccination with highly irradiated somules, albeit low in terms of worm burden, would result in a significant protection against the lethal effects of a challenge.

Experiment 31.

21 C B A mice were vaccinated by intramuscular injection of 500 somules irradiated with 40 kr. 8 weeks later these mice, together with 19 challenge control mice, were challenged with 200 cercariae. Half of the mice, 11 vaccinated and 9 controls, were perfused 7 weeks post challenge (one challenge control mouse died prior to perfusion). Livers and guts were removed from these animals and tissue egg counts performed. The rates of death of the mice in the other half of the experiment were recorded.

The worm and egg recoveries are presented in Table 23. Both the worm burden and the tissue egg counts were significantly lower in the vaccinated animals than the controls. The percent reduction of worm and egg burdens were very similar (37% and 39% respectively) and correspondingly the mean number of eggs/worm pair in the vaccinated and control animals were very similar. The survival time course, presented in figure 6 shows that death of the vaccinated mice was somewhat delayed. The mean survival times post challenge of the vaccinated and control mice, 60 ± 7.8 and 53.2 ± 4.9 days respectively, were significantly different ($P < 0.05$).

Thus mice vaccinated with highly irradiated somules can show significant protection against challenge insofar as they harbour significantly fewer eggs and worms and also live significantly longer.

TABLE 23. Experiment 31.

Worm and tissue egg recoveries from mice vaccinated with 500 somules irradiated with 40 kr, challenged with 200 cercariae 8 weeks later and perfused 7 weeks post challenge

W O R M

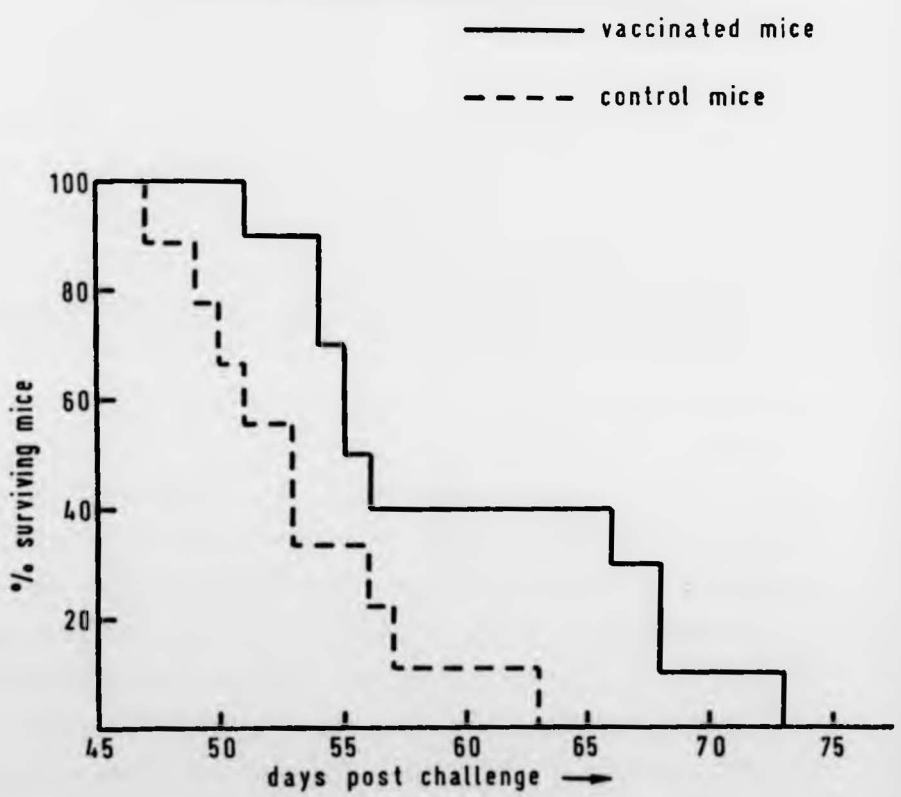
Group	Number of mice per group	Mean worm recovery (S.D.) ♂	♀	Σ	Percent reduction	Significance (P.)
1. Vacc. + Chall.	11	32.5(9.5)	23.6(8.2)	56.0(16.5)		
2. Chall. Co.	9	50.2(9.4)	39.0(7.1)	89.2(13.1)	37	<0.001

E G G

Group	Mean number of eggs/organ (S.D.) Liver	Gut	Liver + Gut	Mean number of eggs/worm pair (S.D.) Liver	Gut	Liver + Gut	Percent reduction total eggs	Significance
1. Vacc.+ Chall.	13,358(3,998)	45,406(20,282)	58,764(23,539)	583(70)	1,917(424)	2,500(403)		
2. Chall. Co.	20,978(6,303)	75,496(19,047)	96,474(24,189)	548(98)	1,987(226)	2,534(258)	39	<0.01

FIGURE 6 Experiment 31

Mortality time course, post challenge, of vaccinated (500x40kr. somules) and control mice.



6.10 Discussion.

Perlowagora-Szumlewicz and Olivier (1963) and Perlowagora-Szumlewicz (1964a) conluded that their results showed that the "resistance" induced by vaccination of mice with irradiated cercariae merely represents a delay in migration of the challenge-derived parasites. This conclusion was based on the observations that the "resistance" assessed by perfusion 27 or 33 days post challenge was greater than that apparent when perfusion was delayed until 60 or 80 days post challenge. Doenhoff et al. (see Appendix 1) have presented evidence of a delay in the migration of a challenge infection in previously infected mice insofar as the "resistance" as assessed by perfusion 21 days post challenge is somewhat greater than the resistance assessed by perfusion at 5 or 8 weeks post challenge. Similarly, in the present studies, although direct comparisons were not made, the levels of "resistance" as assessed by perfusion 21 days post challenge were somewhat higher than the levels assessed at 6 or 8 week perfusion of experiments of comparable design. However, whilst the levels of "resistance" arrived at by 21 day perfusions may include some element of delay it is considered that any conclusions derived from comparisons of experimental groups within one experiment are valid. The challenge that the "resistance" assessed by perfusion at 6 or 8 weeks merely reflects a delay could not be directly tested using the same format as was employed in the majority of these studies as mice challenged with 200 cercariae would not survive longer. However, in both experiments 25 and 30 in which resistance was assessed by perfusion 7 - 8½ weeks post challenge, there was a very good correlation between the resistance assessed by both worm burden and tissue egg burden. Thus the mean number of eggs

per worm pair in the vaccinated and partially resistant animals was not significantly different from that in the controls. If, in the vaccinated animals, challenge derived organisms were delayed such that they arrived in the mesenteric veins and commenced egg laying later than in the controls, then the mean number of tissue eggs per worm pair would have been lower in the vaccinates than in the controls. As this was not the case, it is considered that data based on perfusions performed 6 weeks or later after challenge (eggs start to be produced 5-6 weeks post infection) give an accurate measure of resistance to challenge.

The results presented in section 6.3 show that, although significant resistance can be stimulated by cercariae or somules exposed to 2.3 - 160 kr, the degree of resistance is influenced by the time of challenge post vaccination. When resistance was assessed by challenge of mice 3 weeks after infection, maximum resistance was stimulated by 20 kr parasites, higher and lower radiation doses resulting in lower levels of resistance. Large numbers of unirradiated cercariae of one sex or small numbers of cercariae of both sexes failed to stimulate any resistance at this time. The resistance induced by 20 kr parasites appeared to decline somewhat between 3 and 16 weeks though remained highly significant. The low level of resistance induced by 40 kr somules remained constant between 2-16 weeks. The level of resistance induced by 10 kr parasites was lower than that induced by 20 kr parasites at 3, 8 and 17 weeks and also showed some decline with time. In contrast, the resistance of mice vaccinated with 2.3 kr parasites increased with time such that by 8 weeks post vaccination such mice were comparably resistant to mice vaccinated with 20 kr parasites and by 17 weeks somewhat more resistant. While the resistance of mice

infected with small numbers of unirradiated worms of both sexes increased dramatically between 3 and 8 weeks, mice infected with large numbers of unirradiated male cercariae failed to show any resistance to challenge either 3 or 8 weeks post infection.

As a consequence of this variation of resistance with time, the results of the experiments in section 6.2, in which challenge was performed 8-12 weeks post vaccination, did not reveal any major consistant differences in the efficacy of cercariae or somules exposed to doses in the range 2.3-160 kr in inducing resistance. However, Radke and Sadun (1963) reported that vaccination of mice with cercariae exposed to the ill-defined dose of 12.5-40 kr failed to stimulate significant resistance to challenge 8 weeks later while significant resistance was conferred by cercariae exposed to 2.5-10 kr. Villella et al. (1961) also reported that vaccination by intraperitoneal injection of cercariae exposed to 2.5 kr, a proportion of which survived to become stunted adults, stimulated markedly greater resistance than cercariae exposed to 5 or 7.5 kr. Erickson and Caldwell (1965) and Perlowagora-Szumlewicz and Olivier (1963), however, reported the opposite trend viz. doses of 3 or 4 kr failed to stimulate resistance to challenge 6-7 weeks post challenge, whereas doses of 6-10 kr generally stimulated significant resistance. It is difficult to comment on the results of these authors, based as they are on only one or two experiments, but any apparent discrepancies with the results presented here may reflect differences in the mouse or schistosome strains with regard to the relationship between radiation dose/immunizing interval in the development/persistence of resistance.

With regard to the time of development of resistance following infection with cercariae exposed to differing doses of radiation the studies performed by S.Y. Li Hsü and H.F. Hsü and their co-workers on S. japonicum in the rhesus monkey provide interesting parallels to the present observations. Their early studies (Hsü et al., 1962 and 1963b) concentrated on repeated vaccination with cercariae exposed to low doses of radiation in the range 1.7-4.0 kr and challenges were given 130-468 days after the first vaccination and from 18-343 days after the last. They concluded that radiation doses in the range 1.7-3.0 kr were slightly more effective than those in the range 3.5-4.0 kr and that resistance was greater in monkeys challenged 18 days or 140-343 days after the last vaccination than at the intervening times. An essentially similar relationship between the degree of resistance and the interval between the last vaccinating exposure and challenge was observed in experiments involving the Formosan strain of S. japonicum which fails to reach the adult stage in the rhesus monkey (Hsü and Hsü, 1965). Consequently, they developed the theory that there was an 'early non-specific inhibiting reaction' maximal at 8-15 days after the last vaccinating exposure and a 'late specific reaction' developing at around 90 days. Following up this theory (Hsü et al., 1965b) they demonstrated virtually complete protection in monkeys challenged 182-653 days after the last vaccination and concluded: "Although other factor(s) may also contribute to the success of immunization it is evident that a period of 6 months between the last immunizing innoculation and the challenge is a primary requirement for the rhesus monkey in reaching a very high resistance to challenge". An explanation for the requirement of a long immunizing interval was offered by Smithers (1976) who commented, with reference to the resistance induced by

irradiated cercariae "... in order to obtain the highest degree of immunity a long immunizing period is necessary which suggests that the immunizing effect is predominantly due to the few stunted worms which survive the lethal effects of radiation". However, in the paper by Hsü et al., (1965b), comparable resistance with that achieved by exposure to cercariae irradiated with 2 kr was afforded one monkey vaccinated with cercariae exposed to 12 kr which do not survive to become stunted adults (Hsü et al., 1963a). Furthermore, it was subsequently demonstrated (Hsü et al., 1969 and 1975) that very high partial resistance could be induced by exposure to even more highly irradiated cercariae (24 or 48 kr) and that with such vaccination long immunizing intervals were no longer required e.g. virtually complete resistance was achieved by Hsü et al. (1975) in 2 monkeys vaccinated on 4 occasions with either 16,000 or 45,300 cercariae irradiated with 36-48 kr. These two monkeys were challenged 25-27 days after the last immunization and 136-138 days after the first.

Hsü et al (1969) also demonstrated that the rhesus monkey could develop strong resistance to S. mansoni following vaccination with cercariae irradiated with 24 or 48 kr but in this experiment the monkeys were challenged 145 and 194 days after the last vaccination. Sadun et al. (1964) reported strong partial resistance to S. mansoni in rhesus monkeys challenged 9 weeks after the first of five weekly exposures to 5,000 cercariae irradiated with 2.5 kr whereas monkeys vaccinated in a similar fashion but with cercariae irradiated with 4 or 10 kr exhibited markedly lower resistance. Smithers (1962) similarly reported partial resistance in monkeys challenged thirteen weeks after the first of two exposures totalling 13,000 cercariae irradiated with 2.0 or 3.0 kr.

There is little to be gained from comparing these isolated experiments on S. mansoni with the more extensive studies on S. japonicum owing to the marked variation in experimental designs. Nevertheless, the results obtained in the rhesus monkey with both S. mansoni and S. japonicum are in accord with those obtained in the present investigations insofar as resistance has been demonstrated following vaccination with cercariae exposed to both low (2.0-2.5 kr) and high (24-48 kr) doses of radiation. Furthermore, although direct comparisons have not been performed, marked resistance to S. japonicum in the rhesus monkey can be demonstrated relatively early post vaccination with highly irradiated cercariae but appears to take longer to develop to a maximal level with cercariae exposed to the lower doses close to the minimum sterilizing dose.

The early onset of resistance in mice following vaccination with highly irradiated parasites together with the finding that such early resistance was greater in mice vaccinated with parasites irradiated with 20 kr than with those exposed to the lower dose of 2.3 kr and negligable in mice infected with large numbers of unirradiated parasites of one sex indicates that the radiation treatment is in some way necessary for the expression of such resistance. Although somewhat fewer unirradiated cercariae (300) were used than the 20 kr-irradiated cercariae (500), the results of the dose dependancy studies (section 6.5) indicate that such a difference is unlikely to have accounted for the differences in resistance produced. It is also considered unlikely that the success in vaccination with 20 kr cercariae and failure with unirradiated cercariae was influenced by the fact that the irradiated infection comprised cercariae of both sexes while the unirradiated

infection comprised only male cercariae. This is because of the early appearance of resistance prior to the expression of major sexual differences by the vaccinating worms and also because the results presented in Chapter 5 indicate that cercariae exposed to 20 kr do not survive to express sexual differences. However, the influence of subtle differences between the sexes can obviously not be excluded. In this context, mention should be made of the experiments performed by Perlowagora-Szumlewicz (1964c and 1966) in which 300 or 500 male cercariae irradiated with 2 kr failed to induce resistance in mice while comparable vaccination with female cercariae did stimulate resistance. Surprisingly, however, female cercariae irradiated with 1.7 kr, unirradiated female cercariae and 1500 cercariae of mixed sex irradiated with 2 kr failed to stimulate resistance. In general, these results are confusing and inconsistent with those obtained herein. It is perhaps relevant that the strain of Swiss albino mouse used throughout the studies of Perlowagora-Szumlewicz failed to develop a significant level of resistance to reinfection (Perlowagora-Szumlewicz, 1964a).

The apparently critical role played by radiation treatment in the induction of resistance by irradiated cercariae or somules demands some explanation. It is possible that the irradiation in some way alters the intrinsic antigenicity of the larvae such that the antigens involved in protection become per se more immunogenic. Such a hypothesis could be investigated by trying to vaccinate with extracts of irradiated parasites. In this context it is interesting to note the results of experiment 14 in which the 40 kr somules killed as a consequence of combination with C. parvum or B. pertussis, possibly by the thiomersil preservative in these preparations, failed to stimulate significant

resistance while the living parasites administered with B.C.G. or medium alone did stimulate significant resistance. Whilst it must be accepted that the putative altered antigens on the surface of the parasites could have been rendered ineffective by the thiomersil or that the adjuvants themselves may have interferred with the development of resistance, it is considered more likely that this result indicates that the irradiated parasites must be alive in order to stimulate resistance and do not merely possess radiation-altered antigens.

The failure to demonstrate resistance in mice infected with large numbers of unirradiated parasites of one sex indicates, however, that the irradiated parasites must die in order to stimulate resistance. Thus the optimal resistance manifest 3 weeks after vaccination with 20 kr somules may reflect a balance between a requirement for the parasites to survive for a period of time and the requirement for them to die. The question thus arises as to how death of the parasites could be involved in the induction of resistance. The studies presented in Chapter 5 show that a small percentage of the 20 kr parasites escape from the site of infection in the skin or muscle and reach the lungs. Thus, it could be postulated that the "resistance" produced by infection with such irradiated parasites is a non-specific phenomenon arising because the irradiated parasites die in the lungs producing inflammation which interferes with the migration of challenge-derived schistosomula. Smith et al. (1975) have reported that inflammation in the lungs of hamsters produced by intra cardiac injection of formalin killed E. coli can result in a reduced recovery of parasites if administered whilst the migrating schistosomula are in the lungs. However, the results of

Chapter 5 show that only a small proportion of the schistosomula derived from an infection with 20 kr cercariae or somules can reach the lungs. Similarly, Hsü et al. (1963) reported that infection of mice with S. japonicum cercariae irradiated with 24 kr produced negligable pathology in the lungs. Furthermore, when 40 kr somules were injected into the tail vein of mice and could thus have been expected to cause marked pathology in the lungs, the resistance stimulated was not significantly different from that produced following intramuscular injection. Finally, the persistance of resistance (demonstrable 17 weeks post vaccination) and the lack of dose dependance indicates that the resistance is not due to an obstruction of challenge derived parasites caused by transient inflammation associated with pathology produced by death of the irradiated parasites in the lungs. The demonstration of high partial resistance in rhesus monkeys challenged 145 or 194 days after the last vaccination with 24 kr S. mansoni cercariae supports this conclusion (Hsü et al., 1969).

The granulomatous reaction around dying irradiated parasites may, however, be important in facilitating host recognition of larval antigens. Hsü et al. (1963) described the inflammatory reactions in the skin of mice around S. japonicum larvae irradiated with 24 kr. The numerous densly cellular nodules were composed of neutrophils, eosinophils, mononuclears, giant cells, pigment granules, degenerate schistosomula and cellular thrombi. An essentially similar cellular infiltration encloses irradiated somules dying in the lungs whether they be exposed to 2.3, 4 or 10 kr (see Chapter 5). Thus the radiation-damaged schistosomulum becomes enclosed in a granulomatous response rich in cells

which could be involved in antigen recognition, in which site it will die and disintegrate presumably releasing both metabolic and somatic products.

The generation of an immune response in this way would explain the resistance demonstrable early after vaccination. If such an immune response is generated by death (or a period of life surrounded by immunocompetant cells) of the irradiated parasites, the increase of resistance with time in mice vaccinated with cercariae or somules exposed to 2.3 kr could be a reflection of their longer mean survival time (see Chapter 5). An alternative explanation for the increase in the level of resistance following vaccination with 2.3 kr parasites is that the sterile worms which survive the radiation treatment stimulate a concomitant type of immunity (Smithers and Terry, 196b) as is described in the studies of resistance to reinfection in Chapter 3. However, the failure to demonstrate resistance in mice infected with worms of one sex is inconsistent with this theory.

The studies described in this chapter have shown that mice can be partially protected against S. mansoni in terms of a reduced worm burden, egg load and consequently in terms of survival, by what appears to be a relatively non-pathogenic live vaccine. Increasing the number of larvae in the vaccination, giving multiple vaccinations or administering the larvae by different routes all failed to produce an enhancement of resistance. Thus, at the present time, none of the parameters studied would seem to warrant further investigation in a primate such as the baboon which is regarded as being similar to man immunologically and in its response to schistosome infections. Although

the rhesus monkey can be rendered almost solidly resistant to both S. mansoni and S. japonicum by vaccinating with irradiated cercariae, the failures to demonstrate resistance in the baboon (Taylor et al., 1976) and in the chimpanzee (Hsü, 1970) indicate that the possibility of developing a live vaccine is still remote. It is considered that the most hopeful approach is to look for acceptable adjuvants that could be used in conjunction with a live vaccine and the vaccination protocols described for the mouse in this chapter may prove useful in this context.

Although it has not been conclusively demonstrated that a number of the vaccinating parasites exposed to such high doses of radiation as 20 or 40 kr do not survive somewhere in the body, the results presented in Chapter 5 and by Hsü et al. (1963a) indicate that this is not the case. Thus the demonstration of resistance following vaccination with highly irradiated parasites indicates that resistance to S. mansoni in the mouse can persist in the absence of the living parasites. If it can be demonstrated that such resistance is immunologically mediated it follows that the relevant antigens are present on, or in, the relatively simple larval stages and renewed investigation of such antigens may be warranted. The apparent requirement for the presentation of such antigens in a living form may prove crucial in this context possibly indicating the involvement of excretory or secretory products or indicating that the manner in which antigens are presented to the host is a key factor.

CHAPTER 7. STUDIES ON THE DEVELOPMENT OF RESISTANCE FOLLOWING EXPOSURE TO RADIATION ATTENUATED S. MATTHEEI INFECTIONS.

7.1 Introduction

The experiments described here were undertaken in the hope that studies in the mouse would complement those aimed at the development of a live attenuated vaccine against S. mattheei and S. bovis in sheep and cattle (Taylor et al., 1976b). At the time of their inception Taylor (1975) had demonstrated that vaccination of sheep with 4 monthly doses of 10,000 S. mattheei cercariae irradiated with 6.0 kr resulted in marked protection in terms of worm and tissue-egg burdens against a challenge of 5,000 cercariae given 4 weeks after the last immunization. The subsequent studies on sheep (Taylor et al., 1976b; Bickle et al., Appendix 4) were carried on in parallel with those reported below. Also included in this chapter is an experiment designed to compare the degrees of protection against S. mattheei and S. mansoni afforded by homologous and heterologous vaccination with irradiated cercariae.

7.2 In view of the reports in the literature indicating that cercariae exposed to low doses of radiation stimulate maximum resistance in the mouse against S. mansoni, studies were undertaken to find the minimum dose of radiation that would result in a sterile S. mattheei infection (see Chapter 5). The first experiment, performed before this minimum sterilizing dose had been established as 2.7 kr, involved a comparison of the resistance induced by vaccination with cercariae irradiated with 3.0, 6.0 and 10.0 kr. Taylor (1973) had shown that 6.0 kr prevented the establishment of adult S. mattheei in the mouse while 3.0 kr allowed a small proportion of stunted parasites to survive.

TABLE 24. Experiment 32

Worm recoveries from mice vaccinated with 150 cercariae irradiated with 3.0, 6.0 or 10 kr, challenged 8 weeks later with 100 cercariae and perfused 8 weeks post challenge.

Group	Number of mice per group	Radiation dose (kr)	Mean worm recovery (S.D.) ♂	♀	⚥	Mean worm recovery from challenge	Percent reduction	Significance (P)
1. Vacc. Co.	5	3.0	1.8(0.8)	3.2(0.8)	5.0(1.2)			
2. Vacc.+ Chall.	20	3.0	10.5(6.0)	17.2(10.0)	27.7(14.2)	22.7	29	<0.05
3. Vacc. Co.	5	6.0	0	0	0			
4. Vacc.+ Chall.	20	6.0	9.5(4.3)	15.0(6.6)	24.5(10.4)	24.5	23	<0.05
5. Vacc.+ Chall.	16	10.0	9.8(6.6)	12.8(8.8)	22.5(14.7)	22.5	29	<0.05
6. Chall. Co.	20	-	13.0(3.5)	18.9(17.2)	31.8(9.1)	31.8		

Experiment 32

Groups of T.O. (A.S.L.) mice were vaccinated with 150 cercariae irradiated with 3.0, 6.0 or 10.0 kr, challenged with 100 cercariae 8 weeks later and perfused 8 weeks post challenge. Appropriate challenge control mice were included and a group of mice vaccinated with 3.0 kr cercariae was unchallenged serving as a vaccine control group.

The worm recoveries are presented in Table 24. All of the vaccine control mice were infected, the mean worm burden being 5.0 worms. No eggs were seen in the liver squash preparations of any of these mice. All three vaccination procedures resulted in significant but low grade resistance. There were no significant differences between the mean worm burdens in any of these vaccinated groups.

Thus, as was observed in the studies with S. mansoni, persistance of stunted worms surviving the radiation treatment did not enhance the resistance apparent 8 weeks after vaccination.

7.3 Effect of size of vaccinating infection.

Experiment 33.

Groups of T.O. (A.S.L.) mice were vaccinated with 600, 300 or 100 cercariae irradiated with 3.0 or 6.0 kr. 8 weeks later the mice were challenged with 100 cercariae as also were the mice in a challenge control group. Groups of mice vaccinated with 600, 300 or 100 cercariae irradiated with 3 kr were unchallenged serving as vaccine controls. The worm recoveries are presented in Table 25. Mean worm recoveries

from the controls vaccinated with 600, 300 and 100 cercariae were 16.3, 8.7 and 3.1 respectively. All of these mice harboured worms apart from 2 of the 7 in the group given 100 cercariae. No eggs were detected in any of the liver squash preparations. Significantly fewer challenge derived worms were recovered from the groups vaccinated with 600 or 300 cercariae irradiated with 3.0 or 6.0 kr than from the challenge controls. There were no significant differences between the recoveries from these four groups of vaccinated mice. The recoveries from mice vaccinated with 100 cercariae were not significantly different from the controls. Of the groups vaccinated with 6.0 kr cercariae the mean worm recoveries from those vaccinated with 600 or 300 cercariae were significantly lower than that from the mice given 100 cercariae ($P < 0.05$ and < 0.02 respectively). Worm recoveries from the 3 groups vaccinated with 3 kr cercariae were not significantly different.

These results indicate a degree of dose dependence in the induction of resistance to challenge given 8 weeks post vaccination in so far as 100 cercariae resulted in a lower degree of resistance than either 300 or 600 cercariae which resulted in comparable levels of resistance.

7.4 Vaccination with intramuscularly injected somules.

Experiment 34

Groups of T.O. (B.K.) mice were injected intramuscularly with 600 somules irradiated with 2.7 or 6.0 kr or with 50 unirradiated somules. The mice were challenged with 100 cercariae 10 weeks later

TABLE 25. Experiment 33

Worm recoveries from mice vaccinated with 100, 300 or 600 cercariae irradiated with 3.0 or 6.0 kr, challenged 8 weeks later with 100 cercariae and perfused 8 weeks post challenge.

Radiation Dose	Group	Number of mice per group	Number of cercariae in immunization	Mean worm recovery (S.D.) ♂	♀	⚥	Mean worm recovery from challenge	Percent reduction	Significance (P)
	1. Vacc. Co.	8	600	6.6(4.1)	10.7(5.1)	16.3(8.2)			
	2. Vacc.+ Chall.	12	600	15.4(9.5)	22.7(8.1)	38.1(17.4)	21.8	39	<0.05
	3. Vacc. Co.	7	300	3.1(2.3)	5.6(2.6)	8.7(5.1)			
3.0	4. Vacc.+ Chall.	11	300	14.1(5.2)	18.7(7.1)	33.8(10.9)	25.1	30	<0.05
	5. Vacc. Co.	7	100	1.4(0.9)	1.7(1.1)	3.1(1.8)			
	6. Vacc.+ Chall.	12	100	16.1(4.8)	17.8(4.4)	33.9(8.3)	30.8	14	>0.2
	7. Vacc.+ Chall	12	600	12.8(6.2)	10.5(4.3)	23.3(10.4)	23.3	35	<0.02
6.0	8. Vacc.+ Chall.	11	300	11.0(4.1)	11.1(4.7)	22.1(8.2)	22.1	38	<0.01
	9. Vacc.+ Chall.	10	100	17.3(7.6)	17.4(6.0)	34.7(12.2)	34.7	3	>0.8
	10. Chall. Co.	11	-	16.8(6.7)	19.0(6.2)	35.8(11.3)			

together with a group of challenge controls and perfused 6 weeks post challenge. Groups of mice injected with unirradiated somules and 2.7 kr somules were left unchallenged serving as controls for the initial infections.

The worm recoveries are presented in Table 26. No eggs were seen in the liver squash preparations of the mice vaccinated with 2.7 kr somules. The mice infected with unirradiated somules showed the greatest resistance. The mean challenge derived worm recovery from the mice vaccinated with 2.7 kr somules was not significantly different from that of the challenge controls. However, one of the mice in this group harboured 120 worms which considerably raised both the mean and the standard deviation of the worm burden in this group. It is considered possible that this mouse was inadvertantly given a double dose of challenge cercariae. A low but significant degree of resistance was apparent in the group of mice vaccinated with 6 kr somules, the mean worm burden in this group not being significantly different from that in the 2.7 kr group.

These results showed that intramuscularly injected somules could be used to induce resistance in the mouse and such a method of vaccination was subsequently employed in the sheep (Taylor et al., 1976).

7.5 Heterologous vaccination.

Nelson et al. (1969) and Amin and Nelson (1969) reported that mice infected with S. mattheei strongly resisted a challenge with S. mansoni. The development of such heterologous protection with un-

TABLE 26. Experiment 34

Worm recoveries from mice given a primary infection with 50 unirradiated larvae or vaccinated with 600 somules irradiated with 2.7 or 6.0 kr, challenged 10 weeks later with 100 cercariae and perfused 6 weeks post challenge

Group	Number of mice per group	Radiation dose (kr)	Mean worm recovery (S.D.) ♂	♀	♂+♀	Mean worm recovery from challenge	Percent reduction	Significance (P)
1. 1^{o} Co.	9	0	8.4(2.8)	7.9(2.6)	16.3(5.0)			
2. 1^{o} + Chall.	9	0	20.9(6.7)	16.6(4.4)	37.5(8.6)	21.2	50	<0.001
3. Vacc. Co.	8	2.7	7.0(2.9)	8.9(5.1)	15.9(7.7)			
4. Vacc.+ Chall.	12	2.7	22.8(13.1)	23.9(12.6)	46.7(24.8)[1]	30.8	27	>0.1
5. Vacc.+ Chall.	14	6.0	13.1(6.8)	17.3(5.7)	30.4(11.7)	30.4	28	<0.01
6. Chall. Co.	12	-	18.2(4.2)	24.2(7.2)	42.4(8.3)			

Note - 1. Very high standard deviation due to one mouse harbouring 120 worms.

irradiated infections was confirmed in Chapter 3. Vaccination with heterologous irradiated infections have not previously been investigated and such was the purpose of the following experiment.

Experiment 35

Groups of T.O. (A.S.L.) mice were vaccinated with 200 cercariae of S. mansoni or S. mattheei or with 100 cercariae of both species, all cercariae being irradiated with 3.0 kr. These mice, together with a group of challenge controls, were challenged 9 weeks later and perfused 8 weeks post challenge. Appropriate groups of vaccinated mice were left unchallenged to serve as vaccine controls.

The worm recoveries are shown in Table 27. Sterile, stunted worms of both species survived the irradiation, though as would be expected from the results presented in Chapter 5, considerably more S. mattheei worms survived (13.2 $\pm$ 6.8) than S. mansoni (1.6 $\pm$ 1.4). Significant partial resistance following homologous challenge was found with both S. mattheei (32% fewer worms than the controls) and S. mansoni (41% fewer worms), these degrees of resistance being roughly comparable with those reported above. However, no significant heterologous protection was evidenced. The mixed species vaccination resulted in significant partial protection against S. mansoni but not against S. mattheei.

The results of experiments 1 and 4 in Chapter 3 indicate that mice infected with unirradiated S. mattheei or S. mansoni can develop comparable levels of resistance to challenge with S. mansoni. However, the above result suggests that the resistance stimulated by irradiated

TABLE 27. Experiment 35

Worm recoveries from mice vaccinated with 200 S. mattheei or S. mansoni cercariae or 100 S. mattheei plus 100 S. mansoni cercariae irradiated with 3.0 kr, challenged 9 weeks later with 100 cercariae and perfused 8 weeks post challenge

	Group	Number of mice per group	Challenge species	Mean worm recovery (S.D.) ♂	♀	♂♀	Mean worm recovery from challenge	Percent reduction	Significance (P)
Vaccinated with S. mattheei	1. Vacc. Co.	8	-	4.7(2.9)	8.5(4.9)	13.2(6.8)			
	2. Vacc.+ Chall.	14	S. mattheei	14.8(5.9)	21.5(5.6)	36.3(9.6)	23.1	32	<0.02
	3. Vacc.+ Chall.	15	S. mansoni	14.3(5.6)	15.7(4.6)	30.0(9.4)	16.8	17	>0.2
Vaccinated with S. mansoni	4. Vacc. Co.	6	-	0.8(0.8)	0.8(0.6)	1.6(1.4)			
	5. Vacc.+ Chall.	12	S. mansoni	6.5(4.0)	7.1(4.2)	13.6(7.5)	12.0	41	<0.01
	6. Vacc.+ Chall.	12	S. mattheei	20.5(6.3)	17.7(4.3)	38.2(8.5)	36.6	-8	>0.4
Vaccinated with S. mattheei & S. mansoni	7. Vacc. Co.	7	-	3.5(2.0)	5.2(2.9)	8.7(4.4)			
	8. Vacc.+ Chall.	13	S. mattheei	20.2(6.8)	18.8(6.0)	39.0(11.3)	30.3	10	>0.4
	9. Vacc.+ Chall.	13	S. mansoni	10.5(3.7)	10.5(2.7)	21.0(5.6)	12.3	39	<0.01
	10. Chall. Co.	14	S. mattheei	17.6(6.8)	16.2(5.7)	33.8(11.7)	33.8		
	11. Chall. Co.	14	S. mansoni	9.5(2.9)	10.7(4.4)	20.2(6.6)	20.2		

infections is more specific, homologous but not heterologous protection being demonstrated.

7.6 Discussion.

Considering the generally poor protection stimulated against S. mattheei by irradiated infections in mice, and the rapid development of successful vaccination procedures in sheep (Taylor et al., 1976; Bickle et al., Appendix 4), no further attempts were made to optimize vaccination procedures in mice. However, some general conclusions arising from these studies may have relevance to those in the sheep.

It appears that a minimum of 150 cercariae irradiated with 3 or 6 kr are required to stimulate significant resistance but that increasing the number up to 600 does not affect the degree of resistance. This apparent dose dependancy in the induction of resistance is somewhat more clear cut than the results obtained with S. mansoni. The failure to protect sheep with 1 or 2 doses of 10,000 irradiated (6 kr) cercariae compared with the successful protection afforded by 4 doses of 10,000 cercariae (Taylor, 1975 and Taylor et al., 1976b) may be a reflection of a similar threshold requirement rather than a requirement for multiple vaccination.

Comparable levels of protection can be afforded mice by vaccination with percutaneously applied cercariae and intramuscularly injected somules. Similar findings have been reported in sheep challenged with S. mattheei (Taylor et al., 1976b) and in mice challenged with S. mansoni (see Chapter 6).

With regard to the optimal radiation dose, the results contained herein indicate that when challenge is administered between 8-10 weeks post immunization, comparable protection is afforded by cercariae or somules irradiated with 6 or 10 kr that fail to persist in the host and by larvae irradiated with 2.7 - 3.0 kr which produce a sterile worm infection. Essentially the same results were obtained with S. mansoni. Similarly, Taylor et al. (1976b) obtained comparable resistance in sheep vaccinated with cercariae irradiated with 6.0 and 3.0 kr and challenged 22 weeks after the last vaccination (2-4 weeks after the first). In a recently completed experiment (Bickle et al., Appendix 4) comparable levels of resistance were apparent in sheep vaccinated with somules irradiated with 2.7 or 6.0 kr and challenged about a year post vaccination. Vaccine controls were not included in this experiment and therefore it is not certain that any vaccinating parasites did survive as stunted adults although the results presented in Chapter 5 show that 2.7 kr allows a percentage survival in mice. The results of this latest sheep experiment show that the resistance conferred by the 6.0 kr infection which probably survives only a few weeks is a long lasting resistance which clearly indicates that it is immunologically mediated.

In view of the success in vaccinating mice against S. mansoni with highly irradiated cercariae and somules (Chapter 6), it is likely that such procedures would be effective in protecting mice and probably sheep against S. mattheei. However, as there are no stringent regulations concerned with the use of live vaccines in animals and as we have no indication that vaccination with larvae exposed to low radiation doses produces any significant pathology, there seems little point in

pursuing the use of such highly irradiated cercariae or somules in domestic animals.

The fact that homologous but not heterologous protection was observed in mice vaccinated with irradiated S. mansoni or S. mattheei indicates that such resistance is species specific. This contrasts with the demonstration that an unirradiated S. mattheei infection stimulates comparable protection against challenge with either S. mattheei or S. mansoni (see Chapter 3). Although based on isolated, unconfirmed results this apparent anomoly suggests that, in addition to differences in the kinetics of development of resistance following vaccination with irradiated cercariae and following unirradiated infection (see Chapter 6), the mechanisms of resistance and/or the relevant antigens may also differ.

Similarly, recent studies in the sheep have shown that homologous vaccination with irradiated somules is markedly more effective than heterologous vaccination (Bickle et al., - see Appendix 4).

CHAPTER 8. SUMMARY AND CONCLUSIONS

1) The literature review covered the state of knowledge of resistance to S. mansoni in man, subhuman primates and mice and suggested not only that the prospects of developing a highly effective human vaccine are remote at present but also that our knowledge of the mechanisms of resistance in any of these hosts is limited. One of the more successful approaches to vaccination, investigated in both primate and murine hosts is the use of radiation-attenuated infections, but despite interest in this during the 1960's, the parameters involved in the induction of this resistance have never been fully investigated. This was therefore one of the main areas covered in the present investigations, and was combined with studies on the effects of radiation on parasite migration and death in the host. There have been numerous reports that the mouse, chosen as the host in the present studies, can develop resistance to S. mansoni following both attenuated and unattenuated infections. However, other workers employing similar experimental protocols have failed to demonstrate resistance even in mice harbouring unattenuated adult worm infections. It was thus considered advisable at the outset to establish that the particular mouse/schistosome strains employed in our laboratories would demonstrate resistance following unattenuated infections. Such investigations constituted the preliminary studies of this thesis. Despite the fact that it has been demonstrated that resistance to reinfection in the rhesus monkey can be stimulated by infections with worms of one sex, i.e. that resistance is stimulated by the adult worm alone, there is disagreement as to the efficacy of single sex infections in stimulating resistance in the mouse. It was thus decided to investigate the protection afforded by single sex infections which, if effective, would provide a model in which the mechanisms

of resistance could be investigated uncomplicated by the host mortality and immune responses associated with the schistosome egg. Finally, vaccination with radiation-attenuated organisms has been successfully employed in protecting sheep against the sheep and cattle schistosome S. mattheei: Preliminary experiments were also undertaken to investigate the use of the mouse as a model in which to study vaccination against this parasite.

2) It was shown that the Swiss albino, T.O., strain developed significant resistance to challenge with S. mansoni administered 8 weeks post primary infection and that comparable levels of resistance resulted following primary exposure to 20-80 cercariae. Comparable resistance was also developed following primary exposures established by intramuscular injection of syringe-transformed 'schistosomula' ('somules') and by 'schistosomula' prepared by penetration of isolated mouse skin, indicating that the penetration enzymes released during skin penetration are not necessary for the stimulation of resistance. However, it was found that T.O. mice obtained from 3 different animal suppliers responded differently to infection, showing differences in mortality rate and markedly different levels of resistance to challenge. It is suggested that similar differences could at least in part account for certain of the anomalous results that exist in the literature on resistance in the mouse. It was demonstrated that T.O. mice can develop resistance to reinfection with S. mattheei and confirmed that mice infected with S. mattheei display heterologous resistance to S. mansoni.

3) Studies performed in collaboration with Dr. M.J. Doenhoff, presented as the preprint of a paper in Appendix 1, demonstrated that

the CBA mouse also develops resistance to reinfection with S. mansoni. However, it was found that such mice infected with either male or female worms alone develop significantly lower resistance to challenge 8 weeks post primary infection than mice harbouring an egg producing infection of worms of both sexes. Increasing the mean single sex (male) worm burden from 20.4 - 138.7 worms significantly increased the resistance to challenge but this was still only about half that manifest by mice harbouring a mean of 16.1 worms of both sexes. It is concluded that some factor(s) associated with a reproductively competent infection, possibly the eggs, are involved in the resistance manifest 8 weeks post primary infection. However, injection of eggs into the lungs via the tail vein failed to confer any resistance to challenge, suggesting that in this system there may be a requirement for both adult worms and eggs in the induction of resistance.

4) Prior to investigating the resistance-inducing potential of cercariae or 'somules' exposed to varying doses of radiation, the fate of such larvae and the pathological consequences to the host of their migration and death were investigated by a combination of recovery of the parasites and histology of the organs through which they pass. The minimum dose of radiation allowing persistence of a small proportion of parasites as sterile adults was found to be 2.3 kr for S. mansoni and 2.7 kr for S. mattheei although neither dose is an absolute sterilizing dose, producing a range of attenuation. Female worms predominate in such persistent infections. By infecting mice with irradiated worms of one sex together with unirradiated worms of the opposite sex it was found that the sterility of the 2.3 kr S. mansoni infections was due predominantly to sterility of the females. Any eggs produced by the 2.3 kr infections almost invariably lacked ova and

were found predominantly in the liver. Exposure of cercariae to 40 kr resulted in a very small proportion reaching the lungs whether infection was percutaneous or intramuscular and it is concluded that such schistosomula failed to penetrate blood vessels and died at the site of infection. A greater, though still small, percentage of parasites exposed to 20 kr reached the lungs though very few reached the liver and it is concluded that the majority died at the sites of infection, the remainder dying in the lungs and in other organs of the body to which they travel during their systemic migration. Infection with somules irradiated with 2.3 - 10 kr resulted in a relatively normal lung migration as compared with an unirradiated infection indicating minimal radiation-induced death at the injection site. The low recovery of 10 kr parasites from the liver, however, indicated that the majority of such schistosomula must have died in the lungs and possibly in other organs on their systemic migration route. Exposure to 4 and 2.3 kr resulted in a greater proportion of parasites reaching and dying in the liver and,while most of the parasites exposed to 2.3 kr died in the first 2-4 weeks post infection, producing lesions in both the lungs and liver, a small proportion (1-4%) were capable of surviving at least up to 200 days post infection. The nature and resolution of the schistosomular granulomas in the lungs and liver were found to be essentially as described by other authors. Lesions in both the lungs and liver associated with dead parasites exposed to 2.3 - 10 kr showed complete resolution without fibrosis and by 5-6 weeks post infection both organs were essentially normal although sporadic lesions associated with dying 2.3 kr parasites were noted as late as 140 days after infection.

5) The various parameters involved in the induction of resistance to S. mansoni by irradiated infections were investigated.

(a) Radiation dose

Significant resistance was demonstrated following exposure to cercariae or somules irradiated with 2.3 - 160 kr, persistence of stunted survivors of the radiation treatment not being necessary for the development of resistance. Vaccination with parasites exposed to 2.3 or 40 kr was shown to result in reduced worm and egg burdens following challenge and increased survival time post challenge was conferred by vaccination with 40 kr somules.

(b) Time of onset and duration of resistance

Resistance was apparent just 3 weeks post vaccination at which time maximal resistance was stimulated by larvae exposed to 20 kr, larvae exposed to both higher and lower doses producing lower levels of resistance. The resistance manifest by mice vaccinated with 40 kr parasites was shown to persist at a relatively low level until at least 16 weeks post vaccination. Resistance induced by 20 kr larvae declined a little with time. However, the level of resistance induced by larvae exposed to 2.3 kr increased such that by 8 weeks post vaccination it was comparable with that induced by 20 kr larvae and by 17 weeks somewhat greater. Vaccination with 10 kr parasites produced somewhat inconsistent results.

(c) What stimulates resistance?

Whereas vaccination with 500 cercariae irradiated with 20 kr stimulated marked resistance, manifest at both 3 and 8 weeks post

vaccination, mice infected with 300 unirradiated male cercariae failed to develop significant resistance to challenge at either of these times while mice exposed to 35 unirradiated cercariae of both sexes showed a marked increase in resistance between 3 and 8 weeks. It is concluded that the resistance induced by irradiated infections is stimulated in a fundamentally different manner from that induced by a primary bisexual infection and depends upon the death of the irradiated parasites. Thus the slower development of resistance in mice vaccinated with 2.3 kr parasites may be a reflection of their longer mean survival time. Vaccination with 40 kr somules killed as a consequence of mixing with the adjuvants C. parvum and B. pertussis (death possibly caused by the thiormersil preservative) failed to induce significant resistance indicating the necessity for the somules to be alive in order to stimulate resistance. It is postulated that a period of life, albeit in a moribund state, surrounded by the cellular components of the schistosomular granuloma may be involved in the stimulation of such resistance.

(d) Dose of vaccine

Comparable resistance was stimulated when T.O. mice were vaccinated with between 200 and 500 cercariae irradiated with 2.3 kr, resulting in mean sterile worm burdens of 3.5 - 9.3 worms. Similarly, increasing the number of 40 kr somules from 500 to 2000 failed to result in increased resistance in C B A mice although resistance was less consistently produced by 50-200 somules.

(e) Frequency of vaccination

A single vaccination with 600 cercariae was as effective as 2 or 3 vaccinations comprising the same total number of cercariae.

(f) Routes

Comparable resistance was induced following intradermal, intramuscular and intravenous administration of 40 kr somules but subcutaneous vaccination failed to stimulate significant resistance.

(g) Adjuvants

The level of resistance could not be enhanced by administering the irradiated somules together with B.C.G., B. pertussis or C. parvum although the somules mixed with the latter two adjuvants were dead on injection. It is considered, however, that such vaccination procedures as have been demonstrated here should be tested in combination with the ever increasing number of adjuvant preparations in the hope that resistance can be markedly improved.

6) These studies have clarified the parameters involved in the induction of resistance against S. mansoni in the mouse and resulted in the development of vaccination procedures that consistently produce partial protection. The stimulation of resistance by relatively simple, short-lived larval parasites offers a convenient model in which the mechanisms of resistance can be studied and, if found to be immunological, may lead to an analysis of the antigens involved.

7) Mice vaccinated with irradiated S. mattheei larvae were shown to be partially resistant to homologous challenge administered 8-10 weeks later. Comparable levels of resistance followed vaccination with percutaneously-applied cercariae and intramuscularly-injected somules irradiated with 2.7-3.0 kr, which persisted as stunted sterile adults or with 6.0-10.0 kr which did not produce persistent infections. The induction of resistance appeared to be dose dependant, vaccination with 150-600 irradiated cercariae resulting in comparable levels of resistance but 100 cercariae failing to induce any significant protection. The level of protection afforded mice by such vaccination was generally weak (20-40%) and, in view of the rapid development of successful vaccination procedures in sheep, it is concluded that the mouse is not a particularly useful host in which to try and develop optimal vaccination procedures against the ovine and bovine schistosomes.

In a comparative experiment, mice vaccinated with irradiated S. mansoni or S. mattheei cercariae developed significant resistance to challenge with the homologous but not the heterologous species. This apparent species specificity contrasts with the demonstration of heterologous resistance following primary unirradiated infection (see above) and suggests that the mechanisms and/or the relevant antigens involved may differ in the resistance induced by irradiated as compared with unirradiated infections.

APPENDIX 1.

Preprint of a paper to be published in the Journal of Helminthology comprising collaborative studies on resistance to reinfection in C B A mice.

Factors affecting the acquisition of resistance against *Schistosoma mansoni* in the mouse. I. Demonstration of resistance to reinfection using a model system that involves perfusion of mice within three weeks of challenge.

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ABSTRACT

The degree of resistance acquired by Schistosoma mansoni-infected mice against homologous challenge has been determined by perfusion of the animals within three weeks of the challenge, at which time the challenge-derived organisms were morphologically distinguishable from the primary infection which induced the resistance. The method has been compared with assays based on determination of the number of organisms migrating through the lung, and with perfusions at a later time when the challenge has matured. The results obtained with the three week perfusion method, showing that resistance was acquired by eight weeks after a primary infection, were confirmed by the longer survival of, and reduced egg excretion rates and tissue egg burdens in the experimental animals relative to respective challenge control animals. However, some discrepancy in challenge-derived worm numbers was found between animals perfused three weeks after challenge and those autopsied at later times, and the possible reasons for this difference are discussed. The degree of resistance that was acquired was to some extent dependent on the size of the challenge infection.

Resistance to reinfection with schistosomes in experimental animals is usually assayed by determining the number of challenge-derived organisms which survive in primarily-infected animals relative to appropriate challenge controls. At various stages during its migration from the skin to the portal system the challenge infection lends itself to enumeration. Thus, a technique for isolating lung borne schistosomula, first described by Olivier (1952) and used in resistance studies by Olivier and Schneidermann (1953), was reintroduced as a method by Clegg (1965) and used for assaying resistance within a week of challenge in the rat by Perez, Clegg and Smithers (1974) and in the mouse by Sher, Mackenzie and Smithers (1974), Sher, Smithers and Mackenzie (1975), and Mahmoud, Warren and Graham (1975).

By perfusing mice from two to four weeks after challenge, Olivier and Schneidermann (1953) were able to isolate surviving challenge-derived organisms, the majority of which have by this time probably reached the portal system (Olivier, 1952), and could distinguish them from worms of the primary infection by the immaturity of the former. Thompson (1954) used a similar ploy in some of his experiments. More recently the two populations have been distinguished from each other by radiolabelling and subsequent auto-radiography (Reid, Phillips and Roscinsky, 1977). However, in the majority of studies on resistance, perfusion of the experimental animals has been delayed until the challenge has matured, and the degree of resistance in these animals has been calculated relative to the number of organisms found in two control groups given either the primary infection or challenge alone.

In this study we reinvestigate the potential usefulness of perfusing animals before the challenge infection has matured, and discuss some of the advantages of this method compared with the more widely used assays based on detection of immature challenge-derived organisms in the lung or perfusion of fully mature organisms from the portal system. We have found the system particularly useful in assessing the effects of a variety of parasite- and host-related factors on the degree of resistance acquired against S. mansoni by mice. Parameters which we have investigated include the size of the primary infection, its sex and its route of administration, the time at which resistance is acquired (see following paper), and the effect of immunosuppressing the host before and after administration of the primary infection. The results of these investigations will be given in subsequent papers of this series.

MATERIALS AND METHODS

Mice

Two inbred syngeneic strains of C B A mice were used for resistance studies, these being C B A /Lac or C B A /H-T6T6. The latter differs from the former in possessing two minute marker chromosomes which arose as a result of an irradiation-induced translocation (Ford, Hamerton, Barnes and Loutit, 1956), but both strains are H-2^k and neither rejects skin grafts from the other. These mice were either bred on site, or obtained from Chester Beatty Research Institute, London, or Bantin and Kingman, Aldbrough. No differences were observed between experiments that could be attributed to the interchange of the two strains or the three suppliers, and the mice will here be referred to only as C B A.

Use was made of mice aged between eight and twenty weeks of either sex, and no differences attributable to age or sex were discerned between experiments.

Parasite

A Puerto Rican strain of Schistosoma mansoni was maintained by laboratory passage in Biomphalaria glabrata snails, each infected with up to twelve miracidia, and random bred T.O. mice infected with 50 cercariae (Taylor, Amin and Nelson, 1969). Miracidia for infecting snails were hatched from liver derived eggs. Infected snials were collected into dechlorinated water and induced to shed cercariae phototropically at 30° C. The parasite larvae were used for infection within three hours of emergence (Olivier, 1966).

Method of infection

Mice were infected percutaneously through a shaven abdomen or flank by the method of Smithers and Terry (1965). Animals were anaesthetized with sodium pentobarbitone (0.06 mg/g body weight, Sagatal, May and Baker Ltd., Dagenham, England) and the cercariae placed in a nickel-plated brass ring which was retained on the skin with the aid of transparent self-adhesive tape. The volume of fluid placed in the ring varied according to the concentration of cercariae obtained on the day of infection, but was never less than 0.1 ml nor more than 0.9 ml. When checked, at least 90% of cercariae had penetrated after twenty minutes, at which time excess fluid and rings were removed and the animals allowed to recover over gentle warmth.

Perfusion technique

Infected mice were perfused according to the method of Smithers and Terry (1965). Mice were killed with 12 mg pentobarbitone in solution containing heparin, and the thoracic and peritoneal cavities opened. 25 ml of perfusion fluid (8.6 g NaCl, 15 g trisodium citrate, 2000 units heparin, 0.2 g Merthiolate/litre water) was injected into the right ventricle, and the perfuseate emerging from an incision in the hepatic portal vein was drained into a conical-based plastic "Universal" container. Fresh solution was used to rinse out the peritoneal cavity into the same vessel. After allowing the parasites to settle excess solution was removed and the erythrocytes in the remaining 1 - 1.5 ml of fluid were lysed with a few drops of 2.5% Saponin solution.

Perfuseates containing only mature worms were poured into a Petri dish and the organisms counted and sexed under a dissecting microscope. Fluid from vessels containing both adult and immature parasites was emptied into Sedgewick Rafter chamgers, and the "Universals" rinsed out with 0.5 ml perfusion fluid which was emptied into the same chamber. The adults were removed for counting separately and the chambers scanned at a magnification of 32x for determination of the number of immature worms. The side and base of emptied "Universals" were examined under a dissecting microscope for the presence of attached immature parasites.

Isolation of migrating schistosomula from the lungs and liver

The method used for lungs was the same as that described in James and Taylor (1976) which in turn was adapted from descriptions by Sher et al. (1974) and Clegg, (1965). Essentially the same method was used in an attempt to extract from the liver oganisms which had failed to emerge from the portal system with the perfusion fluid.

Experimental design and computation of results

In the earlier perfusion experiments three groups of mice were set up in the conventional manner; namely, Group A mice were control animals given a primary infection alone; Group B, experimental mice given a primary infection and challenge; and Group C, challenge controls. The adopted practice of perfusing mice by 25 days after challenge allowed morphological differentiation between mature worms derived from the primary infection and immature challenge-derived

organisms, and because no consistent difference was found in the number of mature worms perfused from Groups A and B in any one experiment, Group A mice were in some experiments considered superfluous and dispensed with.

The mice used in an experiment were all of the same C B A substrain, sex and age, and obtained from the same supplier. In general experiments were initiated with 10 mice/group, and situations in which mice died during the course of the experiment, particularly where this is likely to affect interpretation of the results, will be indicated in the text.

Results of perfusion experiments have been presented in tabular form. The percentage reduction (%r) in the number of challenge-derived organisms perfused from an individual group B animal (n), relative to the mean number of organisms/mouse in Group C (N) has been calculated according to the formula

$$\%r = 100 - \left(\frac{n}{N} \times 100\right).$$

The mean value of %r for B animals as a group was then determined and is given in the table columns headed "% reduction". When a Group B animal was found to have a greater number of immature challenge-derived organisms than the mean number found in Group C it was considered to have a %r of 0. For this reason the figures given for the % reduction of the challenge in Group B are sometimes greater than the results of the calculation

$$100 - \left(100 \times \frac{\text{mean number of challenge-derived organisms in Group B}}{\text{mean number of organisms in Group C}}\right)$$

This is particularly so when an experimental group as a whole has acquired only a relatively low degree of resistance. Because it has been found that mice given cercariae of one sex alone only developed minimal resistance to challenge relative to animals harbouring bisexual infections (Bickle et al., in preparation), all Group A and B mice which on perfusion had no worms or worms of only one sex, and no macroscopic evidence of liver granulomas, were excluded from the further calculations performed on the group to which they belonged. Such mice were generally only occasionally found in groups primarily-infected with fewer than 35 cercariae. The percentage reduction for Group B animals has only been calculated relative to Group C mice challenged with the same batch of cercariae.

The mean numbers of parasites perfused from each group are given with an indication of the group variability in terms of $\pm$ one standard deviation.

Faecal egg counts

Mice were placed individually in 400 ml plastic beakers and allowed to defaecate. Single faecal pellets of between 10 and 40 mg were weighed to the nearest milligram and placed in 10 ml isotonic saline solution. After approximately 30 minutes the pellets were disrupted by aspiration in a 10 ml plastic syringe without needle, and the larger undigested food particles removed by filtration through a 320 μ metal sieve. Each filtrate was passed through a Whatman No. 4 filter paper and the eggs retained on the paper were stained with saturated Ninhydrin solution as described by Bell (1963). Dried

papers were examined at a magnification of 32x. Results are expressed as the number of eggs/100 mg faecal matter or Log_{10} (x + 1) number of eggs/100 mg.

Tissue egg counts

Following perfusion of infected mice, the large intestine, caecum, small intestine and the liver (the latter always excluding the two lobes encompassing the gall bladder, these having been sometimes taken for histological examination) were removed and stored at -20° C until required. The gut and liver were digested in either 10 or 20 ml of 5% potassium bydroxide solution at 37° C for 16 hours (Cheever, 1968). Fifty or 100 µl aliquots of the digests were placed under coverslips on microscope slides and the eggs counted at 32x magnification. Each digest was examined in triplicate and the mean of the results expressed as eggs/tissue or egg/worm pair/tissue. No correction was made for the eggs that were present in the portion of liver removed for histology.

Statistics

The Student "t" test was used to determine the significance of the difference between experimental groups.

RESULTS

Table 1 gives the results of an experiment in which mice were perfused at intervals between 7 and 24 days after challenge. Although in both Groups B and C there was a marked increase in the number of

perfuseable challenge-derived organisms between 7 and 11 days after challenge, it appears that the majority of the survivors of the challenge had all reached the portal blood system in both types of mice some time between 11 and 24 days after challenge. The percentage reduction in the number of challenge organisms in the primarily-infected mice remained relatively constant throughout the time course, and the number of mature worms derived from the primary infection was no different in unchallenged compared with challenged animals (Groups A and B respectively).

In an attempt to determine whether the percentage reduction of challenge organisms in primarily-infected mice relative to challenge control animals (Groups B and C respectively, Table 1) was to any extent due to a delay in the migration of challenge organisms in the former mice, two further experiments were performed in which the results of perfusion 3 weeks after challenge were compared with perfusion 5 or 8 weeks post-challenge (Table II). In both instances the percentage reduction in the challenge was less at the later perfusion time than at the 3-week perfusion, suggesting that at the earlier an element of delay contributed to the degree of resistance observed. Nevertheless even at the later perfusion times in both experiments there was a significant difference between the primarily-infected and challenge-control animals with respect to the number of challenge-derived organisms which survived. The results in Table III substantiate the figures for the worm counts of the second experiment given in Table II, in as much as the faecal egg output of the primarily-infected and challenged mice was intermediate between those of the two control groups on the day before perfusion. Thus the egg excretion rate was

reduced by 83% in the experimental animals relative to challenge controls and at the time of perfusion the number of parasite eggs found in the liver and gut in the same animals was approximately 65% lower than in the challenge controls, in both instances the result having been corrected for eggs derived from the primary infection Table III).

The discrapancy between the results of perfusing mice 3 weeks and 5 or 8 weeks after challenge (Table II) may be attributable to a proportion of the challenge being trapped at the time of perfusion in the microvasculature of the granuloma-damaged livers of primarily-infected animals. To investigate this possibility the livers of primarily-infected challenged mice were subjected to the same treatment as lungs in the assay for detecting schistosomula during their lung migration stage (Sher et al., 1974; Clegg, 1965). The results for the number of organisms which were perfused from the portal systems and extracted from the livers of these animals as well as respective challenge control mice are given in Table IV. The number of perfuseable challenge-derived organisms was reduced by approximately 84% in the primarily-infected mice relative to the challenge controls, and there was little difference in the number of immature worms detected in the livers of the two groups.

The results of an extended time-course experiment on the egg excretion rate in an experimental group and two control groups of mice infected similarly to those in Table III are given in Figure 1. The mean number of eggs detected in the faeces of the experimental group tended to increase throughout the time course, whereas the number had "plateaued" in the challenge controls by approximately 50 days after

challenge (Figure 1b). However, interpretation of these results is complicated by the death of some animals in all three groups in this experiment during the period of study (Figure 2). The protection afforded by the primary infection against the lethal effects of the challenge was confirmed by the extended survival rate of animals in the experimental group relative to the challenge controls (Figure 2). The real extension of the life span of the primarily infected and challenged mice relative to the challenge controls is difficult to calculate, since in the time interval between the deaths of the first and last of the challenge controls 7 mice given the primary infection alone also succumbed.

In a further experiment the results obtained by perfusion of animals before maturation of the challenge infection were directly compared with the lung migration technique by performing both assays on the same animals. The results for the absolute number of organisms detected in the experimental and challenge control animals are given in Figure 3. In this experiment it appears that there was no increase in the number of perfuseable challenge-derived organisms in the primarily-infected group between days 10 and 25 after challenge (Figure 3b). The number of challenge organisms detected in the lungs of primarily-infected animals was lower on days 3-6 post-challenge relative to the control group, but the number was approximately the same in both groups thereafter (Figure 3b). The number of mature worms perfused from the experimental animals remained relatively constant throughout the time course (Figure 4a). When the percent reduction in the number of challenge organisms in the primarily-infected animals relative to challenge controls was calculated with respect to lung-borne schistosomula alone,

the result was highly variable between days 3 and 7 post-challenge, and thereafter the difference between the two groups was minimal (Figure 4c). In contrast, by 10 days post-challenge the percent reduction in the number of perfuseable challenge-derived organisms had reached a level which was retained relatively constantly until day 25 (Figure 4d).

The results given in Table V are from an experiment designed to determine the effect of varying the number of challenge cercariae in the model system described here. It is apparent that by increasing the size of the challenge from 100 to 800 cercariae, the degree of resistance was decreased to a level that was not significant.

DISCUSSION

Advantages which accrue from the technique of perfusing primarily-infected and challenged animals before the latter infection has matured and become indistinguishable in size from the former include (a) the ease with which "single-sex"-infected animals (which have been shown to develop only a poor degree of resistance - Bickle *et al.*, in preparation) can be recognized, (b) the earlier termination of experiments and (c) the potential for analysing the degree of resistance in individual animals. Furthermore, the early perfusion method has shown that the number of mature organisms derived from the primary infection in Group B mice (primary infection and challenge) is no different from that found in Group A (unchallenged) animals, thus corroborating the hypothesis of "concomitant immunity", that is, that adult worms from a primary infection can survive in the presence of a

host response that prevents the maturation of a challenge infection (Smithers and Terry, 1969).

Our results indicating that the degree of resistance is greater when animals are perfused 3 weeks after challenge instead of at 5 or 8 weeks (Table II), may be explained in terms of a delayed migration of challenge organisms in primarily-infected mice. Thus Sher et al. (1974) observed two phases in the acquisition of resistance; (1) an early phase of resistance 3 weeks after infection, which could be detected as a delay in the migration of schistosomula through the lungs of infected mice relative to challenge controls, but there was little difference in the number of challenge-derived organisms which eventually matured, and (2) in mice infected for 12 to 15 weeks there was a reduction in both the number of schistosomula migrating through the lung and in the size of the perfuseable worm burden. The pattern observed here for the migration of challenge organisms through the lung (Figure 3b) appears to have a form that is intermediate between the 3 week-infected and 12 week-infected animals of Sher et al. (1974) relative to the respective challenge controls in the two studies.

In the present study nearly as great a total number of challenge-derived schistosomula were eventually found in the lungs of both infected and control animals, but since relatively few challenge organisms were subsequently able to emerge into the portal system of the primarily-infected mice by 25 days (Figure 3c) it appears that the majority were eliminated in the infected animals either in the lungs or fairly soon afterwards. This interpretation may be contrasted with the evidence of von Lichtenberg, Sher and McIntyre (1977) which

suggests that the destruction of the challenge in resistant animals occurs before the parasites reach the lungs. This discrepancy may again depend on the different time intervals between infection and challenge in the two studies.

According to our calculations, the degree of resistance varied markedly between days 3 and 7 post-challenge, the majority of challenge organisms being detected in the lungs at this stage. In contrast, the degree of resistance with respect to perfuseable challenge orgaisms was relatively constant from day 7 onwards. Estimates of acquired resistance based on the lung migration assay at a single time point after challenge may therefore be misleading.

The disparity between the results in Figures 3 and 4, which give no evidence of a delay in migration of the challenge to the portal system of previously infected mice, and those in Table II may be due to a very much reduced rate of migration of challenge organisms from the lungs to the portal system of primarily-infected animals compared with the rate of migration in challenge control mice. A greater number of schistosomula were detected in the lungs of the former animals 18, 21 and 25 days after challenge (Figure 3b) and these organisms may be representatives of a population which as a result of slow maturation are of a size which allows them to remain in the general circulation for a greatly extended period.

It is not known to what extent the results given here on host survival (Figure 2) and egg excretion rates (Figure 1) were affected by the parasite egg-induced granulomatous reaction being less severe

(Andrade and Warren, 1964; Cheever, 1965) and the rate of egg destruction being greater (Cheever and Anderson, 1971) in chronically-infected mice than in mice with early post-patent infections.

It is apparent from Table IV that the degree of resistance detected in primarily-infected animals may depend on the size of the challenge that has been administered, with larger challenges to some extent over-riding the protective mechanisms that have been induced. In contrast, Hunter, Crandall, Zickafoose and Purvis (1962) concluded that variation in the number of challenge larvae made little difference to the demonstration of resistance in mice 8 weeks after primary infection, though a reduced degree of resistance has been noted in rats exposed to 5000 cercariae compared with those challenged with only 500 to 1000 organisms (Knopf, Nutman and Reasoner, 1977). In the following paper, which investigates the time at which resistance to homologous reinfection is acquired in the mouse, a challenge of 200 cercariae has been used.

LEGENDS

Table 1

Mice were infected percutaneously with 50 S. mansoni cercariae (Groups A and B) and 8 weeks later challenged, together with age- and sex-matched uninfected control groups (C), with 200 homologous larvae. Mice were perfused 7, 11, 15 or 24 days after challenge. (1) Number of mice per group at time of perfusion. (2) Number of worm pairs = number of mature male or female worms, whichever was the lower, derived from the primary infection. (3) Number of challenge-derived organisms, distinguishable in terms of size from the adult worms of the primary infection. (4) % reduction = mean of the values obtained for the calculations of $100 - (\frac{n}{N} \times 100)$ where n = the number of challenge-derived organisms perfused from each Group B mouse and N = the mean number of organisms perfused from Group C as a whole. (5) Derived from the Student 't' test and calculated using the actual number of challenge-derived organisms perfused from respective Group B and C animals. (6) Perfused on the same day as the Group B and C mice which were perfused 7 days after challenge.

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TABLE I

Group	No. of mice (1)	Day of Perfusion	No. of Adult Worms	No. of Worm Pairs (2)	No. of Immature Worms (3)	% Reduction(4)	p.(5)
A	10	7(6)	7.5 ± 4.3	2.5 ± 1.4	-	-	-
B	10	7	8.3 ± 3.8	3.1 ± 1.7	3.7 ± 2.1	56.0 ± 25.1	<0.01
C	10		-	-	8.4 ± 4.0		
B	10	11	6.4 ± 2.8	2.4 ± 0.8	17.4 ± 9.1	56.3 ± 22.9	<0.001
C	9		-	-	39.8 ± 10.5		
B	10	15	7.1 ± 4.1	2.1 ± 1.4	25.8 ± 10.4	30.6 ± 25.5	N.S.
C	10		-	-	36.0 ± 14.6		
B	9	24	6.4 ± 2.8	2.3 ± 1.0	23.6 ± 15.0	50.7 ± 30.2	<0.01
C	10		-	-	46.8 ± 16.3		

Table II

Three groups of CBA mice (one Group A and two Group B) in each of two experiments were given three percutaneous injections of 15, 15 and 10 S. mansoni cercariae respectively, each infection separated by an interval of one week. Eight weeks after the first of the primary infections the three groups of animals were challenged with 200 homologous cercariae, together with two uninfected control groups (C). Twenty-one days after challenge one Group B and one Group C in each of the experiments were perfused, and 35 days (Experiment 1) or 53 days (Experiment 2) after challenge the remaining three groups were perfused. For notes (1), (2), (3) and (5) see Legend to Table 1. (4) The degree of resistance in animals perfused 3 weeks after challenge was calculated as in note 4, Table 1; for animals perfused 5 or nearly 8 weeks after challenge, it was calculated by subtracting the mean number of worms/mouse found in Group A from the number found in each Group B animal, and subsequently calculating the percentage reduction in each B animal relative to the mean number of worms perfused from Group C mice.

TABLE II

Expt.	Group	No. of Mice(1)	Day of Perfusion	No. of Adult Worms	No. of Worm Pairs(2)	No. of Immature Worms (3)	% Reduction (4)	p.(5)
1	B	9	21	10.0 ± 3.0	4.3 ± 1.4	18.3 ± 14.7	76.6 ± 18.8	<0.001
	C	10		-	-	78.4 ± 14.9		
	A	21		8.6 ± 3.2	3.5 ± 1.5	-	-	
	B	22	35	53.7 ± 28.1	20.1 ± 12.7	-	52.2 ± 29.3	<0.001
	C	12		93.8 ± 11.8	40.8 ± 6.7	-		
2	B	9	22	13.0 ± 4.6	5.0 ± 1.7	13.3 ± 7.6	64.6 ± 20.1	<0.001
	C	10		-	-	37.7 ± 10.6		
	A	17		10.7 ± 4.7	4.4 ± 2.1	-	-	
	B	20	53	34.2 ± 17.0	15.1 ± 7.9	-	48.0 ± 32.6	<0.001
	C	13		42.0 ± 11.0	19.3 ± 5.3	-		

Table III

The number of worms perfused from the mice autopsied 8 weeks after challenge in Experiment 2, Table II, together with the number of eggs found in the liver, intestine and faeces of these animals. % reduction in each instance was calculated according to the formula

$$100 - \left(\frac{\text{mean of Group B} - \text{mean of Group A}}{\text{mean of Group C}} \times 100\right)$$

Faecal egg determination was made on the day before perfusion.

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TABLE III

Group	No. of Mice	No. of Adult Worms	% Red'n	No. of eggs in liver	% Red'n	No. of eggs in gut	% Red'n	No. of eggs/ 100mg faeces	% Red'n
A	17	10.7 ± 7.7	--	16463 ± 4691	--	27874 ± 17926	--	94.1 ± 72.6	--
B	20	34.2 ± 17.0	44.1	21467 ± 6549	67.2	48513 ± 15749	62.6	129.3 ± 68.6	83.4
C	13	42.0 ± 11.0	--	15241 ± 4582	--	54149 ± 18808	--	212.6 ± 141.2	--

Table IV

Mice infected percutaneously with 100 S. mansoni cercariae were challenged 7 weeks later together with uninfected controls with 200 homologous larvae. Immediately following perfusion 21 days after challenge the livers from both groups of mice were finely minced in medium and incubated for three hours at 37° C for extraction of challenge-derived organisms which had not been perfused. Notes (1), (3), (4) and (5) as in Table I.

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TABLE IV

Group	No. of mice(1)	No. of adult worms	No. of perfused immature worms(3)	No. of immature worms in liver	Total No. of immature worms	% (4) Reduction	p.(5)
B	9	26.9 ± 6.6	6.2 ± 3.6	1.1 ± 1.3	7.3 ± 4.5	83.7 ± 9.9	<0.001
C	9	--	44.0 ± 16.5	1.0 ± 1.0	45.0 ± 16.8	--	--

Table V

CBA mice were given a primary infection of 35 S. mansoni cercariae (Groups A and B) and the Group B mice, together with the respective challenge controls (C) were challenged 8 weeks later with 100, 200, 400 or 800 cercariae. All groups were perfused 28 days after challenge. Notes (1), (3), (4) and (5) as in Table I.

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TABLE V

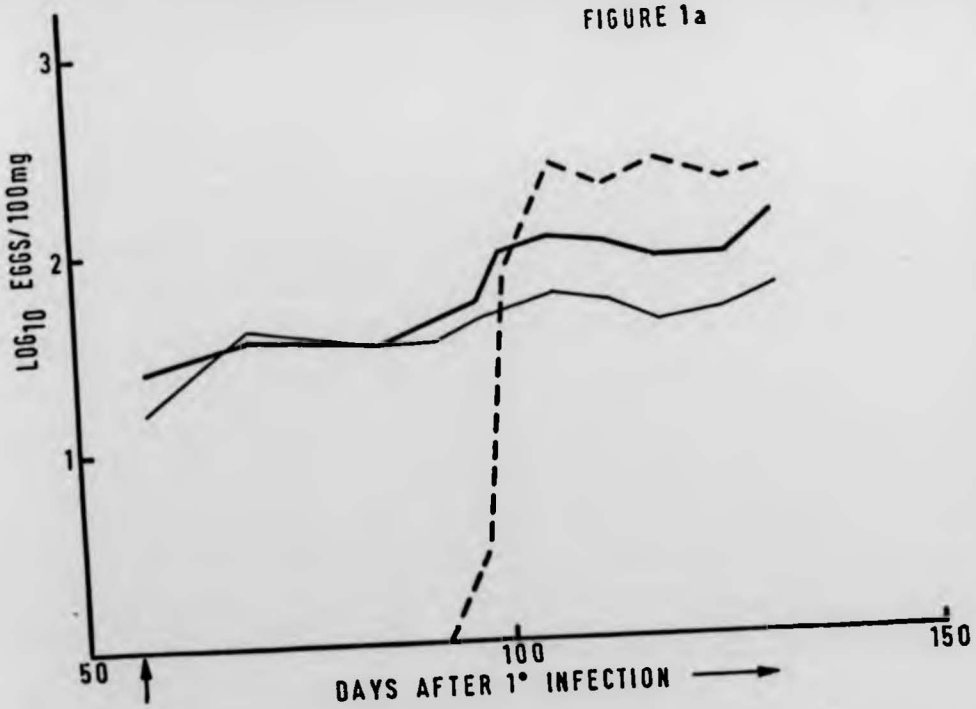
Group	No. of mice(1)	No. of Challenge cercariae	No. of Adult worms	No. of Immature worms (3)	% Reduction(4)	p.(5)
A	21	-	7.9 ± 4.4	-	-	-
B	10	100	6.8 ± 1.2	9.6 ± 5.2	63.9 ± 19.4	<0.01
C	10		-	26.6 ± 13.2		
B	11	200	5.3 ± 3.5	22.8 ± 16.1	47.6 ± 33.7	<0.05
C	11		-	41.3 ± 23.7		
B	11	400	7.4 ± 4.1	57.7 ± 35.5	45.2 ± 32.5	<0.02
C	11		-	103.1 ± 45.5		
B	9	800	9.0 ± 4.9	113.7 ± 66.3	38.3 ± 34.7	N.S.
C	10		-	179.5 ± 74.8		

Figure 1

(a) The rate of S. mansoni egg excretion (mean number of eggs/100 mg faecal matter) in Group A (thin continuous line), B (thick continuous line) and C (dashed line) mice given a primary infection of 40 S. mansoni cercariae over a two week interval (A and B) and a challenge (arrow) of 200 cercariae 8 weeks after the first of the primaries (B and C), according to the protocol given for a different set of similarly infected experimental mice in the Legend to Table II.

(b) The mean number of eggs/100 mg faecal matter found in the mice of Group A, Figure 1a, has been subtracted from the faecal egg count in Group B and the Log_{10} transformed result plotted (continuous line) relative to the Group C result (dashed line).

FIGURE 1a



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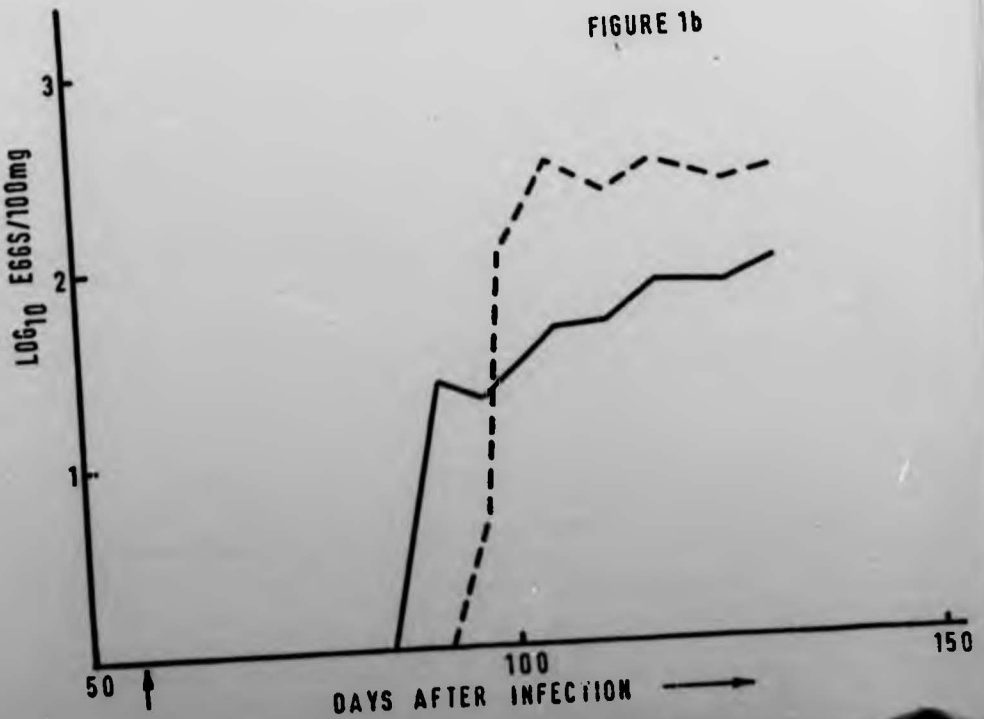

Figure 2

(a) The survival time course of CBA mice primarily infected with 40 S. mansoni cercariae over a period of 2 weeks (Groups A and B, see Legend to Table II) and challenged (arrow, Groups B and C) with 200 cercariae 8 weeks after the first of the primary infections. Group A, primary infection alone, = thick continuous line; Group B, primary infection and challenge = thin line; Group C, challenge alone = dashed line.

(b) The mortality rate of the 11 mice in Group B, Figure 2a, which were alive on the day on which the first challenge control mouse died, has been replotted (thin line) with respect to the challenge control Group from Figure 2a (dashes).

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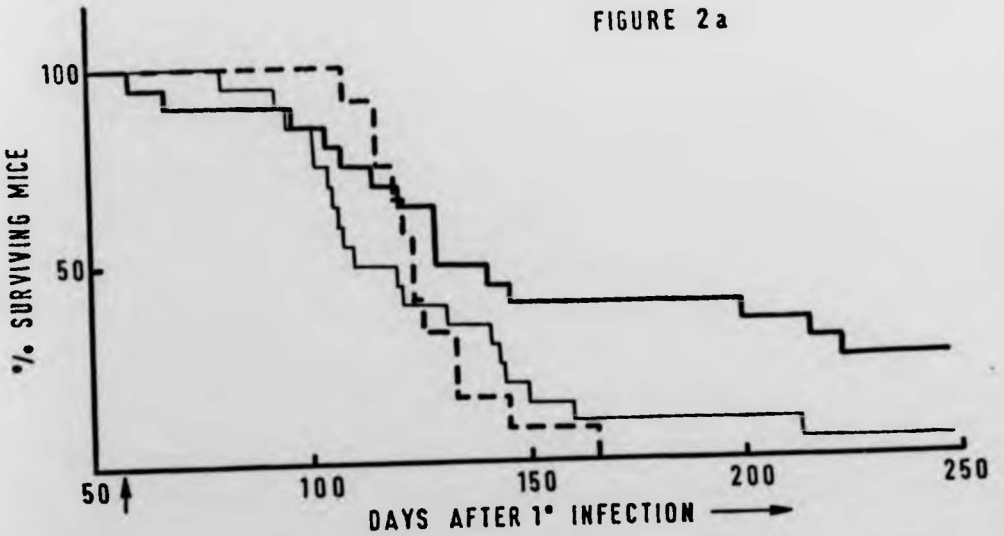

FIGURE 2b

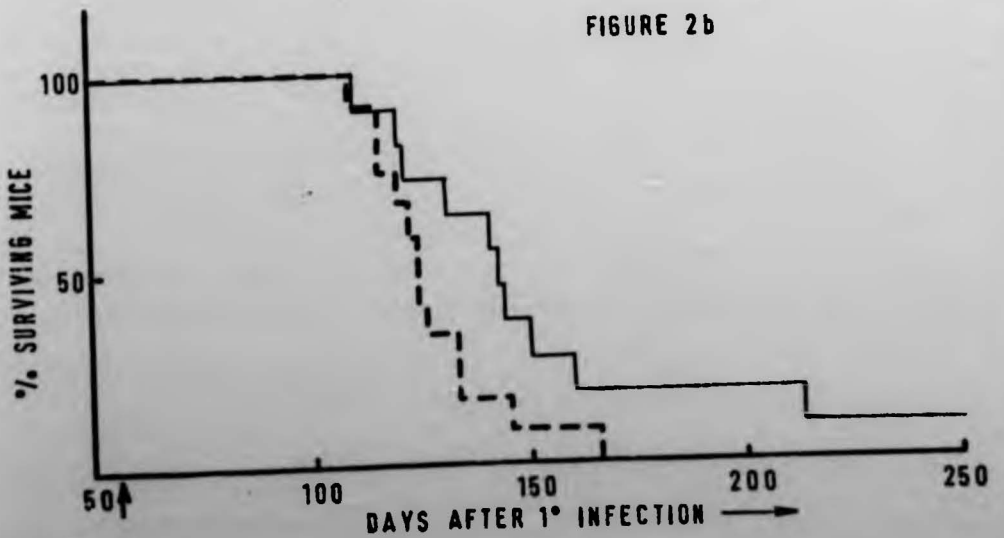

Figure 3

CBA mice were given three percutaneous primary infections of 25, 35 and 35 S. mansoni cercariae respectively, each infection separated by one week, and both they and age- and sex-matched uninfected control mice were challenged with 500 homologous cercariae 8 weeks after the first of the "trickle" primary infections. The total number of challenge-derived organisms detected in both the lungs and perfuseate of the primarily infected mice (thin line) or control mice (thick line) is given in Figure 3a. Figures 3b and c respectively give the number of organisms detected in the lungs and liver perfuseates separately. Five primarily infected and five control mice were assayed at each time point after the challenge except on day 21 when only three of the former animals were available.

Figure 4

(a) The number of mature worms perfused at each time point from the primarily infected animals depicted in Figure 3.

(b) The degree of resistance to challenge in the primarily-infected animals of Figure 3 calculated with respect to the total number of lung-borne and perfuseable challenge-derived organisms shown in Figure 3a.

(c) & (d) The degree of resistance with respect to lung-borne and perfuseable organisms, calculated from the results given in Figures 3b and c respectively.

Figure 3

CBA mice were given three percutaneous primary infections of 25, 35 and 35 S. mansoni cercariae respectively, each infection separated by one week, and both they and age- and sex-matched uninfected control mice were challenged with 500 homologous cercariae 8 weeks after the first of the "trickle" primary infections. The total number of challenge-derived organisms detected in both the lungs and perfuseate of the primarily infected mice (thin line) or control mice (thick line) is given in Figure 3a. Figures 3b and c respectively give the number of organisms detected in the lungs and liver perfuseates separately. Five primarily infected and five control mice were assayed at each time point after the challenge except on day 21 when only three of the former animals were available.

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FIGURE 3

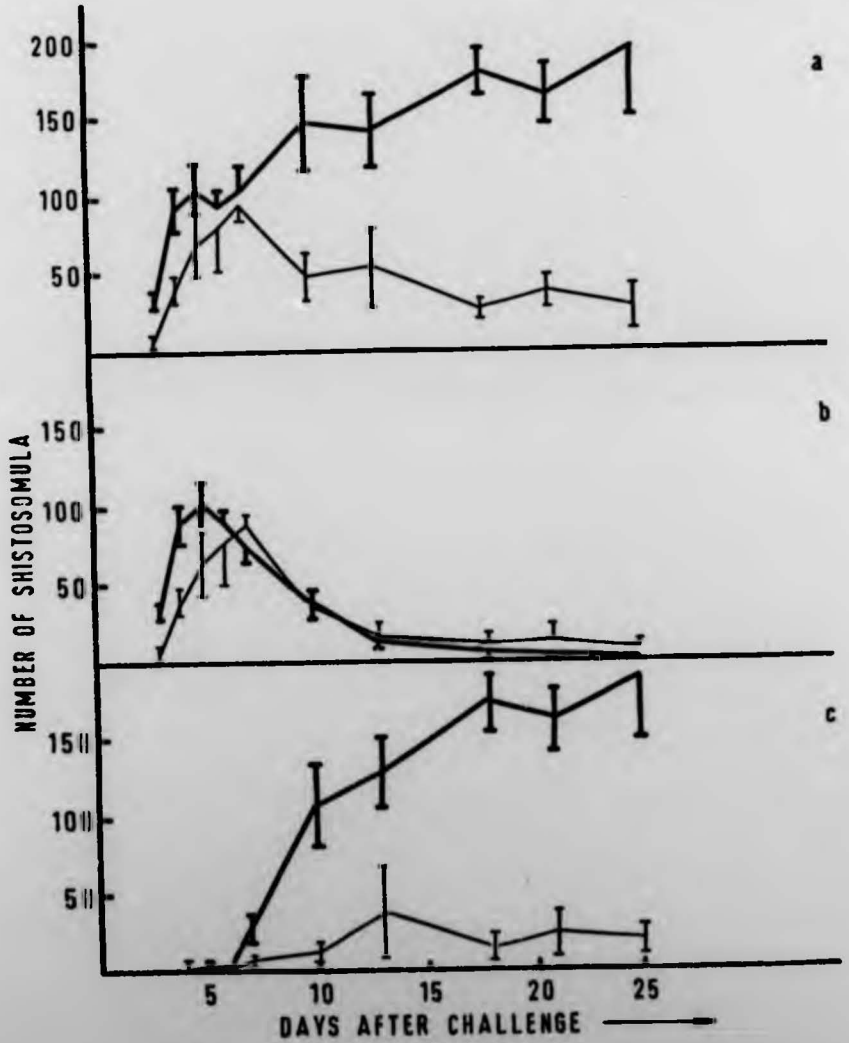
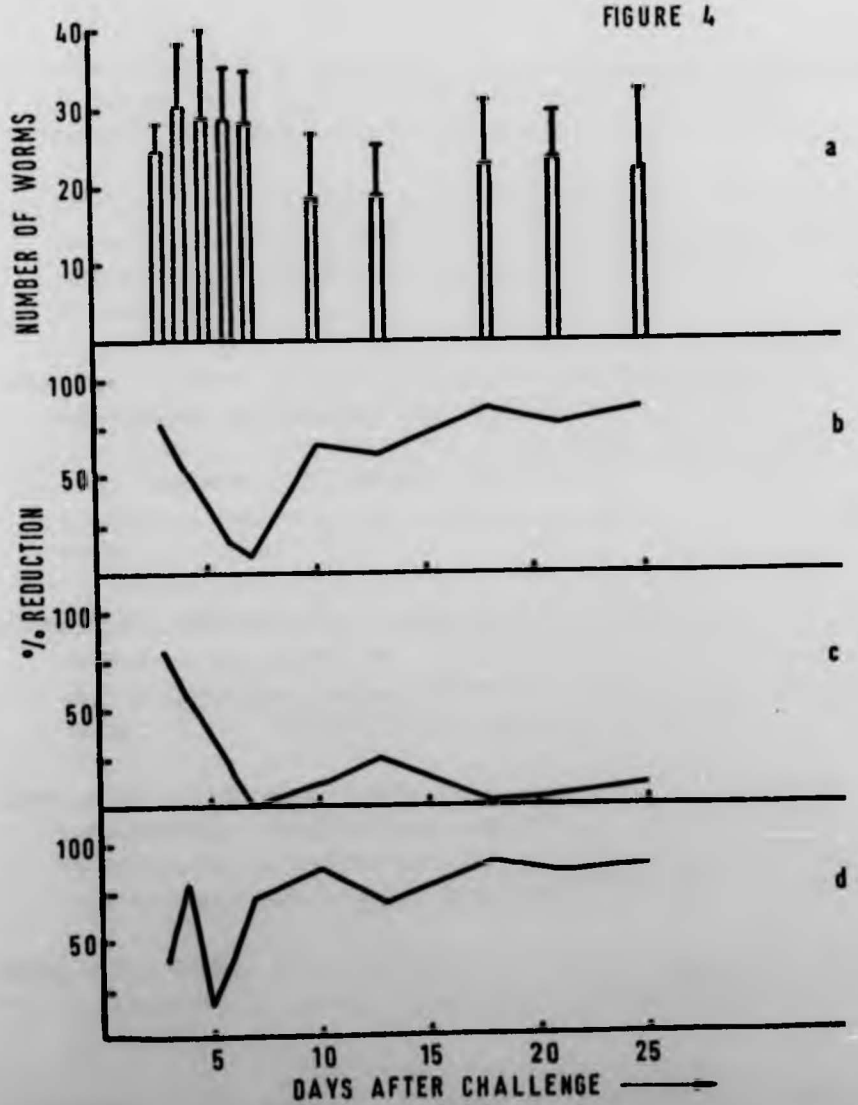

200
150
100
50
a
150
100
50
b
NUMBER OF SHISTOSOMULA
15
10
5
c
5
10
15
20
25
DAYS AFTER CHALLENGE

FIGURE 4


NUMBER OF WORMS
40
30
20
10
a
% REDUCTION
100
50
b
100
50
c
100
50
d
5
10
15
20
25
DAYS AFTER CHALLENGE

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APPENDIX 2.

Summary of the experimental date from previous studies on resistance to reinfection with *S. mansoni* in the mouse.

Notes to Tables A & B.

1. Interval to challenge is in weeks unless otherwise stated. When multiple primary infections were given this interval is from the last primary infection (vaccination).

2. Primary infection worm burden has normally been assessed by perfusion of a group of mice which had received the primary infection alone. Olivier and Schneidermann (1953), however, perfused their mice 17-35 days post challenge and could distinguish the older worms of the primary infection from the pre-adults of the challenge.

3. Percentage reduction is calculated with reference to the worm recoveries from a group of mice that had received the challenge infection but not the primary infection (vaccination), thus :-

$$\% \text{ reduction} = 100 \times \frac{\text{Mean number of worms in challenge controls (A)} - \text{Mean number of worms attributable to the challenge in previously infected mice}}{A}$$

4. 'P' values were invariably calculated by Student's 't' test.

5. Some data additional to that published was personally provided by the authoress.

6. Interval between challenge and perfusion.

7. Interval between repeated exposures.

8. Positive values indicate that the calculated challenge-derived worm burdens in superinfected groups were greater than in their challenge controls.

9. Doses of around 3.0 kr and above generally prevented persistence of ancy irradiated worms.

10. Ultra-violet radiation was used. Although no data was presented on worm burden surviving the radiation it was stated that a small proportion survived as stunted adults.

I.P. Cercariae given by intraperitoneal injection.

S.C. Cercariae given by subcutaneous injection.

N.S. Not significant ($P > 0.05$).

Sign. Significant ($P < 0.05$).

N.A. Data not available from the published results.

TABLE A. Summary of published data on resistance to reinfection with S. mansoni in the mouse.

Reference	Mouse strain	Mean number of cercariae in primary infection(s)	Interval to challenge (weeks 1)	Mean number of cercariae in challenge	Mean number of primary infection worms 2	Mean percentage reduction of challenge 3	Significance (P) 4
Stirewalt 5 (1953)	NIH (CFW)	50	1 hr	50	N.A.	57	<0.001
		50	1 day	50	N.A.	49	<0.001
		50	1	50	N.A.	0	N.S.
		50	4	50	N.A.	0	N.S.
		50	8	50	N.A.	37	<0.001
		50	8	50	13.6	65	Sign.
		50	8	50	20.0	93	Sign.
		50	8	50	20.8	93 (6 weeks) 6	Sign.
Gold et al. (1975)	Swiss	100	8	100	23.4	28 (7 weeks)	N.S.
Sher et al. (1974)	C_3H	Small No.	3	75	10.5	12	>0.05
	Parkes	" "	12	75	4.9	74 (6 weeks)	<0.001
Sher et al. (1975)	Parkes	30-45	12-15	125-140	N.A.	74	<0.05
	CBA	30-45	12-15	125-140	N.A.	67	<0.05
	C_3H	30-45	12-15	125-140	N.A.	92 (6 weeks)	<0.05
Ritchie et al. (1963)	Bagg	35	16	50	10	33	N.S.
		25	24	80	13	56 (8 weeks)	<0.002
Mahmoud et al. (1975a)	CF1	10 (S.C.)	38	100	4	69 (6 weeks)	<0.001
Hunter et al. (1967a)	Swiss ICR	75	6	75	14.8	41 (6 weeks)	<0.01
Olivier and Schneidermann (1953)	Swiss	25+30	16	360	3.8	39	<0.001
		20+25	41	318	9.9	19	>0.1
		15+20+20	29	352	13.0	52	>0.05
		112	18	239	20.0	71	<0.001
	A/LN	25+30	13	339	3.1	79	<0.001
		50+25	12	239	14.3	99	<0.001
	C57BL/ 5JN	100	21	239	48.6	92	<0.001
		45	16	342	18.5	28	=0.05
		45 (2-6 weeks) 7	16	348	16.3	25 (17-35 days)	<0.05
Hunter et al. (1961)	CFW	5x10	4-5	50	5.9	25	0.3
		5x10	4-5	50	7.4	+8 8	0.7
		5x10 (10-14 days)	4-5	50	10.9	24 (6 weeks)	0.2
Hunter et al. (1962)		4x15	30 days	100	11.4	33	0.1
		4x15	60 "	100	11.4	40	0.025
		4x15 (10 days)	90 "	100	11.4	28	0.025
		3x20	0 "	100	9.1	+15	0.1
		3x20	30 "	100	9.1	42	0.005
		3x20	60 "	100	9.1	47	0.005
		3x20 (15 days)	90 "	100	9.1	57	0.005
		60	30 "	100	10.8	+12	0.25
		60	60 "	100	10.8	77	0.005
		60	90 "	100	10.8	77	0.005
		60	120 "	100	10.8	65	0.005
		3x25	56 "	50	16.2	74	0.005
		3x25	56 "	100	16.2	85	0.005
		3x25 (10 days)	56 "	200	16.2	85 (8½ weeks)	0.005
	Swiss ICR	5x10	50 "	100	13.2	33	0.005
		2x25	47 "	100	14.0	0	0.75
		4x25 (7 days)	47 "	100	24.0	73 (7 weeks)	0.005
Hunter et al. (1967b)	C57BL/ 6S	4x15 (10,1, 1 week)	7	100	17.5	50	<0.05
Murrell et al. (1975)	NIH (CFW)	9x10-15 (1.P.) (7 days)	1	50	N.A.	57	<0.01
		50	24	50	N.A.	79 (7 weeks)	<0.001
Frick et al. (1965)	Bagg	10x10	4	100	34.8	74	<0.001
		10x10 (7 days)	4	100	19.7	90 (4 weeks)	<0.001
		10x10 (14 days)	4	100	24.0	85 (8 weeks)	<0.001

APPENDIX 3.

Summary of the experimental data from previous studies on resistance following infection of mice with radiation-attenuated S. mansoni cercariae.

TABLE B.

Summary of the published data on resistance to S. mansoni induced by infection of mice with irradiated cercariae

Reference	Mouse strain	Mean number of irradiated cercariae	Radiation dose (rads)	Interval to challenge[1]	Mean number of cercariae in challenge	Mean number of worms from irradiated infection[2]	Mean percentage reduction of challenge[3]	Significance (P.)[4]
Villella et al. (1961)	Swiss	300(I.P.)	0	8	300	52	100	N.A.
		300 "	1000	8	300	29	92	N.A.
		300 "	2500	8	300	1-2	88	N.A.
		300 "	5000	8	300	0	36	N.A.
		300 "	7500	8	300	0	34	N.A.
		200 "	3000	10	300	1	88	N.A.
		2x200 " ($7\frac{1}{2}$ weeks)[7]	3000	6	300	>1	92 (28 days)[6]	N.A.
Radke and Sadun (1963)	Swiss	200	950-2000	8	200	20	100+	<0.001
		200	2500-10000	8	200	N.A.	50	<0.001
		200	12500-40000	8	200	N.A.	13	>0.1
		200	3000	8	200	N.A.	41	<0.001
		7500	3000	8	200	N.A.	12	>0.1
		15000	3000	8	200	N.A.	37	<0.05
		200	5000	8	200	-[9]	61	<0.001
		500	5000	8	200	-	43	<0.02
		1000	5000	8	200	-	51	<0.01
		2000	5000	8	200	-	43	<0.01
		1000x3	5000	8	200	-	26	<0.01
		10000x3	5000	8	200	-	48	<0.001
		1000x3	5000	1	200	-	19	>0.05
		1000x3	5000	13	200	-	25	<0.01
		10000x3	500	1	200	-	33	<0.001
		10000x3 (7 days)	5000	13	200	-	39 (8 weeks)	<0.001
Erickson and Caldwell (1965)	Bagg-Swiss	100	5000	8	100	-	11	>0.05
		100	6000	8	100	-	16	>0.05
		100	8000	8	100	-	25	<0.01
		100	10000	8	100	-	27	<0.01
		4x1000	4000	6	100	-	+33	N.A.
		4x1000	6000	6	100	-	36	<0.01
		4x1000	8000	6	100	-	49	<0.01
		4x 250 (I.P.) (2-4 weeks)	7000	6	100	-	33 (8 weeks)	<0.01
Murrell et al. (1975)	NIH	5000	N.A.[10]	4	50	N.A.	66 (7 weeks)	<0.01
Perlowagora-Szumlewicz and Olivier (1963)	Swiss	130	3000	7	130	0	0(+36)	N.A.
		130	6000	7	130	0	43(31)	N.A.
		130	9000	7	130	0	56(41)	N.A.
		130	12000	7	130	0	56(38)	N.A.
		130	15000	7	130	0	76(31) 33(60) days	N.A.
Perlowagora Szumlewicz (1964a)	Swiss	300	2000	6	70	1-3	40(11)	N.A.
		300	2500	8	70	1	39(+20) 27(82) days	N.A.

APPENDIX 4.

Preprint of a paper submitted to Parasitology comprising collaborative studies on immunization of sheep against S. mattheei and S. bovis using radiation attenuated parasites.

Further observations on immunisation of sheep against Schistosoma mattheei and S. bovis using irradiation-attenuated schistosomula of homologous and heterologous species

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Short (running) title:

'Immunisation of sheep against schistosomiasis'

SUMMARY

Previous experiments have shown that sheep can be protected against S. mattheei or S. bovis infection by immunising them with irradiated cercariae or schistosomula. In these S. mattheei experiments, a laboratory strain of parasite of rather low pathogenicity had been used, throughout. One of the aims of the current study was to see whether equally strong protection could be demonstrated when a more virulent, freshly isolated strain of S. mattheei was used for the challenge. The duration of resis-

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tance was also investigated, by challenging sheep over a year after the last vaccination. The results were assessed by bodyweight-gains, worm and egg counts, and histopathology. It was found that the vaccine was highly effective against the virulent strain, and that protection lasted over a year. As there was no evidence that the irradiated parasites were able to persist this long, it was concluded that the vaccine had induced a 'sterile' resistance. An experiment was also carried out on heterologous immunisation, to determine whether the S. mattheei vaccine could protect sheep against challenge with the sibling species S. bovis, but little evidence of protection was demonstrated. Finally, an attempt was made to protect sheep against S. mattheei infection by prior immunisation with irradiated S. mansoni schistosomula. The results again showed that much lower levels of protection were obtained when an heterologous system was used.

INTRODUCTION

In previous experiments sheep were protected against the most severe manifestations of subsequent S. mattheei infections following immunisation with irradiated S. mattheei cercariae or schistosomula (Taylor, James, Nelson, Bickle, Dunne and Webbe, 1976), and similar protection has been produced against S. bovis after immunisation with irradiated S. bovis cercariae or schistosomula (Hussein and Bushara, 1976; Taylor, James, Bickle, Hussein, Andrews, Dobinson and Nelson, 1978). Protection was shown to last at least 37 weeks in the case of S. bovis (Hussein and Bushara, 1976) but the duration of protection against S. mattheei was not investigated; this

was therefore one of the objectives of the present study. Two different irradiation doses were used for the vaccines; 6 krad as in the previous study (Taylor et al., 1976) but also a lower dose (2.7 krad) which was expected, on the basis of work in mice, to result in the establishment of long-lived sterile adult worms, and perhaps therefore a more durable resistance.

In the earlier S. mattheei experiments the strain of parasite used both for vaccinations and challenges was of low pathogenicity for sheep, having been attenuated by repeated passages in hamsters (Taylor, James, Nelson, Bickle, Dunne, Dobinson, Dargie, Berry and Hussein, 1977). Sheep chronically infected with normal (non-irradiated) parasites of this attenuated strain showed few signs of disease (Dargie, Berry, Holmes, Taylor, James and Nelson, 1977) but nevertheless had considerable resistance against a challenge with cercariae of a highly virulent strain (Dargie, Berry, Holmes, Reid, Breeze, Taylor, James and Nelson, 1977). In the present experiment we investigated whether similar protection against challenge with the virulent strain could be obtained when irradiated, rather than normal parasites of the attenuated strain were employed for the immunisations.

Two studies on heterologous vaccination are also reported here. In the first of these, we have investigated the question of whether vaccination of sheep with irradiated S. mattheei schistosomula provides any cross-protection against S. bovis, the other important schistosome parasite of domestic animals in Africa. Secondly, we wanted to see whether irradiated vaccination with the readily available larvae of S. mansoni would protect sheep against S. mattheei. Effective heterologous

resistance occurs between many different species of non-irradiated schistosomes in several different types of experimental host, including sheep and cattle (Hussein, Saeed and Nelson, 1970; Massoud and Nelson, 1972; Preston, Nelson and Saeed, 1972) but cross-protection experiments using irradiated larvae have not been reported. In this experiment we included a highly-irradiated vaccine group (20 krad) as well as a group given a vaccine irradiated at a level (2.3 krad) designed to give rise to irradiation-sterilised adult worms. It has been reported that, in mice, highly irradiated S. mansoni vaccines can induce higher levels of protection than vaccines irradiated at near the minimum dose level required for parasite sterilisation (Minard, Dean, Jacobson, Vannier and Murrell, 1978).

MATERIALS AND METHODS

Parasites and snails

The attenuated strain of S. mattheei used for the vaccinations was originally isolated from snails collected at Komatipoort, South Africa in 1957 and maintained in South Africa for two years in mice and then in Mastomys natalensis (Pitchford, personal communication). We were sent infected snails by Dr. Pitchford in 1973 and subsequently passaged the strain in hamsters and Bulinus globosus snails from Nelspruit.

The S. mattheei challenges were carried out with cercariae shed by Bulinus globosus sent to us by Dr. Pitchford in January, 1977 which had been infected with miracidia derived from naturally-infected cattle (Pitchford, personal communication).

The cercariae of S. bovis used were shed by B. truncatus collected from Ed Duem, a town in the White Nile Province of the Sudan. These snails had been screened to eliminate already-parasitised snails and then infected in the laboratory with S. bovis miracidia obtained from experimentally-infected calves in Khartoum.

The strain of S. mansoni used was originally isolated from a patient in Puerto Rico and has been maintained at Winches Farm since 1964 in mice and hamsters.

Sheep

Forty-eight five months old Border Leicester x Suffolk wethers were obtained commercially, given anthelmintic treatment and vaccinated against clostridial diseases and foot rot. Throughout the experiment they were maintained outdoors at Winches Farm, St. Albans, on grass with supplementary concentrates.

Parasitological techniques

The method used for production of schistosomula is described by James and Taylor (1976). Techniques for irradiation, for infection of the sheep and for worm and egg counts were those of Taylor et al., (1976).

Plan of Experiments

The lambs were grouped and vaccinated as follows:

group 1: vaccinated with 40,000 6 krad irradiated S. mattheei schistosomula produced by syringe transformation and injected intramuscularly in four doses given over a five-month period;

group 2: as above, but with 30,000 2.7 krad irradiated schistosomula;

group 3: vaccinated with 40,000 2.3 krad irradiated S. mansoni schistosomula, given by intramuscular injections, in four weekly doses of 10,000;

group 4: as for group 3 above, but with 20 krad irradiated schistosomula;

group 5: non-vaccinated, S. mattheei "challenged controls";

group 6: non-vaccinated, S. bovis "challenged controls";

group 7: uninfected controls (experiment 2).

RESULTS

Experiment 1: Duration of resistance to S. mattheei following homologous vaccination

Eight sheep from group (1), eight from group (2) and five from group (3) were challenged with 3,400 normal S. mattheei cercariae each, applied percutaneously, 55 weeks after the last vaccination. The sheep were weighed weekly after challenge and faecal egg counts were performed four times after challenge on faeces taken from the rectum. The sheep were necropsied 15 weeks after challenge.

Results

Between weeks 5 and 15 after challenge infection, the sheep vaccinated with the 6 krad-irradiated schistosomula gained in bodyweight by 15% and with the 2.7 krad schistosomula the weight gain was similar (12%), whereas the controls lost weight (see Figure 1). Analysis of variance showed that the differences between the weight gains of the vaccinated and non-vaccinated

sheep are significant ($p < 0.001$). There were also significant reductions in the worm counts and tissue and faecal egg counts ($p < 0.001$), as shown in Table 1.

Histopathological studies showed that most of the vaccinated sheep had very small, fibrous granulomas in their livers and generally reduced hepatic lesions compared with the non-vaccinated controls. However, in about a third of the vaccinated sheep, and in one of the controls, there was a massive periportal eosinophilic reaction with the development of large, eosinophilic, necrotic nodules sometimes around old granulomas already in the fibrotic stage. The sheep showed severe vascular lesions even when hardly any eggs occurred in the sections.

On the other hand, despite some variation in the intensity of pathological reactions seen in different parts of the bowel from the same animal, all the vaccinated sheep showed considerably milder and fewer granulomas and eggs both in the small and large intestines, and hence much fewer inflammatory, destructive, or hyperplastic lesions than the controls.

In all groups, the granulomas varied from the ordinary giant-celled and fibroid types to highly eosinophilic, exudative or necrotic types and were almost confined to the mucosa, except in non-vaccinated sheep where they also occurred in other layers of the intestinal wall. In the latter animals, too, an important feature was the presence of numerous intact eggs, often without reaction, in the intestines; this was a rare finding in the vaccinated sheep. Lesions in the kidneys and spleen were slight and similar in the two groups of sheep while pulmonary involvement was more frequent in the non-vaccinated controls.

Experiment 2: Sheep vaccinated with S. mattheei and challenged with S. bovis

Seven group (1) sheep and seven group (2) sheep were challenged with 4,400 S. bovis cercariae 86 weeks after the last vaccination, along with five group (6) controls, and three uninfected controls (group 7) were also included in this experiment. Faecal egg counts, packed cell volumes and bodyweights were determined weekly and the sheep were necropsied 15 weeks after challenge.

Results

Bodyweight measurements showed no evidence of protection in this experiment and packed cell volume determinations showed that all the experimental groups did equally poorly compared with the uninfected controls (Figure 2). Faecal egg counts are shown in Table 2. The average logarithmic counts, taken over the 6 to 8 determinations for each sheep, show a statistically significant difference between controls and vaccinated animals ($p < 0.05$) but none between the two vaccination treatments. Worm and egg counts showed no statistically significant evidence of resistance.

Experiment 3: Sheep vaccinated with S. mansoni and challenged with S. mattheei

Four sheep from group (3) and four from group (4) were challenged nine weeks after the last dose of vaccine with 3,400 normal S. mattheei cercariae, employing the same challenged controls as in Experiment 1. Faecal egg counts and weighings were carried out four times and the sheep were necropsied 15 weeks after challenge.

Results

Bodyweight gains of 4% with the 2.3 krad vaccine and 9% with the 20 krad vaccine were recorded, whereas the non-vaccinated 'challenged controls' lost weight by 2% (Figure 1). These differences between vaccinated and non-vaccinated sheep were statistically significant ($p < 0.05$). No significant differences were found between the worm or egg counts (Table 1).

DISCUSSION

The first experiment showed that the irradiated S. mattheei vaccine provided good protection against a virulent strain of S. mattheei recently isolated from the field as well as against our more benign laboratory strain. This protection lasted for at least one year in the absence of re-exposure within that time and was at a level comparable to that demonstrated when challenge took place 2 to 4 weeks after the last vaccination (Taylor et al., 1976). Protection was demonstrated by worm and tissue egg counts, histopathology, and weight gain studies. This suggests that the vaccine might confer economic benefits in enzootic areas. These results are also of theoretical interest because they strongly suggest that this irradiated vaccine produces a "sterile immunity". It seems highly unlikely that any of the 6 krad irradiated organisms could have survived the full year before the sheep were challenged. In fact no typical irradiation-deformed worms were recovered from any of the vaccinated sheep, and in a previous study no worms were recovered from mice infected percutaneously with 6 krad-irradiated cercariae and perfused 8 weeks later (Taylor, 1975).

Immunization with either minimally or highly irradiated heterologous S. mansoni schistosomula was not as effective as the homologous vaccine. A similar result was obtained when we used normal, unirradiated S. mansoni cercariae previously in sheep as a vaccine against S. mattheei (Taylor et al., 1976), although previous workers had been able to demonstrate some heterologous resistance when a much longer period was allowed to lapse between immunisation and challenge.(Preston et al., 1972). Neither did vaccination with irradiated S. mattheei schistosomula induce a high level of protection against S. bovis challenge. These results suggest that a species-specific irradiated vaccine might be necessary to control domestic animal schistosomiasis in Africa.

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TABLE 1

Worm and egg counts in sheep vaccinated with either irradiated S. mattheei schistosomula (Experiment 1) or irradiated S. mansoni schistosomula (Experiment 3) and challenged with normal S. mattheei cercariae.

	Experiment 1				Experiment 3	
	No Vaccine	4,000 6 krad S. mattheei		30,000 2.7 krad S. mattheei	40,000 2.3 krad S. mansoni	40,000 20 krad S. mansoni
Individual worm recoveries	1301	112	750	273	632	1455	1798
	1583	117	1042	451	882	1393	1382
	1767	320	1487	475	1600	1953	1663
	1858	327		507		2055	1875
	1905	554		543			
mean	1682		589		670	1714	1434
% red'n c.f. controls	-		65		60	0	15
Individual tissue egg counts x 10^{-3}	19113	1857	9608	5421	8872	15307	32084
	27732	1705	23912	13389	18701	16304	17598
	32347	2101	16501	3368	31465	27110	17103
	19814	3031		3397		23760	20678
	22515	7071		4231			
mean	24304		8223		11105	20620	21866
% red'n	-		66		54	15	10
Individual mean faecal egg count (e.p.g.)	20	3	8	8	5	91	28
	152	3	29	18	13	8	30
	19	2	33	13	15	44	21
	51	9		2		47	52
	66	16		15			
mean	62		13		11	48	33
% red'n	-		79		82	23	47

TABLE 2

Faecal egg counts (e.p.g.) in sheep vaccinated with irradiated *S. mattheei* schistosomula and challenged with *S. bovis* (Experiment 2). The means of eight determinations between days 46 and 102 post-challenge are shown

	S. mattheei vaccine		Challenged controls
	6 krad	2.7 krad	
Mean counts from individual sheep	25.4 7.9 19.4 16.6 6.7 37.1 44.6	6.4 32.5 54.4 64.1 47.6 10.0 6.8	57.1 32.2 37.8 39.4 65.7
Overall mean	22.5	31.7	46.4
% reduction	52%	32%	-

TABLE 3

Worm recoveries and tissue egg counts in sheep vaccinated with irradiated S. mattheei schistosomula and challenged with S. bovis (Experiment 2)

	Worm Recovery	TISSUE EGG COUNTS (e.p.g.)		
		Liver	Small Intestine	Large Intestine
6 krad S. mattheei vaccine	1312	1314.6	6380.2	2286.4
	1046	370.9	7843.3	4798.7
	613	173.4	6799.3	1678.8
	631	382.1	4141.6	2943.4
	397	367.0	3817.1	1663.0
	282	253.5	4139.3	2087.4
	840	2804.5	9501.5	3009.8
mean	731.6	809.4	6088.9	2638.2
% red'n	30	0	26	6
2.7 krad S. mattheei vaccine	283	226.6	1678.2	829.5
	455	715.6	10083.4	4013.7
	-	705.2	4417.5	3420.5
	180	247.7	2219.1	968.2
	448	327.2	5258.4	2047.4
	1536	213.8	6328.8	2672.1
	553	157.9	8039.0	4088.7
mean	575.8	370.6	5432.1	2577.2
% red'n	45	45	34	10
Challenged controls	1380	491.3	8474.7	4647.6
	626	621.2	12466.1	1883.3
	628	1203.2	4856.3	2103.8
	1343	695.0	7427.8	3316.3
	1261	371.8	7827.1	2304.0
mean	1047.6	676.5	8210.4	2851.0

CAPTIONS FOR FIGURES

Figure 1

Bodyweight gains in sheep vaccinated with either irradiated S. mattheei schistosomula (Experiment 1) or irradiated S. mansoni schistosomula (Experiment 3) and challenged with normal S. mattheei cercariae.

Figure 2

Packed cell volumes in sheep vaccinated with irradiated S. mattheei schistosomula and challenged with S. bovis (Experiment 2)

Figure 1.

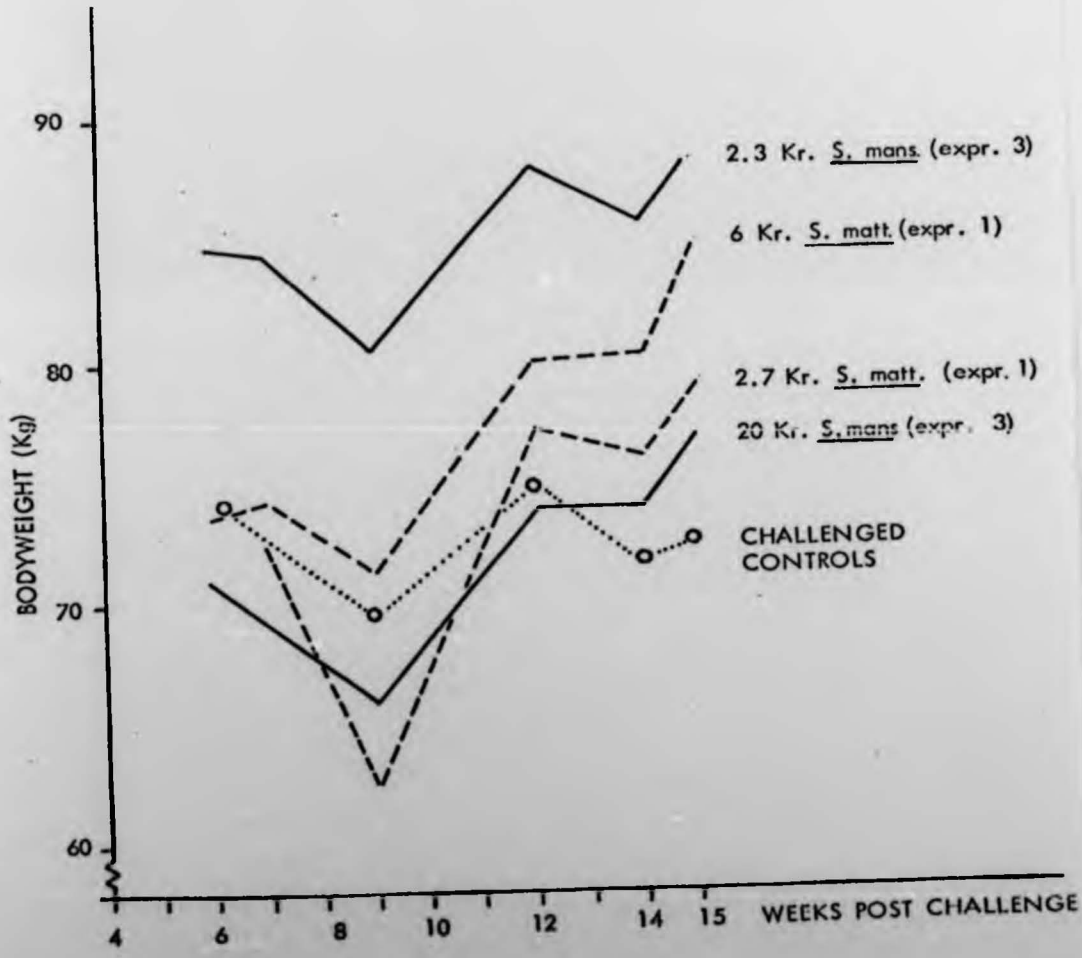
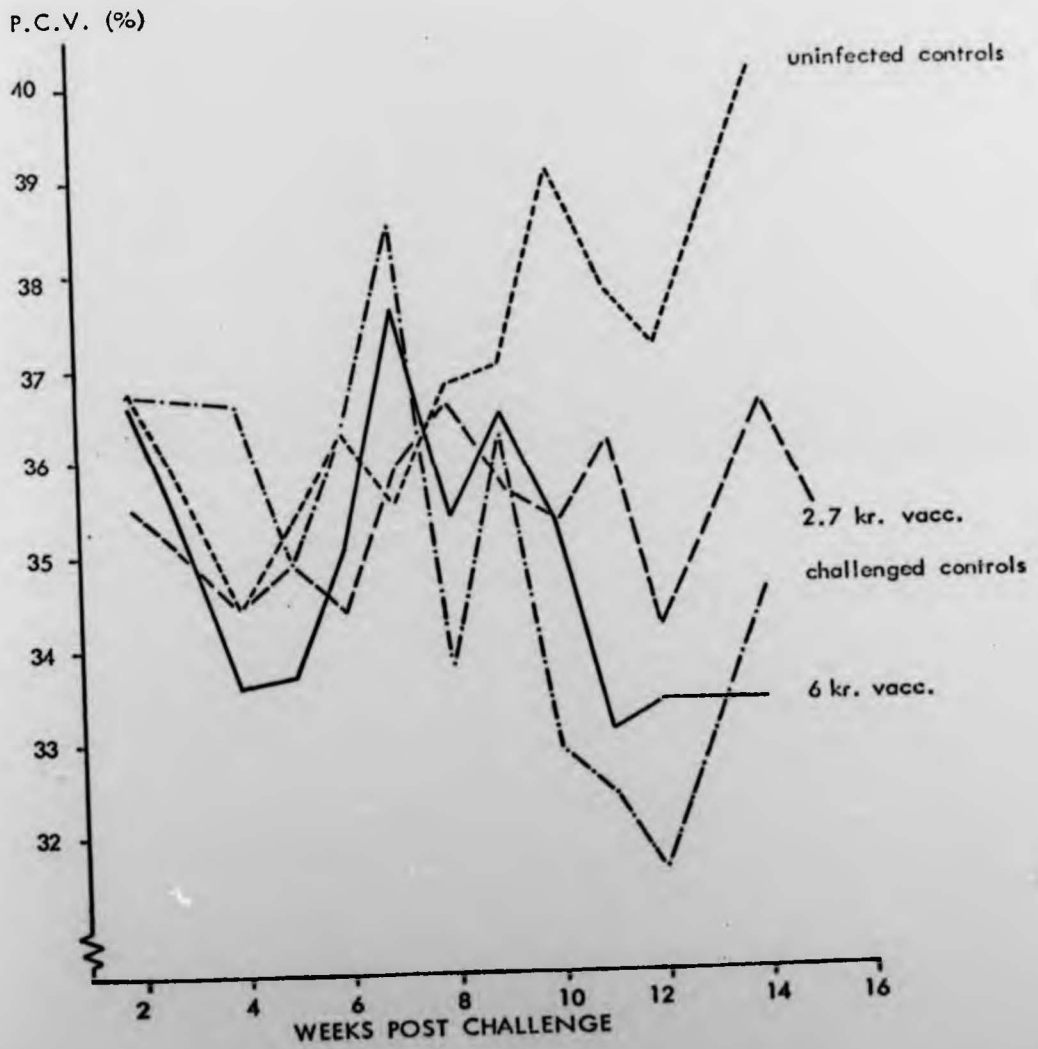

90
80
70
60
BODYWEIGHT (Kg)
2.3 Kr. S. mans. (expr. 3)
6 Kr. S. matt. (expr. 1)
2.7 Kr. S. matt. (expr. 1)
20 Kr. S. mans (expr. 3)
CHALLENGED
CONTROLS
4
6
8
10
12
14
15
WEEKS POST CHALLENGE

Figure 2.


P.C.V. (%)
40
39
38
37
36
35
34
33
32
2
4
6
8
10
12
14
16
WEEKS POST CHALLENGE
uninfected controls
2.7 kr. vacc.
challenged controls
6 kr. vacc.

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