THE DEVELOPMENT, HISTOPATHOLOGY AND *IN VITRO* CULTIVATION OF *Schistosoma margrebowiei*

by

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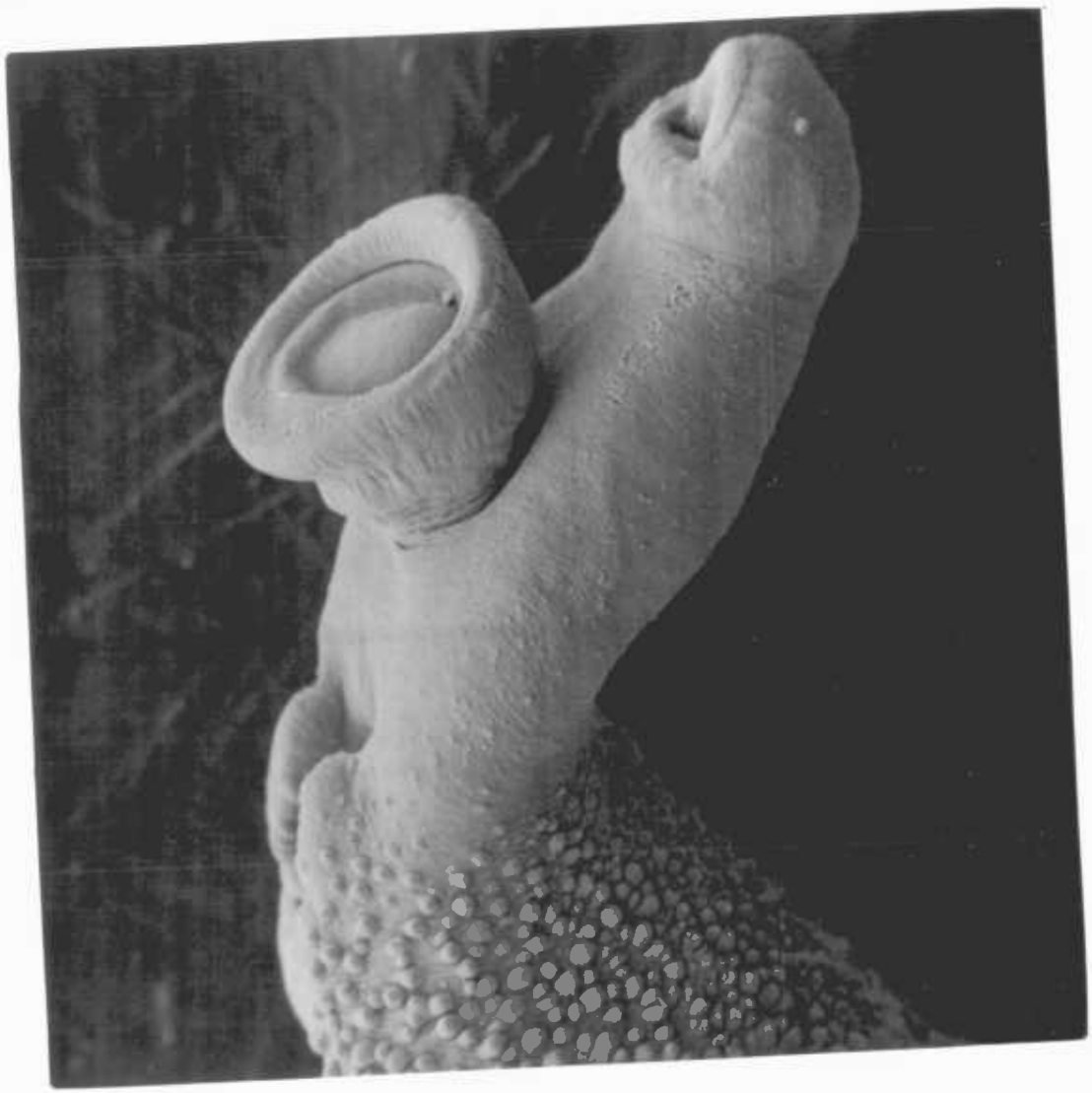
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F R O N T I S P I E C E

Scanning electron micrograph of the anterior end of adult male *S. margrebowiei*. x650



ABSTRACT

Schistosoma margrebowiei Le Roux, 1933 has been recorded in a number of mammals in Africa. The parasite was maintained in the laboratory using Bulinus natalensis as intermediate host and hamsters and mice as definitive hosts.

Parasitological features associated with Schistosoma margrebowiei infection in hamsters, mice and gerbils were determined and compared. High adult worm recoveries were observed by day 50 postinfection, up to 60% in hamsters, 50% in mice and 30% in gerbils. The growth and development of the parasite in these three hosts were studied and shown to be similar. The parasite attained maturity in these experimental hosts, the prepatent period being about 33 to 35 days.

The scanning electron microscope (SEM) was used to show the microtopographical details of the tegument of immature and adult worms in order to study the developmental changes. The observations revealed structural differences between migratory stages and adult worms, and also between adult male and female worms. The SEM was used to show the microtopographical details of the egg.

The histopathology of the infection in the experimental

hosts was studied. Unisexual and bisexual infections were used to show the pathology due to worms in the mesenteric-portal veins as well as that of deposited eggs in the host tissue. The main pathology was associated with deposited eggs in the liver and colon in the three hosts though pulmonary and splenic involvement was observed in mice with late infections.

Attempts were made to culture *in vitro*-derived schistosomula in a range of culture media. The transformation of cercariae to schistosomula was achieved either mechanically by shaking packed cercariae on a Rotamixer, or by incubation in Hanks' saline containing 50% rabbit serum at 37°C. The parasite developed to the 'closed-gut' stage in 24 days in culture medium NCTC 135 containing 50% rabbit serum and 1% rabbit red blood cells. There was no further development in organisms that lived in the culture for up to 40 days.

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1 INTRODUCTION AND REVIEW

1.1 INTRODUCTION

Trematodes of the genus Schistosoma cause the disease schistosomiasis in man and lower mammals. Schistosoma margrebowiei, a member of this genus was first described by Le Roux in 1933, who found it in a number of ungulates in Zambia. The type specimens on which the description of the parasite was based are presently in the collections of the Commonwealth Bureau of Helminthology, St. Albans, Hertfordshire : Slides 195, 196, 197; bottles 1025, 5023.

Pitchford (1977) in a check list of definitive hosts exhibiting evidence of the genus Schistosoma Weinland, 1858 acquired naturally in Africa and the Middle East, showed that S. margrebowiei occurred in a variety of mammals in Mali, Zambia, Zaire, Botswana, Tchad and South West Africa. He also mentioned that the parasite occurred as a mixed infection in some of these areas with two other species of schistosome, S. leiperi and S. mattheei. S. leiperi, like S. margrebowiei has been recorded from naturally infected cattle, lechwe, uku and the reedbuck in Zambia and both parasites have been shown to have a similar geographical distribution (Pitchford, 1976). S. mattheei is a member of the terminal

spined-egg complex' of schistosomes responsible for disease in man and lower mammals.

In the past, much attention has been given to the three schistosome species which are of major public health significance, S. haematobium, S. japonicum and S. mansoni. The possibility that human exposure to animal schistosomes might influence the severity of natural infection by the more pathogenic human species by a process of non-specific resistance, has led to more research work on animal schistosomes. Le Roux (1961) suggested that animal schistosome cercaria may immunize man against human species.

Based purely on epidemiological studies, Pitchford (1976, 1977b) has provided evidence to suggest that S. margrebowiei and S. leiperi, ungulate schistosomes and S. mansoni and S. haematobium, human species are mutually exclusive in their distribution due to the process of non-specific resistance. The immunological studies on school children, by Pitchford & Wolstenholme (1977) in an area where S. margrebowiei and S. leiperi are endemic, have shown that a large percentage were positive for schistosomal intradermal and complement fixation tests although they were free of schistosome infection by the standard urine and stool parasitological examination. This suggested that some form of

heterologous immunity may exist between these animal schistosomes and the human species.

The growing importance in research on animal schistosomes and the fact that little is known of S. margrebowiei has led to the present investigation on aspects of its biology. Snails infected with Schistosoma margrebowiei, which form the original stock of this investigation, were supplied by the Experimental Taxonomy Unit of the British Museum (Natural History), London in October 1977. The parasite was maintained in the laboratory using TF1 mice, hamsters and mongolian gerbils as definitive hosts and Bulinus natalensis as intermediate host.

The parasitological features in relation to the duration of unisexual and bisexual infections were evaluated and compared in three definitive laboratory hosts. The growth and development of the parasite in these three hosts were also determined.

The scanning electron microscope (SEM) has been used to study the microtopography of adult, immature worms and eggs of S. margrebowiei. This part of the study was undertaken to provide a detailed account of the microtopographical variation along the length of adult worms and the changes that occur on the surface from the

time the worms appear in the liver to when they mature and migrate to the hepatic portal and mesenteric veins.

As information on the definitive host-parasite relationship is still superficial, the present study is also a contribution to the histopathology of the infection. Gross and microscopic changes associated with the duration of the infection in the three hosts were compared.

Some preliminary studies on *in vitro* cultivation of the parasite starting with the cercaria, were undertaken.

1.2 LIFE CYCLE

Schistosomes are digenetic trematodes which develop in a complicated life cycle through a succession of stages : egg; miracidium; mother sporocyst; daughter sporocyst; cercaria; schistosomulumand adult. An alternation of generation occurs in which there is the sexual generation parasite in the definitive vertebrate hosts (mammals and birds) and the asexual generation parasite in the intermediate invertebrate hosts (molluscs).

The life cycle is briefly summarized here, a detailed review of the literature is given in Section 1.4. *Schistosoma margrebowiei* adult worms, like other mammalian intestinal schistosomes, reside in the hepatic

and mesenteric veins. The paired worms in these blood vessels produce eggs which must be passed out of the vertebrate host to complete the life cycle. The passage of eggs from the blood vessels to the lumen of the gut is probably facilitated by enzymatic secretion from the eggs and the peristaltic movement of the gut. A large proportion of the eggs may end up in the tissues of the gut. However, many eggs do not reach the intestinal lumen but are retained in the intestinal wall itself or may spread to other organs or tissues not related to the portal venous system.

The viable eggs that pass out with faeces hatch rapidly in fresh water, releasing the miracidium. The miracidium is a relatively short-lived free-living larval stage that dies if a suitable intermediate host is not encountered during its short life-span. In the appropriate molluscan host (Bulinus sp.), the miracidium develops to the mother sporocyst close to its site of penetration and some days later gives rise to the daughter sporocysts which migrate to the digestive gland. In 30 to 35 days, the numerous cercariae, developed from the daughter sporocysts, are shed by snails. The cercaria which is a free-swimming larval stage is infective to the definitive host (vertebrates).

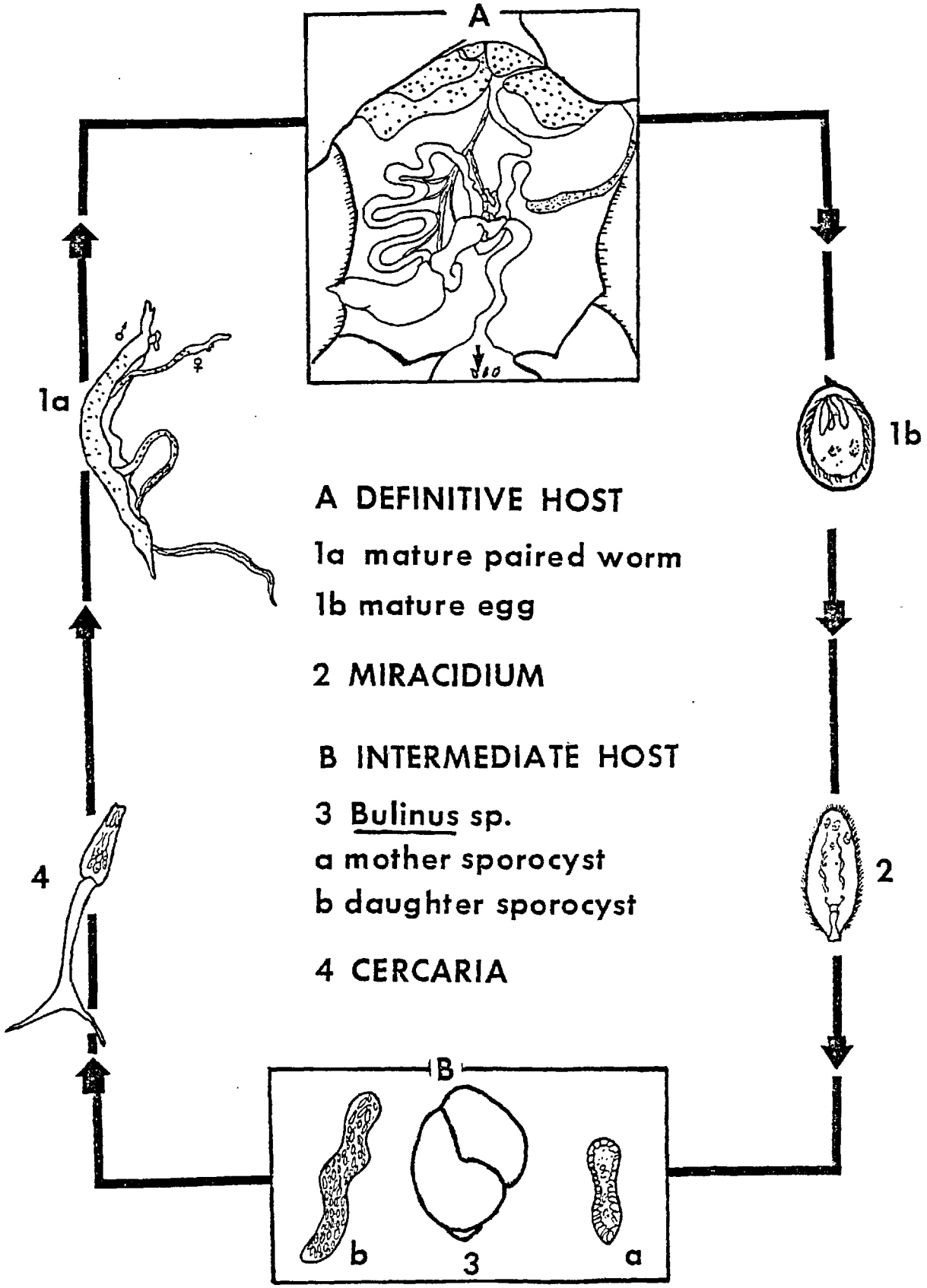
The cercaria penetrates the vertebrate skin, this is

accomplished by attachment to the skin with the aid of suckers and by mechanical action eased by enzymatic secretion. On entry, the cercaria leaves its tail outside the skin and becomes the schistosomulum.

The schistosomulum migrates first to the lungs from the skin, either through the blood-stream or the lymphatics, then it migrates to the liver. In the portal veins, worms pair, attain maturity and move against the blood flow to their final site, the hepatic and mesenteric veins. The mature paired worms produce eggs in about 30 to 35 days after penetration. Like S. japonicum, eggs of S. margrebowiei are laid in aggregates. Figure 1.1 is a diagrammatic representation of the life cycle of S. margrebowiei.

FIG. 1.1

LIFE CYCLE OF S. margrebowiei



1.3 GEOGRAPHICAL DISTRIBUTION

At present two areas of schistosomiasis due to *S. margrebowiei* are known to occur in Africa. One is in South East Africa, through Zambia, spreading from Katanga (van den Berghe, 1934) to Botswana (Pitchford, 1974). The other is in the Tchad Republic (Graber, 1969) where the schistosomes, in small numbers were collected in 1965, 1967 and 1968 from the hepatic portal and mesenteric veins of two waterbuck (*Kobus defassa*) and two buffon cobs (*Adenota cobs*).

The record of *S. margrebowiei* in *Kobus* sp. in Tchad by Graber (1969, 1978) is beyond the known distributional range of *Bulinus tropicus* group of snails. With the first report of *Bulinus forskali* and *Bulinus scalaris* for *S. margrebowiei* from Zambia by Wright, Southgate & Howard (1979), *B. forskali* was suggested by Wright *et al.* (1979) as the likely snail host in Tchad as it was reported to be present there by Birgi and Graber (1969).

The relative susceptibility of potential intermediate hosts will obviously play a part in the distribution of schistosomes. Pitchford (1975a) working in Chobe Game Reserve in Botswana, showed that the *Bulinus tropicus* group of snails from Chobe, Nelspruit and Northern Zululand were susceptible to *S. margrebowiei* from Chobe.

Bulinus natalensis used in this study, which was shown to be suitable for the parasite passage by Southgate & Knowles (1977) come from Sibaya, Natal. Figure 1.2 is a map of Africa showing countries and areas mentioned in relation to the geographical distribution of Schistosoma margrebowiei.

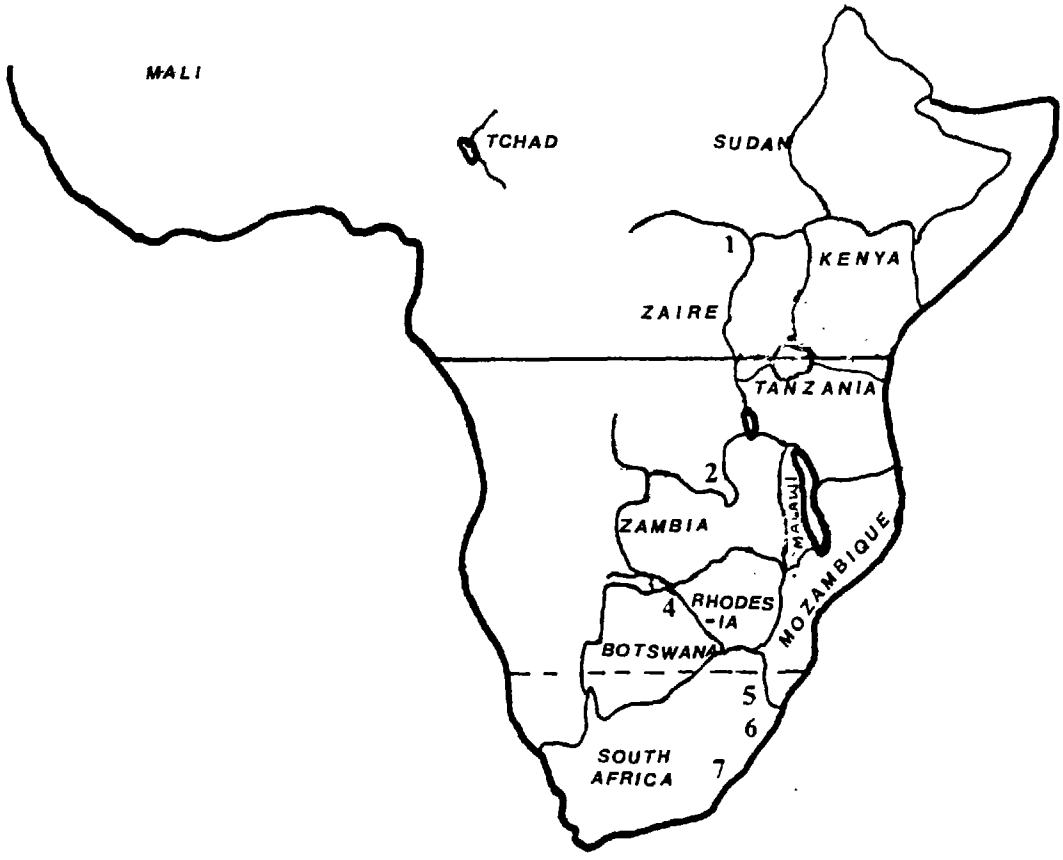
Pitchford (1976) attempted to define the southern limits of the distribution of S. margrebowiei and S. leiperi and also discussed their apparent limited distribution in relation to their potential intermediate host.

Schistosoma japonicum-like ova recovered by Walkiers (1928) from Upper Uele, North-East Zaire, from the faeces of five fishermen suffering from diarrhoea, and named as Schistosoma faradjei may have been spurious infections with S. margrebowiei. Lapierre and Hien (1973) reported the possible occurrence of eggs of S. margrebowiei in association with eggs of S. mansoni and S. haematobium in rectal biopsy material in an inhabitant from Mali.

The possibility of the development of S. margrebowiei in man, considered with the development in intermediate hosts from various parts of Africa and the report that the Bulinus forskali group of snails are natural hosts, suggest that the distribution of the parasite may be

greater than that described by Pitchford (1976).

The restricted distribution of the ruminants from which S. margrebowiei has been recorded, might account for the restricted distribution of the parasite(Pitchford, 1976). Moreover, Pitchford (1976) in a review of previous work on S. margrebowiei showed that the transmission of the parasite occurs on a vast plateau about 1,000 metres above sea level and it is possible that transmission may not be favoured in areas of greater temperature variations.



1 North East Zaire
2 Katanga
3 Eastern Caprivi
4 Chobe Game Reserve
5 Nelspruit
6 Northern Zululand
7 Natal

Fig. 1.2 Map showing the countries and places mentioned in the text.

1.4 GENERAL REVIEW ON *S. margrebowiei*

The bibliography on schistosomiasis in general is very large but well documented. Warren and Newill (1967) produced a useful bibliography that covered the period 1852 to 1962. Warren (1973) and Hoffman & Warren (1978) produced a series of selected abstracts and citations that cover the periods 1852 to 1972 and 1963 to 1975 respectively. A more recent quarterly publication, the 'Schisto Update', which gives a comprehensive coverage of the literature of schistosomiasis, is produced by the Edna McConnell Clark Foundation in cooperation with the National Library of Medicine, Bethesda, Maryland, USA.

The present literature review is intended to cover *Schistosoma margrebowiei* and work done on schistosomiasis that hasadirect relationship with the present study.

S. margrebowiei is a plathyhelminth worm of the class Trematoda, order Digenea, family Schistosomatidae. The anatomy, histology and life cycle of schistosomes are reviewed by Hyman (1951) and Jordan & Webbe (1969).

S. margrebowiei was first discovered in Zambia and described in 1933 by Le Roux. The specimens on which the classification was based, were collected from a wide

range of definitive hosts : cattle, zebra (Equus sp.), lechwe (Kobus leche), puku (Kobus vardoni), reedbuck (Redunca arundinum), blue wildebeast (Connochaetes equinus), situtunga (Tragelaphus spekei) and roan antelope (Hippotragus equinus). Le Roux (1933) also reported that S. margrebowiei resembled S. japonicum in the design of egg.

Little further work was carried out on this parasite after the description by Le Roux (1933) until Pitchford (1974) found the eggs in the droppings of four species of antelope in Chobe National Park, Botswana. Pitchford (1975) succeeded in establishing the parasite in the laboratory using laboratory bred snails (Bulinus tropicus and B. depressus) and rodents, Saccostomus campestris and Praomys (Mastomys) natalensis. This was the first report of the passage of S. margrebowiei from any snail host.

Pitchford (1976a) investigated the shedding pattern under outdoor conditions of snails experimentally infected with S. intercalatum, S. leiperi and Schistosoma margrebowiei and showed that the shedding of Schistosoma margrebowiei was not restricted and occurred throughout the day and night. This might give an ample opportunity for transmission to man and a number of other animals.

The preliminary work on S. margrebowiei was reviewed by Pitchford (1976b), who discussed the distribution, definitive hosts and relationship with other schistosomes. He suggested that the restricted distribution of the main definitive hosts, Kobus leche and Kobus vardoni and the likelihood of most 'common' game being unable to maintain the parasite indefinitely outside the confines of K. leche and K. vardoni distribution in South Africa might account for the restricted distribution of the parasite.

The next major contribution to the biology of this parasite was by Southgate and Knowles (1977). Their study entailed the morphology of the egg, miracidium, cercaria, and the parasite's compatibility with species of Bulinus and the development in the golden hamster. The authors showed that the parasite developed well in diploid, tetraploid, and octoploid snails of the Bulinus truncatus/tropicus complex and B. reticulatus group of snails (Bulinus wrighti). The parasite was reported to be pathogenic in hamsters with the peak death rate occurring by the 50-60th day of infection which coincided with the peak of egg production.

Graber (1978) recovered S. margrebowiei in small numbers in Tchad where the then known compatible intermediate hosts for the parasite (Bulinus depressus, B. natalensis

and B. tropicus, Pitchford, 1976; Pitchford and Du Toit, 1976) were never recorded. Studies in Tchad, (Birgi and Graber, 1969) revealed the presence, in most districts of the country, of Bulinus species belonging to the sub genera Physopsis (B.P. jousseaumei), Bulinus (B.B. truncatus) and Pyrgophysa (B.P. forskali). The first recovered natural snail host for S. margrebowiei was by Wright, Southgate and Howard (1979). They recorded natural infections in Bulinus forskali group of snails (Bulinus forskali and B. scalaris). This finding by Wright et al.(1979) led to the suggestion that Bulinus forskali might be the snail host responsible for the transmission of the parasite in Tchad.

Wright et al.(1979), working in Lochinvar National Park, Zambia, where naturally infected snail hosts for S. margrebowiei were found, reported that the most abundant host in the Park for S. margrebowiei and S. leiperi was the lechwe (Kobus leche). The examination of a few samples of the lechwe in their investigation showed that animals less than one year old had pure infections of S. margrebowiei and those over five years old had S. leiperi only. This finding was related to the different habitats occupied by the snail hosts for the two parasites and the annual movement of lechwe herds in response to the local flood cycle. The possibility of some cross-immunity was discussed in

their report.

1.5 REVIEW ON GROWTH AND DEVELOPMENT IN DEFINITIVE HOST

Schistosome cercaria penetrates the skin of the definitive host and becomes the schistosomulum. The conversion of cercariae to schistosomula has been reviewed by Stirewalt (1974). She is of the opinion that the mechanism involved in the conversion under natural conditions is rapid rather than the progressive process reported by Clegg and Smithers (1968) under *in vitro* conditions.

The development of the schistosomula within the definitive host has been described by several workers (Faust, James and Hoffman, 1934; Yolles, Moore and Meleney, 1949 and Clegg, 1965). Quantitative changes of the postcercarial growth and development of *S. mansoni* was reported by Lennox and Schiller (1972). The precise measurements of dry weight and glycogen content of individual worms were the criteria used to determine growth. The growth rate and weight at maturity of the male worm exceeded that of the female, but it was only in the male worm that the two parameters showed significant differences that could be related to the number of cercariae used to infect the host.

Wilson, Draskau, Miller & Lawson (1978) described the activity patterns and changes in shape of schistosomula recovered from various locations during migration from the skin to the hepatic portal system. They observed maximum increase in length of worms recovered from the lungs and suggested it facilitated migration through the blood capillaries. They also observed that when worms arrive in the hepatic portal system, they undergo a progressive shortening and suggested that this shape terminated migration by preventing escape of worms from the portal system via liver sinuses. The stimulus which triggers this response is thought to be the high nutrient levels present in the hepatic portal system and it probably terminates migration as well as initiates growth.

The measurement of the wet weight of schistosomula recovered during development by Lawson & Draskau (1977) shows that the increase in length of worms recovered from the lungs is not accompanied by worm growth. Gain in weight was only observed when worms reached the liver.

Bell and Smyth (1958) in a report on the cytological and histochemical criteria for evaluating development of trematodes and pseudophyllidian cestodes in vivo and in vitro, recognized seven developmental stages : 1. cell multiplication; 2. segmentation or body shaping;

3. organogeny; 4. early gametogeny; 5. late gametogeny; 6. egg-shell formation; and 7. oviposition.

Based on the study by Bell and Smyth (1958), Clegg (1965) demonstrated six developmental stages in S. mansoni : 1. lung form; 2. gut formation; 3. organogeny; 4. gametogeny; 5. egg-shell formation. and 6. oviposition. After this report, several workers have studied the development of other species of schistosomes (Smith, Clegg and Webbe, 1976; Ghandour, 1978 and Ghandour & Babiker, 1978).

The growth of paired S. margrebowiei in hamsters (Southgate and Knowles, 1977) showed that in about 31 days, the length of the paired female exceeded that of the male. This point on the growth curve, termed the 'cross-over point', is an indication of growth rate and is about 80 days in S. haematobium, 61 days in S. mattheei, 49 days in S. intercalatum, and 40 to 58 days in S. bovis.

1.6 REVIEW ON ULTRASTRUCTURE

1.6.1 SCHISTOSOME TEGUMENT

Schistosoma mansoni was the first digenetic trematode to be examined with the electron microscope by Gonnert (1955) and by Senft (1959). Since then various workers have examined several other species of schistosomes using electron microscope techniques.

In Fasciola hepatica, Threadgold (1963) first observed that the outer layer of the trematode tegument was a living, anucleate, cytoplasmic structure continuous with nucleated cell bodies located beneath the superficial muscle fibres. In order to emphasize that the tegument was not an inert structure secreted by the cell bodies as formerly thought, he proposed that the term cuticle was inappropriate and suggested the term tegument should be used. This term has now been generally accepted. Several other names have been suggested for both the tegument and the cell bodies. The term integument is commonly used in the United States of America (Burton, 1964) with internuncial processes connnecting the integumental epithelium to integumental cytons (Smith, Reynolds & von Lichtenberg, 1969). The suggestion by Lee (1966) to use the simpler name epidermis has not been generally adopted possibly because it does not fully

illustrate the structure of the tegument. The syncytial tegument has been found in all adult Digenea examined and it was first reported in S. mansoni by Lee (1966). Not only does the tegument have this unusual gross cytological structure but it also has unique cytoplasmic inclusions. Hockley (1973) suggested that these unique features of the trematode tegument are presumably related to its role as part of the host-parasite interface.

The schistosome cercaria, which is the free-swimming larval stage, penetrates the skin of the definitive host and becomes the schistosomulum. Tegumental changes associated with the transformation in S. mansoni have been described by Hockley (1973), Hockley & McLaren (1973) and the following description is a summary of their work. Figure 1.3 is a diagrammatic representation of these changes based on the results of Hockley (1973).

In free-swimming cercaria, the external tegument is bounded by trilaminate membrane surrounded by a thick fibrous coat or glycocalyx. Immediately beneath the membrane is a prominent dense layer. Hockley (1973) speculated that both the surface coat and the dense layer may be concerned with ionic regulation in a hypo-osmotic environment. These two structures are lost after penetration of the vertebrate skin. The dense cytoplasm of the cercarial tegument contains spherical and enlongate

membrane-bound bodies (Fig. 1.3 (a) & (b)). The schistosomula, unlike the cercariae, are adapted to a hyper-osmotic environment and therefore, are water intolerant. Such physiological alteration is reflected in the changes in tegumental ultrastructure. The tegumental distal cytoplasm of the schistosomulum still has a surface coat and a trilaminate membrane, but multilaminate bodies comprising concentric whorls of trilaminate membrane bodies are now present (Fig. 1.3 (b)). These bodies move up cytoplasmic connectives from the tegumental cell bodies; Hockley & McLaren (1973) suggest that these connections with the distal cytoplasm develop only after penetration. The surface of the tegument presents a mosaic appearance one hour after penetration and some parts retain the surface coat although these are cast off later (Fig. 1.3 (c)). At the same time, in other areas, the multilaminated bodies open out at the surface and add multiple stacked membrane sets to the pre-existing external trilaminate membrane. As a result of this process, most of the outer membrane is heptalaminate and in some parts, membrane fragments occur on the surface (Fig. 1.3 (c) & (d)). The heptalaminate limiting membrane body or vacuole thus becomes the outer membrane of the tegument. At the same time, the original trilaminate tegumental membrane is formed into microvilli which are cast off and thus the cercarial outer membrane is lost (Fig. 1.3 (c)).

FIGURE 1.3 Diagrammatic representation of tegumental changes from cercaria to schistosomulum, to adult and the structure of a sensory organelle. (Based on Hockley, 1973).

(a) Observation on the body tegument of the cercaria. Note the thick surface coat (sc) and the presence of trilaminate structures (ts) within it.

(b) Observation on the tegument of a 30 minute schistosomulum. Note reduction in the surface coat, the connection with subtegumental cell (stc) and the presence of numerous trilaminate bodies (lb).

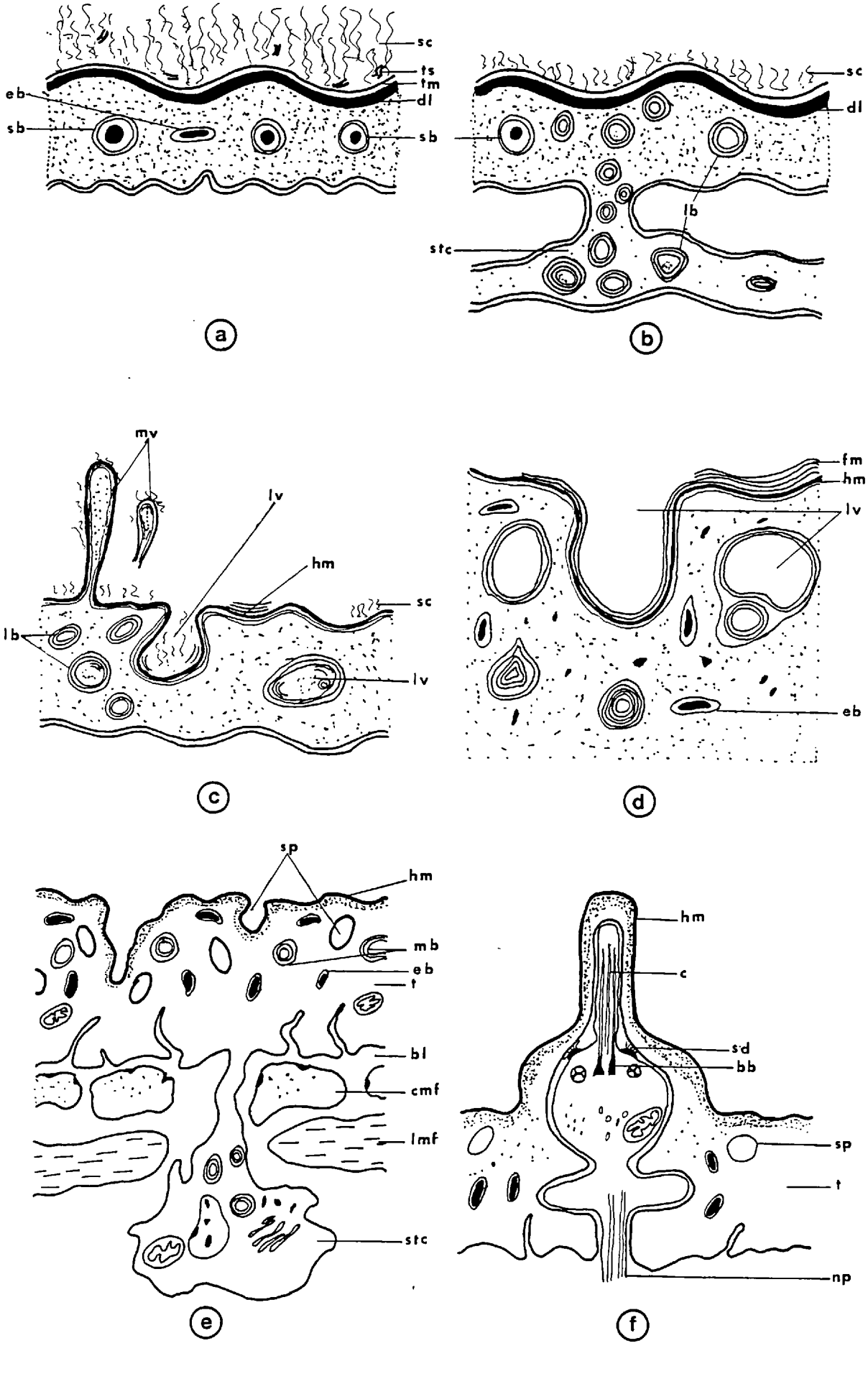
(c) Observation on the tegument of a 60 minute schistosomulum. Note the presence of large membranous vacuoles joined to the outer membrane and the dense stacks of membrane on the outer surface. The outer surface is hepta-laminate (hm). There is also the cast off of microvilli (mv) with trilaminate membrane.

(d) Observation on the tegument of 7 day schistosomulum. The surface is hepta-laminate and there are large surface pits (sp).

(e) Diagram of a typical part of the adult tegument. The outer surface is hepta-laminate and is pitted.

(f) Diagram of a sensory organelle of an adult. Note that the surface like the rest of the tegument, is heptalaminate.

KEY bb, basal body; bl, basal lamina; c, cilium; cmf, circular muscle fibres; dl, dense layer; eb, elongate body; fm, fragement of membrane; hm, hepta-laminate membrane; lm, laminated body; lmf, longitudinal muscle fibre; lv, large vacuole; mv, microvilli; np, nerve process; sb, spherical body; sc, surface coat; sd, septate desmosome; sp, surface pit; stc, subtegumental cell body; t, tegument; tm, trilaminate membrane; ts, trilaminate structure.


sc
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f

Schistosomula usually have a heptalaminate outer membrane within three hours of penetration. After this period, large vacuoles are replaced by smaller membrane bodies which probably contribute to the outer membrane during the growth of the schistosomulum. These membranous bodies are also present in the tegument of the adult worm (Fig. 1.3 (e)). Hockley & McLaren (1973) have shown evidence that the outer surface is continually renewed. Stirewalt (1961 & 1963) has described rapid changes in the antigenic nature and permeability of cercaria after penetration of the host and these may be connected with the renewal of the outer membrane. Smithers, Terry & Hockley (1969) also suggested that it was likely that the production of the multiple membranes during development is somehow related to the parasite's evasion of the host's immune response.

The formation of the heptalaminate membrane of the adult worm commences immediately after the cercaria has penetrated the vertebrate host. The tegumental surface of the worm is greatly increased during development because the outer membrane is invaginated and forms complex infoldings (Hockley, 1973). It is this outer surface of the adult worm that is observed with the scanning electron microscope and has revealed structural differences in the different schistosomes already examined (Miller, Tulloch & Kuntz, 1972; Silk, Spence &

Buch, 1970; Hicks & Newman, 1977; Sakamoto & Ishii, 1977).

The sensory organelles on the surface of adult schistosomes have been described in detail by Morris & Threadgold (1967) and Silk & Spence (1969) and briefly by Hockley (1970). The organelles are all of one type and they have the same basic structure as the uniciliated papillae on schistosome cercaria (Hockley, 1973). Fig. 1.3 (f) is a diagrammatic representation of the sensory organelle of an adult worm. The organelle consists of a bulb bearing an apical cilium which projects from the surface of the tegument and Morris & Threadgold (1967) speculated that the unit may function to detect the direction of the flow of the surrounding medium.

Scanning electron microscopy has been greatly employed for the characterization of helminth morphology especially since the improvement of scanning electron microscope preparation techniques. The external appearance of various species of schistosomes for example, has been studied using the SEM. The appearance of S. mansoni, for example, has been described by Hockley (1970), Silk et al. (1970) and Race, Martin, Moore and Larsh (1971). These authors have shown the variation that occurs on the surface of the male and female worms, described the spination of S. mansoni and provided

a valuable new view of the surface pits that occur on the worms.

Hicks & Newman (1977) studied the surface of S. haematobium and showed that the dorsal surface of the male was studded with prominent, spine-covered tubercles or bosses, not found in the female. The structural details of the oral and ventral suckers and local variations in the tegument were also described.

S. japonicum, which was studied by Sakamoto & Ishii (1977), was shown to present a spongy appearance due to the absence of bosses and spines on the dorsal surface of the male worm. Spines occur on the inner surface of the oral sucker, acetabulum and the lining of the gynaecophoric canal. The tegument of the female worm on the other hand was shown to be ridged and pitted with a tegumental surface devoid of spines except in its posterior end.

Tulloch, Kuntz, Davidson & Huang (1977) scanned the surface of critical point dried S. mattheei and observed marked differences in surface features of male and female worms as well as different regions on the same parasite. Their scanning electron microscope studies revealed surface structures which suggest basic adaptations for attachment or anchorage of the worms in the

blood vessels of the definitive host.

The dorsal surface of the adult male schistosome is in intimate contact with the host and is probably the initial target for host immunity. To provide an accurate basis for localizing and assessing tegumental damage and surface changes which accompany the immune response, Hockley & McLaren (1977) compared the worms of eight Schistosoma spp. Three basic types of dorsal tegumental surfaces were recognizable : i) tuberculate with spines; ii) tuberculate without spines; iii) non-tuberculate. S. mansoni was shown to be a typical example of species with spiny tubercles , S. haematobium and S. intercalatum also belong to this group. S. mattheei, S. bovis and S. margrebowiei were examples of non-spiny tuberculate schistosomes. The non-tuberculate dorsal tegument occurs in S. japonicum in which the dorsal surface is highly folded and formed into microvillus-like projections. Tuberculate species have a characteristically pitted surface between the tubercules and in these areas there are many sensory organelles and a few spines. Sensory organelles but not spines also occur on the dorsal surface of S. japonicum. Hockley & McLaren (1977) suggested that the presence or absence of tubercles and spines may be related to the age, the environment of the parasite or strain or species difference. This report by Hockley & McLaren

(1977) was short and did not contain micrographs.

Voge, Price & Bruckner (1978) studied the changes that occur in the tegument of S. mansoni during development in an experimental host using the SEM. They reported that the surface of immature male and female worms bear no resemblance to that of adult worms and that it is characterized by having many tegumental folds. The tegumental surfaces of immature male and female worms are similar and the dorsal and ventral surfaces of the male are similar before the formation of the gynaecophoric canal. Transition of the tegumental surface from juvenile to adult form begins after worms are 'in copula' and have grown several millimeters in length.

1.6.2 THE EGG

Smyth and Clegg (1959), showed that the egg of some trematodes is composed of sclerotin, or 'tanned' protein. Stenger, Warren & Johnson (1967) reported the presence of microspines covering the surface of schistosome eggs using the transmission electron microscope. Since then various workers (such as Race, Micheals, Martin, Larsh & Matthews, 1969; Race, Martin, Moore & Larsh, 1971) have confirmed the presence of microspines using transmission and scanning electron microscopes. Hockley

(1968) reported that the egg of S. mansoni, characterized by the backwardly pointing spine, seen under the SEM, is itself covered with minute spines which measure 0.28 x 0.5μm. He also observed that similar microspines covered the surface of S. haematobium eggs. Schnitzer, Sodeman, Sodeman, Durkee & Durkee (1971) using the transmission electron microscope reported microspines on the eggs of S. japonicum. These microspines which cannot be removed by enzymatic digestion are continuous with the main part of the shell and are probably composed of the same material (Hockley, 1968).

Ford, & Blankenspoor (1979) using the SEM have examined and compared the egg shell of the three human schistosomes, S. haematobium, S. japonicum and S. mansoni. They found that the microspines on S. mansoni and S. haematobium are essentially similar and that in S. japonicum, they were smaller and more densely distributed. They observed that the fibrous matrix on the surface of the eggs was not a host derivative and that it might account for the stickiness of the eggs and the microspines may function to hold the matrix in place.

1.7 REVIEW ON HISTOPATHOLOGY OF EXPERIMENTAL SCHISTOSOMIASIS

Warren (1973) reviewed the pathology of schistosome infection in both man and animals (experimental, domestic and wild). The main part of his review consisted of a series of descriptions of the gross and microscopic pathology of the major organ systems involved in schistosomiasis - the liver, spleen, intestine, urinary tract, nose, lungs and central nervous system - and the relationship of pathological changes to overt manifestation of disease. This review therefore, is only a summary of the histopathology of schistosomiasis.

The histopathology of schistosomiasis will vary depending on the species of worm or host and the site infected. In man, in the case of S. haematobium, it is the bladder which is mainly involved, but in S. mansoni and S. japonicum, the disease is mainly associated with the bowels. The disease results from : i) a general and localized reaction to the by-products of growing and mature worms, ii) haemorrhage when the eggs escape from the venules, and iii) abscess and tubercle formation around eggs trapped in the perivascular tissue.

The fate of eggs deposited by the female worm in the venules differs. Some escape through the tissue of

the appropriate organ, i.e. intestine or bladder and pass to the exterior; others however drift to the deeper viscera and become retained in submucosal tissue. In this way, eggs trapped in the liver, submucosal tissue and lung arterioles become the centres of areas of inflammation (El Mofty, 1962; Lincicome, 1962). Their presence may lead to the formation of sclerosing granular tissue which, when healed, is followed by fibrosis.

Fairley (1920) infected monkeys with S. mansoni and S. haematobium and studied the lesions produced up to 14 weeks after infection. He stated that the eggs of the two species produced identical types of lesions and he described the structure of the typical pseudotubercle and its development as far as early fibrosis. He described the early lesions in the liver, spleen and kidneys of monkeys dying of heavy infection, before the deposition of eggs, apparently caused by toxic products of the worms.

Brumpt and Chevallier (1931) studied the histopathology of S. mansoni and S. haematobium infection in mice and found them essentially similar, consisting of two types, granuloma produced by eggs and those by toxic products of worms.

Koppish (1937) followed in great detail the development of lesions produced by S. mansoni in rabbits and albino rats. Unfortunately, the two hosts which he employed were not favourable for normal development, reproduction and long survival of S. mansoni. Most of the pathology described by Koppish were produced by worms congregating and dying in the liver. Eggs were not found in large numbers and often were merely empty shells rather than normal eggs containing miracidia. No eggs or pathological lesions were found in the intestine.

Krakower, Hoffman and Axtmayer (1943) also described the course of lesions produced in guinea pigs harbouring massive infections of S. mansoni. The authors were mainly interested in the basis for production of portal hypertension which led to "congestive splenomegaly" and the development of large porto-caval anastomosis by which adult worms left the portal-mesenteric veins and reached the right heart and the lungs. They described the egg lesions in the liver from the acute stage through the tubercle and fibrosis stages until the egg shells and even the fibrosis disappeared. They also described the lesions produced by dead worms in the portal venules. Since the guinea pig is a relatively unfavourable host for S. mansoni, these authors found that most of the worms had died and had been absorbed by about

nine months after infection, leaving the tissues of the liver, intestine and lungs practically normal in appearance.

Faust and Meleney (1924) described the pathology produced by S. japonicum in experimental animals. The period of egg deposition was studied in dogs. They described the "schistosomiasis abscess" as an acute reaction to the eggs and its transition to a pseudo-tubercle. No lesions directly associated with adult worms were described, and only insignificant cellular infiltration independent of egg lesions were found.

Meleney, Sandground, Moore, Most and Carney (1953) described lesions encountered in mice harbouring a bisexual infection of long duration, that is after the beginning of egg deposition, and pointed out the similarities and differences in the lesions found in different experimental hosts harbouring the same species of worm, and the same species of host harbouring different species of schistosomes.

Hsü, Davis, Hsü and Lust (1970) in comparative studies of pathogenicity of schistosome egg lesions, suggested that as certain schistosome egg-host tissue relationships are better shown in special experimental animals, a comparative study of the lesions in various

animal species of different susceptibilities should throw more light on the pathobiology of the disease.

Hsü, Hsü, Davis and Lust (1972) carried out a comparative study of the lesions caused by eggs of S. japonicum and S. mansoni in livers of mice and rhesus monkeys and established a general model of the biological process of egg lesions. It consisted of five stages : non-reactive or weakly reactive; exudative; exudative-productive; productive and involutional. They also observed that some characteristics of egg lesions were controlled by the host species, i.e. they were common to lesions in both albino mice and rhesus monkeys; while some were determined by the parasite species, i.e. they were common to lesions elicited by S. japonicum and S. mansoni.

The only record in the literature on the pathobiology of S. margrebowiei was by Southgate and Knowles (1977) who reported that the parasite was pathogenic in hamsters.

1.8 REVIEW ON *IN VITRO* CULTURE OF SCHISTOSOME FROM CERCARIA

The *in vitro* cultivation of helminths in general has been reviewed by several workers (Guevara, 1971; Silverman, 1965; Silverman & Hansen, 1971; Weinstein, 1966; Smyth, 1976 & 1980; and Taylor & Baker, 1968 & 1978).

A number of workers have attempted to culture *S. mansoni* through the stages of development which occur in the vertebrate host (Senft & Weller, 1956; Robinson, 1957; Cheever & Weller, 1958 and Clegg, 1959). Apart from the works of Mao and Lyu (1957) and Fu, Chow and Chiu (1976) on *S. japonicum*, only a few attempts have been made on the *in vitro* cultivation of other human and animal schistosome species.

Significant growth of *S. mansoni* in culture was first recorded by Cheever & Weller (1958), who used young schistosomes recovered from the liver or lungs of experimentally infected mice. In a medium containing horse or human serum, Hanks' saline, chick embryo extract and red blood cells, the schistosomula increased in size considerably, but the rate of growth was relatively slow and sexual maturity was not attained. More rapid growth occurred in a similar medium where

the chick embryo extract was replaced by a mixture of amino acids, which were supplied by 0·25% lactalbumin hydrolyzate (Clegg, 1959). Schistosomula from the lungs of mice developed up to the point of sperm formation in males, but females, although forming a small ovary, did not develop vitellaria or lay eggs. The rate of development of schistosomes during cultivation was assessed by reference to the optimum rate of growth in the white mouse.

The *in vitro* cultivation of *S. mansoni* by Clegg (1965) has produced the best result on post-cercarial development. He used the Clegg's Medium II (inactivated rabbit serum and Eagle's saline 1:1) and the simple cellulose sac culture tube. Development to near-sexual maturity occurred after 42 days compared with 30 days *in vivo* in mice. Michalick, Gazzinella & Pellegrino (1979) have recently repeated the *in vitro* culture of *S. mansoni* from cercarial bodies to a stage of development at which worms are nearly sexually mature.

Taylor & Baker (1968) in their review showed that a number of workers had reported on cercariae transformation to schistosomula. Stirewalt (1966) established criteria to monitor cercariae to schistosomula transformation; i.e. loss of cercarial tail, evacuation of acetabular gland secretion, saline or serum tolerance

(cercariae are water tolerant and saline or serum intolerant) but water intolerance of the schistosomula. Stirewalt & Uy (1969) provided details of an artificial skin membrane and collection chamber for obtaining schistosomula following penetration of the membrane. A disadvantage is that the artificial preparation can only be used once or twice. Clegg & Smithers (1972) reported the use of an artificial membrane that yeilded only about 20-30% schistosomula. Eveland & Morse (1975) suggested that the use of an artificial membrane would not allow for the analysis of the precise steps occurring during cercariae-schistosomula transformation.

The conversion of cercariae into schistosomula was found to occur when cercariae were implanted into the peritoneal cavities of mice (Eveland, 1972). This study indicated that tissue contact was not a pre-requisite for conversion. Furthermore, Gilbert, Rosa, Borojevic & Pellegrino, (1972) showed that transformation occurred <u>in vitro</u> without a tissue stimulus and that a change was initiated by a phospholipid fraction containing a crude lecithin extract. Eveland and Morse (1975) however found that lecithin was highly toxic to cercariae.

A number of techniques have been used to induce cercarial transformation <u>in vitro</u>, for example, high

speed centrifugation (Gazzinelli, De Oliveira, Figueiredo, Pereira, Coelho & Pellegrino, 1973); vortex mixing (Tiba, Holanda, Ramalho-Pinto, Gazzinelli & Pellegrino, 1974) and the passage of cercariae through a narrow bore hypodermal needle (Colley & Wikel, 1974). Although these techniques appear effective, they may produce a traumatic physical stress and may damage the cercariae (Eveland & Morse, 1975).

Eveland and Morse (1975) determined the rate and extent of conversion of S. mansoni cercariae to schistosomula after incubation for 3-5 hours at 37°C in various test solutions. The criteria used to identify schistosomula included : 1) loss of cercarial tail; 2) viability of organisms in saline but not in water; 3) pre-acetabular gland evacuation; and 4) ability to survive in culture. They showed that although cercariae incubated at 37°C for three hours in Eagle's saline converted to water sensitivity, tail loss and pre-acetabular gland emptying did not occur. However, when Eagle's saline was supplemented with 25% or 50% rat serum, both conversion to water sensitivity and tail loss occurred and pre-acetabular gland emptying was initiated. Intestinal contents were apparent after seven to nine days and after two weeks in culture 95% of the organisms remained alive. Their best results were obtained when cercariae were incubated in a

mixture of Eagle's saline and rat serum (1:1) at 37°C for three hours, followed by cultivation in Clegg's Medium II.

Smith, Clegg and Webbe (1976) in a report on the *in vivo* and *in vitro* culture of *S. haematobium* showed that the rate of development in culture was the same as in the hamster up to day 31 when pairing first occurred and male produced spermatozoa. Worms in their culture did not pair and females did not complete sexual maturation as occurred in hamsters between days 57 and 65.

Yasuraoka, Hata and Saito (1978) reported the conversion of both *S. japonicum* and *S. mansoni* cercariae to schistosomula in serum supplemented media and subsequently cultured them *in vitro*. The transformation was achieved by the incubation of the cercariae at 37°C in NCTC 109 containing 2-50% human or 50% rabbit serum. The resultant schistosomula were grown *in vitro* to adults which mated, although females did not produce eggs. They also observed that the cercaria-schistosomulum transformation took place at a slower rate in heat inactivated serum than in unheated serum.

2 MATERIALS AND METHODS

2.1 MAINTENANCE OF *S. margrebowiei* IN THE LABORATORY

The maintenance of experimental schistosomiasis in the laboratory in non-endemic areas greatly depends upon the availability of large numbers of the intermediate host of the schistosome species involved. *Schistosoma margrebowiei* was maintained in the laboratory using *Bulinus natalensis* as intermediate host and hamsters and mice as definitive hosts.

2.1.1 REARING OF SNAILS (*Bulinus natalensis*)

Most laboratories engaged in schistosome studies rear snails in balanced aquaria of various types using snail diets of varying components. In this study, the snails were maintained as simply and economically as possible. The method used is similar to that used by the Experimental Taxonomy Unit, British Museum (Natural History), London.

The snails were kept in groups of ten in non-aerated plastic trays containing 1 litre of 'aged water' (i.e. London water containing guppies, allowed to stand in the laboratory, in large plastic containers, for at least

one month). The snails were fed mainly on sycamore leaves (Acer pseudoplatanus) which were dried and soaked in London tap water for at least three days before being used. Malek (1962) suggested that the soaking of the leaves in water removed the tannic acid and wax. Algae were allowed to grow on mud and this was baked dry. A small amount of this mud was added to supply the snails with trace elements. As a supplement, a very small amount of fish food (Bemax) and non-toxic blackboard chalk (as a source of calcium) was added everytime the trays were cleaned. The trays were covered with glass plates to prevent the snails from crawling out. They were maintained under artificial light at 24-26°C. The set-up is shown in Plate 1.1. All but 100ml of the water in each tray was replaced with fresh 'aged water' every fort night, as faeces, growth and reproductive inhibitors would otherwise accumulate.

Under these conditions, mature snails deposited gelatinous egg masses on plastic rafts placed in the trays or on the sides of the trays. Hatching occurred about ten days after oviposition and the newly hatched snails were put in separate trays and fed on the diet described above. Infected and uninfected snails were both maintained in the same way.

2.1.2 INFECTION OF LABORATORY REARED SNAILS

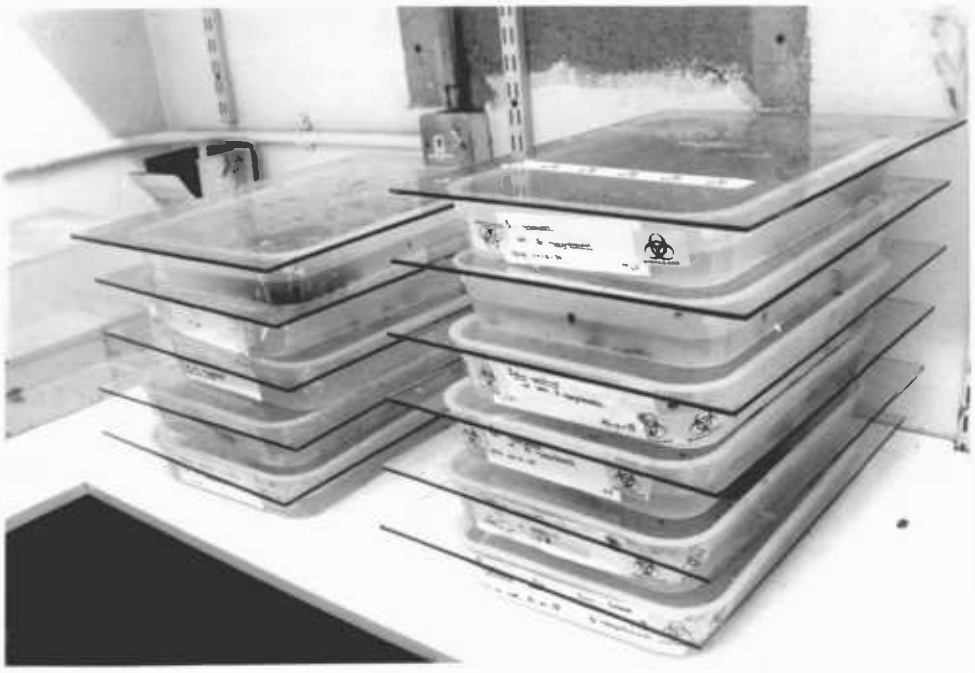
Laboratory reared Bulinus natalensis snails were infected with miracidia obtained from the eggs deposited in the liver or intestine of hamsters or mice with 50 to 70 day old S. margrebowiei infection.

The deposited eggs in the liver or intestine were obtained by the digestion method, as follows : The whole liver or intestine of the infected host was placed in a Waring blender with 100ml of 0·85% saline, minced for two minutes and then centrifuged. The sediment was transferred to a 250ml screw-cap bottle containing 1mg trypsin dissolved in 1N phosphate buffer (pH 7·8). To facilitate the digestion of host tissue by trypsin, this was agitated in a water bath at 37°C for three hours. The content of the bottle was centrifuged, the sediment washed several times in dechlorinated water and then transferred to the side-arm flask (Plate 1.2) used for hatching of eggs and collection of miracidia. The side-arm flask system described by Rau, Bourns and Ellis (1972) is an improved method for the recovery of newly hatched miracidia. This method exploits the negative-geotaxis and positive-phototaxis of the free-swimming larval stage. Large numbers of miracidia appeared in the vertical component of the arm as it was close to the light source. The miracidia were collected

in a beaker by opening the tap on the horizontal component of the arm.

Snails of about 2-2·5mm long were placed singly in wells of a multiwell dish containing 5ml of dechlorinated water (Plate 2.1). Under a spot lamp, miracidia were picked out from the beaker by means of a fine-drawn glass pipette and about three to five miracidia transferred to each well containing a snail. Some batches of snails were infected with single miracidium and cercariae from such infections were used for unisexual infections in hamsters and mice. To ensure infection, snails were left in the multiwell dishes overnight and then transferred to the prepared plastic trays as described in Section 2.1.1.

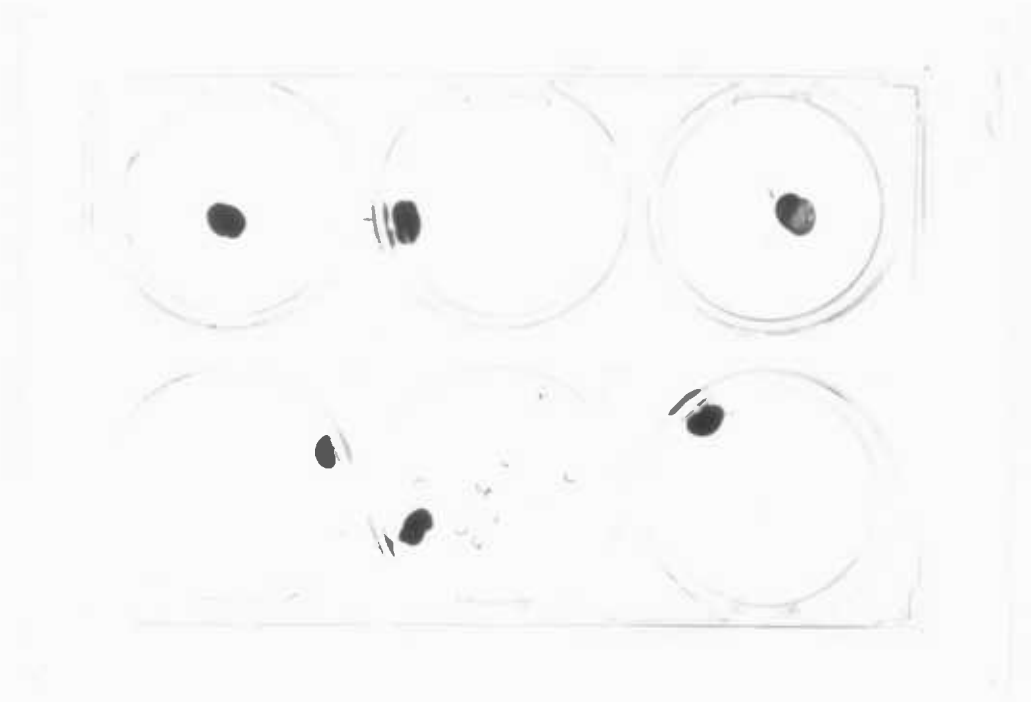
The inspection of snails for cercarial production commenced 30 days after infection. To isolate individual snails producing cercariae, all snails were removed from the trays, placed singly in separate dishes containing dechlorinated water and exposed to light at 26°C. Shedding occurred between 35 and 40 days after infection. After the onset, shedding usually continued for about a month. Snails exposed to three to five miracidia had an infection rate of about 20-40% while snails with single miracidial doses showed a lower infection rate, 0-10%.



1.1 Set-up used for rearing of snails in the laboratory. Plastic trays covered with glass plates.



1.2 Side-arm flask used for the recovery of newly-hatched miracidia (Rau et al, 1972).
A - aluminium foil
L - light source
S - side-arm
T - tap



2.1 Mini Petri plate used for infecting snails. Well surface area is 9·6cm^2. Each well contains 5ml of water and one snail.



2.2 System used during the infection of experimental hosts. A - set-up for infection; B - crystallizing dish which holds the cercarial suspension; C - 500ml plastic jar with holes in the bottom and on the sides to hold the host.

2.1.3 INFECTION OF LABORATORY MAMMALS

Different techniques have been recommended by various workers for the experimental infection of laboratory mammals. In most cases, it has been the procedure in such infections to apply water containing the cercariae by partial immersion, or by dropping it on the mucous membrane of the lip or by applying to the skin a teased-out preparation of the digestive gland of infected snails kept continuously moist (Fairley, 1920). Workers have now obtained greater control by applying water containing a known number of cercariae to a shaved area of the skin by means of a glass tube. This method allows the cercaria to penetrate the skin in the natural way. The injection method has been employed and in this case, the cercarial suspension is injected either intraperitoneally (Cram & Bozicevich, 1944) or subcutaneously (Peters & Warren, 1969).

Watson and Azim (1948) in a report on the comparative efficiency of various methods of infecting mice with S. mansoni concluded that the partial immersion of animals in water containing cercariae was most satisfactory. Christensen and Frandsen (1977) in experiments on the host-finding capacity of schistosome cercariae, also compared various methods of mouse infection and found the ring method, the immersion technique and the paddling

method most effective.

In this study, the immersion technique was employed as it more nearly parallels the mode of natural infection. Six to eight weeks old hamsters, mice and gerbils were infected. To ensure bisexual infection, cercariae pooled from 20-30 infected snails were used. In the case of unisexual infections in hamsters and mice, cercariae were obtained from single snails that had single miracidial infection. For comparative purposes, each animal was exposed to 100 cercariae.

The animals were put individually in 500ml plastic jars, which were large enough for the animals to remain in an upright position. The lid of the jar was held in position with sellotape. The set-up for the infection is shown in Plate 2.2. Animals in the jars were first subjected to paddling in warm water 2cm deep for about 30 minutes and this induced the discharge of stored urine and faeces thus reducing their contamination of the cercarial suspension during the infection process. The jars with the animals were then transferred to crystallizing dishes containing cercarial suspension 2cm deep. The animals were allowed to paddle in the cercarial suspension for one hour and at the end of the infection were kept in groups in the animal house.

This method of infection was safe, easy to manipulate and prevented splashing of water containing cercariae during the whole operation. It was also advantageous in that a large number of animals could be infected at the same time.

2.2 WORM RECOVERY FROM THE LABORATORY MAMMALS

The maximum rate of development of the parasite in hamsters, mice and gerbils was determined by examination of the most advanced worm recovered at short time intervals throughout the course of the infection. The worm recovery was also used to determine the suitability of these mammals as experimental final hosts for Schistosoma margrebowiei. Infected animals were killed with chloroform between day 2 and day 180 after infection but some apparently died of schistosomiasis.

2.2.1 SCHISTOSOMULA RECOVERY FROM THE LUNGS

At early stages of the infection, schistosomula were recovered from the lungs by a modification of the technique outlined by Perez, Clegg & Smithers (1974). The thoracic cavity of animals killed with chloroform were rapidly opened to expose the heart and lungs. The thoracic aorta was ligatured and cut close to the heart. The lungs were perfused of blood by injecting 20ml of

Hanks' balanced salt solution (HBSS) at 37°C, pH 7·4 to 7·6, containing 10 units of heparin per ml, into the right ventricle and allowing the perfusate to emerge through a needle protuding from the left ventricle. The perfused lungs were removed, washed in heparinized HBSS (HBSS containing 10 units of heparin per ml), placed in a petri dish and chopped into small pieces with a fine pair of scissors. The pieces of lung tissue were transferred to a 25ml culture tube containing 10ml of heparinized HBSS, capped tightly and incubated for three hours in a water bath at 37°C. Following incubation, the contents of the culture tube were filtered through a 30-mesh stainless steel sieve. This allowed the schistosomula to pass through but retained the lung pieces. The lung peices retained on the sieve were washed twice with 10ml of heparinized HBSS and the filtrate centrifuged at 1000r.p.m. for two minutes. The supernatant was discarded and schistosomula in the sediment examined and counted with the aid of a dissecting microscope.

2.2.2 WORM RECOVERY FROM THE HEPATIC PORTAL SYSTEM

Worms were recovered from the hepatic portal system by a combination of perfusion and dissection. Worms in the intrahepatic, portal and mesenteric veins were collected and counted separately. The method used for perfusion was a modification of that described by

Smithers and Terry (1965). Chloroform-killed animals were dipped in water to wet their hair and then skinned. An incision from the sternal apex to the pubic region and transverse incisions were made to expose the abdominal cavity. The preparation of the animal for perfusion was completed by removing the diaphragm and the rib cage to expose the thoracic cavity. The animal was then clamped on a board fully stretched out in a vertical position. As separate worm counts for the liver and mesenteric veins were required, the hepatic portal vein was ligatured and slit open just below the liver. A 23-guage hypodermal needle was inserted in the left ventricle of the heart and pressurized HBSS from a 40ml syringe injected to dislodge the worms in the veins. At first, only slight pressure was applied from the syringe to prevent an excessive surge of blood, saline and worms. The perfusate was collected in a crytallizing dish. The coils of the intestine were then washed into the dish after perfusion as some worms might have adhered to them. The worms in the mesenteric veins were easily seen under the dissecting microscope and these were dissected out using tweezers. The worms recovered were counted and their sexes and site of recovery noted. In early infections, the counting had to be carried out with the aid of a binocular microscope.

2.3 MEASUREMENT OF POSTCERCARIAL GROWTH

The postcercarial growth of S. margrebowiei in hamsters, mice and gerbils was based on the changes in length of specimens recovered at various time intervals from these experimental hosts that had been exposed to 100 cercariae each by the immersion technique.

Worms recovered at specific times following infection were relaxed by chilling. Specimens were placed in vials of Hanks' saline at $4^{o}C$, in which they became less active and were allowed to relax gradually for about one hour. All measurements were carried out on fresh materials. The worms were pipetted onto a glass microscope slide and measured under the microscope using a calibrated eye-piece micrometer. No attempts were made to use fixed materials for measurements as the fixatives caused the worms to shrink.

Only a relatively small number of hosts were experimentally infected and this was a considerable limitation. The results obtained from such few animals could be affected by host susceptibility or worm burden and would not be detected unless a larger number of animals were used. In an attempt to eliminate these possibilities, three animals of the same age of infection from the same host species were used at each time interval.

The measurement of worms from these were pooled. For each time interval, at least six worms were measured.

The data obtained from these measurements were used to study the comparative growth of paired worms in these experimental hosts.

2.4 POSTCERCARIAL DEVELOPMENT AND MATURATION

The development and maturation of S. margrebowiei in hamsters, mice and gerbils were studied by the examination of the most advanced worms recovered, at short time intervals, during the course of the infection. The morphological criteria used for the classification of the developmental stages were based on those developed for cestodes and trematodes by Bell and Smyth (1958) and which were also adopted for S. mansoni (Clegg, 1965).

Specimens recovered at short time intervals were washed in Hanks' saline and relaxed by chilling as described in Section 2.3. Attempts were not made to wash the specimens in water before fixation as this had been found to produce abnormally long worms. The worms were transferred onto glass slides, using a Pasteur pipette, containing some saline, to avoid damage. The worms were then slightly flattened by withdrawing

some of the saline with a piece of filter paper under a cover-slip. The flattened specimens were fixed in 70% ethyl alcohol.

Development and maturation studies were carried out on whole mount specimems fixed in 70% ethyl alcohol and stained in acidified Gower's Carmine or examined in aceto-orcein squash (Smyth & Howkins, 1966). The aceto-orcein squash was valuable in detecting the early stages of spermatogenesis. The egg-shell formation was followed by staining the worms in Fast Red salt B or Malachite Green (Johri & Smyth, 1956). Details of this method have been described by Johri and Smyth (1956) and the outline is given below :

1. Flatten and fix specimens in 70% ethanol (2-24 hours).
2. Wash in water (15 minutes).
3. Place in 1% Fast Red salt B (freshly prepared and filtered) or 1% Malachite Green. The phenolic shell precursors in the vitelline cells become red within 0·5 to 3 minutes with Fast Red salt B or purple with Malachite Green.
4. Wash in water (15 minutes).
5. Stain in Gower's Carmine (1 to 3 hours), differentiate in acid alcohol.
6. Dehydrate, clear and mount.

At maturity, intra-uterine egg counts from freshly recovered female worms were made with the aid of a dissecting microscope.

2.5 QUANTITATIVE DETERMINATION OF EGG DISTRIBUTION IN HOST TISSUES

A modification of the digestion technique of Cheever (1970) was used to determine the quantitative distribution of eggs trapped in the tissues of infected hosts.

One gram or a known weight of fresh representative tissue of lung, liver, spleen, ileum, colon or caecum was placed in a 20ml specimen tube containing 15ml of 3% potassium hydroxide. This was tightly capped and incubated in a water bath at 37°C for 18 hours. After digestion, the mixture was passed through a sieve and washed through into a 200ml conical flask with saline. Aliquots were spun at 1000r.p.m. for two minutes in 40ml centrifuge tubes. The supernatant was discarded and the eggs in the sediment were diluted with saline to 100ml. Small known volumes (1ml) were taken from the large diluted sample and total egg count was made using the veterinary egg counting slide (McMaster worm egg slide). The total number of eggs in the known weight of tissue was calculated by multiplying the

number of eggs in the examined volume by 100. The value stated in the result represents the mean of three known weight samples.

2.6 DETERMINATION OF PERCENTAGE VIABILITY OF EGGS

After the onset of egg laying by the worms, the percentage of viable eggs in the liver and colon in the three experimental hosts was determined. This was carried out on tissues of the liver and colon as a high concentration of deposited eggs were observed in them. The eggs for the experiment were obtained by the digestion technique described above (Section 2.5). In each instance, a portion of the eggs in fresh water was placed on a microscope slide to determine viability by observation of hatched miracidia or movement of miracidia within the egg shells with the aid of a microscope.

2.7 PREPARATION OF MATERIALS FOR SCANNING ELECTRON MICROSCOPY

The tegumental surfaces of adult, immature worms and eggs of S. margrebowiei were examined with the scanning electron microscope.

2.7.1 WORMS

The worms used for SEM studies were recovered from the hepatic portal system by a combination of perfusion and dissection (described in Section 2.2.2) at different stages of development. The worms were rinsed in Hanks' balanced saline solution (HBSS) and then fixed immediately in 1N phosphate buffered 2% glutaraldehyde (pH 7·2) containing 3% sucrose, for at least one hour at 4°C. The fixed specimens were washed in triple distilled water and held in a small nickel basket. They were then dehydrated by upgrading through 50%, 70%, 90% and absolute acetone spending 30 minutes in each grade of acetone. The worms were processed in a Palaron critical point drier, then orientated and attached with double sided sellotape to aluminium stubs. Critical point dried specimens were coated with gold in a Denton 502 vacuum evaporator sputter coater. Suitable gold coated specimens were selected after examination under the dissecting microscope.

Micrographs of the tegument were made with a Cambridge Stereoscan Electron Microscope at 100 to 10,000 x magnification at an accelerating voltage of 10kv.

2.7.2 EGGS

Eggs intended for scanning electron micrographical examination were obtained from the intestine of mice, hamsters and gerbils having at least 40 day old *S. margrebowiei* infections.

The infected animals were killed with chloroform, their intestines removed and slit lengthwise, cleaned of their contents and then homogenized in 0·95% saline. The homogenate was filtered through sieves (mesh sizes 52 and 85) and the filtrate containing the eggs washed several times with saline. The use of 0·95% saline throughout the procedure prevented the hatching of the eggs. These eggs were fixed in 2% phosphate buffered glutaraldehyde (pH 7·2) for one hour and then washed repeatedly with triple distilled water and centrifuged. The supernatant was discarded after centrifuging.

A drop of water containing a large concentration of the eggs was put on a glass coverslip attached to an aluminium stub with double sided sellotape and freeze dried in liquid nitrogen. The freeze-dried specimens were coated with gold and examined in the same way as the worms (Section 2.7.1). Some eggs were air dried for comparative purposes.

2.8 HISTOPATHOLOGICAL STUDIES

The experiments were undertaken to determine the histopathological changes in hamsters, mice and gerbils as a result of early and prolonged S. margrebowiei infections and to compare the host-parasite relations in such infections. The general pathological picture in these experimental infections was observed macroscopically and microscopically. Unisexual and bisexual infections were carried out in order to determine the pathological changes which occur as a result of the deposition of eggs in the tissues and those due to the presence of worms in the hepatic portal circulation.

2.8.1 MACROSCOPIC EXAMINATION

During autopsy, between ten and 180 days after infection, the macroscopic appearance of the organs of infected hosts were compared to the controls. The following features were taken into consideration :

1 Colour of the liver
2 Size of the liver
3 Texture of the liver
4 Presence of lesions on the liver
5 Size of the spleen
6 Colour of the spleen
7 Presence of lesions on the lungs
8 Changes observed on the surface of the gut.

2.8.2 MICROSCOPIC EXAMINATION

Autopsies were performed immediately the hosts were killed with chloroform. Fresh tissue crushes were examined under the microscope to determine the presence of eggs in tissues and the onset of patency.

Representative tissue samples for histological investigations were fixed in aqueous Bouin's solution for about 24 hours. The intestine was examined separately as ileum, colon and caecum. The tissues were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin wax (melting point 55°C) and 6µm sections were made. The sectioned tissues were stained in haematoxylin and eosin and examined under the microscope. The lesions observed in the tissues of the three hosts were compared and only tissues that showed distinct pathological changes were considered.

2.9 IN VITRO CULTURE

The materials and methods used for the cultivation of S. margrebowiei in vitro from cercaria are described in this section. The preparation of culture media, inoculation and periodic changing of culture media were carried out under sterile conditions. Sterile equipment was used throughout the investigation and all experi-

ments were carried out inside a lamina flow cabinet (SLEE, LONDON). Care was taken not to touch the parts of the sterile equipments which would come in contact with the media or culture. The apparatus used were thoroughly washed, dried and put in appropriate sized sterilization bags for autoclaving at 2kg/cm^2 pressue at 135^{o}C for seven minutes under vacuum for pipettes, and at 121^{o}C for 20 minutes without vacuum for other glassware.

2.9.1 COLLECTION AND CONCENTRATION OF CERCARIAE

The collection and concentration of cercariae were carried out by the method described by Ramalho-Pinto, Gazzinelli, Howells, Mota-Santos, Figueiredo & Pellegrino (1974). Cercariae from Bulinus natalensis were collected in de-ionized water and used within two hours of being shed. Intact cercariae in suspension were pipetted into 25ml conical centrifuge vials and cooled in an ice bath for ten minutes to reduce motility of the cercariae. The chilled cercariae were centrifuged for one minute at 1000r.p.m. in a bench centrifuge. The supernatant was discarded and the cercariae washed twice in cold triple distilled water containing antibiotics (100ug/ml streptomycin and 100Iu/ml penicillin). Cercariae from several centrifuge vials were pooled and re-suspended in 10ml Hanks' saline containing antibiotics.

2.9.2 CONVERSION OF CERCARIAE TO SCHISTOSOMULA

The conversion of cercariae to schistosomula was achieved by the two methods described below :

i) The mechanical method described by Ramalho-Pinto et al (1974). Packed cercariae suspended in 2ml of Hanks' saline in 25ml conical vials were vigorously shaken for one minute on a Rotamixer (Chemlab Instrument Ltd., London).

ii) The incubation method described by Eveland & Morse (1975). Packed cercariae were incubated for three hours in Hanks' saline containing varying concentrations of heat-inactivated rabbit serum or new-born calf serum (FLOW) at 37°C. Varying concentrations of sera were used in order to determine the threshold necessary for conversion of cercariae to schistosomula.

The cercarial bodies were then isolated from the tails by increasing the volume of the suspensions with Hanks' saline and centrifuging for one minute at 1000r.p.m. The tail rich supernatant was discarded and the process repeated before the cercarial bodies were inoculated into the culture media.

2.9.3 CULTURE MEDIA

The culture media used in this investigation were commercially obtained in powder form.

i) HANKS' SALINE (DIFCO)

9·8g of the commercially obtained powder and 0·35g of sodium bicarbonate were dissolved in one litre of triple distilled water (DIFCO). The solution was sterilized by vacuum filtration through a 0·2um millipore filter (GELMAN) into sterile flasks taking precautions to avoid contamination. The solution was then aseptically dispensed into sterile bottles leaving a minimum final air space. 2ml of antibiotics (Penicillin 5000Iu/ml and Streptomycin 5000mcg/ml) per 100ml of medium were added. The solution was stored at 4°C. The components of Hanks' saline are given by Paul (1970).

ii) MEDIUM NCTC 135 (GIBCO)

The components of medium NCTC 135 are given by Paul (1970). 9·3g of the powdered medium (contents of one packet) was dissolved in one litre of triple distilled water. 2·2g of sodium bicarbonate was added to adjust the pH (7·2 to 7·4, orange red colour). The solution was dispensed, and stored like Hanks' saline described above.

iii) MEDIUM RPMI 1640 (GIBCO)

The components of RPMI 1640 are given by Paul (1970). The preparation and storage were as for medium NCTC 135.

2.9.4 OTHER COMPONENTS OF CULTURE COMMERCIALLY OBTAINED

i) SERA (FLOW, BIO-CULT)

The sera used in this study were :

a) new-born calf serum (LOT. 2921119)

b) rabbit serum (LOT. 2922128)

The sera were supplied sterile and were stored at -20°C. Sera were allowed to thaw at room temperature before being inactivated by incubation for one hour in a water bath at 50°C.

ii) ANTIBIOTICS (FLOW)

Penicillin-streptomycin solution was used throughout the investigation. This was supplied as a sterile solution at a concentration of 5000Iu/ml penicillin and 5000mcg/ml streptomycin. The solution was used at a concentration of 2ml per 100ml of media. It was stored at -20°C.

iii) RED BLOOD CELLS

Rabbit and sheep red blood cells were used. The cells were supplied in Alsevers' solution and were washed several times in Hanks' saline before being used. They were stored at 4°C.

2.9.5 PHYSICAL CONDITIONS OF CULTURE

i) CULTURE VESSELS

a) LEIGHTON TUBES

These were 15ml screw-capped bottles, and were sterilized in the laboratory using the method described in Section 2.9.

b) FALCON FLASKS (FALCON PLASTICS)

These were 30ml screw-capped plastic flasks with two flat sides. They were supplied sterile by Flow Laboratories.

ii) GAS PHASE

Sterile gas containig 10% carbon dioxide and 90% nitrogen was bubbled through the stock bottles of the various media before they were used.

iii) AGITATION OF CULTURE

Two different methods were used for the agitation of the culture set-up :

a) SHAKING WATER BATH (GALLENKAMP)

The culture vessels were continuously agitated at about 80 shakes per minute.

b) ROLLER TUBE APPARATUS (LEEC)

Only the Leighton tubes were used on this apparatus. The culture vessels were rotated horizontally at about two revolutions per minute in an incubator.

iv) TEMPERATURE

The cultures were incubated at 37-38^{o}C.

v) pH

The pH was maintained at approximately 7·2 to 7·4 using the colour change of the phenol red contained in the media and the Hanks' saline as indicator. Alkalinity was easily corrected by gasing the medium with sterile gas containing 10% carbon dioxide and 90% nitrogen.

2.9.6 SETTING UP OF CULTURE

Fifty schistosomula were inoculated into 15ml Leighton culture tubes containing 10ml of culture media (NCTC 135 or RPMI 1640) containing 50% inactivated rabbit serum (RS) or new-born calf serum (NCS). One hundred schistosomula were inoculated into a 30ml Falcon flask containing 20ml of culture media containing 50% RS or NCS serum. Each experiment was performed using schistosomula derived from a single cercarial harvest. 1% red blood cells (rabbit or sheep red blood cells) was added after two days (Clegg, 1965; Clegg & Smithers,1972).

2.9.7 ROUTINE EXAMINATION OF CULTURE

The cultures were inspected for bacterial or fungal contamination and the viability (i.e. motility) and development of the worms observed using an inverted microscope (UNION). Contaminated cultures were discarded.

Some organisms were removed, fixed and stained for assessment of development as described in Section 2.4. Morphological criteria used to identify the developmental stages were the same as those employed in *in vivo* culture and in addition the development of the caecum was closely followed.

2.9.8 CHANGING OF CULTURE FLUID

The culture media were replaced every three days. This involved withdrawing the top three-quarters of the old media and replacing it with fresh media. The red blood cells in the culture were only replenished when necessary.

3 QUANTITATIVE PARASITOLOGICAL RESULTS

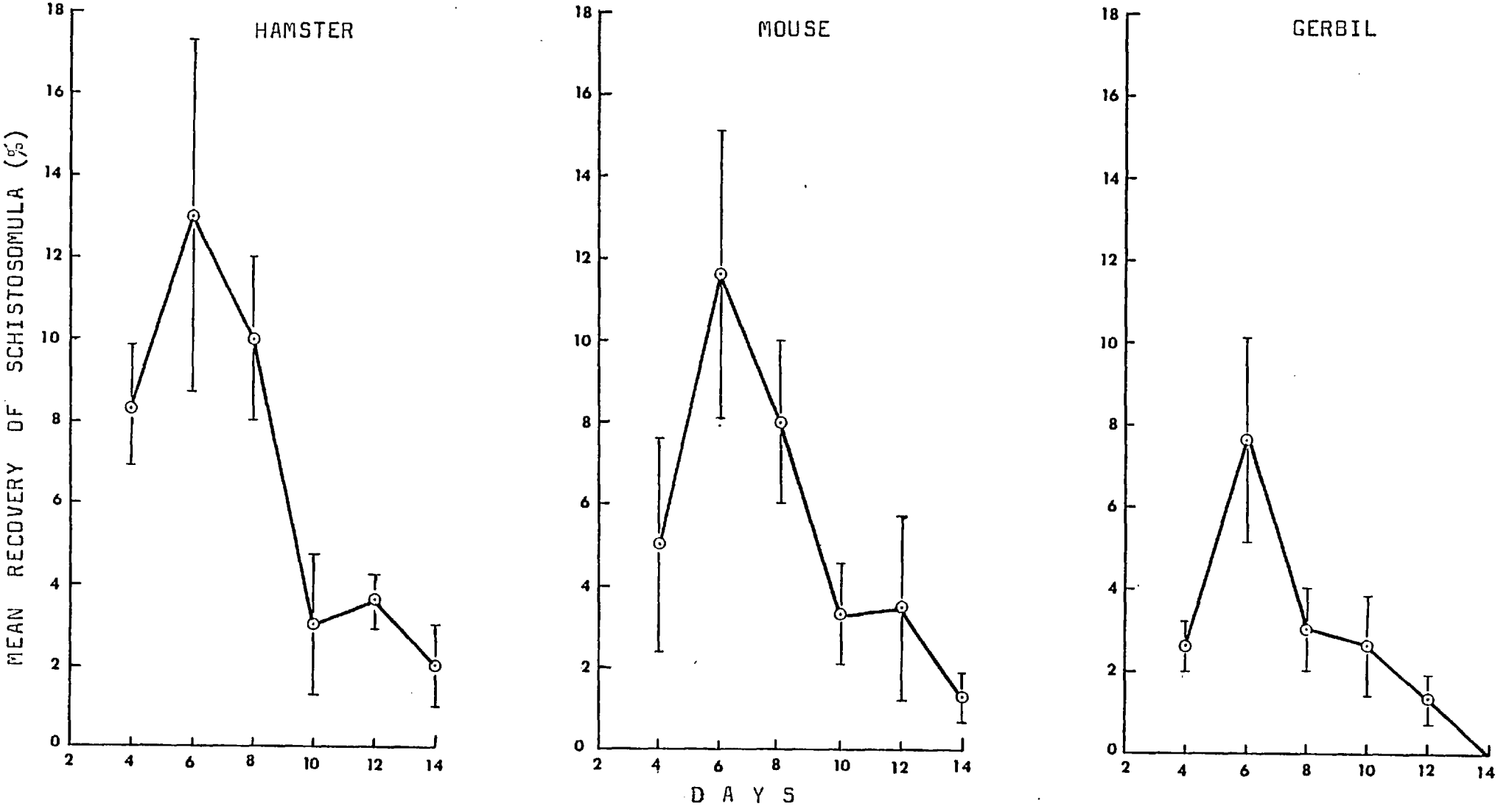
3.1 WORM RECOVERY

S. margrebowiei infections were established in hamsters, mice and gerbils. At early stages of infection, worms were recovered from the lungs and, at later stages, from the hepatic portal system. The worm recovery is expressed as the percentage of cercariae applied. The pattern of the infection in these three experimental hosts was determined by the worm burden and the migration of the worms.

3.1.1 SCHISTOSOMULA RECOVERY

At various times after infection, schistosomula recovery by three hours incubation of lung tissues from three animals of each host species was carried out. The mean of the schistosomula recovery in relation to time is shown in Fig. 3.1. The patterns of recovery were similar in the three hosts. Only a few worms were present in the lungs by day 4 postinfection (p.i.) and the number reached a maximum (13% in hamsters, 12% in mice and 8% in gerbils) by day 6 postinfection. Fig. 3.1 shows a clear decrease in the mean of schistosomula recovery in relation to time after day 6 p.i.

Fig. 3.1 Mean (±SD) of percentage recovery of schistosomula from the lungs of three experimental hosts infected with S. margrebowiei by the immersion technique.



There is a sharp decline in recovery until day 10 p.i., after which it becomes gradual, and by day 14 p.i. no schistosomula were recovered from the lungs of gerbils.

3.1.2 WORM RECOVERY FROM THE HEPATIC PORTAL SYSTEM

Table 3.1 and Fig. 3.2 show worm recoveries from the hepatic portal system in bisexual infections after perfusion and dissection at different time intervals from 20-120 days p.i. Table 3.1 also gives the numbers of paired and unpaired worms in the hepatic portal system. Although the same number of cercariae were applied in all cases, the number of worms that migrated to the portal system varied in the three hosts. At the times indicated on the graphs (Fig. 3.2), groups of three animals were examined, and the worm recovery was expressed as a percentage of the cercariae applied. Only a few worms were recovered from the portal system as early as day 20 p.i. The number increased to a high level by day 50 p.i., then a drastic drop in worm recovery was noticeable by day 70 p.i. Thereafter, only a slight decrease was recorded. Maximum worm recovery (61% in hamsters, 57% in mice and 34% in gerbils) was obtained by day 50 p.i. Infected hamsters and gerbils died of the infection between day 50 and 60 p.i. but the death rate was higher in gerbils. Hamsters that survived this period maintained a low worm burden. All infected gerbils died by day 60 p.i.

Table 3.1 Worm recovery from hamsters, mice and gerbils exposed to 100 *S. margrebowiei* cercariae by the immersion technique. M - male, F - female.

HAMSTERS

Duration of infection (days)	Sex of host	Worm recovery as % of cercariae applied			
		Pairs	Unpaired		Total
			Male	Female	
20	M	-	10	4	14
	M	-	10	6	16
	F	-	4	6	10
30	M	6	10	4	26
	M	9	8	6	32
	F	8	8	6	30
40	M	17	10	11	55
	M	18	12	9	57
	F	15	13	9	52
50	M	19	9	14	61
	M	13	17	7	50
	F	18	12	16	64
60	M	13	14	14	54
	M	18	8	4	48
	F	14	18	4	50
70	M	5	2	1	13
	M	6	4	-	16
	F	4	2	-	10
80	M	4	-	-	8
	M	3	2	-	8
	F	8	3	2	21
90	M	3	1	-	7
	M	3	-	-	6
	F	3	1	1	8
100	M	3	1	-	7
	M	2	-	-	4
	F	3	-	-	6
110	M	4	-	-	8
	M	2	-	-	4
	F	3	2	2	10
120	M	4	2	-	10
	M	2	2	-	6
	F	3	-	-	6

Table 3.1 continued.

MICE

Duration of infection (days)	Sex of host	Worm recovery as % of cercariae applied			
		Pairs	Unpaired		Total
			Male	Female	
20	M	-	13	10	23
	M	-	14	7	21
	F	-	10	8	18
30	M	6	8	4	24
	M	9	6	1	25
	F	6	5	5	22
40	M	15	11	9	50
	M	17	10	7	51
	F	13	10	5	41
50	M	19	12	6	56
	M	17	14	5	53
	F	19	6	8	52
60	M	16	10	6	48
	M	13	8	3	37
	F	11	9	5	36
70	M	5	1	1	12
	M	3	1	-	7
	F	6	2	1	15
80	M	4	1	-	9
	M	4	-	-	8
	F	3	4	2	12
90	M	2	1	-	5
	M	4	1	1	10
	F	3	-	-	6
100	M	2	1	-	5
	M	3	-	-	6
	F	3	1	1	8
110	M	2	1	-	5
	M	2	-	-	4
	F	2	1	-	5
120	M	2	-	-	4
	M	2	-	-	4
	F	1	-	-	2

Table 3.1 continued.

GERBILS

Duration of infection (days)	Sex of host	Worm recovery as % of cercariae applied			
		Pairs	Unpaired		Total
			Male	Female	
20	M	-	8	4	12
	M	-	6	9	15
	F	-	6	5	11
30	M	4	6	2	16
	M	7	1	2	17
	F	3	2	-	8
40	M	7	3	-	17
	M	8	2	-	18
	F	8	2	2	20
50	M	13	5	3	34
	M	8	2	-	18
	F	14	3	1	32
60	M	7	11	2	27
	M	8	5	-	21
	F	6	4	-	16

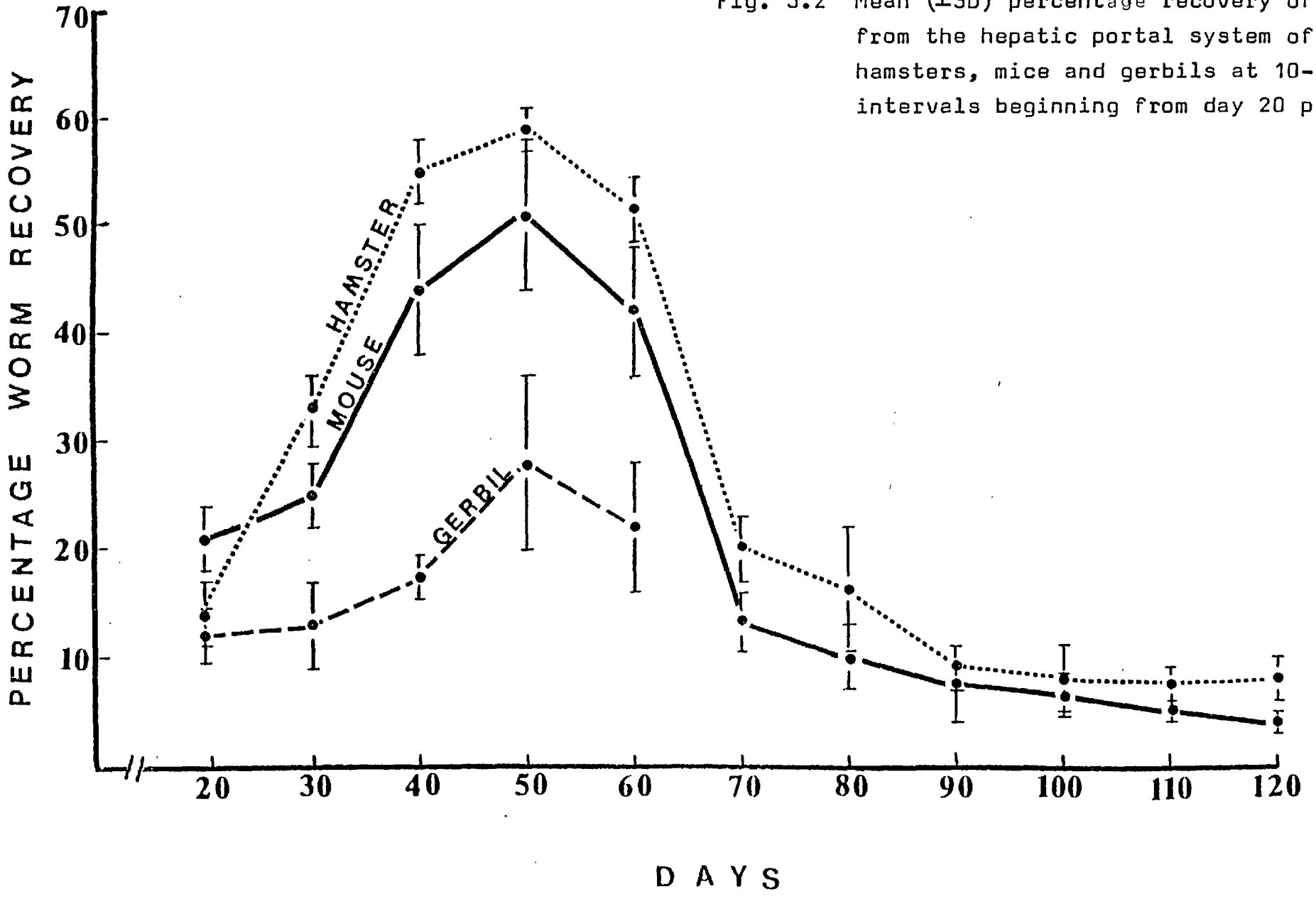


Fig. 3.2 Mean (±SD) percentage recovery of worms from the hepatic portal system of hamsters, mice and gerbils at 10-day intervals beginning from day 20 p.i.

Fig. 3.3 shows the worm recovery from hamsters subjected to bisexual and unisexual infections of S. margrebowiei. There was an increase in worm recovery with time in both bisexual and unisexual infections up to day 50 p.i. A decline in worm burden was common in both bisexual and unisexual infections and the highest worm recovery from day 60 p.i. occurred in hamsters with male unisexual infections. Female unisexual infections gave the lowest yield throughout the period of observation. Apart from a few male worms in the hepatic portal vein, most worms were recovered from the liver in all unisexual infections. Pairing and migration to the hepatic portal and mesenteric veins were only observed in bisexual infections. Hamsters did not die as a result of unisexual infections.

3.2 WORM DISTRIBUTION IN THE HEPATIC PORTAL SYSTEM

In bisexual infections in the three experimental hosts, the worms developed to maturity and migrated to the liver, the hepatic portal and the mesenteric veins. Table 3.2 shows the distribution of worms in the hosts. A high percentage of worms recovered at the early stages of the infection were from the liver in the three hosts. There was a decrease in the percentage recovered from the liver in the three hosts by day 40 postinfection and this was followed by an increase in the percentage

recovered from the hepatic portal and the mesenteric veins. Except for a few unpaired male worms, the worms in the hepatic portal and the mesenteric veins were paired. The data indicate that the worms were almost equally distributed between the hepatic portal vien and the veins of the intestine after day 40 p.i. A large number of the unpaired male worms and the fewer unpaired female worms were recovered from the liver by day 60 p.i.

Fig. 3.3 Mean percentage recovery of worms from the hepatic portal system in hamsters at 10-day intervals. Bisexual and unisexual infections are compared.

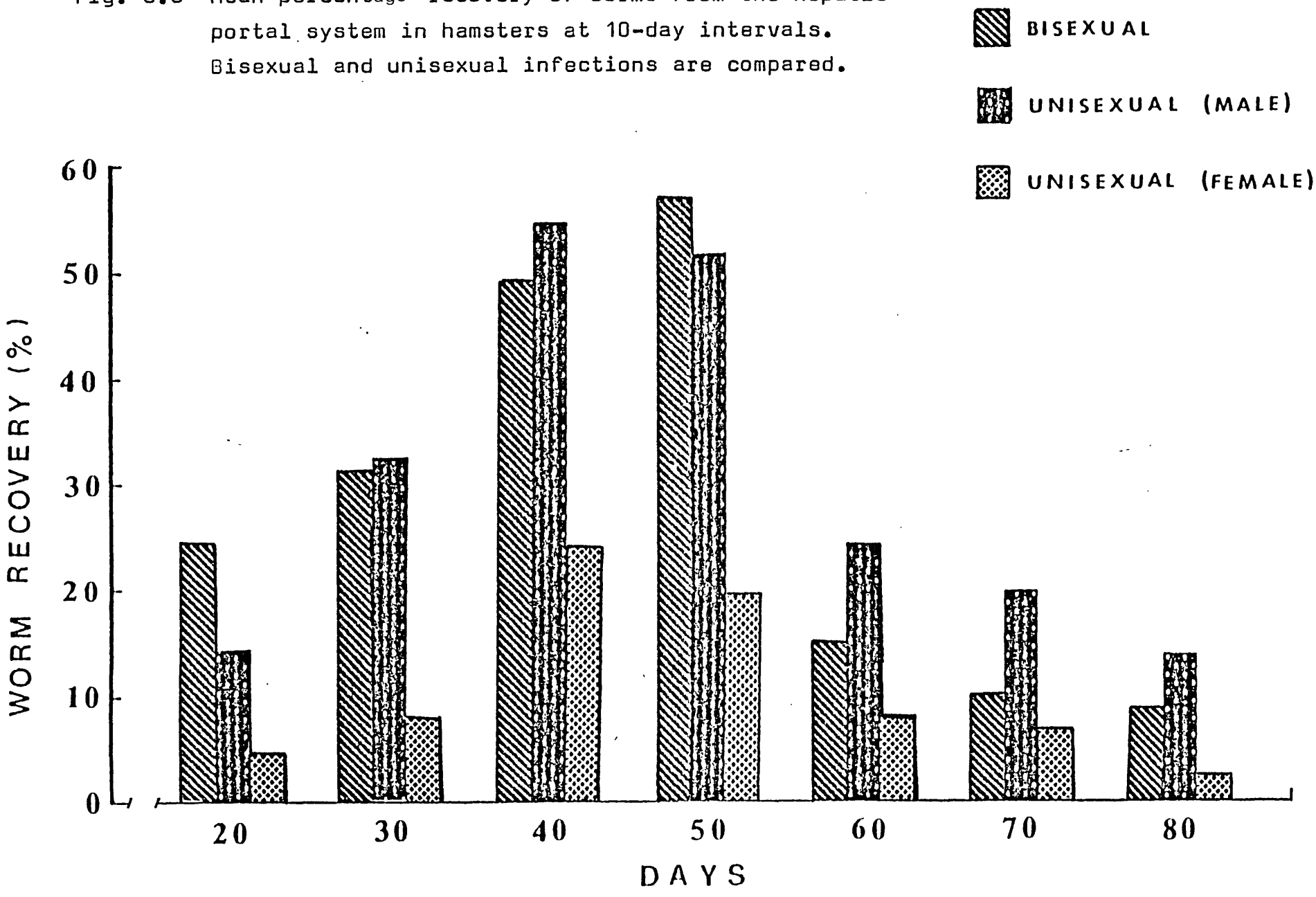


Table 3.2 Worm distribution in the portal system of hamsters, mice and gerbils from day 20 to 60 p.i. The values are percentages of the total number of worms found.

Duration of infection (days)	Hamsters			Mice			Gerbils		
	Liver	Hepatic portal vein	Mesenteric vein	Liver	Hepatic portal vein	Mesenteric vein	Liver	Hepatic portal vein	Mesenteric vein
20	71•42	28•58	0	100•00	0	0	100•00	0	0
30	25•00	38•06	36•94	84•00	16•00	0	50•00	50•00	0
40	37•86	28•57	33•57	65•00	25•00	10•00	10•00	50•00	40•00
50	37•70	10•47	52•45	17•24	55•18	27•59	7•16	57•14	35•72
60	31•54	13•59	54•87	22•00	31•00	47•00	51•00	25•00	24•00

3.3 GROWTH

Growth curves of paired worms in the three experimental hosts are shown in Fig. 3.4. Only paired worms were considered in the determination of these growth curves. The mean of the lengths of a number of worms was used because of the differences in length of worms recovered from each host at any single examination. In all the three curves, each point represents the mean length of five to ten worms drawn at random from the total yield from three animals with the same age of infection.

Rapid and about equal rates of growth were observed for male and female worms in both hamsters and gerbils from day 25 to 30 p.i. The rate of growth of the worms for the same period in mice was slower. The 'cross-over point' which has been used as a measure of the rate of growth of schistosomes, is reached at about day 28 p.i. in hamsters and gerbils but at about day 30 p.i. in mice.

During the next ten days (from day 30 to 40 p.i.), the rate of growth of female worms was faster than that of male worms in all the three hosts. Female worms recovered at the later stages of the infection were therefore longer than the male worms. The growth rate

of the males decreased after day 40 p.i. and this was maintained for the remaining period of the observation. The maximum length attained by an individual male worm in the hamster was 17·5mm; in the mouse, 12·6mm; and in the gerbil, 15·6mm; while that for a female worm was 28·2mm in hamster; 22·8mm in mouse and 20·1mm in gerbil.

The following were apparent from the growth curves :

i) the rate of growth of the paired male worms was greater than that of paired female worms until about day 30 p.i.;

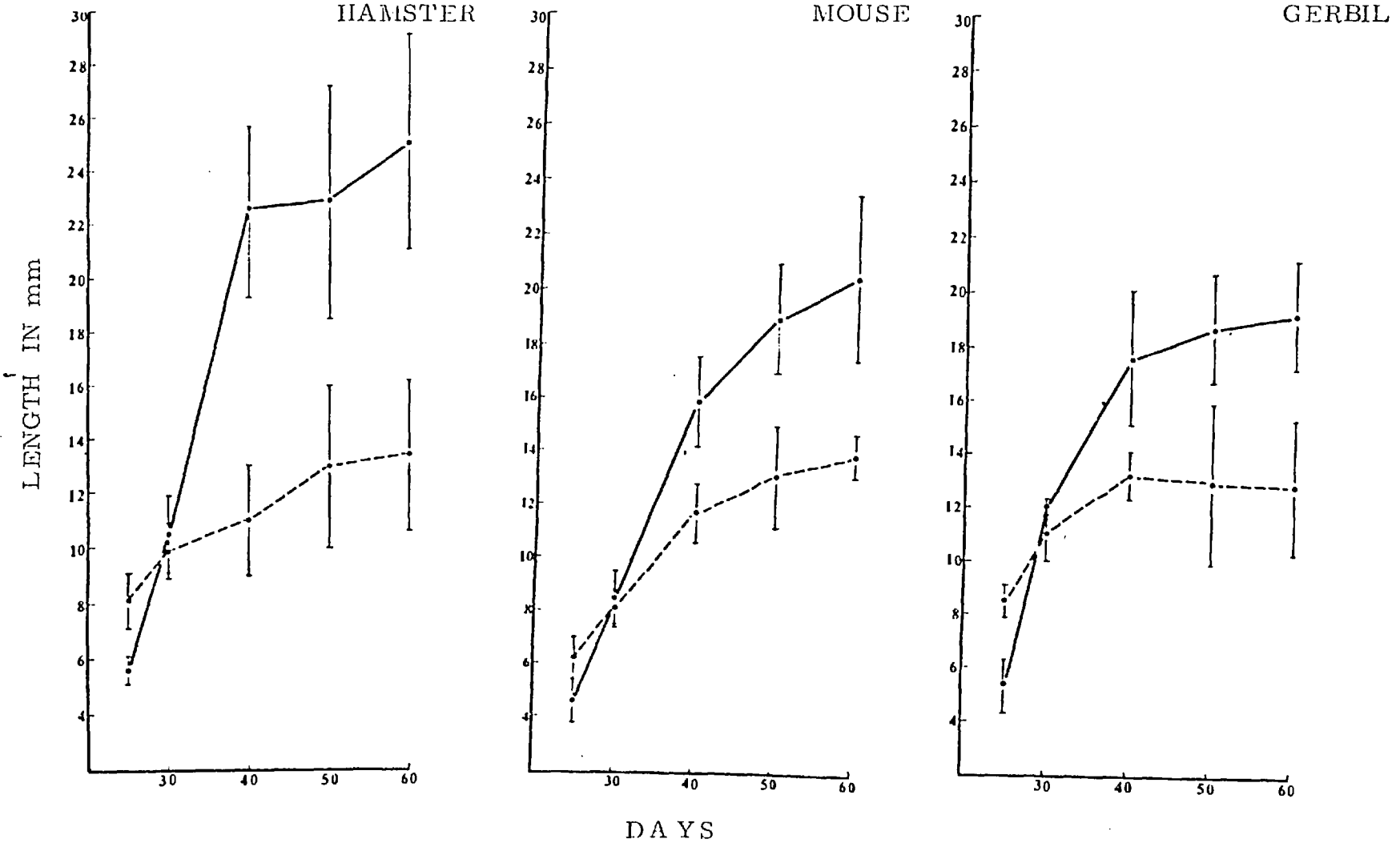
ii) a 'cross-over point' was reached in hamsters and gerbils by day 28 p.i. and by day 30 p.i. in mice;

iii) during the ten days between day 30 and 40 p.i., the female worm showed a faster rate of growth than the male worm;

iv) there were differences in the sizes of the worms from these three experimental hosts with the longest worms occurring in the hamsters and the shortest in the gerbils;

v) worms recovered from the same hosts with the same age of infection, showed considerable variation in length as illustrated by the standard deviation.

Fig. 3.4 Mean (±SD) growth curve of paired worms in hamsters, mice and gerbils. (Solid lines - female; broken lines - male).



3.4 OBSERVATIONS ON DEVELOPMENT

The development of schistosomes can be divided into six stages and the criteria utilized here are based partly on those developed for cestodes and trematodes (Bell & Smyth, 1958) and for S. mansoni (Clegg, 1965). The times shown for the attainment of each stage of development represents the earliest time at which the particular stage was recovered during the course of infection. Worm development was not uniform throughout the population in any host at any period of the investigation and the extent of development was determined by the most advanced worm recovered at any time. Fig. 3.5 is a diagrammatic representation of the developmental stages of S. margrebowiei from cercaria to adult. The six stages are :

1. The cercaria penetrates the skin of the experimental host, loses its tail and is transformed into the schistosomulum.

2. The lung forms were first recovered from the lungs on day 2 p.i. in hamsters and mice but not until day 3 p.i. in gerbils. A peak in worm recovery from the lungs was reached by day 6 p.i. in all the hosts (Fig. 3.1). The digestive tract in this form appears to be functional as dark pigments indicating the beginning of the caecum were observed in some worms. This dark brown haematin-like pigment in the caecum indicates that some of the

worms had fed on red blood cells.

3. The closed-gut form was observed in the three experimental hosts by day 15 p.i. The stage is characterized by the linking of the bifurcated caeca just below the acetabulum.

4. The organogeny stage occurred by day 20 p.i. in the three hosts. The linked caeca had grown longer and the male worm at this stage was longer than the female worm. The male had developed the lateral extensions which form the gynaecophoric folds and one or two testes appeared behind the acetabulum. The female possessed a narrow uterus behind the acetabulum at this stage.

5. Gametogeny occurred by day 25 p.i. in the three hosts. The male worm was still longer than the female worm and had four fully developed testes (Plate 3.1). Aceto-orcein squashes at this stage showed that the testes contained spermatozoa. The female had developed a small ovary and a mature uterus. Worms paired at this stage. Plate 3.2 shows the posterior end of paired worms recovered from the hepatic portal vein by day 25 p.i. in the hamster.

6. The egg-shell formation stage was observed in the three hosts by day 30 p.i. The vitelline cells immediately posterior to the ovary had developed and were positive to Fast red salt B or Malachite Green indicating that shell precursors (phenol and protein)

were present. Plate 4.1 shows the anterior end of a 30 day old male worm and Plate 4.2 shows the posterior ends of paired worms of the same age.

7. The oviposition stage was reached in the hamsters and mice by day 33 p.i. but not until day 35 p.i. in gerbils. At this stage, the vitelline glands were fully developed and appeared densely packed posterior to the ovary on either side of the body (Plate 4.3). The female uterus contained fully developed eggs at this stage.

The rates of development of the parasite in these experimental hosts were similar. Table 3.3 shows a comparison of the rates of development of S. margrebowiei in these experimental hosts and some other species of schistosomes in experimental infections.

3.4.1 UTERINE EGG COUNT

Eggs were first observed in the uteri of worms in all three experimental hosts by day 30 p.i. Table 3.4 shows intra-uterine egg counts at various intervals p.i. Uterine egg counts showed little variation in worms recovered from the three hosts. The lowest uterine egg counts were obtained in worms recovered from gerbils and the highest from worms from hamsters. Female unisexual infections in hamsters gave rise to females without eggs in their uteri.

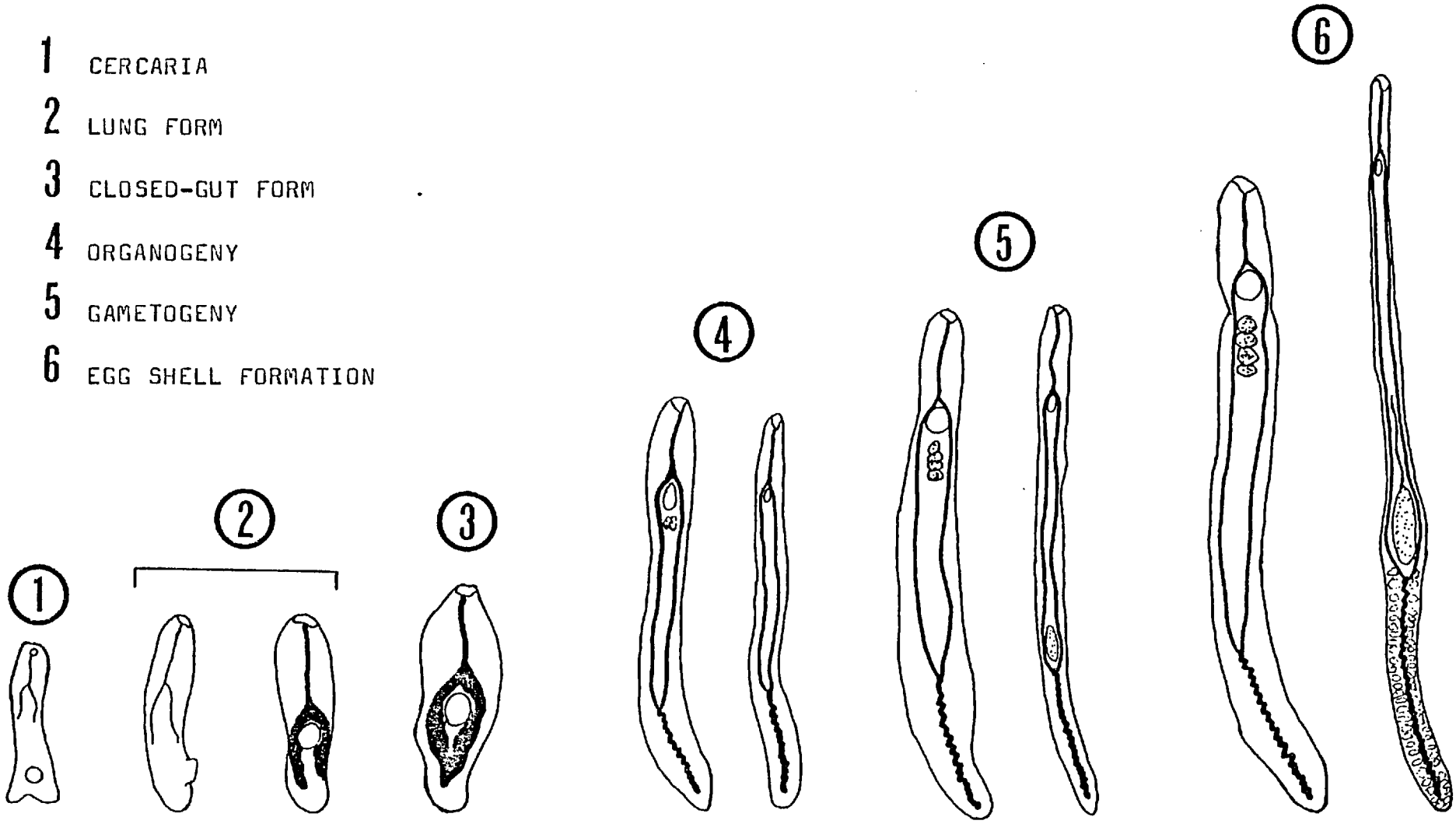
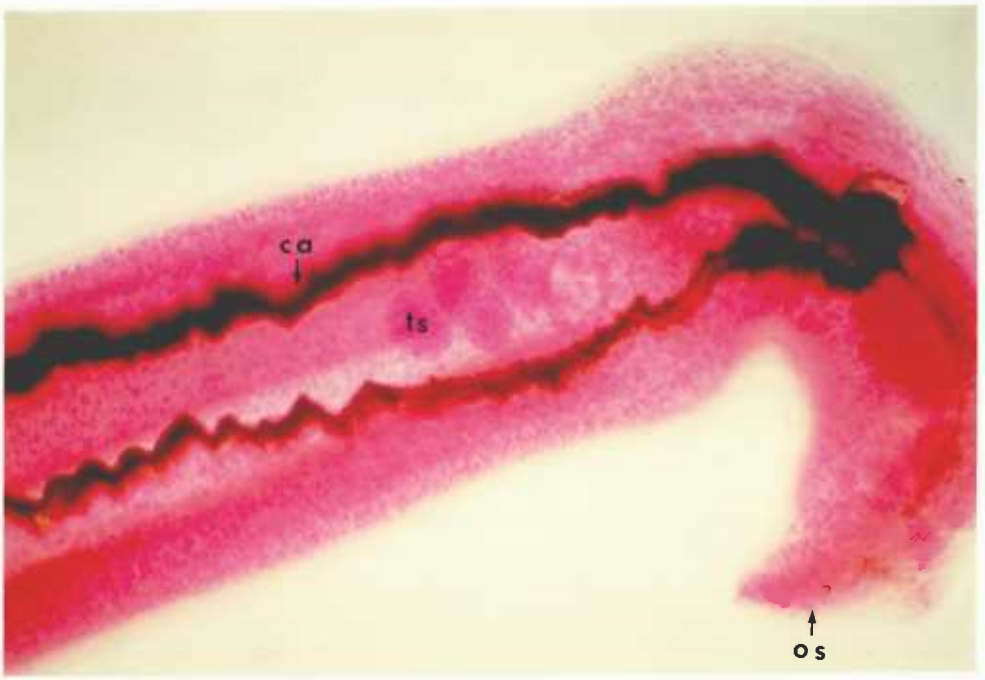
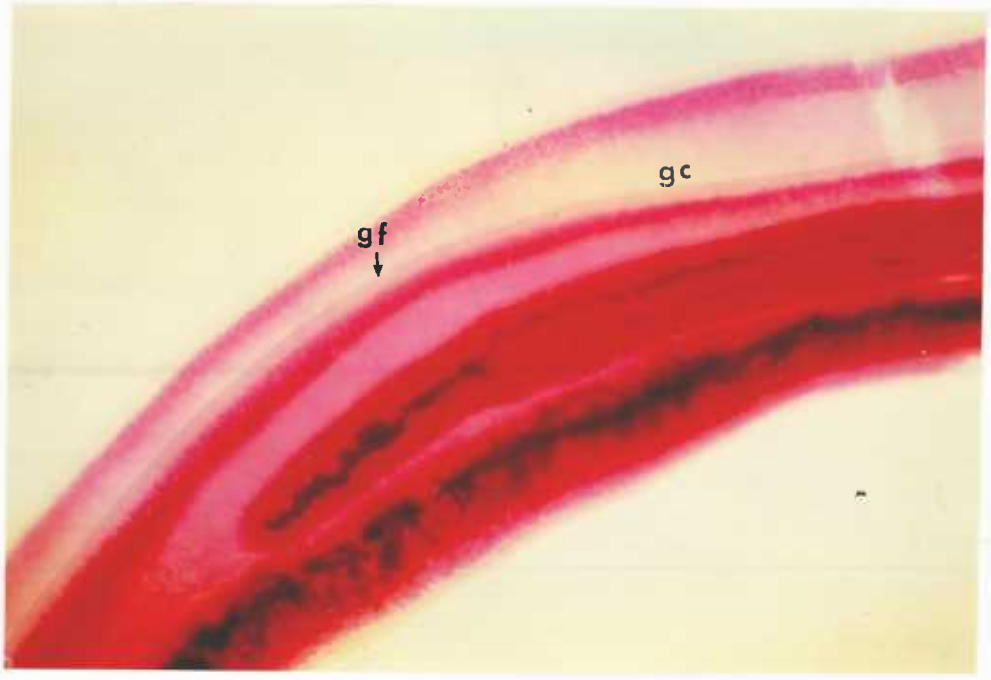


Fig. 3.5 Developmental stages of S. margrebowiei from cercaria to adult.



3.1 Male S. margrebowiei recovered from the hepatic portal vein at day 25 p.i. The four testes are fully developed. ca, caecum; os, oral sucker; ts, testis; x480



3.2 Posterior ends of paired worms recovered from the hepatic portal vein at day 25 p.i. showing the female worm within the gynaecophoric canal of the male worm. gc, gynaecophoric canal; gf, gynaecophoric fold; x480

PLATE 4

4.1 Anterior end of S. margrebowiei male worm showing fully developed testes (ts) below the acetabulum (ac), recovered from the hepatic portal vein 30 days p.i. x240

4.2 Posterior ends of paired worms, showing the female within the gynaecophoric canal, recovered from the hepatic portal vein 30 days p.i. The ovary (ov) has developed. x240

4.3 Posterior end of female worm recovered from the mesenteric vein 35 days p.i. The ovary and the vitellaria are fully developed (stained in Gower's Carmine and Malachite Green); od, oviduct; ov, ovary; vg, vitelline gland. x480

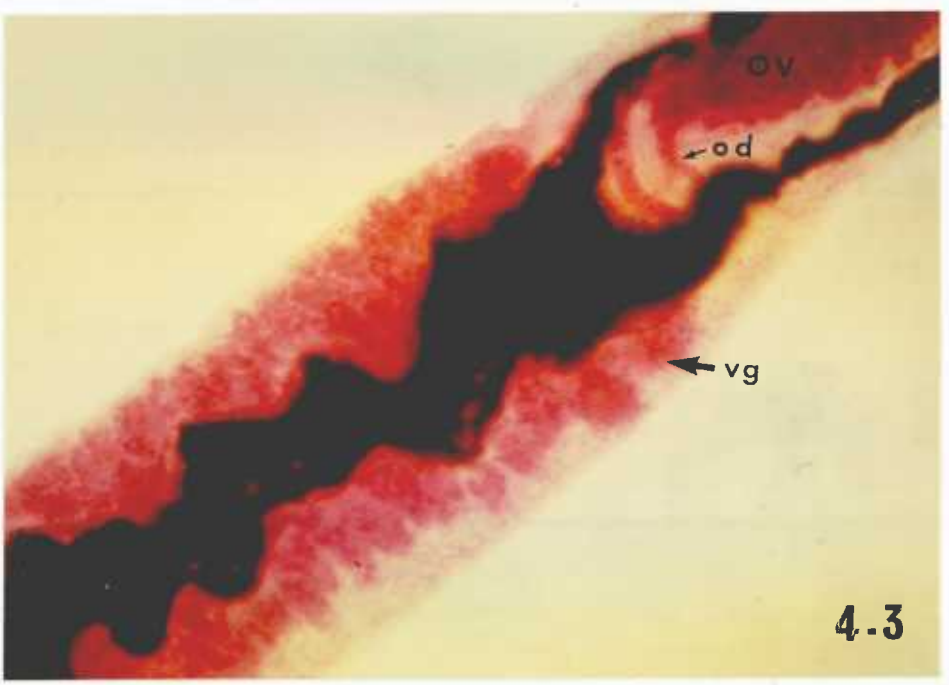
PLATE 4



4.1



4.2



4.3

Table 3.3 Comparison of the rate of development of *S. margrebowiei* with other species of schistosomes. The values are days postinfection when each stage of development was first observed.

Stage of Development	*S. japonicum* Suliman(1974)	*S. mansoni* Clegg(1965)	*S. haematobium* Smith *et al.*(1976)	*S. bovis* Ghandour & Babiker(1978)	*S. margrebowiei* Present study
1. Lung form	4-6	7-8	8	5	5-6
2. Closed-gut	10	15	22	14	15
3. Organogeny	16	21	24-25	17	20
4. Gametogeny	22	28	31	26	25
5. Egg-shell formation	27	30	57	35	30
6. Oviposition	28-29	34-35	65	37	33-35

Table 3.4 Changes in uterine egg counts in the three experimental hosts. Eggs from the uteri of at least five female worms were counted in each case.

Duration of infection (days)	Hamsters		Mice		Gerbils	
	Mean	±SD	Mean	±SD	Mean	±SD
35	28•59	6•99	22•63	3•93	12•71	2•98
40	136•13	23•85	127•88	24•00	83•43	11•93
50	173•00	92•52	132•25	27•82	128•20	22•52
60	210•00	32•35	177•17	29•57	143•50	28•53

3.5 DISTRIBUTION OF EGGS IN THE TISSUES

Fig. 3.6 illustrates egg distribution in the tissues of the experimental hosts, 50, 60 and 70 days p.i. This was determined by the number of eggs per gram of representative tissue digest. Large numbers of eggs were observed in the tissues, mainly in the colon, of hamsters. Gerbils had a similar distribution though fewer eggs were recovered. The mice, however, showed a slightly different distribution as more eggs occurred in the liver and a few were recovered from the lungs and spleen by day 70 p.i.

Although the deposition of eggs in the tissues showed a difference in numbers, the patterns were basically similar. When sections of the intestine (ileum, colon and caecum) were compared, it was found that most eggs were deposited in the colon in the three hosts. However, the presence of eggs in the lungs and spleen of mice made a marked difference in the trend by day 70 p.i.

3.6 VIABILITY OF EGGS

The percentage viability of eggs deposited in the liver and colon of the three experimental hosts in relation to the age of infection was determined and the

result is summarized in Fig. 3.7.

A high percentage of the eggs deposited in the liver and colon of hamsters and mice by day 40 p.i. were viable. In gerbils, a low percentage of viable eggs was observed for the same period. There was a decline in the percentage viability of eggs in the three hosts and that in the hamsters was the most gradual. Gerbils did not retain the infections for more than 60 days and no further observations were carried out on this host. From day 80 to 100 p.i., the percentage of viable eggs in hamsters was greater than in mice.

Fig. 3.6 Egg distribution in tissues of experimental hosts.

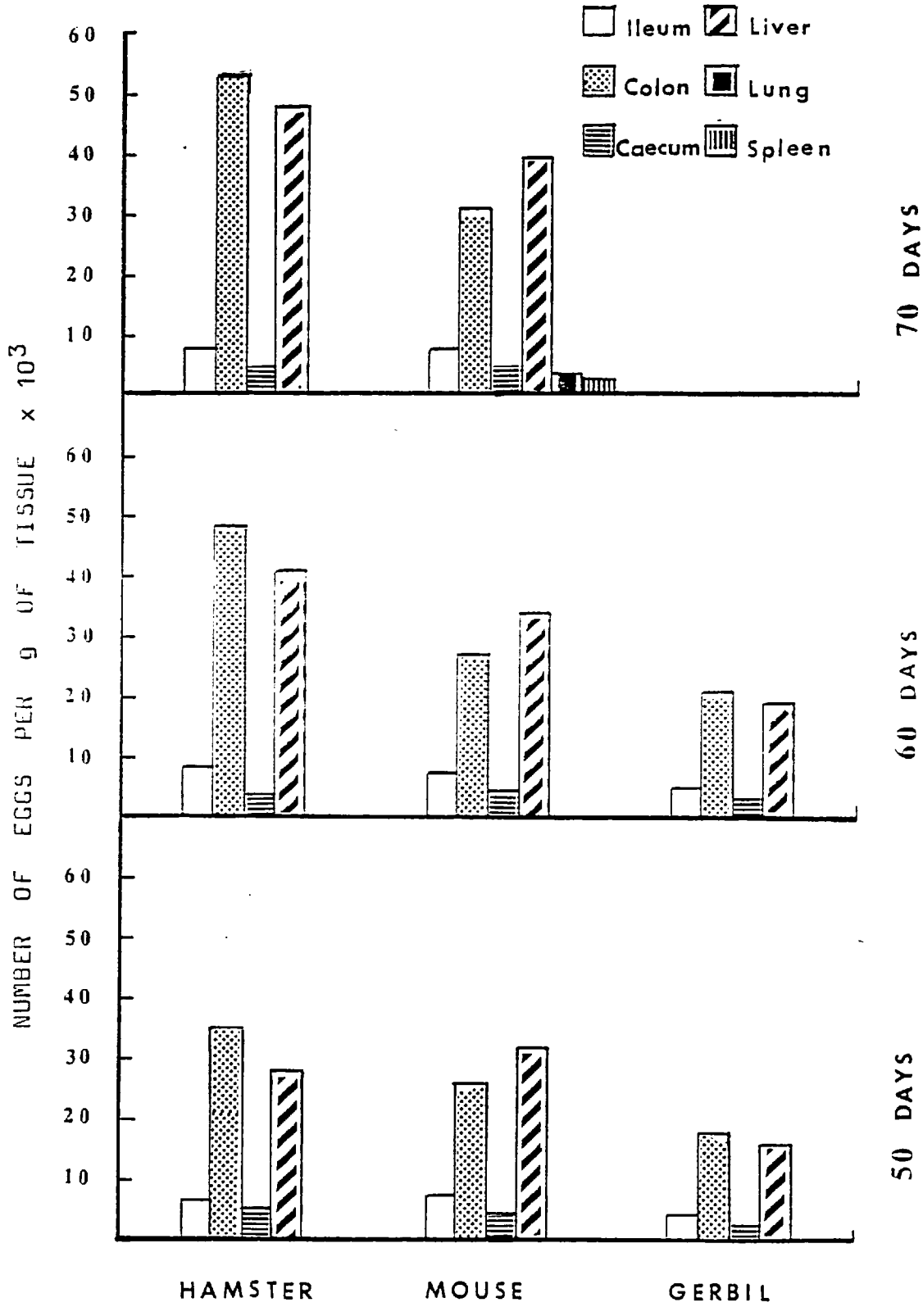
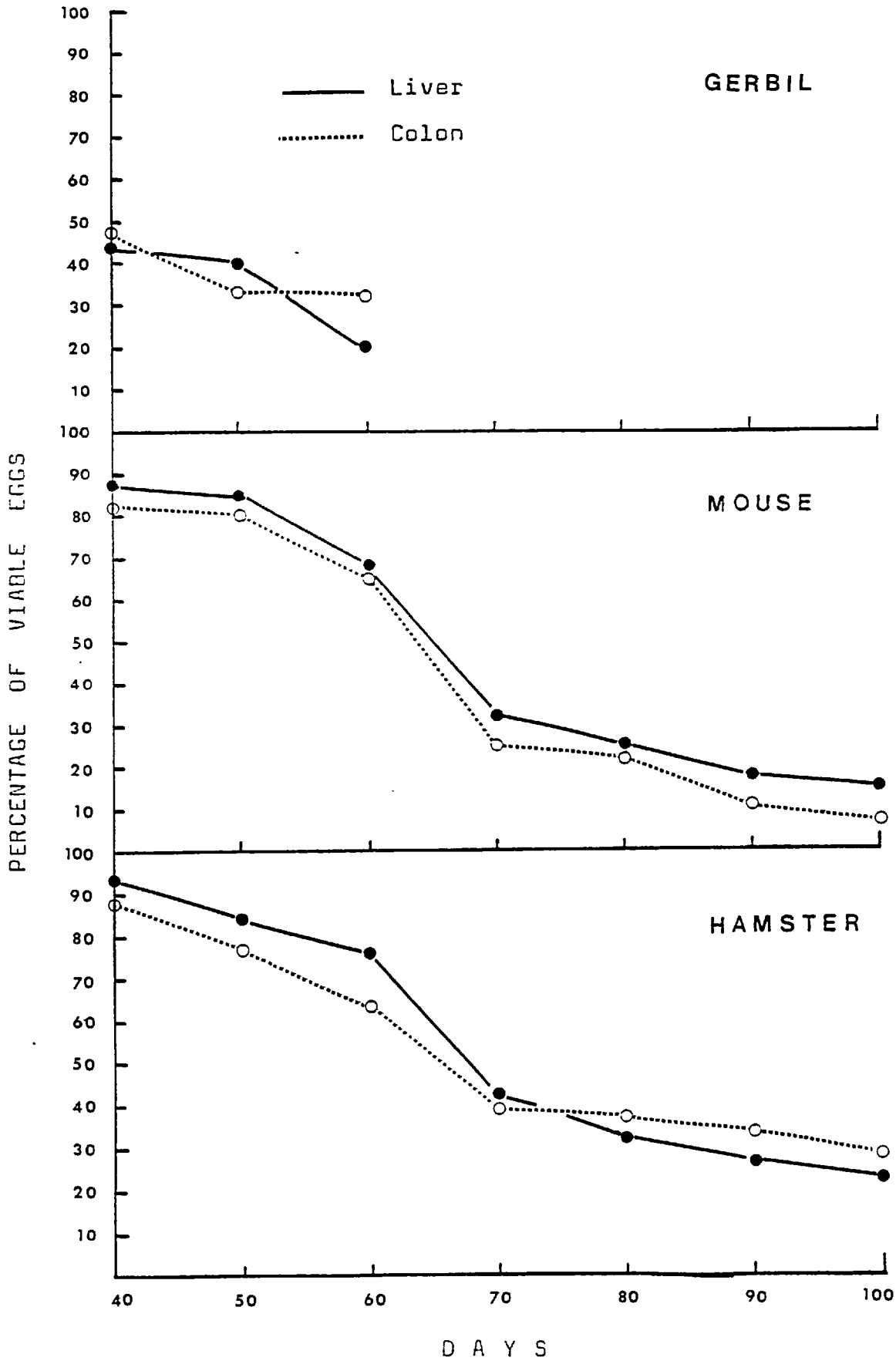


Fig. 3.7 Percentage viability of eggs in the liver and colon of hamsters, mice and gerbils in relation to age of the infection.



3.7 DISCUSSION

The infection of hamsters, mice and gerbils with S. margrebowiei resulted in a pattern of parasite migration, growth and development which is similar to that usually observed in suitable experimental hosts with other species of schistosomes. The quantitative parasitological results of S. margrebowiei infections in the three hosts at autopsy, presented above, allowed a correlation of infection intensity with the host-parasite relationship which is to be discussed later. The limited number of animals used in this investigation, does not allow the interpretation of small variations between the groups.

Considering the mean schistosomula recovery from the lungs of the three hosts, (Fig. 3.1), differences were observed between day 4 and day 6 p.i. This might be due to the differing rates of migration, from the skin to the lungs, of individual schistosomula and therefore, arrival times in the lungs would differ. It is known that migrating schistosomula could be found at different depths of the skin and subcutaneous layer for a week or more p.i., depending on the host and the site exposed (Stirewalt, 1959). This might explain the differences in percentual recovery in the three hosts. Clegg and Smithers (1968) demonstrated that the skin of

some laboratory animals constituted a major barrier to invading cercariae of S. mansoni. They showed that in rats as many as half the cercariae which entered the skin died during the early stages of penetration, in mice about one third, and in hamsters, one tenth of the cercariae died in the skin. Ghandour and Webbe (1976) also showed that 30 to 38% of S. mansoni cercariae died while penetrating the skin of mice. Although the death of S. margrebowiei schistosomula in the skin of the hosts used in this study was not investigated, the variation in lung recovery might be due to the differences in the number which died in the skin of the different hosts as was observed for S. mansoni by previous workers.

There was a difference in the percentage recovery from the lungs in the three hosts but the peak of schistosomula recovery was reached by day 6 p.i. in the three hosts. This shows that the rate of migration is about the same in the three hosts. The percentage recovered at the peak was remarkably low when compared to the peak recovery from the hepatic portal system (Fig. 3.1, 3.2). The small size of these lung forms and the difficulty in recovery, even after perfusion and a long period of incubation of lung tissue, might account for the low number of schistosomula recovered from the lungs.

The appearance of schistosomula in the lungs three days after infection agrees with observations by other workers on other species of schistosomes (Oliver, 1952; Sher, Mackenzie & Smithers, 1974 and Yolles, Moore & Meleney, 1949). The peak recovery was followed by a period of rapid decline in the three hosts, however, a small number were still recovered in the lungs after day 10 p.i. By day 14 p.i., no schistosomula were recorded in the lungs of gerbils. The long stay of schistosomula in the lungs of hamsters and mice is similar to that shown for S. mansoni (Clegg, 1965; Wilks, 1967 and Yolles et al., 1949).

The percentage of worm recovery from the portal system of the three hosts is relatively higher when compared to the schistosomula recovery from the lungs. Generally, high peak recoveries by day 50 p.i. were observed in all the hosts and this again shows that the rate of worm migration was similar in these hosts. The high worm burden in hamsters (61%) and mice (57%) indicates that these animals are more susceptible to infection with S. margrebowiei and might serve as better laboratory hosts than the gerbils where a lower worm burden (34%) was recorded. A sharp decline in worm burden was observed in the three hosts up to day 70 p.i., after which a low worm burden was maintained in hamsters and mice. The death of gerbils between day 50 and 60 p.i.

as a result of the infection, makes this host unsuitable for long term investigation of S. margrebowiei. The pattern of worm burden up to day 50 p.i. and the subsequent decline after then, follows the infection pattern of S. mansoni described by Stirewalt, Kuntz and Evans (1951); Ritchei, Garson & Knight (1963) and Smithers & Terry (1965, a,b). Warren and Peters (1967) showed that with S. mansoni, although the same number of cercariae penetrated the skin of rat, mouse, hamster, guinea pig and rabbit, the number of worms that developed by the fourth week after exposure was different. By the eighth week, the worm burden had fallen in all the hosts. This pattern of infection was common to both bisexual and unisexual S. margrebowiei laboratory infections in hamsters observed in this study, and therefore, reduction in worm burden could not be associated with the period of egg production.

The mechanism of spontaneous reduction in worm burden in schistosome infection which resembles the 'self-cure phenomenon' described by Stoll (1929) for Haemonchus contortus infection in lambs is not understood. The mechanism in rats has been tentatively suggested to be due to an acquired immune response on the part of the host which becomes effective at the fourth week (Smithers & Terry, 1965). Maddison, Norman, Geiger & Kagan (1970) reported that reduction in worm burden

in rats between the fourth and the eighth week does not appear to be associated with hypersensitivity reaction due to reaginic antibody since the peak level is reached after worm loss. Cioli, Blum & Ruppel (1978) studied the relationship between parasite age and time of spontaneous elimination from the rat and presented several hypotheses about the possible nature of this critical event.

Whatever the mechanism responsible for this reduction in worm burden in hamsters, mice and gerbils in this study, and similar observations by other workers with other schistosome species, the occurrence of such reduction is of great practical importance in schistosome development. An understanding of the mechanism involved would throw more light on the development of schistosome infections. The results presented in this report are concerned mainly with the pattern of infection of this parasite in these experimental hosts.

The growth rate of paired worms are considered in this investigation. A number of workers on S. mansoni have evaluated the rate of growth by employing dry-weight measurements (Radke, Schneider & Houghtaling, 1957; Lincicome, Eni & Stirewalt, 1963). Smyth (1966) suggested that the best method for evaluating growth was on a weight basis as this would provide a figure in terms of tissue

synthesis. In this study, length was used for the evaluation of growth rate because pronounced changes in length were observed after the worms had paired and a comparative growth rate of the three hosts was desired. Clegg (1965) examined large numbers of schistosomula from the lungs of mice by the Feulgen staining methods and did not find a mitotic figure. The observation by Clegg (1965), the measurement of the wet weight during development of S. mansoni by Lawson & Draskau (1977) together with the similarity in size and appearance of lung forms has led to the conclusion that little if any growth occurs in schistosomes prior to their arrival in the liver. Wilson et al (1978) suggested that the stimulus in the liver which terminates migration of schistosomula in the liver probably initiates growth.

The pattern of growth of S. margrebowiei in these three experimental hosts was similar to that observed by Southgate and Knowles (1977) in hamsters. The rate of growth was faster in hamsters and gerbils as worms from these two hosts were longer at the early stages of the observation than worms recovered from mice. The point at which the length of the paired female exceeds that of the paired male has been termed the 'cross-over point' and this is an indicator of the rate of development (Southgate & Knowles, 1977). The cross-over point has been determined for various species of schistosomes,

being 80 days for S. haematobium, 61 days for S. mattheei, 49 days for S. intercalatum and 40 to 48 days for S. bovis. The cross-over point was reached in 30 days in mice and in 28 days in hamsters and gerbils. This therefore, indicates that the rate of growth of S. margrebowiei was faster in hamsters and gerbils than in mice. The parasite attained a large size in the three hosts with the longest worm occurring in the hamsters. This fast rate of growth of S. margrebowiei suggests that this might be a suitable schistosome species for in vitro culture. The difference in the sizes of worms by day 60 p.i. from the different hosts might be related to the metabolism of the host or the availability of nutrients to the parasite in the different host species which probably influences the physiological conditions of the parasite. At present, there is no hypothesis to explain this difference in the size of worms from the different hosts.

The increased rate of growth of the female worm after day 30 p.i. could be explained by the hypothesis put forward by Senft (1969). He suggested that the male schistosome may furnish some nutritional substances to the female through the intimate contact within the close confines of the gynaecophoric canal. Lennox & Schiller (1972) in their study of changes in dry weight and glycogen content during postcercarial growth and

development, also suggested that in S. mansoni, a nutritional relationship might be the primary mechanism for the mediation of the growth of female worms subsequent to copulation. This hypothesis might be correct as female worms from unisexual infections in hamsters did not only lack eggs in their uteri, but were also much shorter than paired female worms of the same age. Growth of paired worms continued after sexual maturity had been reached.

The results presented in this study show that hamsters, mice and gerbils favoured the development and maturation of S. margrebowiei. The pattern of development in these hosts was similar to that described for S. mansoni (Clegg, 1965), S. japonicum (Suliman, 1974), S. haematobium (Smith et al., 1976) and S. bovis (Ghandour & Babiker, 1978).

Six developmental stages, distinguished on the bases of morphological and histochemical criteria, were observed in these hosts. Schistosomula were first recovered in the lungs of hamsters and mice by day 2 p.i. and by day 3 p.i. in the gerbils. The digestive tract of these lung forms appeared to be functional at this stage as a few worms showed black caecal content, presumably haematin. The migration time of the lung form was similar to that of S. japonicum (Table 3.3).

The present investigation has shown that the male worm appears to develop faster than the female worm. On day 25 p.i., male worms had four testes while the female reproductive organs were not yet fully developed. The female had developed the ovary by day 25 p.i. in the three hosts but fully developed eggs were first observed in the uterus by day 30 p.i. in the three hosts. Intrauterine egg counts showed that a large number of eggs occurred in the uterus at maturity and only a slight uterine egg count difference was observed between worms from the three hosts. Table 3.3 shows the comparison of the rates of development of a number of schistosome species including S. margrebowiei from the present study. S. margrebowiei, like S. japonicum and S. mansoni, has a fast rate of development.

In unisexual infections in this study, male worms developed to maturity but female worms did not mature. This impediment of maturity of the female schistosome due to the absence of the male has been reported by a number of workers (Severinghas, 1928; Brumpt, 1936; Short, 1952 and Erasmus, 1973). Severinghas (1928) suggested that the male secretes a hormone which stimulates the maturation of the female. Armstrong (1965) suggested that a tactile stimulation was required for the maturation of the female. The situation of the female in the gynaecophoric canal is a further stimula-

tion for the maturation in female S. mansoni (Shaw, 1974).

A few male worms were found in the hepatic portal veins in unisexual infections but all the female worms in unisexual infections were recovered from the liver. Most paired worms were recovered from the hepatic portal and mesenteric veins in bisexual infections and paired worms were first observed in the experimental hosts by day 25 p.i. This led to the conclusion that pairing occurred in the portal vein as worms approached sexual maturity and that mating provided the stimulus for migration to the more distal branches of the portal system. Standen (1949) suggested that the males in S. mansoni were mainly responsible for performing the journey against the bloodstream to the mesenteric veins. This might be the case in S. margrebowiei since a few unpaired male worms occurred in the hepatic portal veins in both bisexual and unisexual infections.

Qualitative and quantitative comparisons based on the number of eggs deposited in the tissues of the infected host species would be difficult since large variations were observed in individual animals. The intensity of the infection, measured in this study by the worm burden, varied in the different hosts, and so did the distribution of paired worms in these hosts. In general, the results of tissue digestion showed that the number of eggs per

gram of tissue paralleled the intensity of the infection - highest tissue egg counts in hamsters and lowest in gerbils. Intestinal involvement, especially in the colon, was common in all the hosts studied. Like S. japonicum, S. margrebowiei produce many more eggs than S. mansoni and this might provide sufficient reason for the severity of S. margrebowiei infection in hamsters, mice and gerbils. The number of eggs deposited in the tissues might be an important parameter when considering the intensity of the infection in relation to tissue damage which will be discussed under histopathology of the infection in these hosts.

A high percentage of viable eggs were observed in the liver and colon of the hamsters and mice. The low percentage in the gerbils might indicate further that the gerbil is a poor host for S. margrebowiei infection. The inhibition of egg development in gerbils may be a highly efficient resistance mechanism. As the infection progressed, the percentage of viable eggs in the colon and liver tissues decreased. The decrease was more in the mice than in the hamsters, and this might indicate that the rate of calcification of eggs was faster in the mice.

These quantitative parasitological results have shown that a high compatibility occurred between the mouse and S. margrebowiei. This combination could be

useful for long term studies in which the main interests are parasite longevity and minimal destruction of the parasite by the host. In hamsters, high worm burden (51 to 61% of cercariae applied) makes this host suitable when large numbers of worms are required. However, the high mortality which occurs between 50 and 60 days after exposure, like in gerbils, make them unsuitable for long term studies.

4 OBSERVATIONS ON SCANNING ELECTRON MICROSCOPY

The features of the tegument of adult and immature male and female Schistosoma margrebowiei as seen with the scanning electron microscope (SEM) are demonstrated in a series of 48 selected micrographs. Plates 5 to 9 are micrographs of adult worms and Plate 10 to 12 are of immature worms. The microtopography of the egg is also illustrated by four micrographs.

4.1 ADULT WORMS

The adult worms are enlongated, the males being dorso-ventrally flattened while the females are cylindrical. The lateral margins of the male curve ventrally to form the gynaecophoric fold that holds the female in a gynaecophoric canal. Plate 5.1 shows an adult pair of worms with the female projecting from the gynaecophoric canal of the male. Apart from the relatively small size of the female, the difference in sex is also illustrated by the absence of any observable surface structure on the female tegument and the male being extensively covered with elevated structures called tubercles or bosses.

The surfaces of the anterior parts up to the acetabulum in both sexes are similar and are devoid of surface

elevations (Plates 5.2, 5.3, 5.4, 6.1). The oral suckers of the male and female, though smaller in the female, are similar and are covered with many sharp pointed spines that appear more closely packed in the male. The rims of the suckers bear numerous sensory organelles (Plates 5.4 & 6.1).

The acetabulum of the male is a more prominent structure than that of the female which is significantly smaller and does not appear elevated above the surface of the worm (Plates 5.2, 5.5, 6.3 & 6.4). In the male, the acetabulum appears as a prominent saucer-like organ with a pronounced rim (Plates 5.5 & 6.3) and at a higher magnification it is characterized by well differentiated areas; a central portion with closely packed spines and an outer rim with spines set in deep sockets. Between the central portion and the outer rim is a narrow area which lacks spines (Plate 5.6). The acetabulum in the female appears less prominent and the surface is not covered with spines (Plate 6.4). The worms feed on the host's blood, and some blood cells can be seen adhering to the spines in the oral sucker of the female (Plate 6.2).

The gynaecophoric canal, which forms the ventral surface of the male, starts from just below the acetabulum, (Plate 5.5) and extends to the caudal end (Plate

7.5); it appears to be deeper around the midbody of the worm (Plates 6.5, 6.6). The canal is bounded on both sides by well-developed gynaecophoric folds which appear rough on the right-hand side due to the presence of tubercles . The rough right gynaecophoric fold when viewed at higher magnification shows scattered spines on the small tubercles . The spines on the rest of the surface of the gynaecophoric fold point towards the canal, sensory organelles also occur around the tubercles The tubercles on the fold measure about 10μm in diameter (Plates 8.1, 8.2). At higher magnification of the square area in Plate 7.5, the gynaecophoric fold is seen as a ridged, porous or spongy surface due to the presence of numerous pits which measure about 3 to 4μm in diameter and the fairly evenly distributed anteriorly directed spines (Plate 7.6). The surface of the gynaecophoric canal is also ridged and pitted and has anteriorly directed spines (Plates 7.1, 7.2). Blood cells are again commonly seen adhering to this surface of the worm (Plate 7.2).

The dorsal surface, posterior to the acetabulum, of the male worm is covered with numerous tegumental elevations called tubercles , that are spine-free and appear to be smaller just below the acetabulum and near the gynaecophoric fold (Plates 6.3, 7.3). At higher magnifications, the tubercles on the dorsal surface which

measure about 30μm in diameter, appear to be slightly rough and sensory organelles project from the ridged tegument between them (Plate 7.4). Towards the posterior end of the male, the tubercules are smaller (20μm in diameter) but similar to those on the midbody (Plates 8.3, 8.4). Plate 8.3 shows the ventral surface of the caudal end of the male reflexed and shows the absence of spines around the excretory pore.

The surface of the adult female which appear relatively smooth at low magnification (Plate 9.1) is seen to be deeply pitted with circumferentially arranged creases around the midbody at a higher magnification (Plate 9.2). The creases are much more prominent on the dorsal surface than on the ventral surface (Plates 9.3 & 9.4). The midbody of the female is devoid of spines but has sensory organelles like those on the surface of the male. The caudal end of the female, like that of the male, tapers rapidly, (Plates 8.5, 8.6) but unlike the male, it has spines which are arranged singly and point away from the excretory pore (Plates 8.3, 9.5). The posterior tip of the female is rounded and around the excretory pore the surface is devoid of spines (Plate 9.5). At higher magnification this surface appears spongy and the spines are seen to be set in deep sockets; sensory organelles also occur in between the spines (Plate 9.6).

4.2 IMMATURE WORMS

The microtopography of worms recovered from the liver at 15 days p.i. shows no resemblance to that of adult worms described above. It is impossible to distinguish between males and females by differences in the surface structure at this stage of development and the dorsal and ventral surfaces were similar in appearance.

The surface of the worms at this stage, is characterized by shallow tegumental folds and pits with wide openings. The folds in the anterior part appear less prominent than those in the posterior. There are more sensory organelles on the anterior surface of the worm (Plate 10.1) than on the posterior surface (Plate 10.2).

At 25 days p.i., the worms are larger (4-8mm in length) and the male can be distinguished from the female by the development of the gynaecophoric canal and also by the appearance of the tegumental surface which now shows great variation along the length of the worm similar to that observed on the mature male worm. Plate 10.3 shows the anterior end of a 25 day old male worm with its well-developed gynaecophoric fold. The micrographs of the magnified areas show that the oral sucker (Plate 10.4) and the acetabulum (Plate 10.6), as in the

adult have well-developed spines that are set in deep sockets and sensory organelles are present. The area between the oral sucker and the acetabulum has flattened tegumental folds that partially obscure the spines as shown in Plate 10.5. The anterior part of the female of the same age is similar to that of the male but with a less prominent acetabulum, resembling that of the adult female. The area between the oral sucker and the acetabulum also has flattened tegumental folds but with more prominent spines (Plate 12.1).

The dorsal surface of the male worm at 25 days p.i. has much increased tegumental folds and small tubercles (about 4μm in diameter) appear on the surface (Plate 11.2). Plate 11.1 shows the dorsal surface and part of the gynaecophoric fold of a male worm about the midbody, and Plate 12.2 shows a magnified view of a part of the dorsal surface to illustrate the pronounced nature of the tegumental folds. Plate 11.4 shows the posterior end of a 25 day old worm with the well formed gynaecophoric canal. The surface of the gynaecophoric fold has shallow tegumental folds and the tubercles are of varying sizes just beginning to develop. Sensory organelles are prominent on this surface (Plates 11.3, 11.5). Plate 11.6 shows a magnified area of the gynaecophoric canal, the tegumental surface is composed of narrow and compressed folds which sometimes obscure the spines.

Both ventral and dorsal surfaces of the midbody in the 25 day old female worm are similar, having less prominent folds than the same section of the male worm. The surface resembles a seive of large mesh size with sensory organelles jutting out at intervals. The posterior end of the 25 day old female worm has flattened tegumental folds with closely packed spines occurring in the more posterior end (Plate 12.2).

At 35 days p.i., worms are fully mature and the tegumental surfaces are similar to the adult surface of 60 day old male and female worms already described. Plates 12.5 and 12.6 show part of the dorsal surface midbody of 35 day old female and male worms respectively.

4.3 EGG

The eggs of S. margrebowiei are rounded to oval in shape. 20-25% of the eggs deposited in the tissues of the experimental hosts possess poorly developed terminal spines (Plates 13.1, 13.3). In some eggs, the spines appear as mere terminal protuberances or knobs. Some eggs have no spines at all (Plate 13.2). The eggs measure about 85µm by 64µm and the spines, about 0·5µm.

At low magnification, the surface of the egg appears granular (Plate 13.3). However, at higher magnification,

the entire surface is observed to be covered with densely packed blunt protuberances or microspines which are uneven in size and round-tipped (Plate 13.4).

During the preparation of the eggs for SEM studies, the eggs were observed to adhere to debris and glassware. This stickiness of the egg was utilized in the preparation of the eggs for freeze-drying in liquid nitrogen by allowing them to adhere to glass coverslips on which they were freeze dried.

4.4 DISCUSSION

The tegumental surface of the male Schistosoma margrebowiei can be seen with the aid of the light microscope to possess small elevations. The present investigation on the microtopography of S. margrebowiei using the SEM, revealed the surface details of both immature and adult male and female worms.

In general, adult worms show a striking difference in the tegumental surfaces of the male and female. Whereas the male has a rough surface which is noticeable at low magnification due to the presence of tubercles, the female is smooth. Higher magnification however revealed that apart from the tubercles in the male, both surfaces are similar in being pitted, ridged and having sensory organelles.

Other workers have also recorded tegumental surface differences between the male and female adult worms of other species of schistosomes, using the SEM. Kuntz et al.,(1976) showed this difference in S. mansoni; Tulloch et al.,(1977) in S. mattheei; Hicks & Newman (1977) in S. haematobium; Kuntz, Tulloch, Huang & Davidson (1977) in S. intercalatum and Kuntz et al.,(1979) in S. bovis. The information from these SEM studies and the present study on S. margrebowiei indicate that the major tegu-

mental difference among the mammalian schistosomes occurs on the dorsal surface of the male worm. Thus the presence of spine-free tubercles on the dorsal surface of S. margrebowiei confirms its similarity with S. mattheei and S. bovis which were observed by Hockley and McLaren (1977).

The tegument of Schistosoma is generally considered to be a protective sheath, but the regional variations observed on the surface of both sexes of S. margrebowiei may be associated with other functions. The spines on the oral suckers of both the male and female worms, and the well-developed acetabulum of the male worm may facilitate rasping and attachment, and help the parasite to withstand the flow of blood in the portal system.

The posterior end of the female worm is always found projecting out of the gynaecophoric canal of the male and has well-developed spines. These spines are anteriorly directed and may be embedded in the epithelial lining of the blood vessels against the direction of the blood flow and thus prevent the worm from being dislodged. The worms are always attached to the walls of the hepatic portal system with the anterior end facing the direction of blood flow.

The roughened lining of the gynaecophoric canal and

the presence of spiny tubercles on the gynaecophoric fold may serve to secure the female in the grasp of the male and could be considered as an adaptation for residence in the blood-stream of the definitive host that ensures continuous pairing of the worms. This modification of the gynaecophoric fold was also reported in S. haematobium by Kuntz et al. (1976).

The sensory organelles which occur all over the surface of both male and female worms appear to be of only one type, as was described by Morris & Threadgold (1967) in S. mansoni. The organelles occur in large numbers on the suckers of the male and female worms and also on the dorsal surface of the male worm. These organelles have been interpreted as tangoreceptors or chemoreceptors which may function to detect the direction of blood flow (Morris & Threadgold, 1967). The increase in number on the suckers and dorsal surface of the male is presumably associated with the fact that these surfaces are in intimate contact with the host and are probably the intial target of the host's response. In these areas, the organelles may function to detect changes in the worm's environment.

The tegumental folds and pits observed on the surface of male and female worms may help to increase the surface area for the uptake of materials in their

blood-stream environment. The two routes of material uptake in trematodes are through the intestinal caecum via the mouth, and through the tegument. Robinson (1961) and Senft (1963) have suggested a transtegumentary absorption of amino acids and carbohydrates in S. mansoni.

The microtopographic observations have also revealed structural differences between the surfaces of migratory stages and adult worms. At early stages of development in the portal system, the tegumental surfaces of male and female worms are indistinguishable. This was also observed in S. mansoni by Voge, Price and Bruckner (1978) who reported pronounced changes on the tegumental surface during the development of the parasite in mice.

The concentration of sensory organelles on the anterior end of 15 day old worms could play a significant role in the migration of the worms as they may help the worms to detect changes in the environment. Similar structures have been associated with chemoreception and attachment by workers on other digenean teguments (Morris & Threadgold, 1967; Erasmus, 1967, 1969). The spines present in between the oral sucker and the acetabulum in immature male and female worms (Plates 10.5, 12.1) but absent in mature worms may function for attaching the anterior end of the worm to the lining of the blood vessels as the worm pulls the posterior end forward

during migration. In mature worms, migration is complete, and attachment to the lining of the blood vessel is probably performed by the well-formed, spine-covered acetabulum of the male.

Differences in the male and female tegumental surfaces are observed in S. margrebowiei by day 25 p.i. when the male develops the gynaecophoric fold and worms are beginning to pair and move into the hepatic portal vein from the liver. In the male worm, the development involves the appearance of tegumental elevations (tubercles) on the dorsal surface and flattening of the greatly increased tegumental folds formed at the early stage. The flattening of the tegumental folds on the ventral surace of the male also makes the spines more apparent in the gynaecophoric canal. The development of the female tegumental surface, however, involves the gradual shrinking and condensing of the tegumental folds with subsequent flattening to give a relatively smooth appearance in the adult. Hence, the surface of the adult female shows no signs of the early tegumental folds.

This investigation has shown that the structure of the tegumental surface of the worm could be useful in determining the extent of development or age of the parasite. The series of microtopographs have also shown the local variations on the tegumental surface along the

length of the worm and this might provide an additional means of identification and description of S. margrebowiei.

Table 4.1 shows structural differences between S. margrebowiei and other species of schistosomes which have been described using the SEM. In S. bovis, S. mattheei and S. margrebowiei, the male worms possess tubercles that are devoid of spines. However, unlike S. mattheei which has flattened nipples near the centre of some of its tubercles, S. margrebowiei has some of its tubercules appearing slightly rough due to the presence of small elevations (Plate 7.4). In the past, differentiation of species of schistosomes was based mainly on the characteristics of internal organs. A detailed description of the surface characteristics, based on the use of the SEM, could be used to complement previous methods of identification and thus provide a more complete description of the different species of schistosomes.

Table 4.1 A comparison of the tegumental structure of *S. margrebowiei* with those of other species of schistosomes. +, present; -, absent.

	S.mansoni Miller et al.,(1972)	*S.haematobium* Kuntz et al., (1976)	*S.japonicum* Sakamoto & Ishii(1977)	*S.intercalatum* Kuntz et al., (1977)	*S.mattheei* Tulloch et al.,(1977)	*S.bovis* Kuntz et al.,(1979)	*S.margrebowiei* Present study
Spines on inner and outer surface of suckers	-	-	+	-	-	-	-
Spines on inner surface only	+	+	-	+	+	+	+
Tubercles on dorsal surface of male	+	+	-	+	+	+	+
Tubercles covered with spines	+	+	-	+	-	-	-
Tubercles with nipples near centre	-	-	-	-	+	-	-
Midbody of female with bosses	-	+	-	+	-	-	-
Midbody of female with spines	-	+	+	-	+	-	-
Posterior end of female with spines	+	+	+	+	-	+	+

A number of workers have published articles with details of the microtopography of various schistosome eggs (Hockley, 1968; Race et al., 1971; Sakamoto & Ishii, 1976 and Ford & Blankespoor, 1979). The micrographs in these articles showed that the surface of the eggs of these schistosome species are covered with microspines. The present SEM micrographs have revealed the presence of similar microspines on the surface of S. margrebowiei eggs. The rounded microspines are similar to those reported on S. haematobium eggs by Hockley (1968). In S. margrebowiei eggs, it was observed that the microspines are closely packed on the surface, a situation similar to that reported by Ford and Blankespoor (1979) for the eggs of S. japonicum.

In the past, S. margrebowiei eggs were practically indistinguishable from those of S. japonicum and this might account for the report of S. japonicum-like eggs from North-East Zaire by Walkiers (1928). The present investigation has provided additional microtopographical differences between the eggs of these two schistosome species. Unlike S. japonicum which has a lateral rudimentary spine on some eggs, the spine on S. margrebowiei eggs is always terminal. The presence of such a rudimentary spine is a variable feature in the S. margrebowiei egg as some eggs lack this spine. This was also observed by Southgate & Knowles (1977) using the

light microscope.

The fibrous net-like matrix which forms a consistent dense coat on the surface of the S. japonicum was suggested by Ford & Blankespoor (1979) to be responsible for the stickiness of the eggs. Such a dense coat of net-like 'fibres' was not observed on the surface of the eggs of S. margrebowiei. Since the stickiness was also observed in S. margrebowiei eggs it is probable that another material other than the fibrous matrix would be responsible for it. However, no other material was observed under the SEM. The eggs of S. mansoni and S. haematobium also display this stickiness, but here the fibrous matrix is generally thin with occasional dense patches (Ford & Blankespoor, 1979).

The microspines which protrude from the shell may serve to maintain a space between the egg and the host's cell membrane and this space was suggested to represent the primary interface of host-parasite contact (Schnitzer et al., 1971). The blood cells observed adhering to the surface of deposited eggs in tissue sections may have been attached to these microspines.

PLATES 5 - 9 Micrographs of 60 day old adult male and female worms.

PLATE 10 Micrographs of 15 day old worm and 25 day old male worm.

PLATE 11 Micrographs of 25 day old male worm.

PLATE 12 Micrographs of 25 day old female worm and 30 day old male and female worms.

PLATE 13 Micrographs of the egg.

PLATE 5



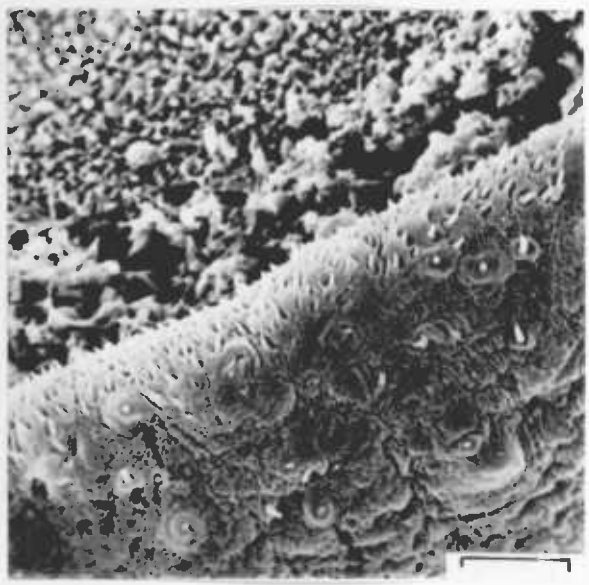
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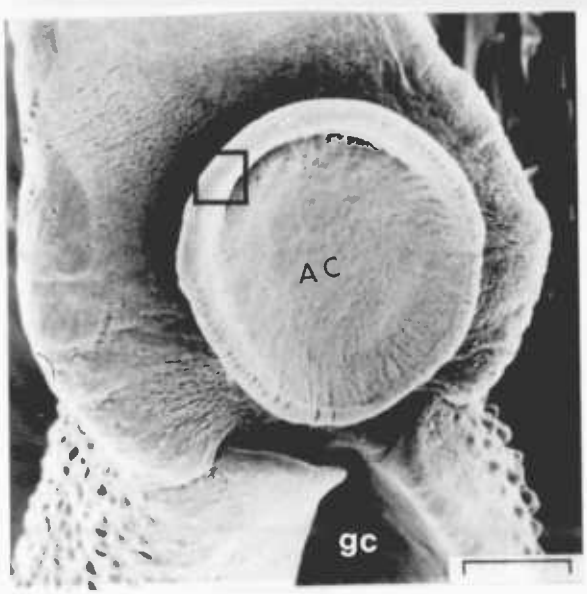
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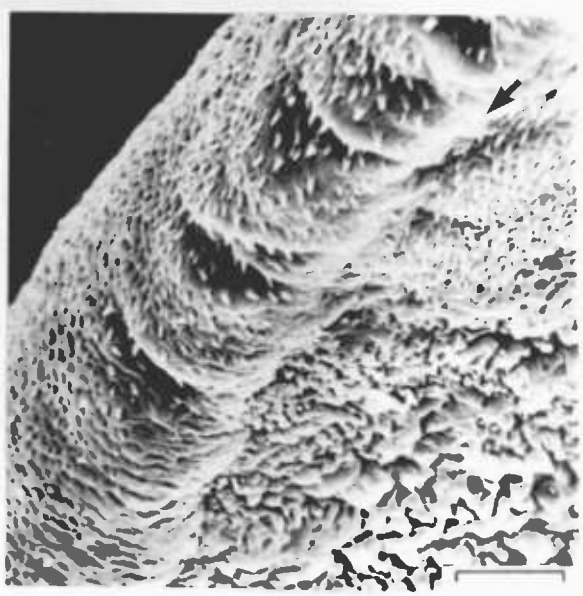
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PLATE 5

5.1 Adult (60 day old) pair of S. margrebowiei, with the female projecting from the gynaecophoric canal of the male. The body of the male is covered with numerous tubercules.
Scale = 200µm

5.2 Anterior end of male showing the oral sucker (OS), the acetabulum (AC), and the absence of tubercles. Scale = 100µm

5.3 Ventral view of the anterior end showing the oral sucker (OS) and the acetabulum (AC).
Scale = 100µm

5.4 A higher magnification view of the square area on Plate 5.3 showing the nature of the spines and the sensory organelles.
Scale = 10µm

5.5 Ventral view of the male showing the acetabulum (AC) and the anterior part of the gynaecophoric canal (gc).
Scale = 100µm

5.6 A higher magnification view of the square area on Plate 5.5 showing the arrangement of spines on the acetabulum. Note the presence of closely set spines on the central portion and the rim, and also a narrow area between them that lacks spines (arrow).
Scale = 20µm

PLATE 6

6.1 Anterior end of the female showing the oral sucker (OS) and the acetabulum (AC). Note the presence of numerous sensory organelles (SO) around the oral sucker.

Scale = 20μm

6.2 A higher magnification view of the square area on Plate 6.1 showing the nature of the spines (S). Note the blood cells (BC) adhering to the spines.

Scale = 2μm

6.3 Lateral view of the anterior of the male worm showing the acetabulum (AC) and the small tubercles (TB) occurring just below the acetabulum. Scale = 100μm

6.4 Ventral view of the acetabulum (AC) of the female. Note that the acetabulum is not raised above the surface of the worm and the absence of tubercles around it.

Scale = 4μm

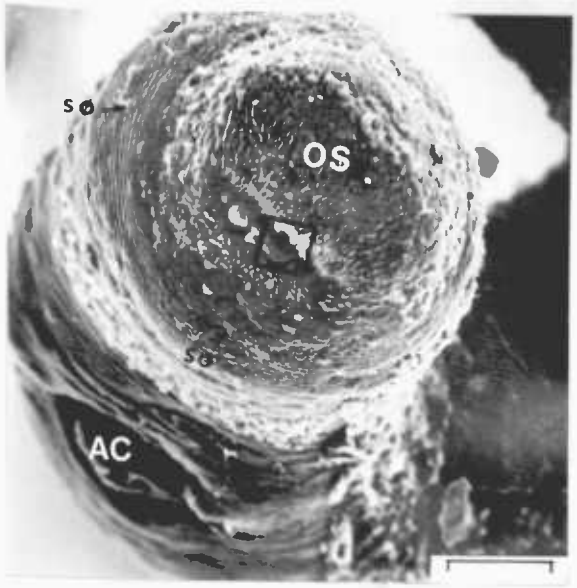
6.5 Ventral view of the male showing the gynaecophoric fold (gf). Note the smooth left fold and the rough right fold due to the presence of small tubercles.

Scale = 200μm

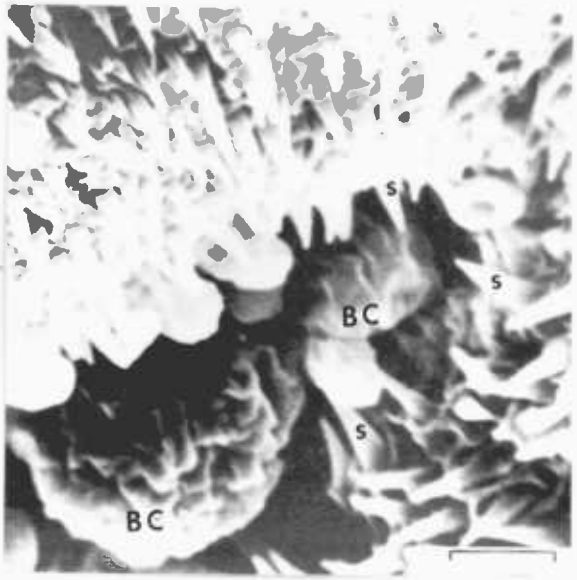
6.6 Ventral view of midbody of the male showing the female in the gynaecophoric canal (gc). Note the smooth left gynaecophoric fold (gf) and the rough right one.

Scale = 200μm

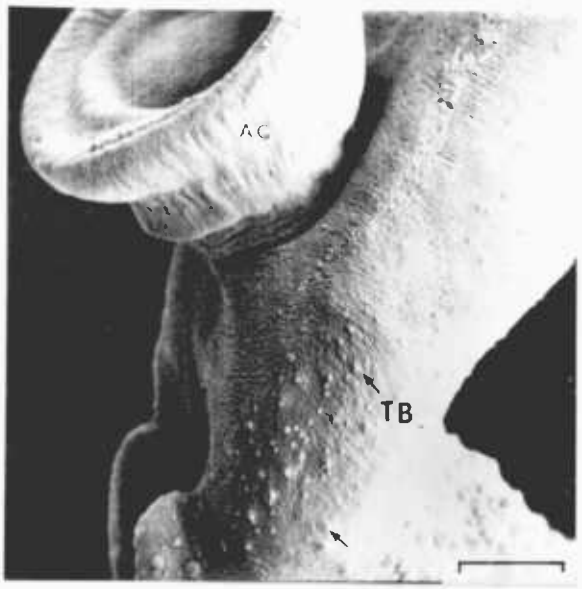
PLATE 6



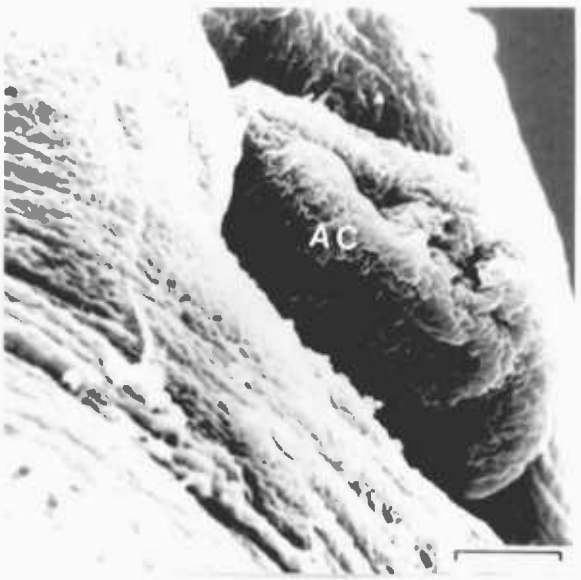
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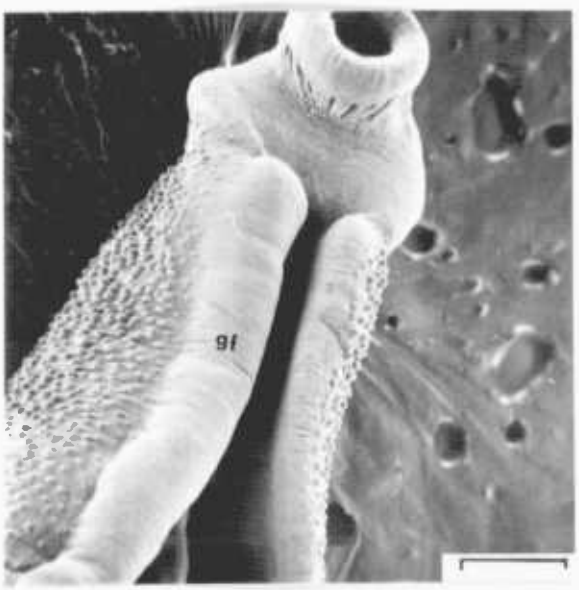
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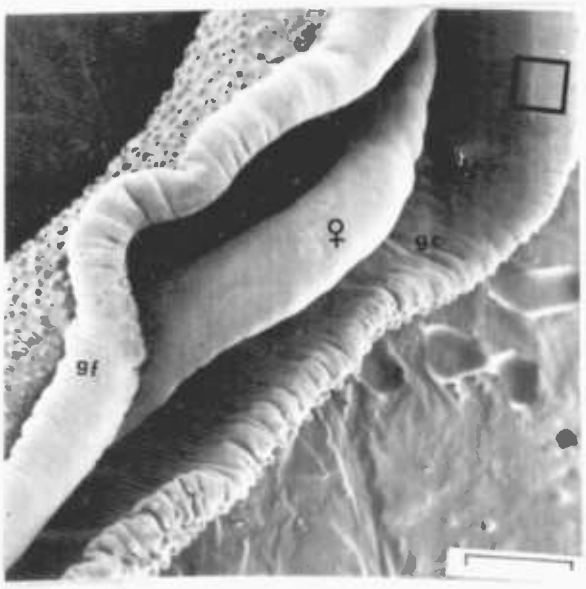
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PLATE 7

7.1 The square area on Plate 6.6 at a higher magnification to show the ridged and spiny surface of the gynaecophoric canal.
Scale = 20μm

7.2 The square area on Plate 7.1 enlarged to show the pits and spines (s) in the gynaecophoric canal. Note the adhering blood cell (BC). Scale = 2μm

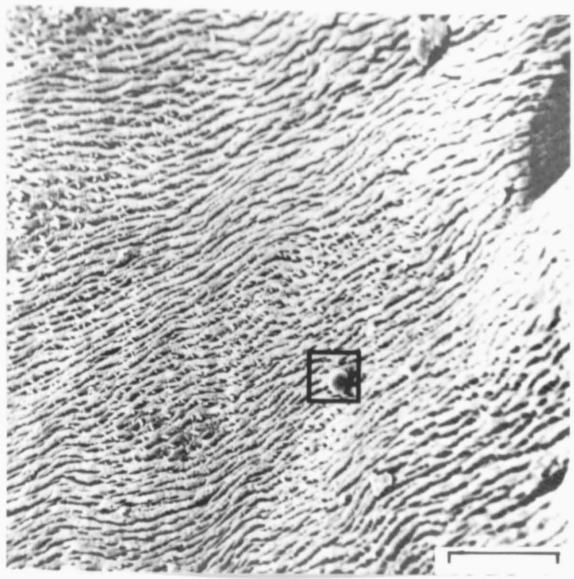
7.3 Dorsal surface of the male midbody showing, the ridged surface with numerous tubercles.
Scale = 100μm

7.4 The square area on Plate 7.3 is magnified to show the ridged and pitted surface. Note the two pronounced tubercles (TB) and the rough surface and the sensory organelles (SO). Scale = 10μm

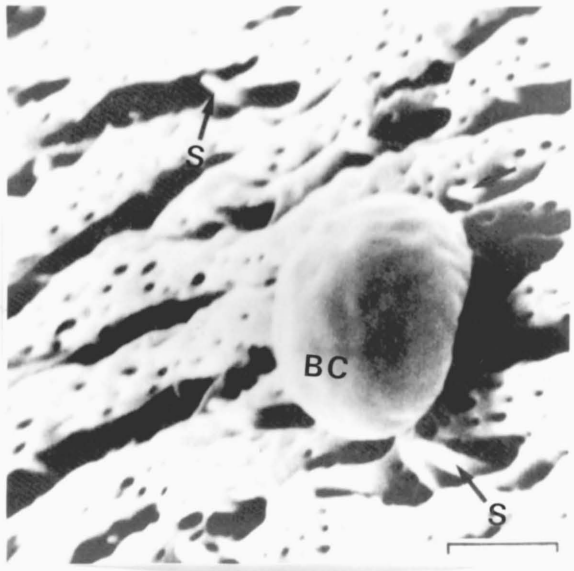
7.5 Posterior end of the male showing the gynaecophoric canal. Scale = 40μm

7.6 A higher magnification of the square area on Plate 7.5 showing details of spines and pits. Note the relatively largre pits (arrows).
Scale = 4μm

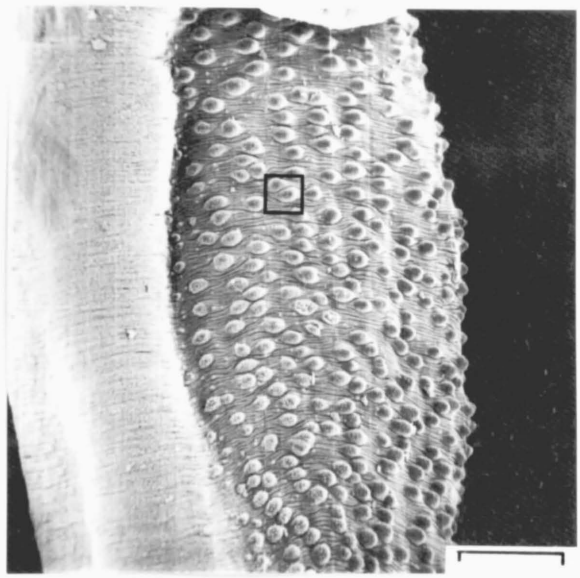
PLATE 7



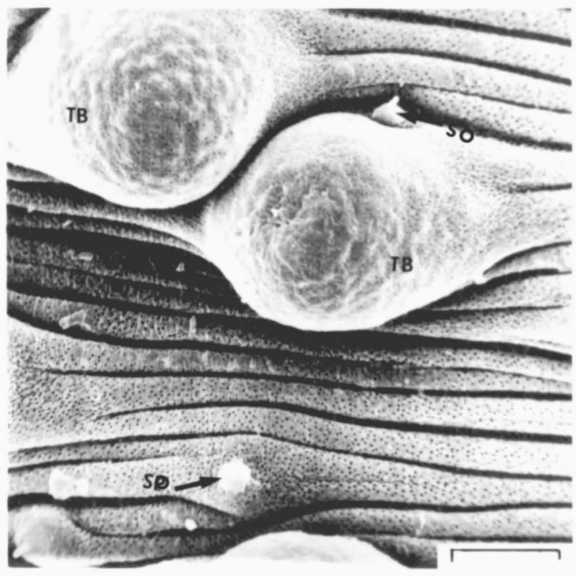
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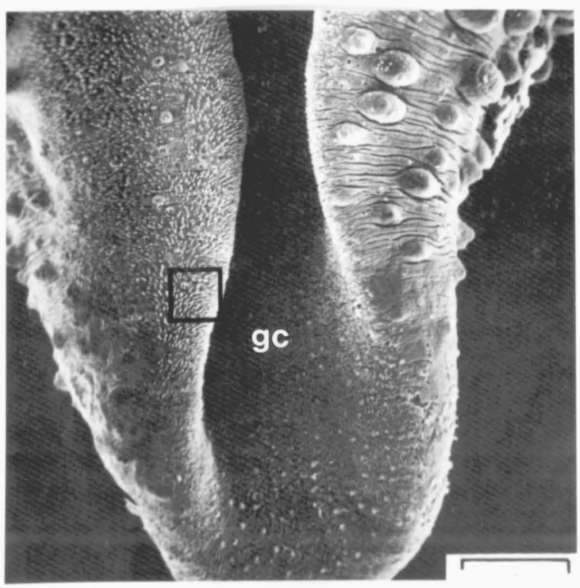
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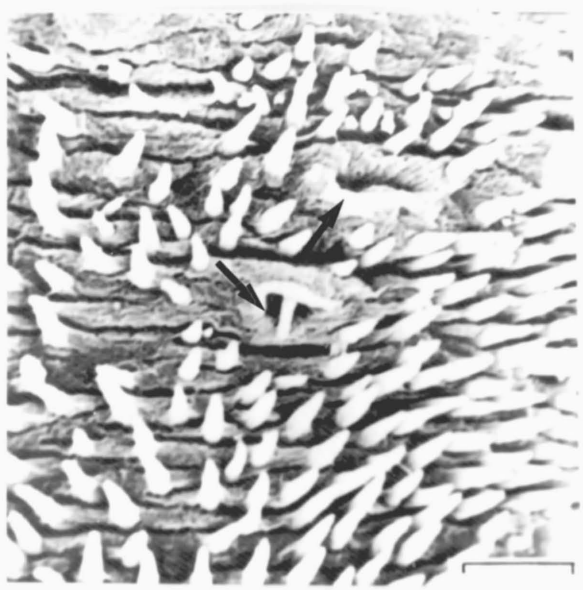
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PLATE 8

8.1 Part of the rough right gynaecophoric fold of the male showing tubercles with spines. Scale = 20µm

8.2 The square area on Plate 8.1 magnified to show one tubercle. Note the spines (s) on the tubercule and on the other parts of the surface and the sensory organelles (SO). Scale = 4µm

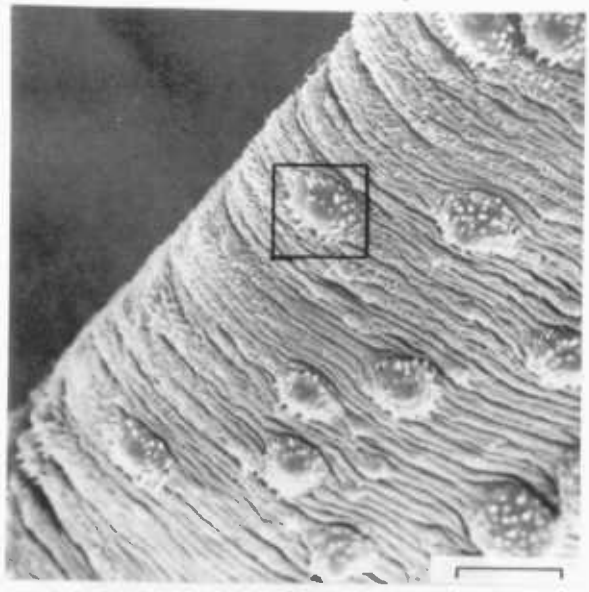
8.3 Dorsal view of the posterior end of the male. The ventral surface is reflexed outwards near the caudal end showing the absence of spines around the excretory pore (EP). Scale = 40µm

8.4 The square area on Plate 8.3 showing tubercles (TB), sensory organelles (SO) and the pitted surface. Scale = 10µm

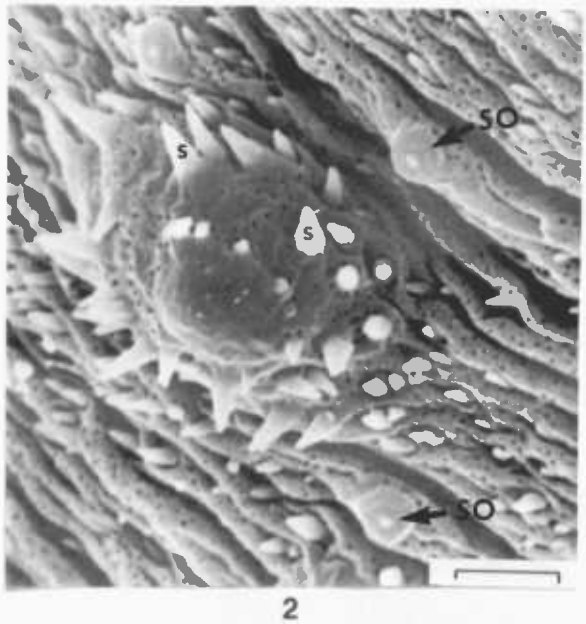
8.5 Dorsal surface of the posterior end of the male showing the tapering and reduction in the number of tubercles towards the posterior tip. Scale = 200µm

8.6 Dorsal surface of the posterior end of the female. Note that it tapers and is covered with spines. Scale = 20µm

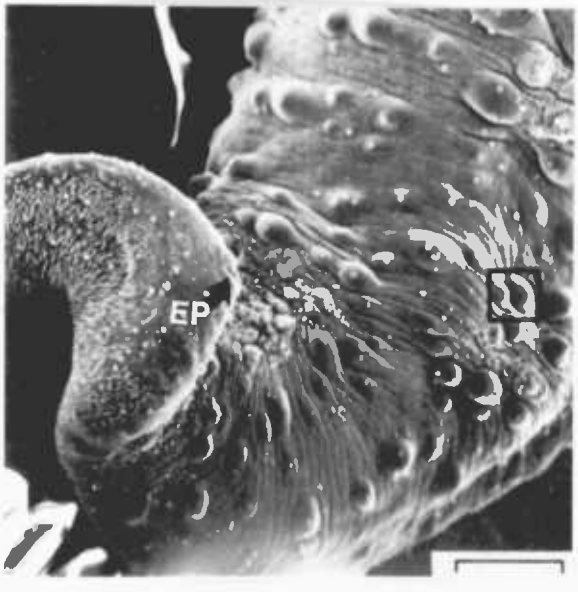
PLATE 8



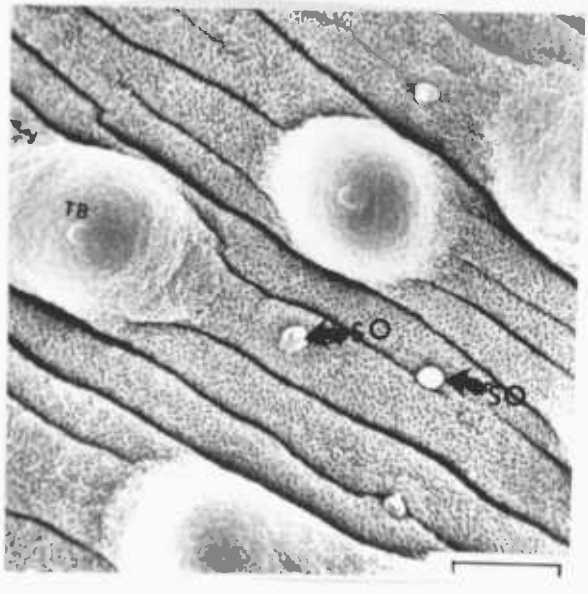
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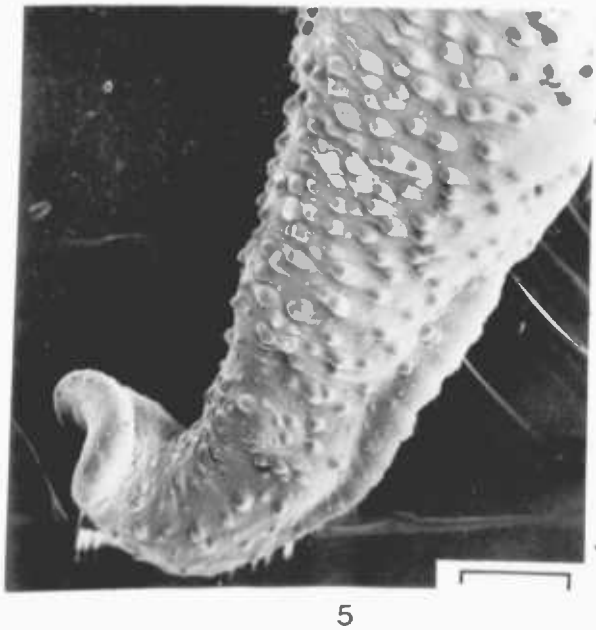
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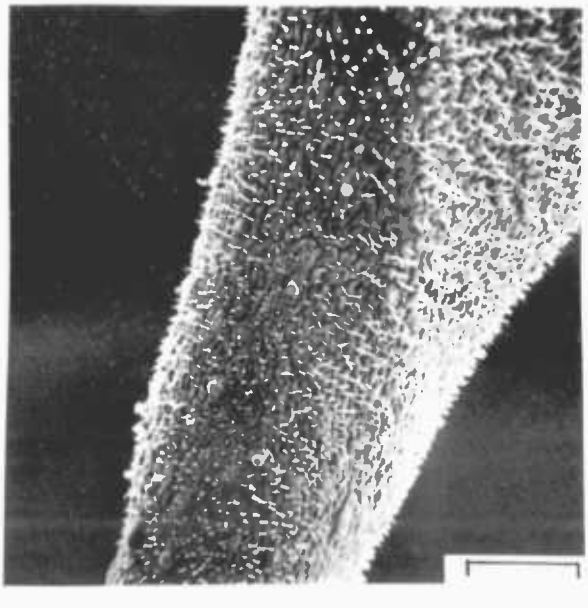
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PLATE 9

9.1 Dorsal surface near midbody of a mature female. Scale = 10μm

9.2 Enlargement of the square area on Plate 9.1 showing the pitted surface with sensory organelles (arrow). Scale = 4μm

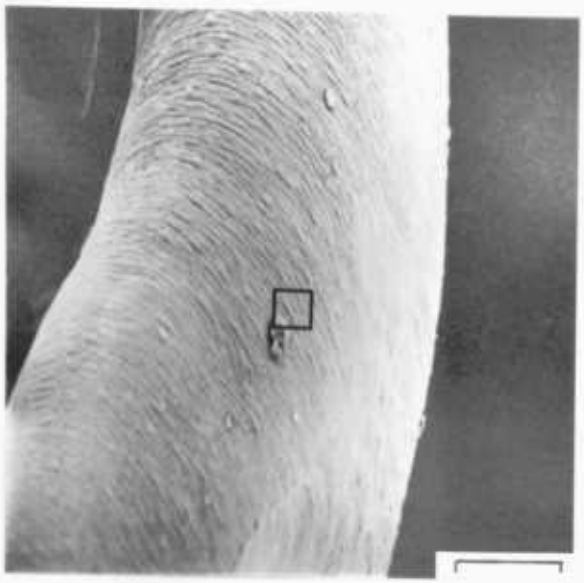
9.3 Ventral surface near midbody of a mature female. Scale = 10μm

9.4 Enlargement of the square area on Plate 9.3 showing pits but no spines. Scale = 4μm

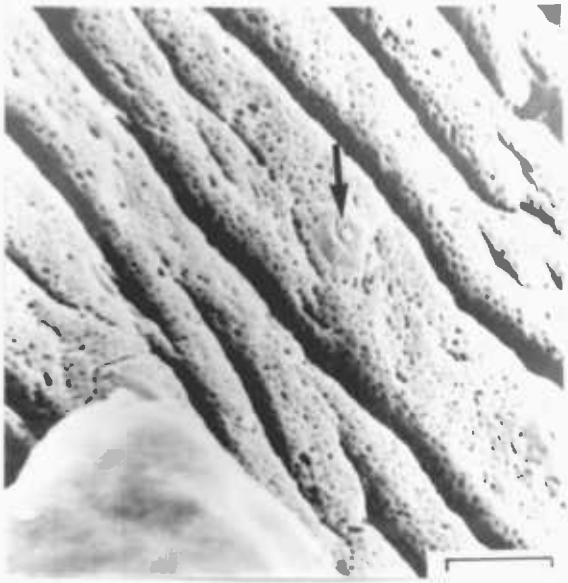
9.5 Posterior tip of mature female showing spines. Note the absence of spines around the excretory pore (EP). Scale = 10μm

9.6 Enlargement of the square area on Plate 9.5. Note the deep socketed spines and a sensory organelle (arrow). Scale = 4μm

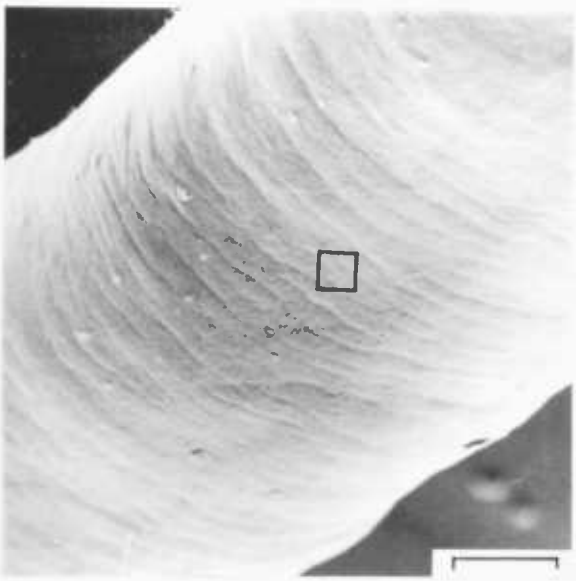
PLATE 9



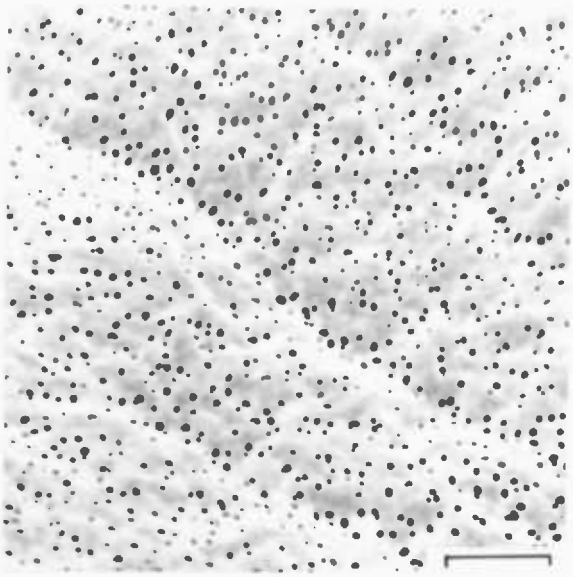
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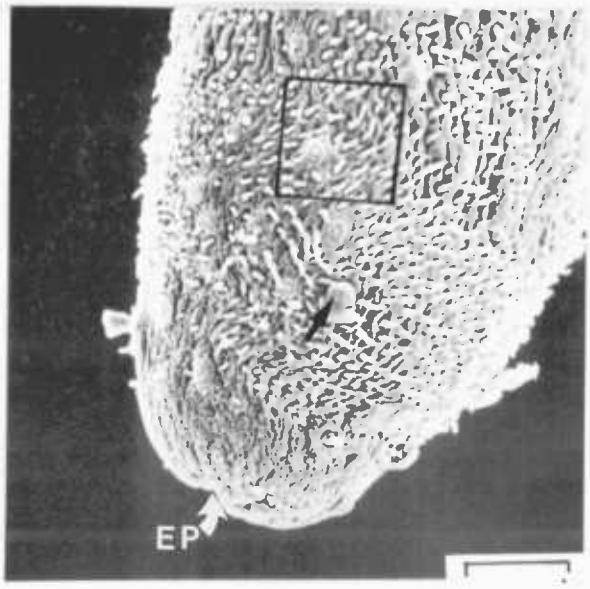
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PLATE 10

10.1 Dorsal surface of the anterior half of a 15 day old worm. Note the presence of numerous sensory organelles (arrows).

Scale = 4μm

10.2 Dorsal surface of the posterior half of a 15 day old worm. Note the presence of few sensory organelles (arrows).

Scale = 4μm

10.3 Ventral view of a 25 day old male worm showing the oral sucker (OS), the acetabulum (AC), and the anterior part of the gynaecophoric canal (gc).

Scale = 100μm

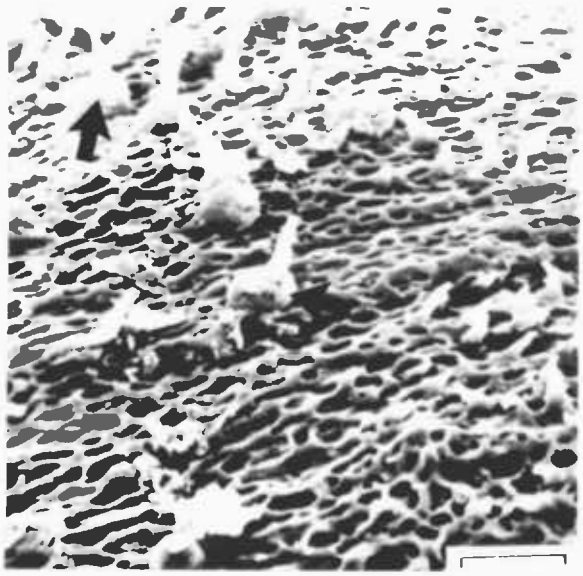
10.4 Enlargement of the square area on the oral sucker in Plate 10.3 showing the nature of the spines and the sensory organelles (SO).

Scale = 4μm

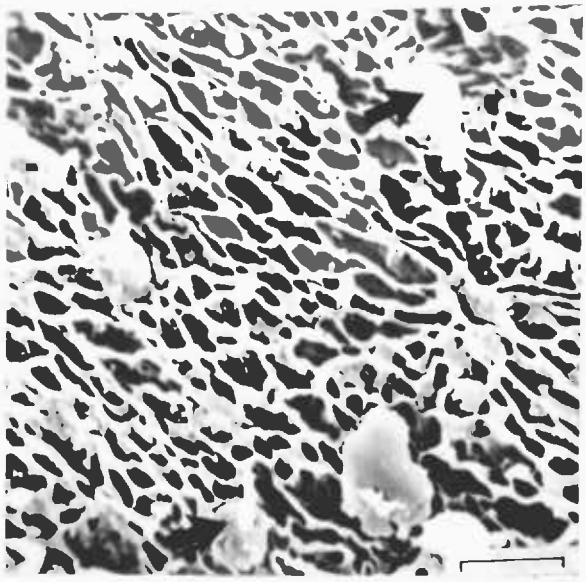
10.5 Enlargement of the square area of Plate 10.3 between the oral sucker and the acetabulum. Note the flattening of the surface and the presence of sensory organelles (SO) and small spines (s). Scale = 4μm

10.6 Enlargement of the square area of Plate 10.3 on the acetabulum showing the nature of the spines. Scale = 4μm

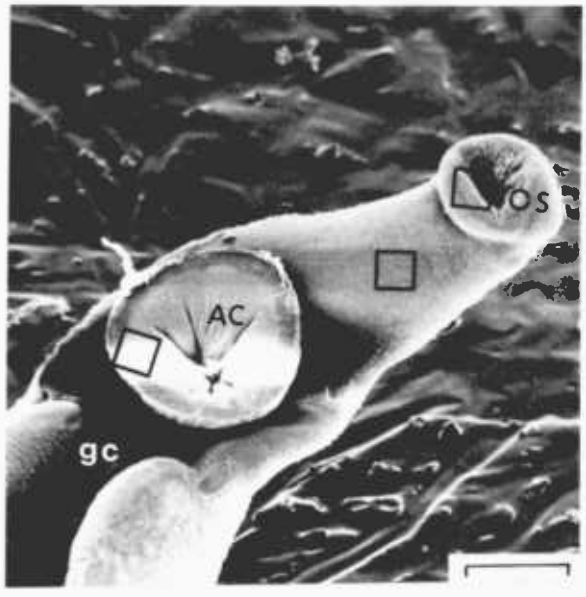
PLATE 10



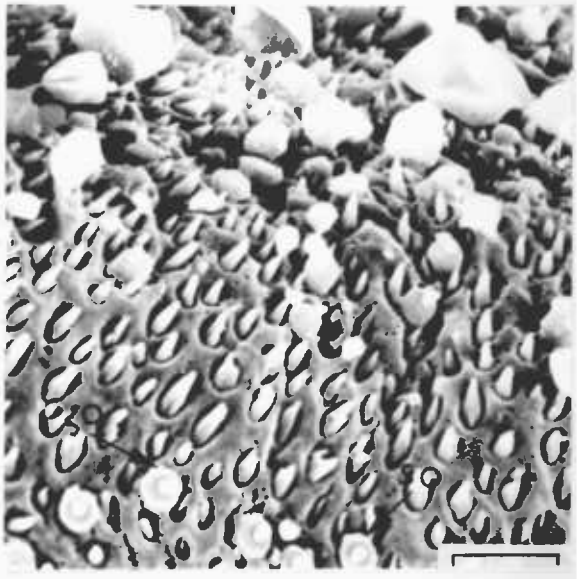
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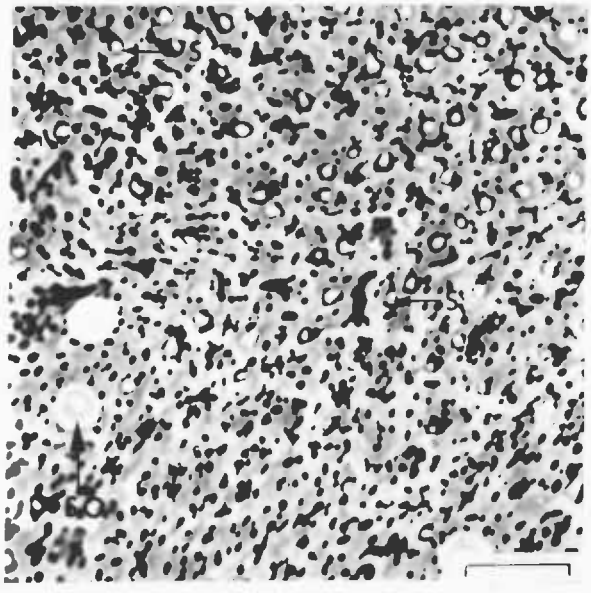
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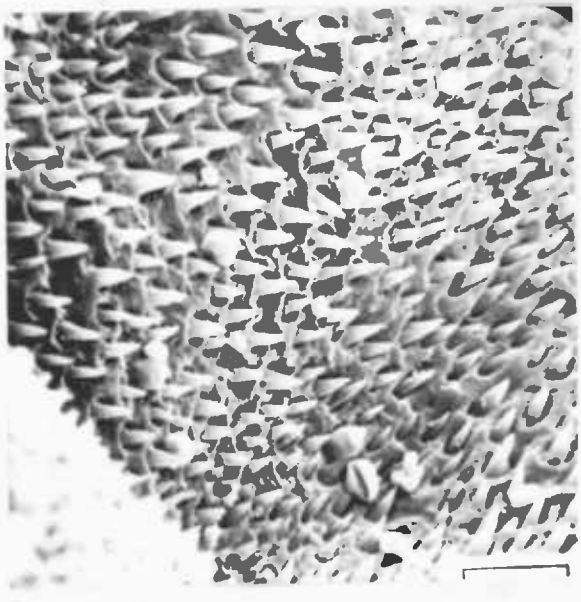
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PLATE 11

11.1 Dorsoventral surface of a 25 day old male worm at the level of the midbody showing a well-developed gynaecophoric fold (gf) and the presence of tubercles (arrows).
Scale = 40µm

11.2 Enlargement of the square area (1) on Plate 11.1, on the dorsal surface, showing the pronounced tegumental folds (tf) and a single tubercle (TB).
Scale = 4µm

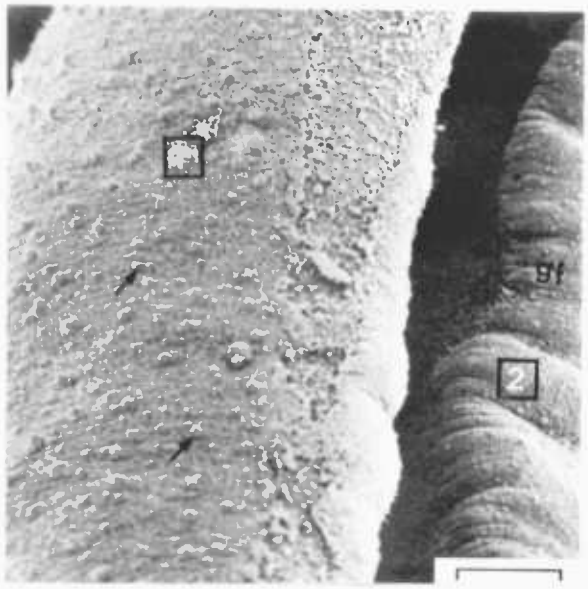
11.3 Enlargement of the square area (2) on Plate 11.1, on the gynaecophoric fold. Arrows show tubercles at different stages of development. Scale = 4µm

11.4 Posterior end of a 25 day old male worm showing the gynaecophoric canal.
Scale = 4µm

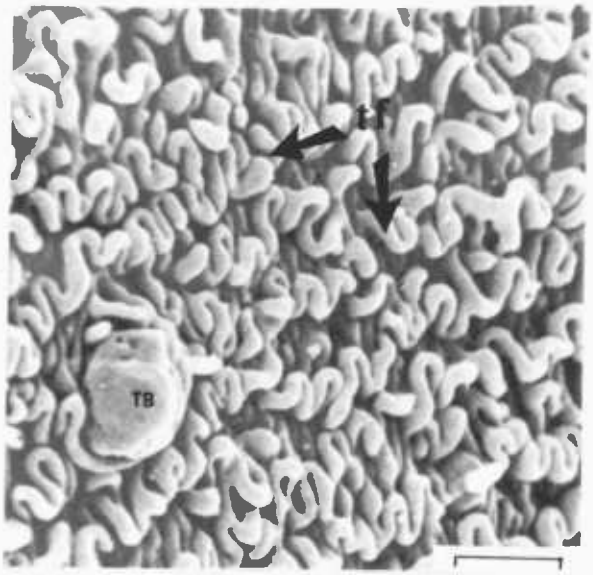
11.5 Enlargement of the square area (1) on Plate 11.4 showing the less pronounced tegumental folds, tubercles (TB) and two sensory organelles (arrows).
Scale = 4µm

11.6 Enlargement of the square area (2) on Plate 11.4 showing the compressed tegumental folds in the gynaecophoric canal with obscured spines (arrows). Scale = 4µm

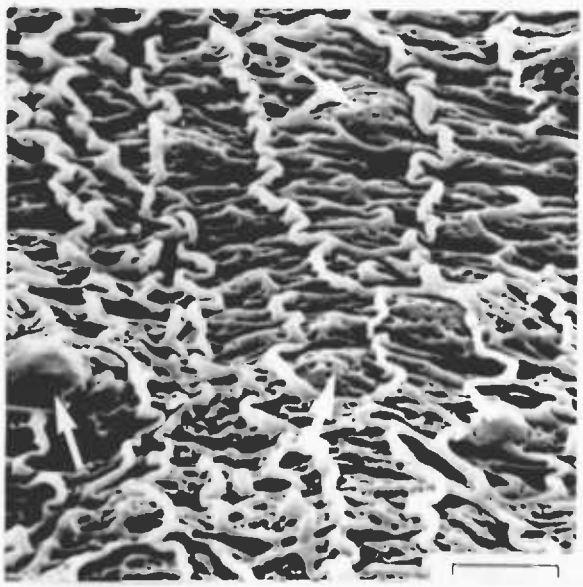
PLATE 11



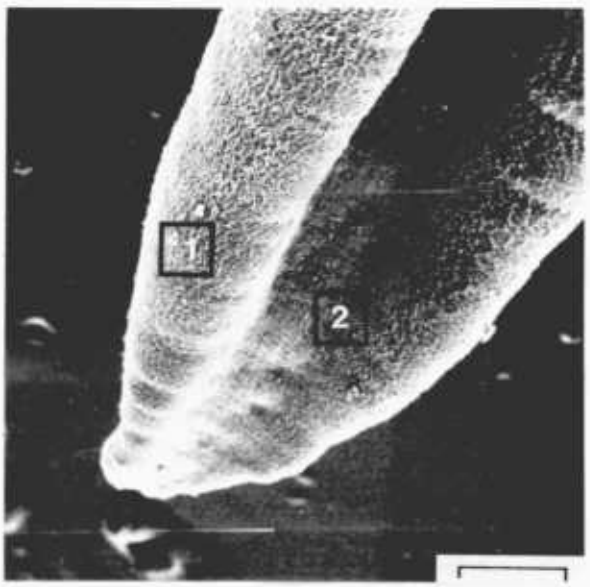
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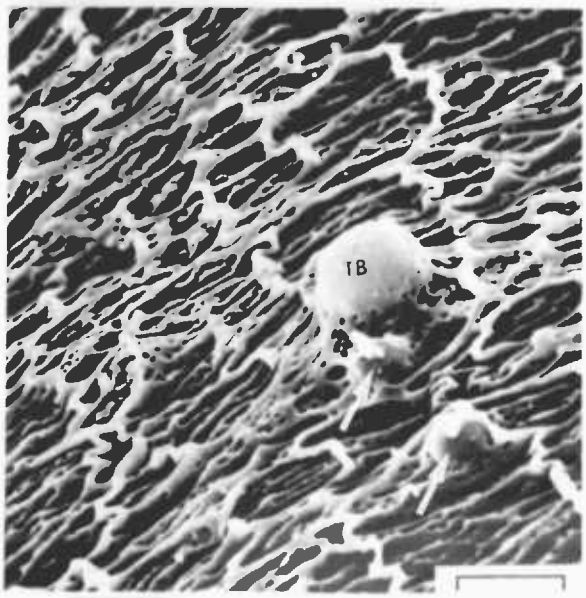
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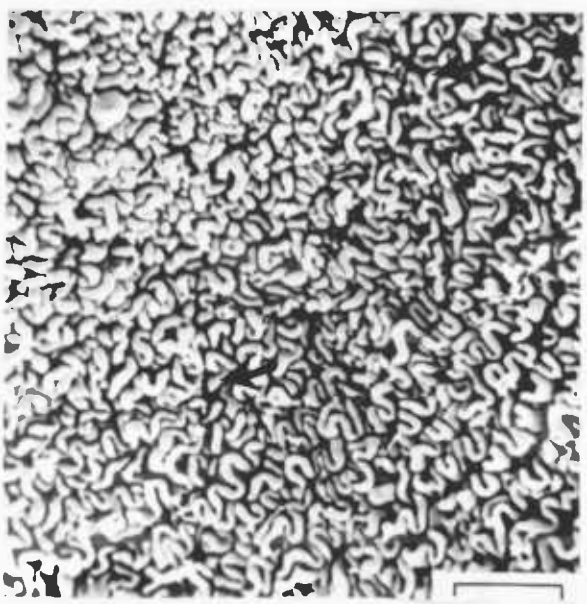
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PLATE 12

12.1 Ventral view of the area between the oral sucker and the acetabulum of a 25 day old female worm. Note the flattening of the surface with projecting spines (arrows).
Scale = 4µm

12.2 Ventral view of the posterior end of a 25 day old female worm. Note the presence of spines only towards the lower end.
Scale = 4µm

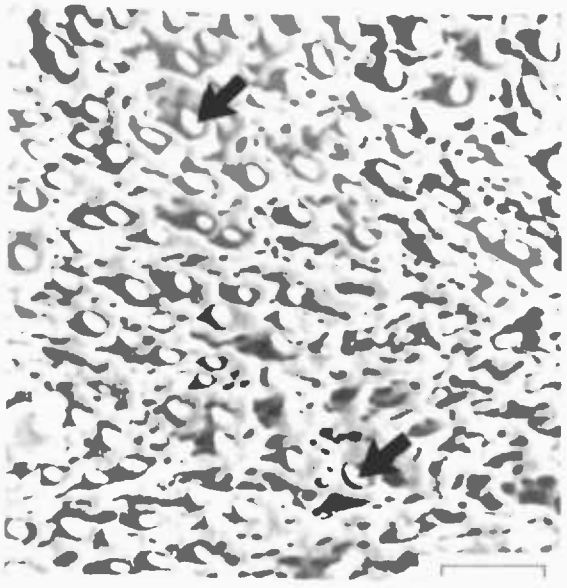
12.3 Dorsal surface of midbody of a 25 day old female worm showing the flattening of the tegumental fold. The surface lacks spines but has sensory organelles (SO).
Scale = 4µm

12.4 Ventral surface of midbody of a 25 day old female worm showing the flattening of the tegumental fold. The surface lacks spines but has sensory organelles (SO).
Scale = 4µm

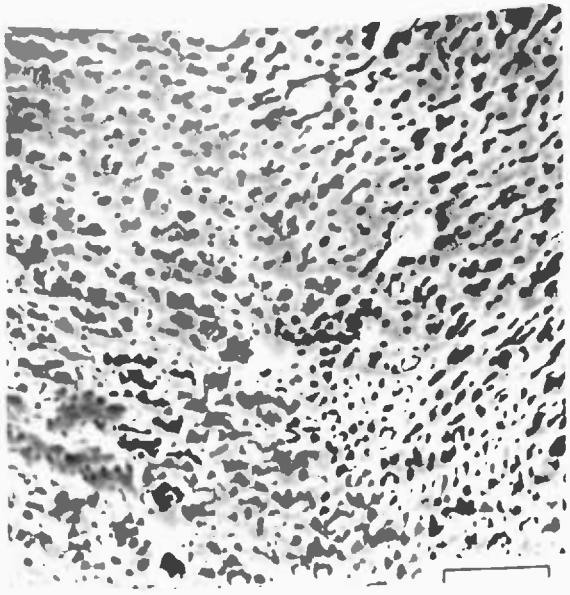
12.5 Dorsal surface of midbody of a 35 day old female worm showing a completely flattened and pitted surface with sensory organelles (SO).
Scale = 4µm

12.6 Dorsal surface of a 35 day old male worm showing the prominent tubercles (TB), sensory organelles (SO) and the ridged surface.
Scale = 40µm

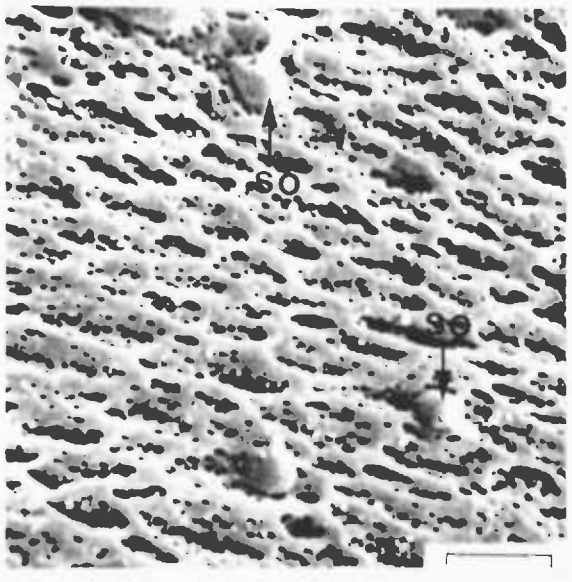
PLATE 12



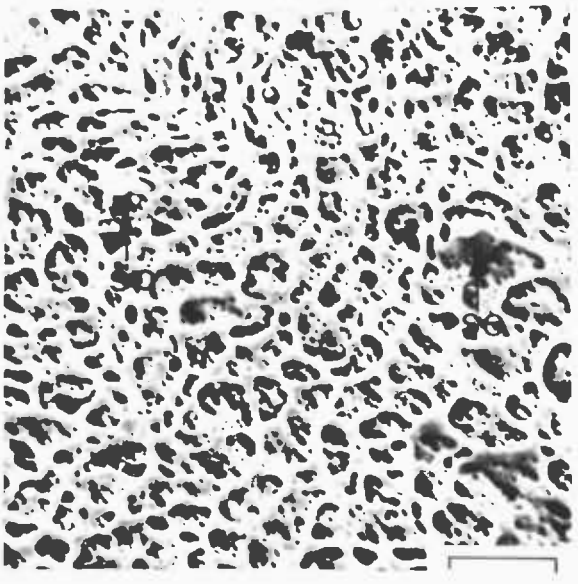
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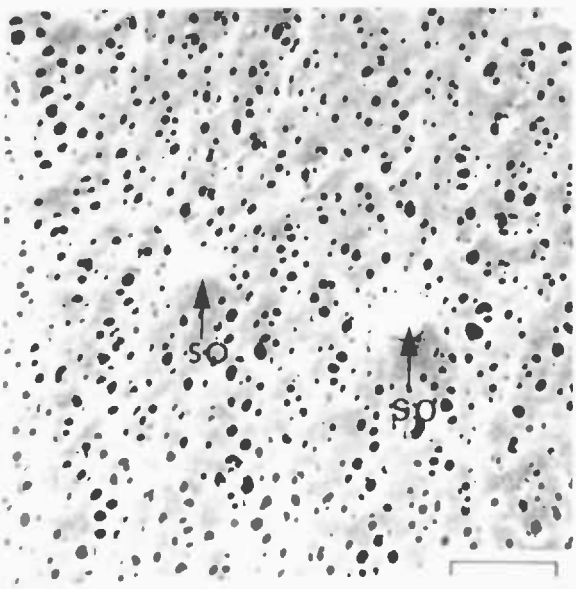
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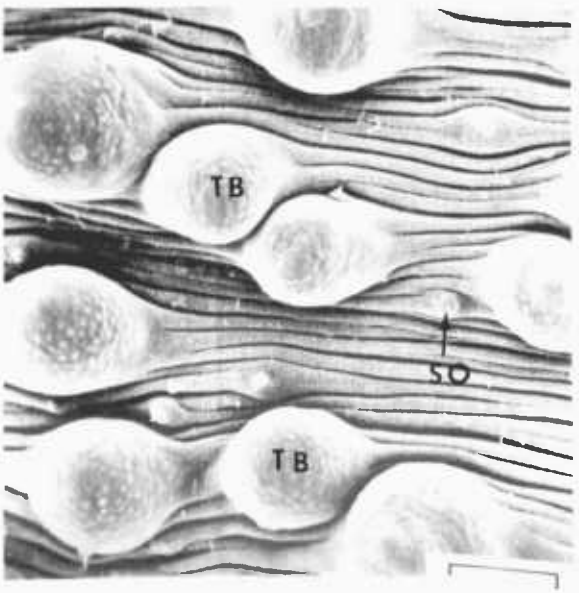
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PLATE 13

13.1 S. margrebowiei egg showing the rudimentary terminal spine (arrow).

Scale = 20µm

13.2 S. margrebowiei egg without a spine.

Scale = 20µm

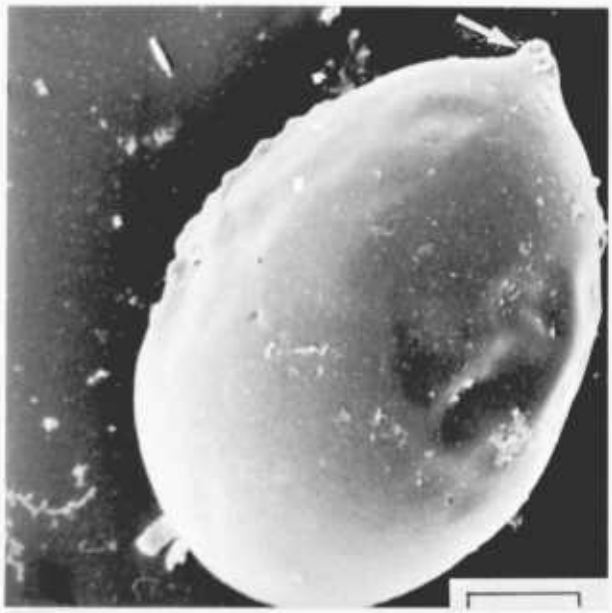
13.3 High magnification of the area around the terminal spine.

Scale = 4µm

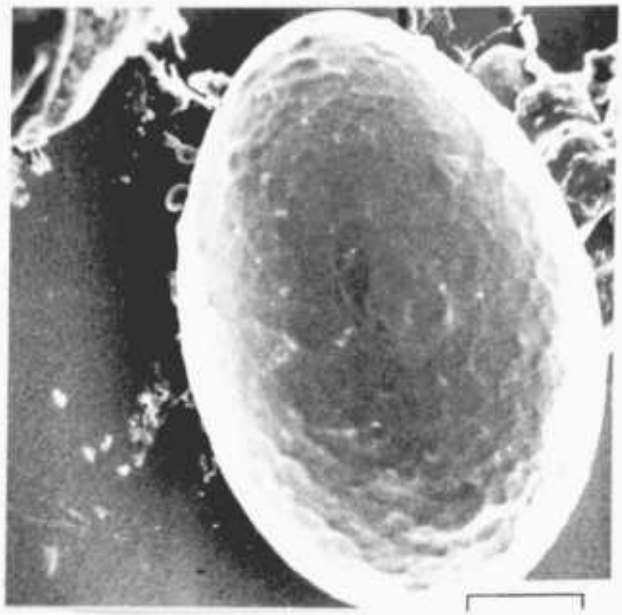
13.4 High magnification of the egg surface showing the microspines which cover it completely.

Scale = 2µm

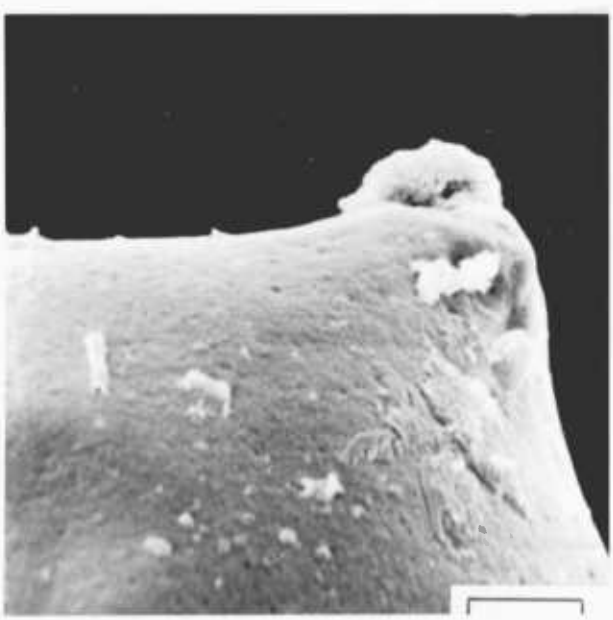
PLATE 13



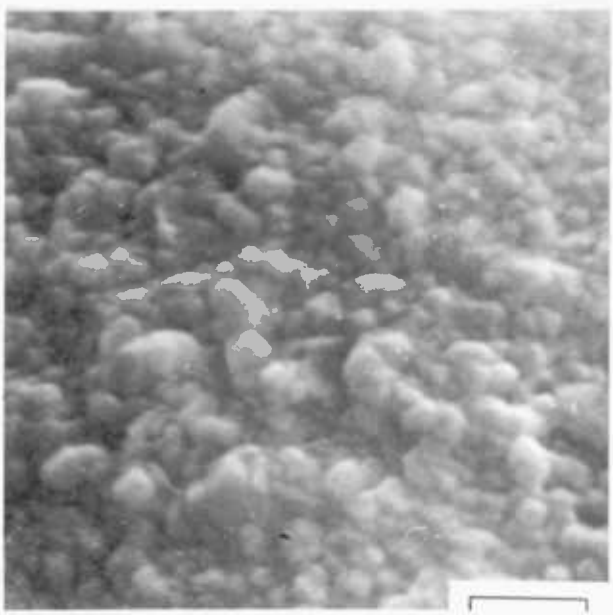
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5 HISTOPATHOLOGICAL RESULTS

5.1 MACROSCOPIC OBSERVATIONS

Macroscopic examination of organs in unisexually infected hamsters and mice during the course of the infection showed no marked differences from those of uninfected control animals. Gross examination of organs in bisexually infected hamsters, mice and gerbils however, showed marked differences by day 50 p.i. These gross pathological changes are outlined in Table 5.1. The following organs were examined : lungs, spleen, liver and intestine.

5.1.1 LUNGS

The gross morphology of the lungs appeared normal in hamsters and gerbils throughout the course of the infection. In mice, the lungs appeared normal until day 70 p.i. when small lesions were observed in the lungs.

5.1.2 SPLEEN

The spleen appeared normal in all the three host species at the early stages of the infection. Slight changes were observed in the spleen of mice and gerbils by day 30 p.i. and this included slight enlargement and

change in colour. The organ had changed from the bright red observed in the uninfected control animals to a dull maroon colour. Further enlargement of the spleen occurred in mice and gerbils with increase in the duration of the infection but this was more pronounced in mice. The size of the spleen in mice with 70 day old infection was about three times those in the control animals. The spleen of mice and gerbils were enlarged in proportion to the duration of the infection. The spleen of hamsters showed no increase in size and no change in colour during the course of the infection.

5.1.3 LIVER

The liver was slightly enlarged in all three hosts by day 40 p.i. Changes in colour and texture were apparent in all the hosts as the infection progressed. This progressive change in texture and colour was more prominent in mice where the liver was characterized by hardening and presence of numerous small greyish spots (lesions) on both surfaces and within the substance of the organ (Plate 14.2). Examination of these spots in squash preparations revealed tissue reaction around deposited eggs. In gerbils, similar but fewer scattered lesions were observed. In the hamsters, hardening and colour change were slight but the surface was extremely rough at later stages of the infection (Plate 15.2).

The liver changed from a pink tan to a dark brown colour by day 70 p.i. in hamsters and mice.

5.1.4 INTESTINE

Grossly visible inflammatory reactions to groups of deposited eggs were observed in the colon of the three hosts 40 days p.i. and were generally characterized by beaded swellings on the surface. More extensive lesions occurred by day 60 p.i. in mice and hamsters (Plates 14.1, 15.1). A few lesions were observed on the ileum in hamsters but these were smaller than those on the colon (Plate 15.2). The lesions on the colon in gerbils were not as extensive as those in mice and hamsters with the same age of infection.

Diarrhoea, blood in the faeces and severe loss in weight were common in gerbils and hamsters between 50 and 60 days p.i. These were not observed at all in mice throughout the course of the infection. Intensive haemorrhage was evident in the intestine of gerbils.

In hamsters, accumulation of a clear fluid in the peritoneum, the thoracic cavity and the pericardial sac was observed by 50 days p.i. This indicated leakage of fluid into these cavities. Death occurred in hamsters and gerbils between 50 and 60 days p.i., and hamsters

surviving beyond this period retained the infection but regained their weight. Gerbils did not survive beyond this period and their high death rate might be attributed to the extensive haemorrhage of the intestinal lining which caused the whole of the intestine to be filled with blood clots. There was also the possibility of concurrent bacterial or viral enteritis though this was not investigated.

Table 5.1 shows macroscopic observations of various tissues in the three hosts compared to those of uninfected controls at different days p.i. The structures are normal if they were similar to those in the controls. ND = no observation.

	Hamster			Mouse			Gerbil		
	30	50	70+	30	50	70+	30	50	70+
Colour of liver	Normal	Dark brown	Dark brown	Normal	Dark brown	Almost black + grey spots	Normal	Pink tan	ND
Size of liver	Normal	Slightly enlarged	Slightly enlarged	Slightly enlarged	Slightly enlarged	Reduced in size	Normal	Slightly enlarged	ND
Texture of liver	Normal	Hard & rough	Hard & rough	Normal	Hard & rough	Hard & rough	Normal	Normal	ND
Lesions on liver	Absent	Absent	Absent	Absent	Numerous	Numerous	Absent	Few	ND
Size of spleen	Normal	Normal	Normal	Slightly enlarged	Enlarged x2	Enlarged x3	Normal	Enlarged x2	ND
Colour of spleen	Normal	Normal	Normal	Normal	Maroon	Dull maroon	Normal	Maroon	ND
Lesions on lungs	Absent	Absent	Absent	Absent	Absent	Few scattered	Absent	Absent	ND
Lesions on intestine	Absent	Nodules in colon	Nodules in colon & ileum	Absent	Few nodules in colon	Numerous nodules in colon	Absent	Absent	ND

5.2 MICROSCOPIC OBSERVATIONS

The main microscopic changes observed in the various tissues as a result of unisexual and bisexual infections of S. margrebowiei are described in this section. The qualitative differences in the host species response were also noted.

5.2.1 UNISEXUAL INFECTION

Microscopic changes were evident in the liver of hamsters and mice with male and female unisexual infections. The changes observed were mainly periportal infiltration of mononuclear and polymorphonuclear leucocytes. There was no significant difference in the extent of cellular infiltration with increase in the age of infection in hamsters and mice (Plates 16.2, 17.1, & 18.2). There was also no difference in the extent of cellular infiltration in the liver of male and female unisexually infected hosts. Plate 17.1 shows four male worms in a section of a portal venule in hamster with 60 day old infection. Increase in the cellular infiltration was not associated with the increase in the number of worms in the venules. The portal venules were sometimes completely surrounded by periportal infiltration (Plate 16.2). The portal infiltration was made up of mononuclear and polymorphonuclear cells and there was no

liver parenchyma involvement.

The liver of uninfected control hamsters and mice examined at the end of the experiment showed no cellular infiltration in the parenchyma and the periportal connective tissue also had no cellular infiltration. (Plate 16.1). Fibrosis was not observed in the liver of unisexual infections of 180 days duration.

Microscopic examinations of sections of the lungs, spleen and intestine revealed no abnormalities when compared to the uninfected controls. The lungs displayed a regular pattern with the air spaces fully open and there was complete absence of areas of tissue damage.

5.2.2 BISEXUAL INFECTIONS

At the early stages of the infection, before the deposition of eggs in the tissues, no marked histopathological changes were observed in the three hosts. In 30 day old infections, paired worms were observed in liver sections in the three hosts but only slight periportal cellular infiltration of mononuclear and polymorphonuclear leucocytes was noticed. This was similar to the reaction associated with the worms in unisexual infections. The cellular infiltration occurred in both venules with paired worms and venules without worms (Plate

18.1). After the deposition of eggs in the tissues, the microscopic changes observed in various tissues differed greatly in the three hosts. The changes observed in the spleen, lungs, liver and intestine are described below.

5.2.2.1 SPLEEN

Microscopic observation of the spleen revealed a marked transformation in the architecture of the organ in mice and gerbils. Plates 19.1, 19.2 and 20.1 illustrate the complete transformation of the spleen by day 60 p.i. The rapid increase in size is also illustrated in these plates. The transformation involves the primary increase in the red and white pulp (Plate 19.2) at early stages of the infection followed by the subsequent depletion of lymphocytes in the white pulp at later stages (Plate 20.1). Severe depletion of leucocytes of the white pulp was maintained in mice and gerbils throughout the infection and by 60 days p.i. splenic nodules appeared as bare germinal centers surrounded by the red pulp (Plate 20.1).

Fresh tissue crushes revealed the presence of eggs in the spleen at 60 days p.i. These deposited eggs might be from worms which had migrated into branches of the splenic vein at the late stages of the infection. The lesions produced by the deposited eggs were similar to

those observed in the liver of mice. There was an initial cellular infiltration of mononuclear and polymorphonuclear leucocytes which was later followed by fibrosis with the degeneration of tissues around the eggs (Plate 20.2). Eggs were not deposited in the spleen of gerbils and hamsters throughout the course of the infection.

The spleen in the hamster showed only slight depletion of cells in the white pulp at the early stages of the infection until 40 days p.i., after which no other remarkable pathological change was observed.

5.2.2.2 LUNGS

There were no remarkable pathological changes in the lungs of all the three hosts at the early stages of the infection though schistosomula were recovered from the lungs at the early stages during their migration from the skin to the mesenteric-portal vein. Apart from the haematin deposits observed in the lungs at the later stages of the infection, the lungs of hamsters and gerbils appeared normal throughout the course of the infection.

At later stages of the infection in mice, both adult worms and eggs were observed in sections of the lungs. This was observed from day 60 p.i. which coincided with the period of rapid reduction in worm burden in mice.

Paired worms were never observed and only male worms were found. The worms and deposited eggs were surrounded by mononuclear and polymorphonuclear leucocytes (Plates 21.1, 21.2). The lesions were prominent at later stages of the infection and consisted of patchy or confluent areas of polymorphonuclear leucocyte infiltration. By day 70 p.i., degeneration of tissue in such lesions and subsequent formation of fibrosis were observed around eggs and worms (Plates 22.1, 22.2). The deposition of large amounts of haematin was a common feature of lungs in which worms were observed.

5.2.2.3 LIVER

The most striking and consistent histopathological change was observed in the liver of bisexually infected mice. There was a progressive development of tissue response to deposited eggs. Similar egg-tissue relationships have been described by Hsü *et al.* (1970, 1972) for other species of schistosomes in experimental infections. They classified the developmental process of egg-granulomatous lesions into five stages. During this study, their classification was followed for the egg lesions in the liver where more characteristic lesions were observed. The general characteristics of these stages are summarized below :

i) Non-reactive or weakly reactive stage. At this

stage, the egg is immature and there is no obvious inflammatory reaction. Mononuclear cells, neutrophils and eosinophils generally appear around the egg when it reaches the late immature phase.

ii) Exudative stage. Here, the egg is mature and acidophilic cephalic glands in the miracidium are usually distinct. Leucocytes accumulate around the egg, forming a micro-abscess. The predominant leucocytes are usually neutrophils or polymorphonuclear cells, although in some instances the predominant leucocytes could be mononuclear cells.

iii) Exudative-productive stage. Histiocytes and epithelioid cells appear outside the leucocytes, with fibrocytes in the zone of histiocytes. The inner leucocyte zone is generally encroached upon by epithelioid cells and histiocytes. Occasionally, degeneration and liquifaction necrosis of the remaining leucocytes occur.

iv) Productive stage. The leucocytes characterizing the exudative stage have disappeared. The inner zone of the granuloma consists of epithelioid cells, histiocytes and occasionally gaint cells; the middle zone, fibroblast and fibrocytes; and the outer zone, lymphocytes, histiocytes, plasma cells and occasionally eosinophils.

v) Involution stage. The egg lesion is greatly reduced in size. A few collagen fibres are observed surrounding the empty shell.

The tissue reaction to the deposited eggs in the liver varied in the three hosts. Gerbils and hamsters showed only slight tissue response to deposited eggs. The granuloma in each of the three hosts are described below :

1. MOUSE

The progressive development of the lesions associated with deposited eggs in mice are illustrated by Plates 23 and 24. Areas of micro-abscesses were observed around eggs by day 40 p.i. Since the eggs in S. margrebowiei are laid in batches, the lesions in the liver are usually not discrete and might contain a number of eggs though single-egg lesions were also common. The infiltrating cells were mainly mononuclear and polymorphonuclear leucocytes and this is the exudative stage of the tissue response (Plate 23.1). As the infection progressed, the inflammatory processes developed to large lesions and necrosis became massive and abscess formation became widespread (Plate 23.2). This represents the exudative-productive stage of the tissue response. By day 60 p.i., the granuloma showed degeneration of leucocytes around the egg, the development of fibrous tissue in the middle zone and the persistence of leucocytes at the periphery (Plate 24.1). At this stage, the productive stage, gaint cells appeared in fibrous tissue and also some were in close contact with the eggs (Plate 26.2). At later stages of the infection, the granuloma were more

advanced and consisted of degenerating eggs surrounded by a few mononuclear leucocytes and concentrically arranged fibrous tissue (Plate 24.2). The lesions at this stage appeared smaller than at the early stages of the infection and may represent the involutional stage of the granuloma development.

2. HAMSTER

Although a large number of eggs were deposited in the liver of hamsters by day 40 p.i., cellular infiltration of leucocytes was generally less intense than was observed in mice (Plate 25.1). There was no increase in intensity of cellular infiltration with increase in duration of infection. There was therefore no difference between lesions from 50 and 70 day old infections (Plate 25.2). The lesions consisted of loose connective tissue and macrophages were predominant, this gave the lesions their foamy appearance (Plate 26.1). Giant cells were also present but no generalized fibrosis in the liver was observed.

3. GERBIL

In gerbils, few eggs were observed in the liver and there was a moderate infiltration of mononuclear and polymorphonuclear leucocytes around deposited eggs. The granuloma were smaller than those observed in mice (Plate 27.1) and at the later stages of the infection, only partial fibrosis occurred. Giant cells, which in some cases completely surrounded deposited eggs, were observed in

the liver of 60 day old infections. As was observed in mice, the exudative-productive stage was reached in the gerbils, but only slight fibrosis was observed (Plate 27.2). The involutional stage was not reached because gerbils did not survive longer than 60 day p.i.

Although living adult worms in the portal blood vessels did not cause any apparent damage and stimulated only slight tissue reaction, dead worms were associated with more intense reactions in the three hosts. In hamsters and gerbils, dead worms in the portal vessels resulted in increased cellular infiltration of leucocytes but did not result in fibrosis. Acute abscesses were not observed (Plate 17.2). In mice, dead worms in the portal vessels stimulated more intense periportal infiltration that resulted in abscesses which at later stages of the infection resulted in fibrosis (Plates 28.1, 28.2). Distinct scars were found around degenerated worm material at later stages of the infection. Although few worms were seen in sections of the liver in the three hosts, large areas of haematin pigmentation were observed at later stages of the infection.

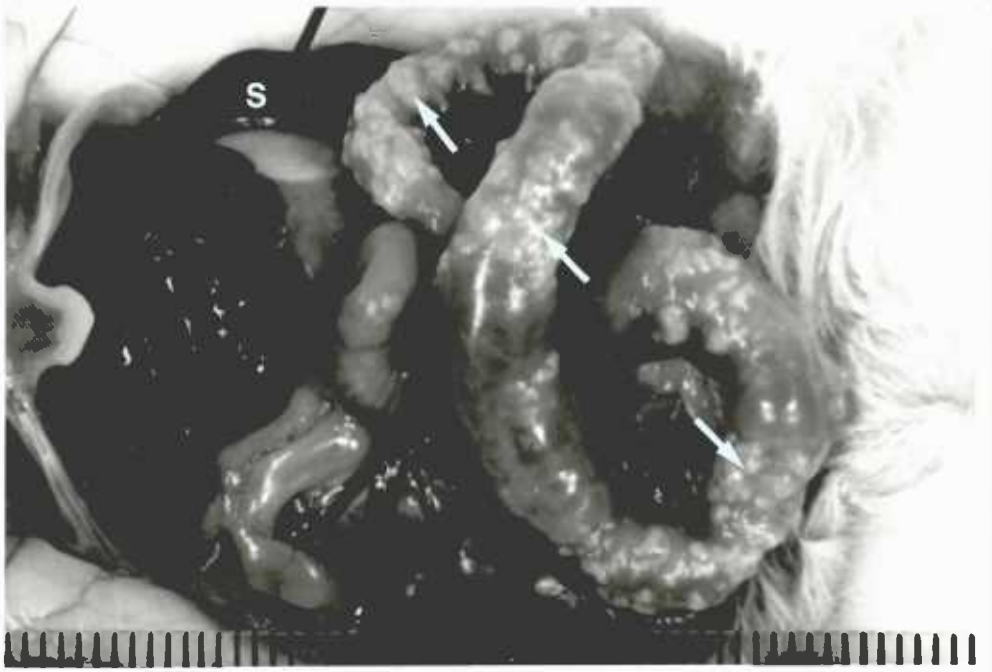
5.2.2.4 INTESTINE

Histopathological changes observed in the intestine seem to be due mainly to deposited eggs as no remarkable changes were observed by day 30 p.i. in bisexual infections and throughout the course of unisexual infections. Egg lesions were observed mainly in the colon in all three hosts by day 40 p.i. Few lesions were seen in the ileum and caecum in these hosts. At this stage of the infection, the eggs were only observed in the mucosa and submucosa and were surrounded by polymorphonuclear leucocytes infiltration which resulted in fibrosis at later stages of the infection (Plate 29.1). Most of the eggs were deposited in the mucosa and submucosa in all three hosts although a few were also deposited in the muscular layer and serosa at the later stages of the infection in hamsters and mice (Plates 30.2, 31.2).

The deposited eggs in the mucosa resulted in thickening and distortion of glands in the three hosts (Plate 30.1). Microscopically, the gerbils and hamsters with blood in their faeces and diarrhoea, had numerous eggs in the mucosa, showed proliferation and distortion of the mucosa and destruction of the lining of the lumen (Plates 29.1, 29.2, 30.1). Eggs were not found outside the mucosa and submucosa in gerbils. The eggs deposited in the muscular layer and serosa resulted in thickening

and nodular masses in hamsters and mice (Plates 30.2, 31.1, 31.2). Cellular infiltration of leucocytes and subsequent fibrosis was common at later stages of the infection.

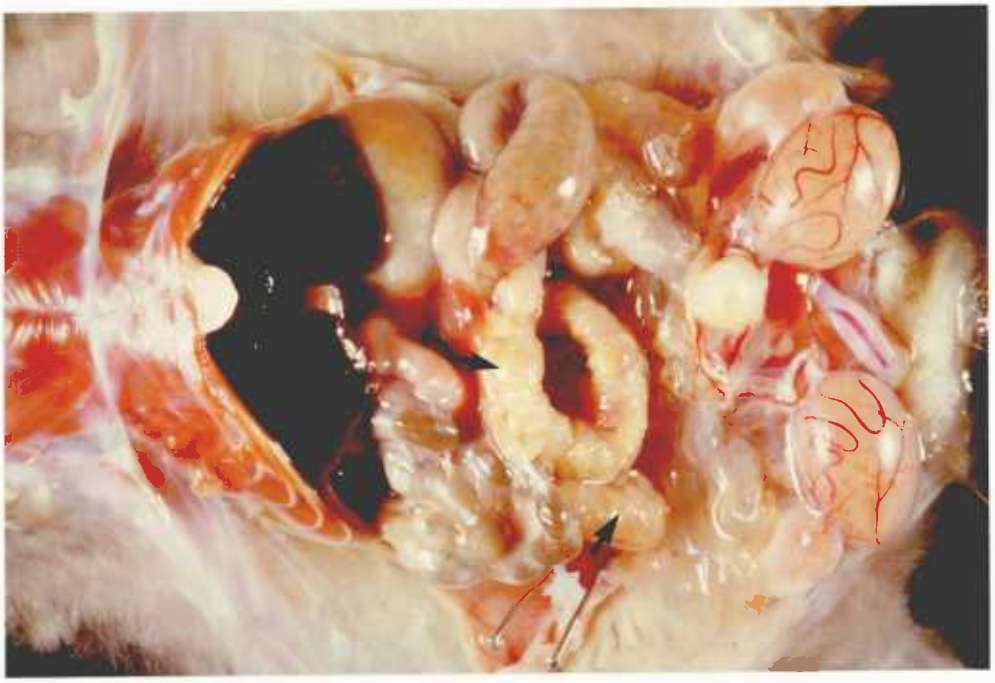
PLATE 14



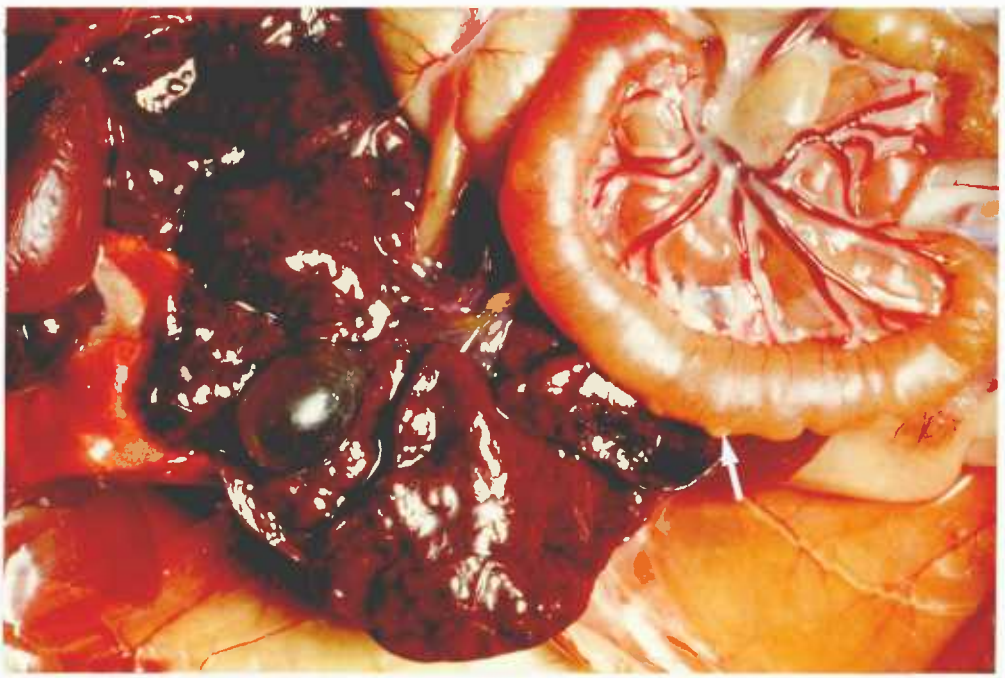
14.1 Mouse, 70 days p.i., bisexual infection. Abdominal cavity showing egg lesions in the colon appearing as nodules (arrows) and the greatly enlarged spleen (S).



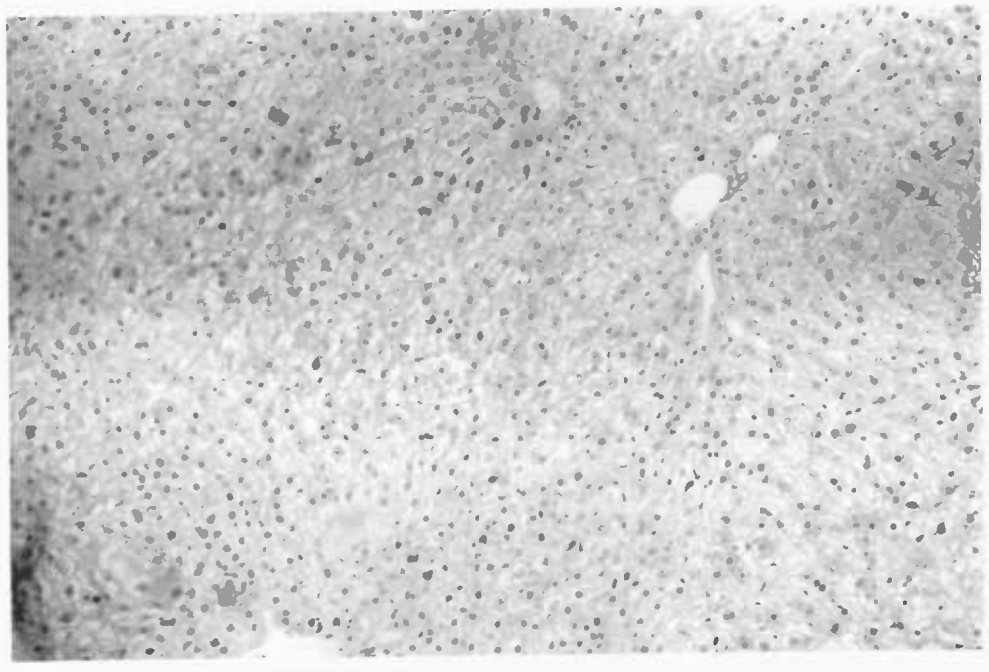
14.2 Mouse, 70 days p.i., bisexual infection. Liver showing numerous lesions as greyish spots in the entire tissue.



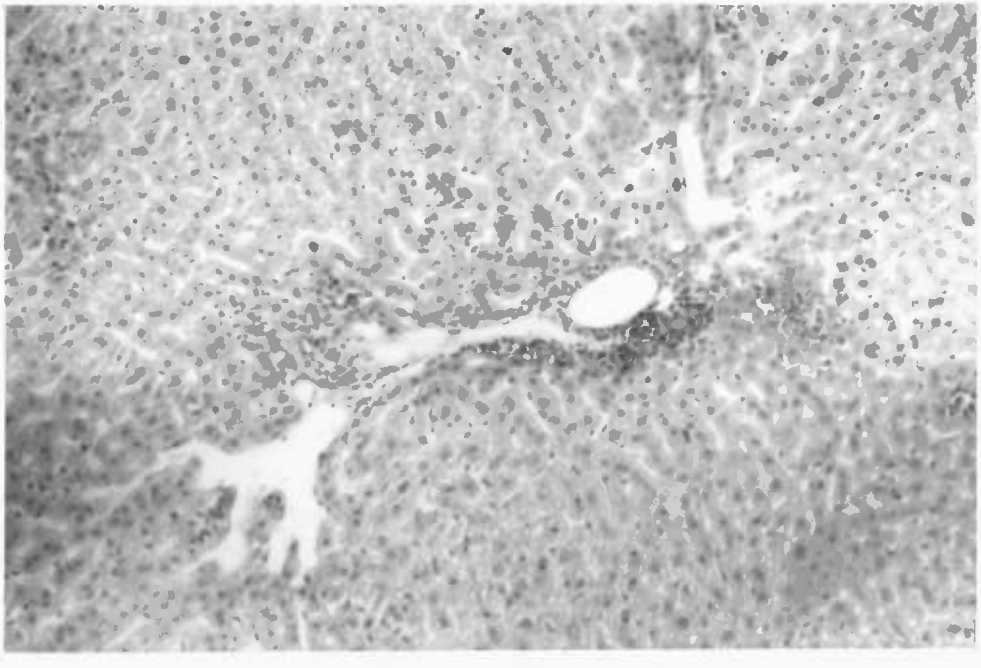
15.1 Hamster, 70 days p.i., bisexual infection. Abdominal cavity showing yellowish egg lesions on the colon (arrows). The hamster had bloody diarrhoea and large amounts of watery effusion in the body cavity.



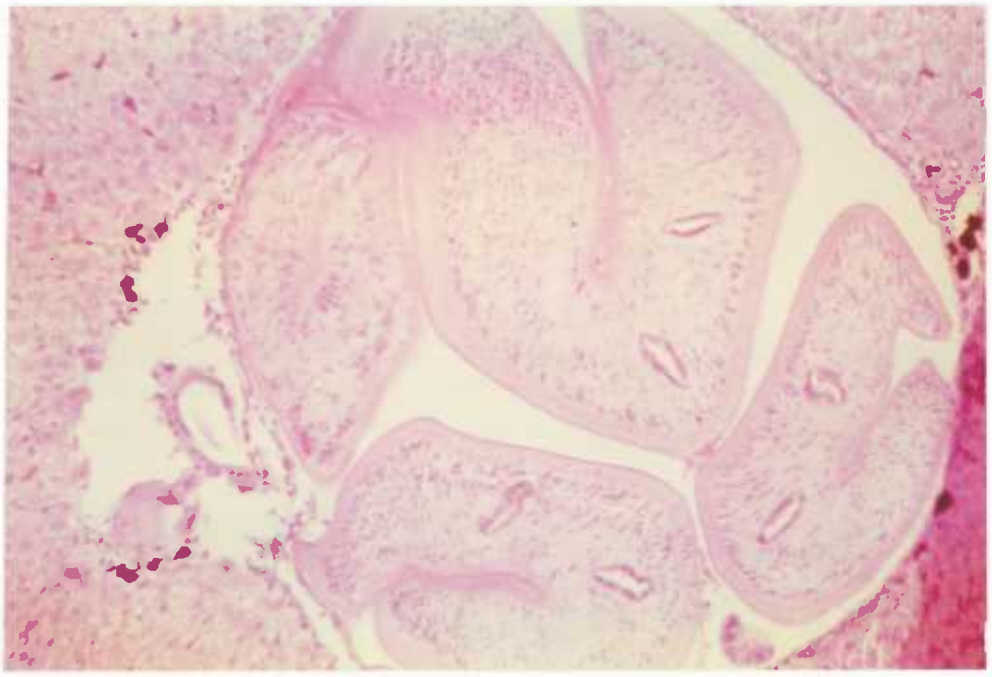
15.2 Hamster, 70 days p.i., bisexual infection. Liver is dark brown in colour, lacks lesions and shows marked rough surface. Few lesions occur on the ileum (arrow).



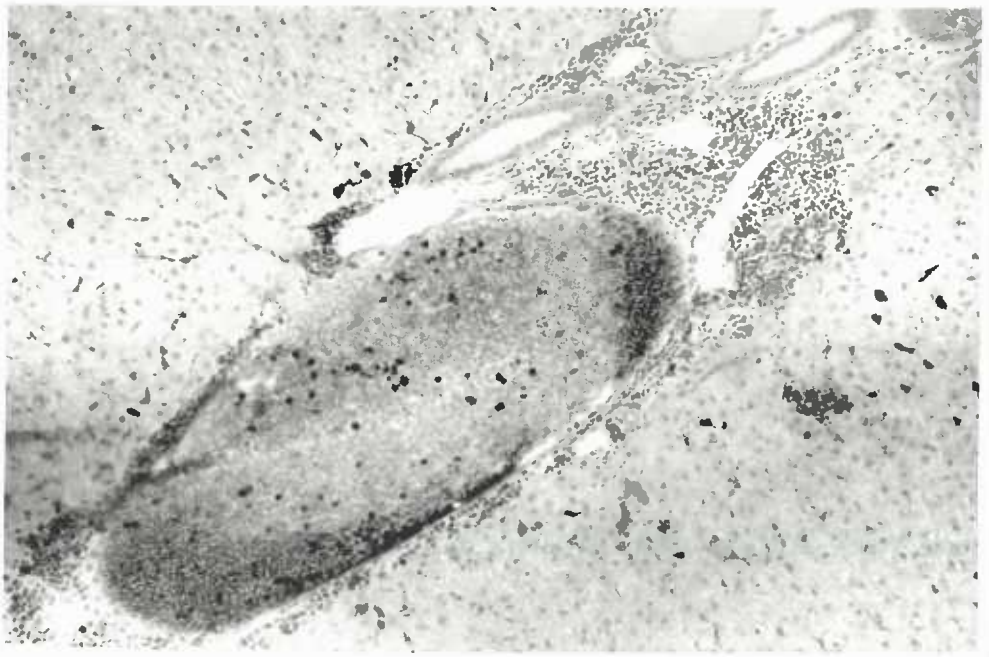
16.1 Hamster, uninfected control, liver examined at the end of the experiment. Note the absence of periportal infiltration of leucocytes. Stains, haematoxylin and eosin (H & E). x150



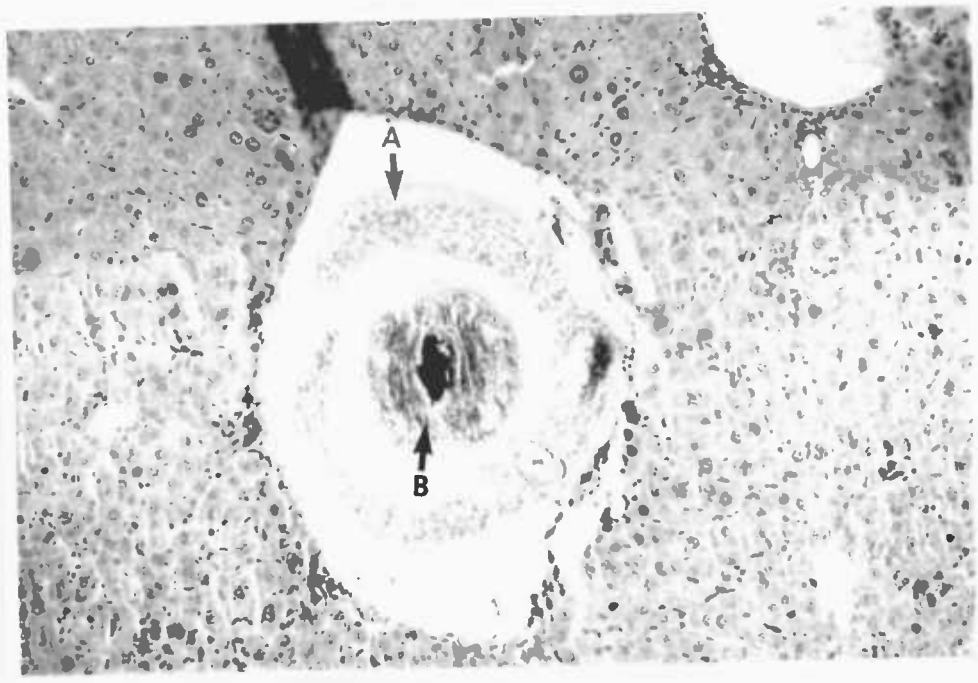
16.2 Hamster, 70 days p.i., liver in female unisexual infection showing slight periportal infiltration of leucocytes. (H & E). x150



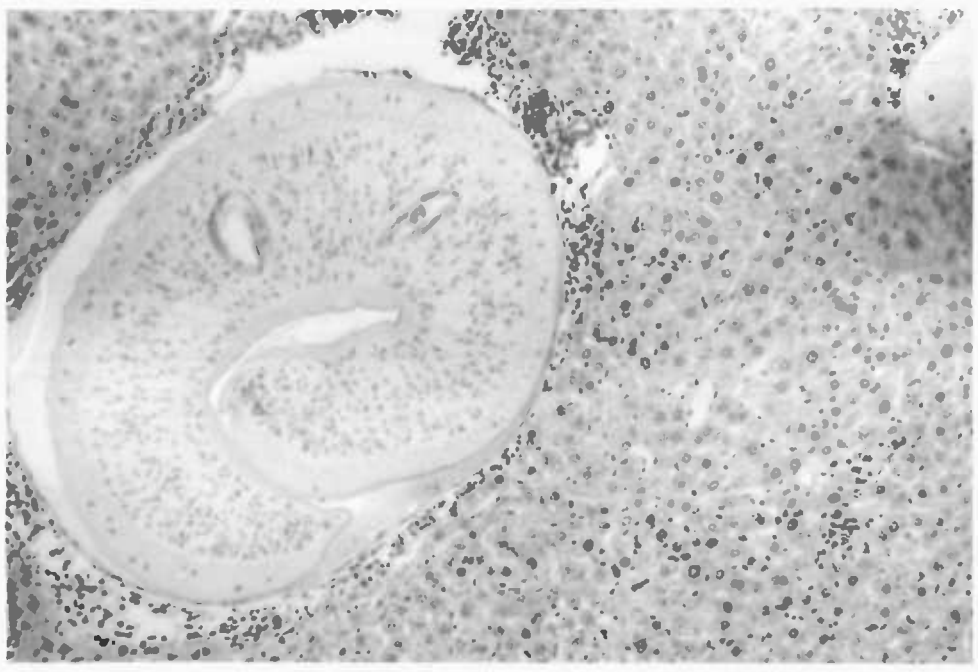
17.1 Hamster, 70 days p.i., liver in male unisexual infection. Note the four worms in the vessel with only slight periportal infiltration of leucocytes. (H & E). x150



17.2 Hamster, 70 days p.i., liver in bisexual infection showing extensive periportal infiltration of leucocytes around dead worm material in the portal vessel. (H & E). x150

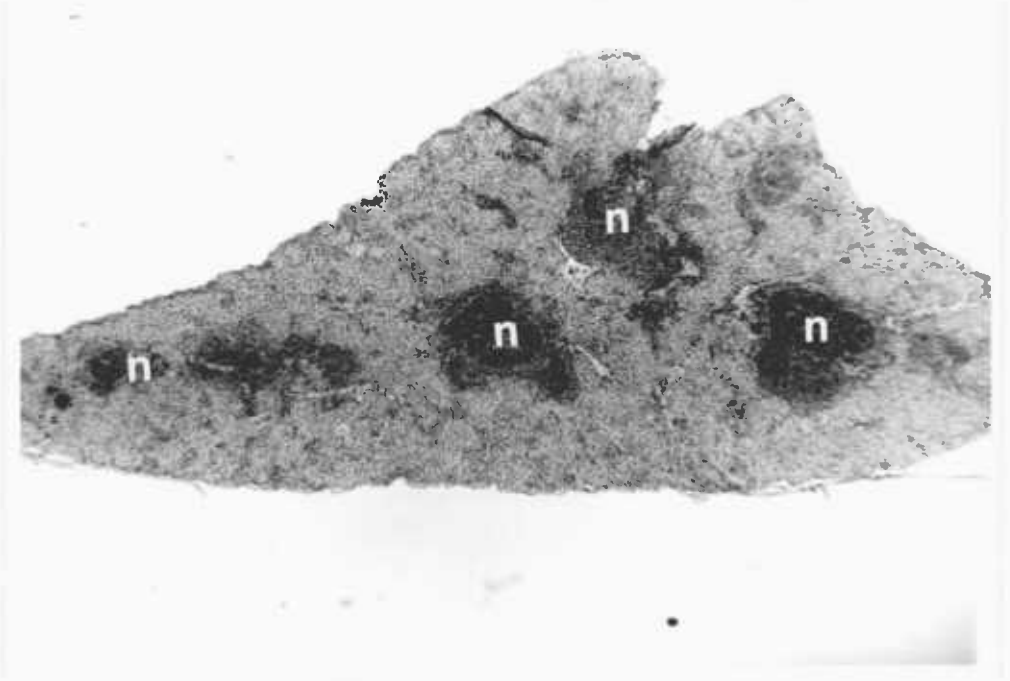


18.1 Mouse, 30 days p.i., liver in bisexual infection showing paired worms and only slight infiltration of leucocytes. Note leucocyte infiltration around the venule in the top right-hand corner without worms. A - male worm, B - female worm. (H & E). x150



18.2 Mouse, 70 days p.i., liver in male unisexual infection showing infiltration of leucocytes around the vessel with the worm and around the vessel without a worm (top right). (H & E). x150

PLATE 19

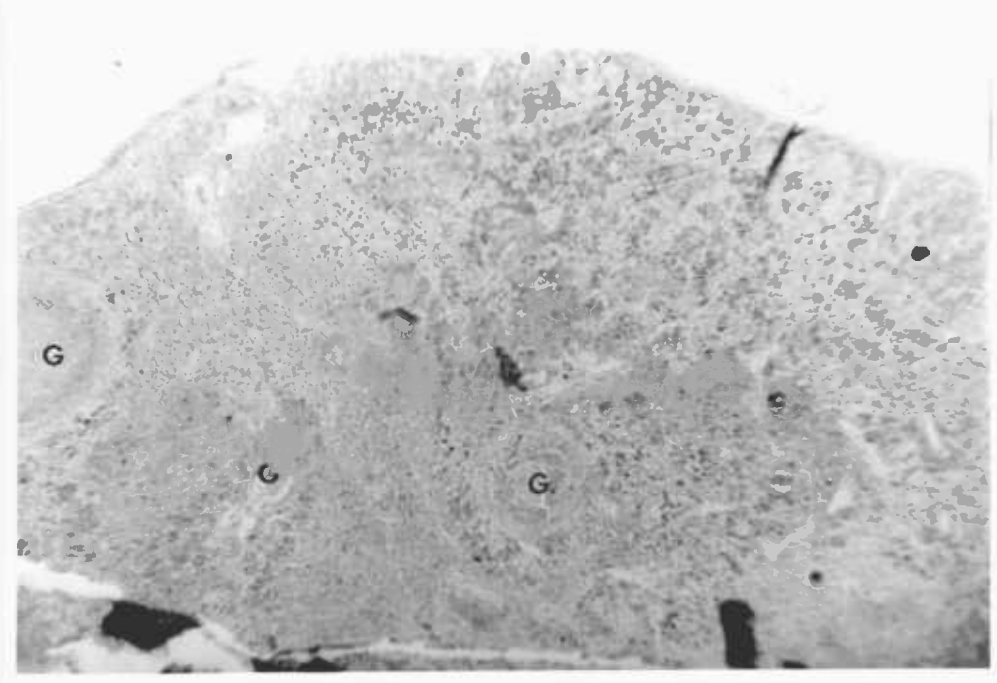


19.1 Mouse, uninfected control. Spleen examined at the end of the experiment, showing distinct lymphatic nodules or white pulp (n). (H & E). x40

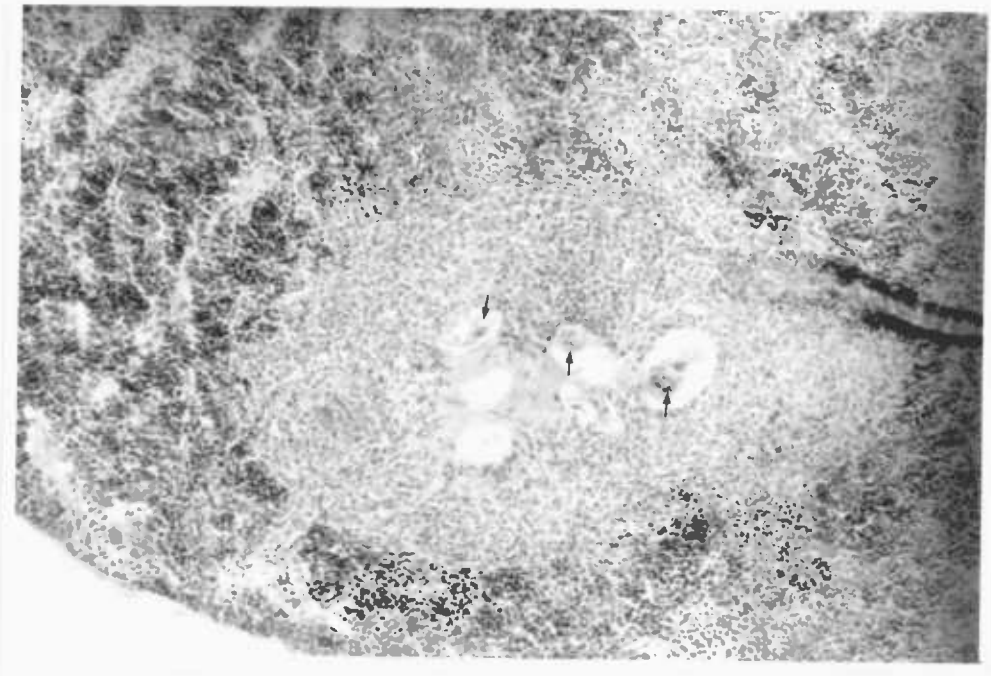


19.2 Mouse, 30 days p.i., bisexual infection. Spleen showing enlargement and depletion of cells in the germinal centre of the lymphatic nodule (n). (H & E). x40

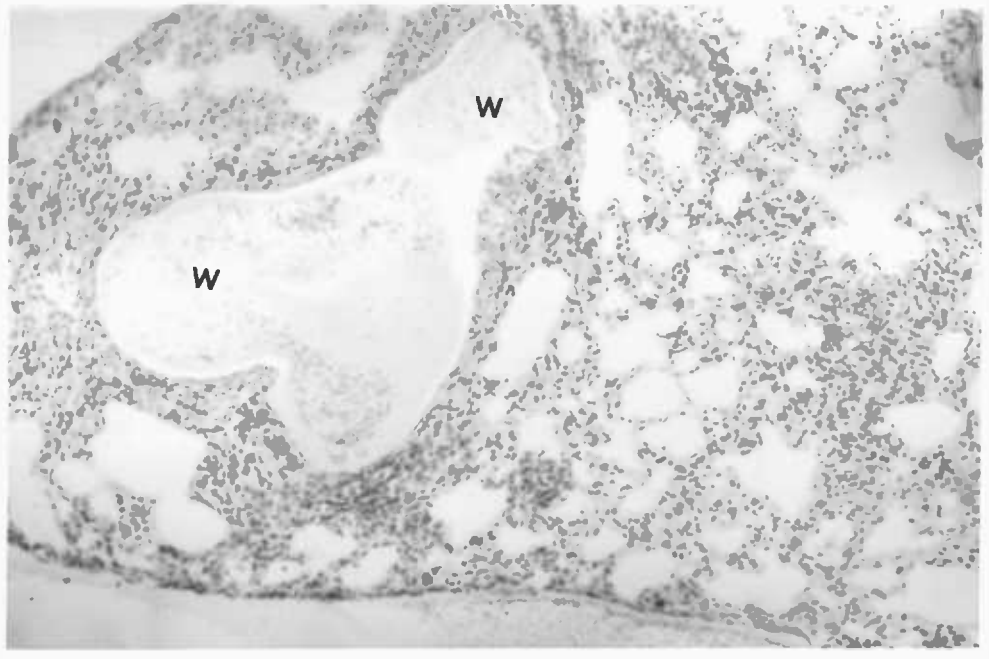
PLATE 20



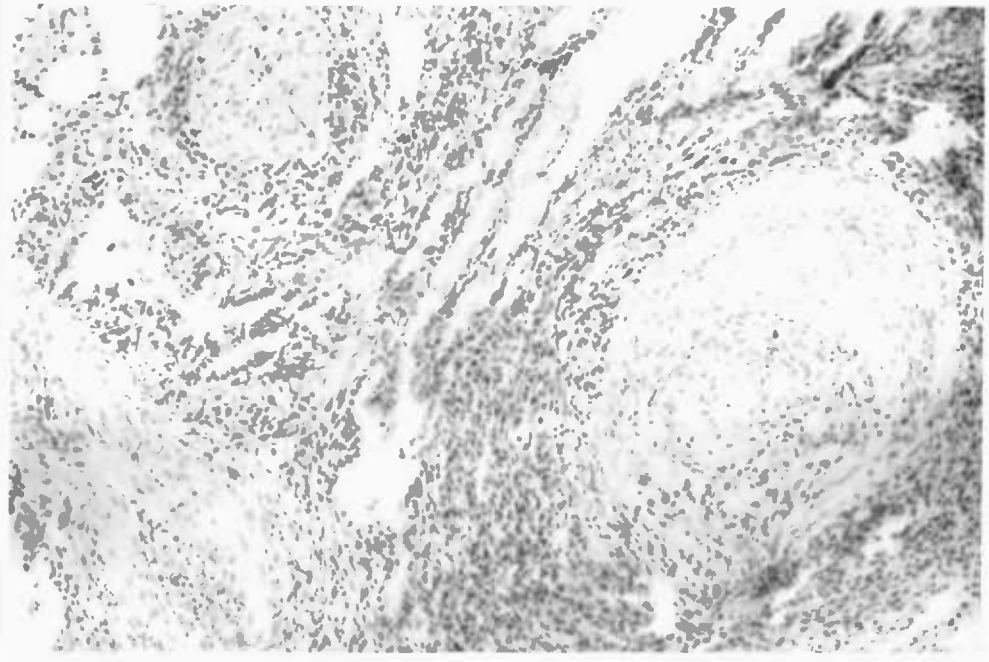
20.1 Mouse, 60 days p.i., bisexual infection. Spleen showing enlargement and bare germinal centres (G). (H & E). x40



20.2 Mouse, 70 days p.i., bisexual infection. Spleen showing granuloma formed around deposited eggs (arrows). The granuloma is made up of leucocytes and fibrous tissue. (H & E). x160



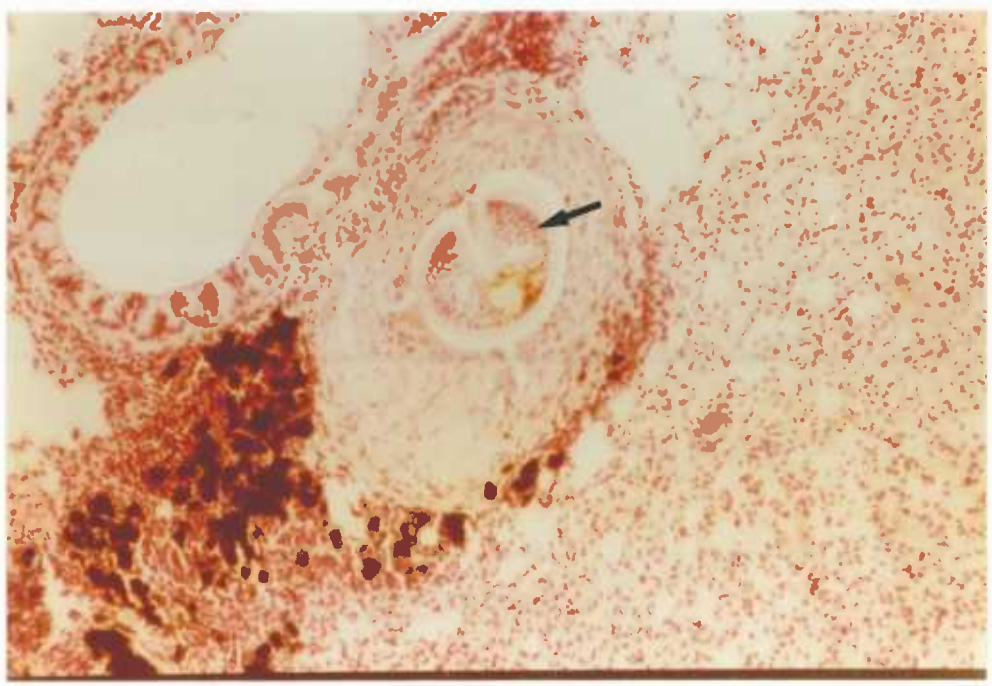
21.1 Mouse, 60 days p.i., bisexual infection. Lung showing two male worms (W) surrounded by inflammatory cells mainly leucocytes, and the collapse of alveolar walls. (H & E). x160



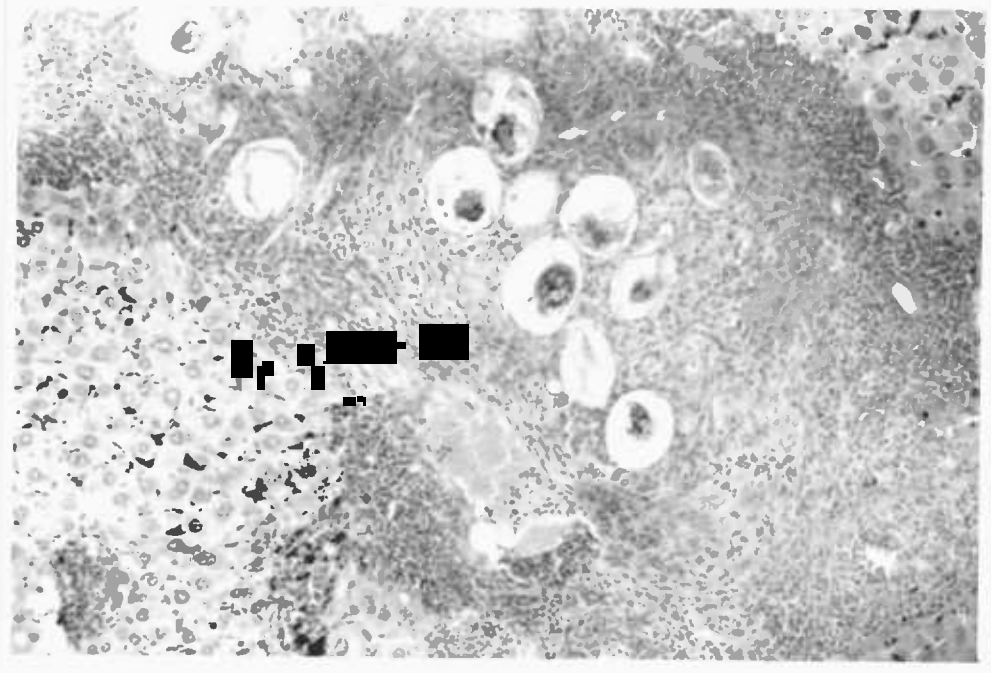
21.2 Mouse, 60 days p.i., bisexual infection. Lung showing extensive inflammatory exudate, and areas of tissue damage attributed to deposited eggs. (H & E). x160



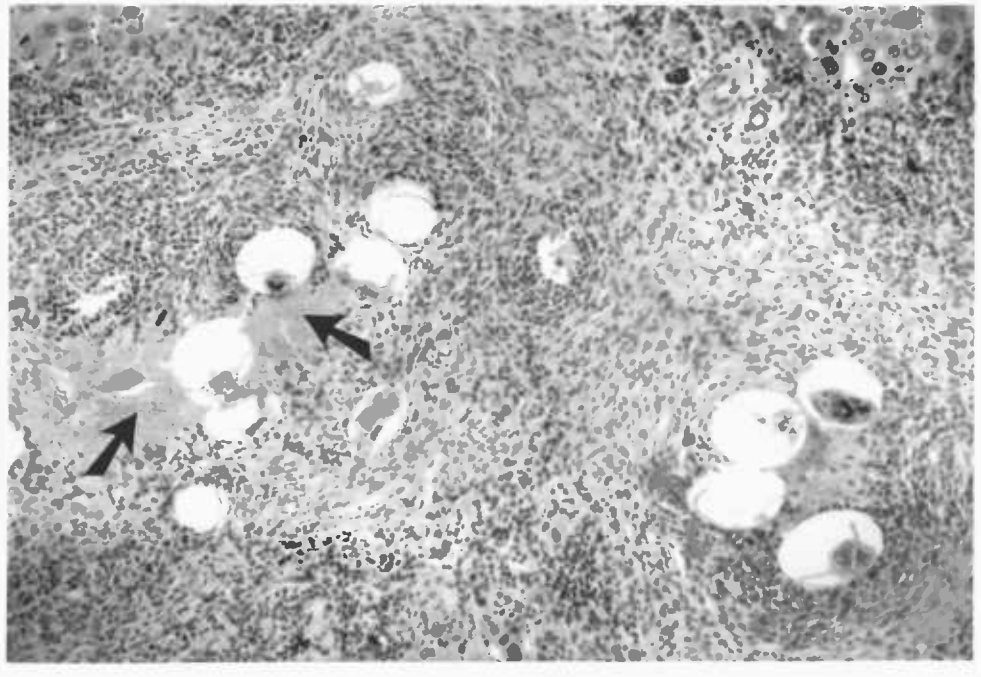
22.1 Mouse, 70 days p.i., bisexual infection. Lung showing granuloma around deposited egg (arrow) with marked fibrosis and inflammation, and areas of tissue damage. (H & E). x160



22.2 Mouse, 70 days p.i., bisexual infection. Lung showing granuloma around a male worm (arrow). Note the worm completely encapsulated and marked fibrosis and inflammation. The black material is deposited haematin. (H & E). x160

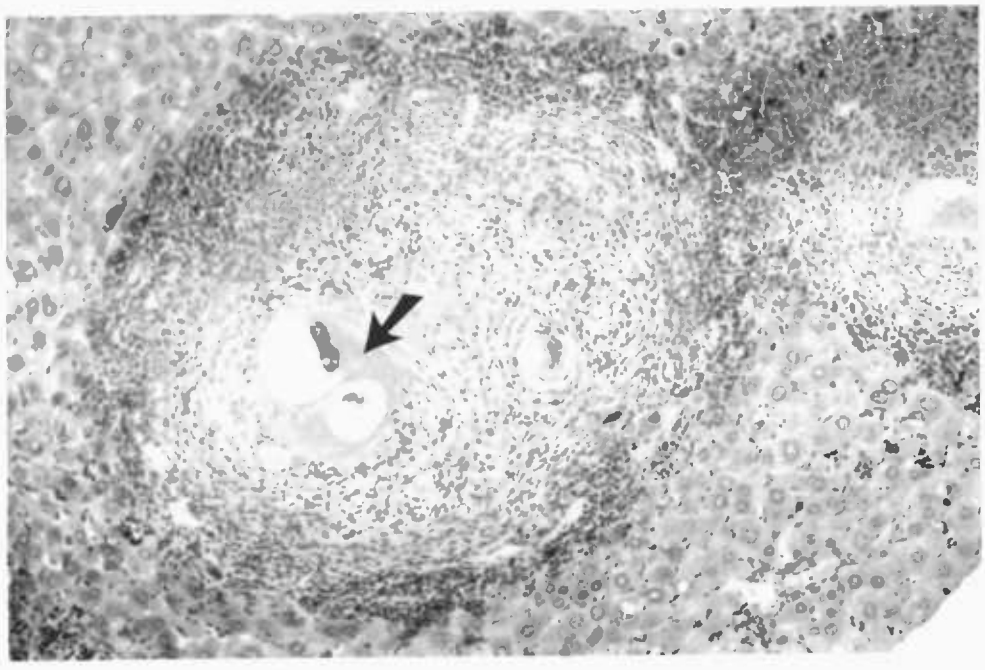


23.1 Mouse, 40 days p.i., bisexual infection. Liver showing micro-abscess around mature eggs, the exudative stage of granuloma formation. (H & E). x160



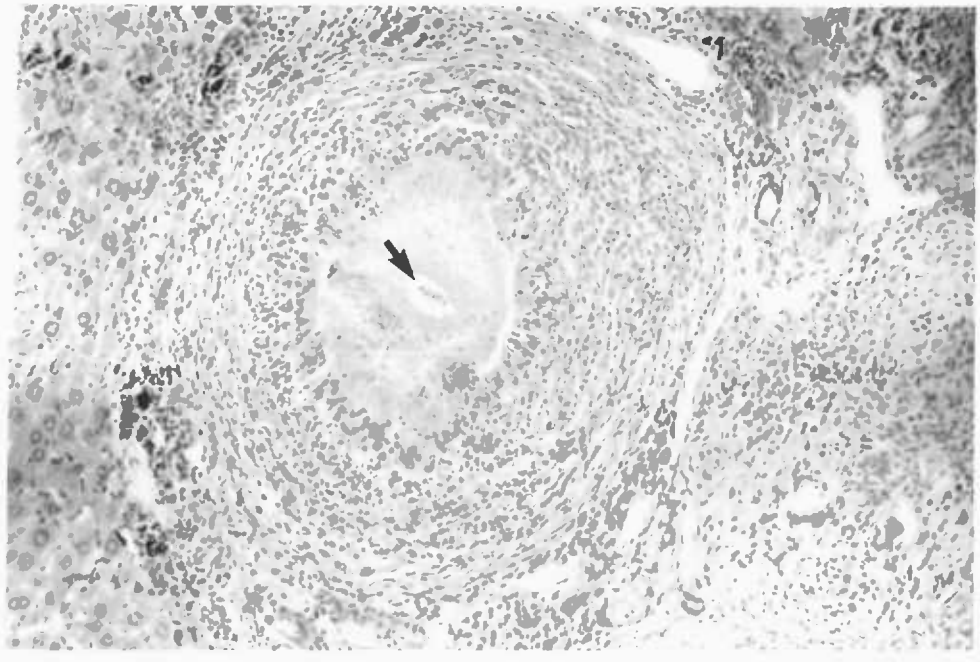
23.2 Mouse, 50 days p.i., bisexual infection. Liver showing degeneration of leucocytes and development of fibrous tissue, note areas of necrosis (arrows). The exudative-productive stage. (H & E). x160

PLATE 24



24.1 Mouse, 60 days p.i., bisexual infection. Liver showing central necrosis (arrow) and leucocyte concentration in the outer zone of the granuloma, the productive stage of granuloma formation. (H & E).

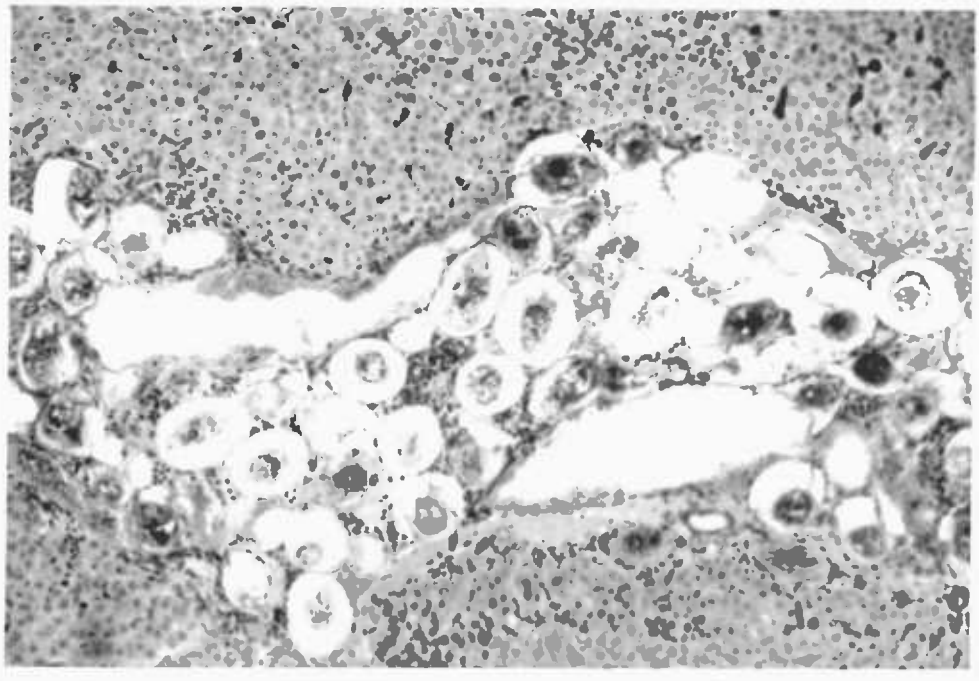
x180



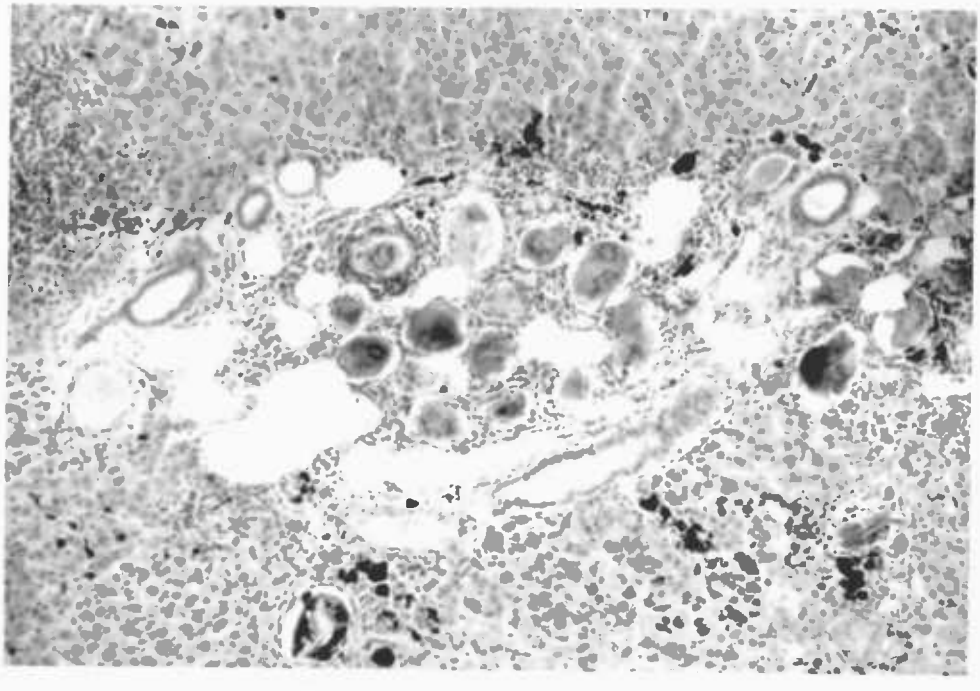
24.2 Mouse, 70 days p.i., bisexual infection. Liver showing concentrically arranged fibrous tissue in the granuloma around a single egg. The involutional stage. (H & E).

x180

PLATE 25

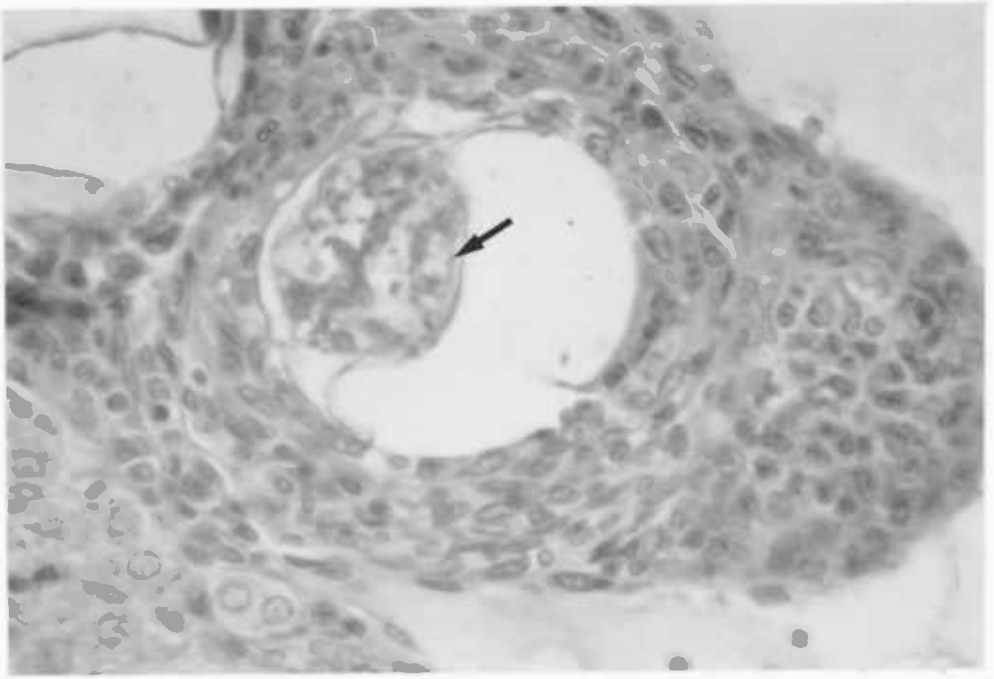


25.1 Hamster, 40 days p.i., bisexual infection. Liver showing numerous mature eggs in the portal vessel with slight leucocyte infiltration. (H & E). x160

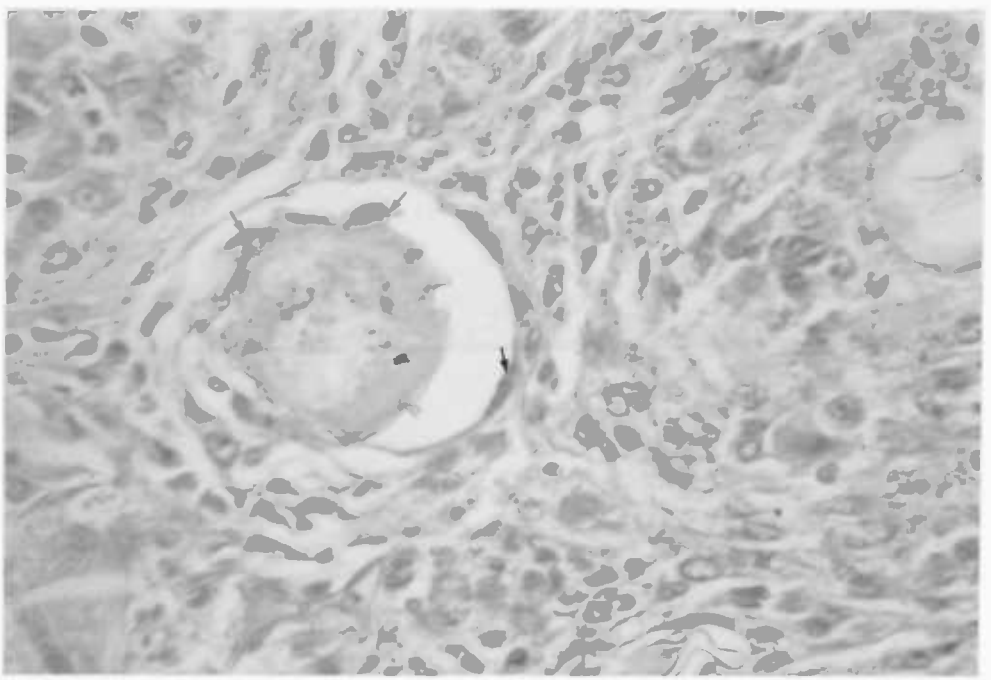


25.2 Hamster, 70 days p.i., bisexual infection. Liver showing leucocyte infiltration around eggs and no fibrous tissue. (H & E) x160

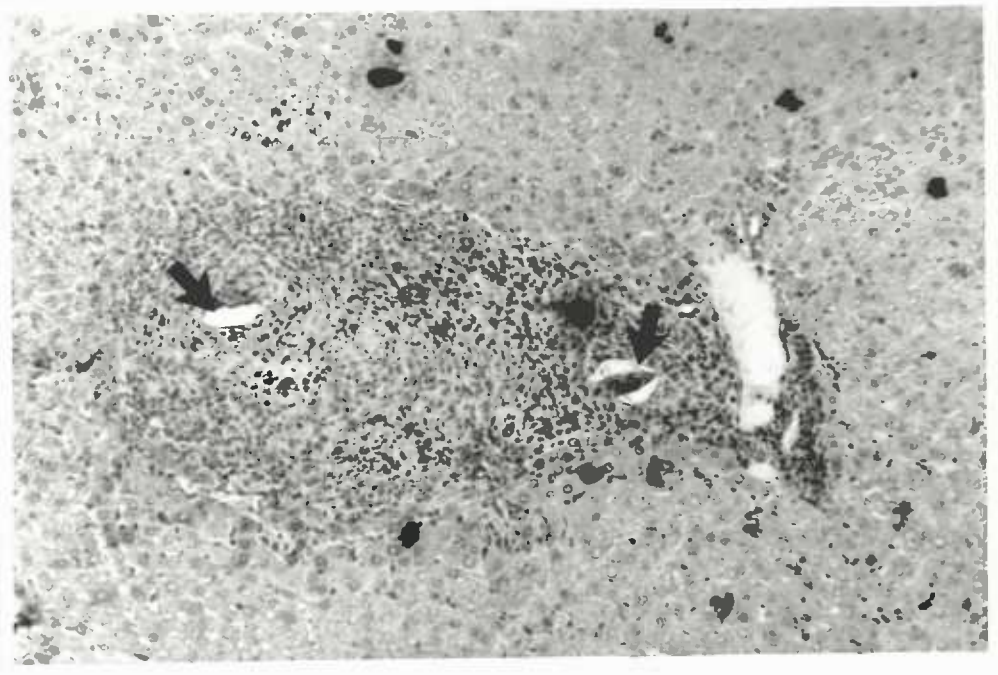
PLATE 26



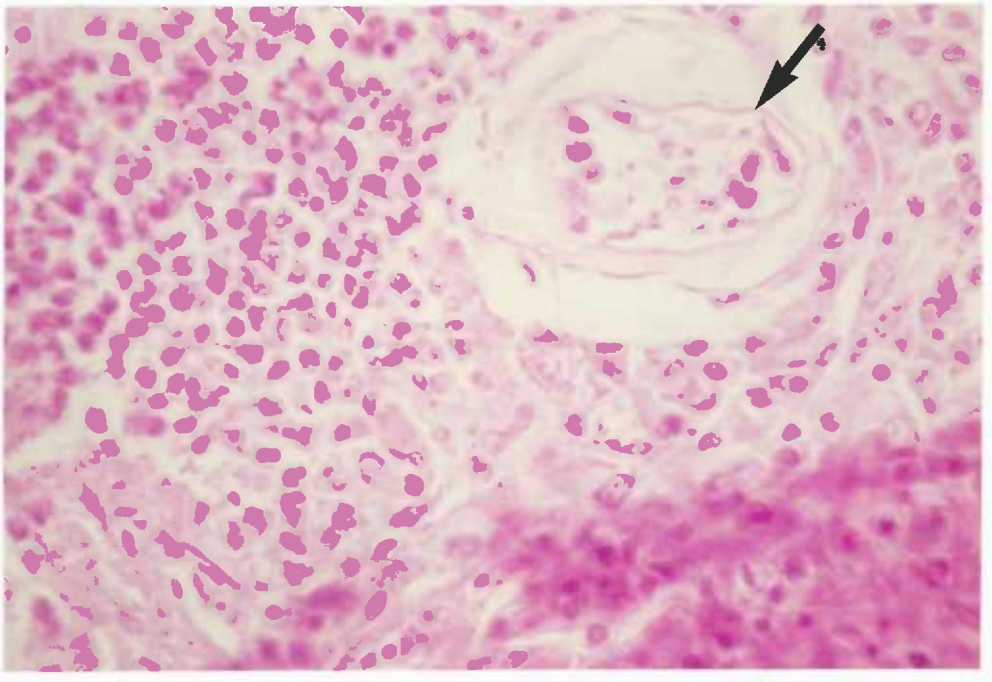
26.1 Hamster, 60 days p.i., bisexual infection. Liver showing egg (arrow) in the centre, surrounded by loose connective tissue and macrophages which gives it a foamy appearance. (H & E). x350



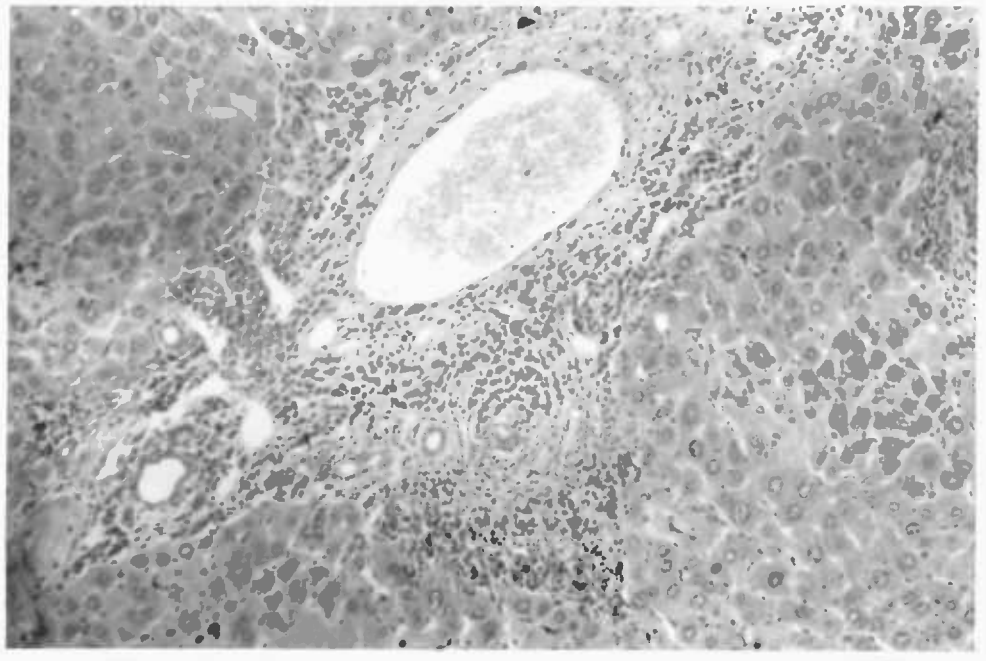
26.2 Mouse, 60 days p.i., bisexual infection. Liver showing mononuclear cells and macrophages surrounding an egg and a number of giant cells, some attached to the egg (arrows). (H & E). x350



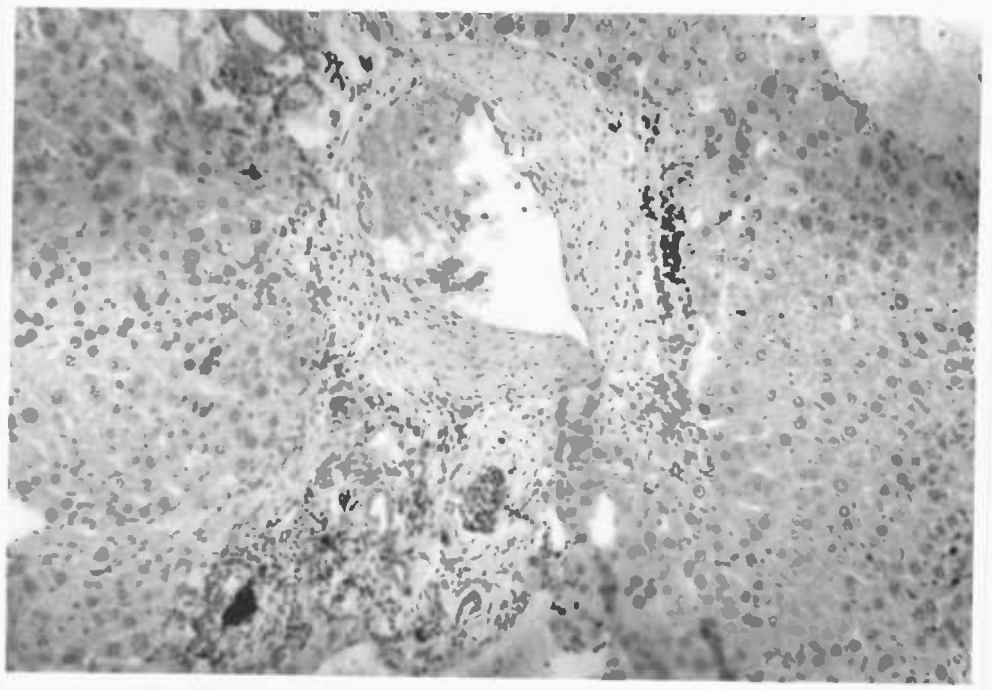
27.1 Gerbil, 60 days p.i., bisexual infection. Liver showing two eggs (arrows) in the granuloma, inflammatory leucocytes with fibrous tissue, the exudative-productive stage. (H & E). x160



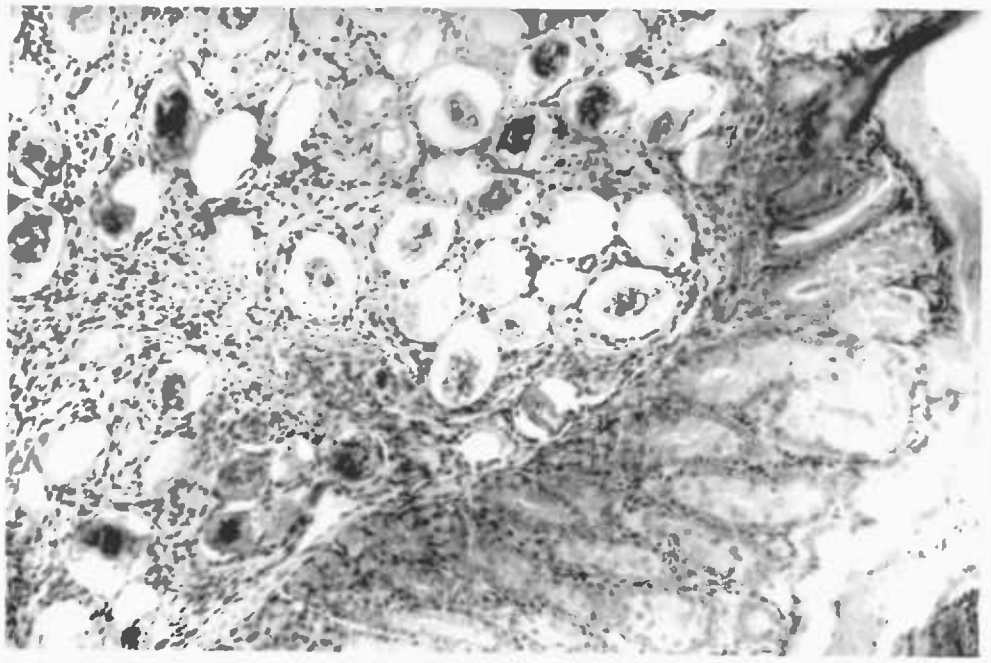
27.2 Gerbil, 60 days p.i., bisexual infection. Liver showing predominantly mononuclear leucocytes to the left of the egg. Gaint cells surround the egg (arrow). (H & E). x350



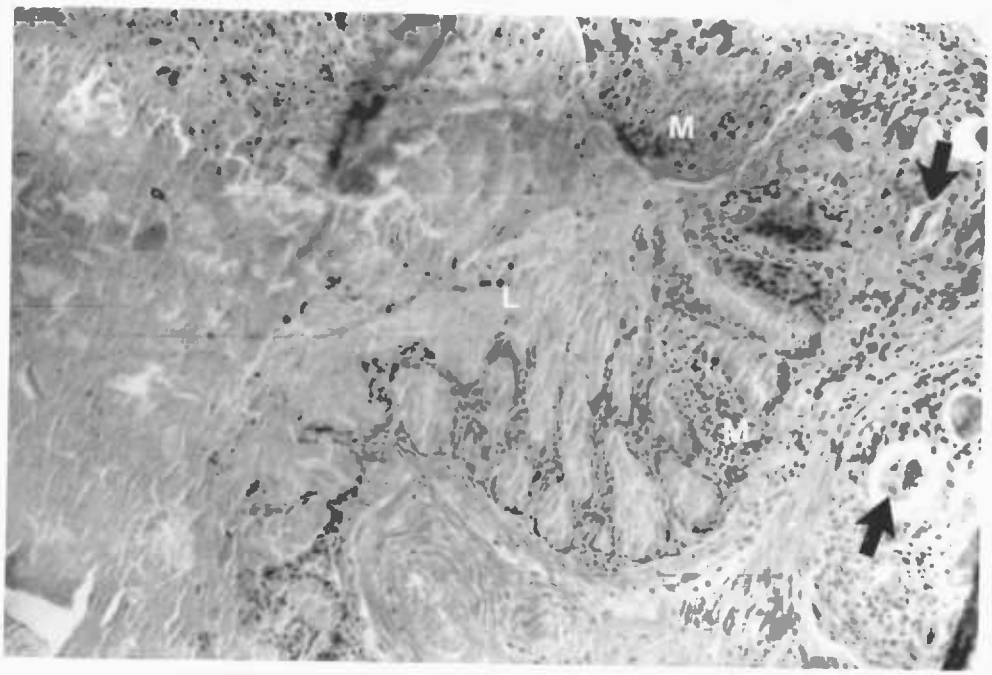
28.1 Mouse, 60 days p.i., bisexual infection. Liver showing periportal infiltration around degenerated worm material and fibrous tissue developing around the portal vessel. (H & E). x160



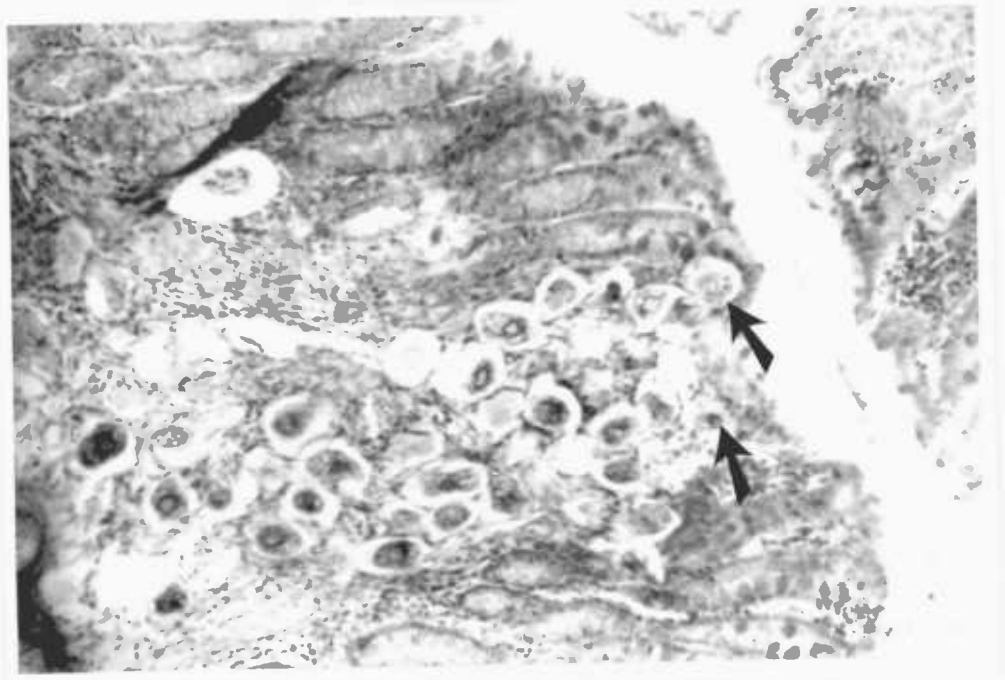
28.2 Mouse, 70 days p.i., bisexual infection. Liver showing fibrous scar formed around degenerated worm material in the portal vessel. (H & E). x160



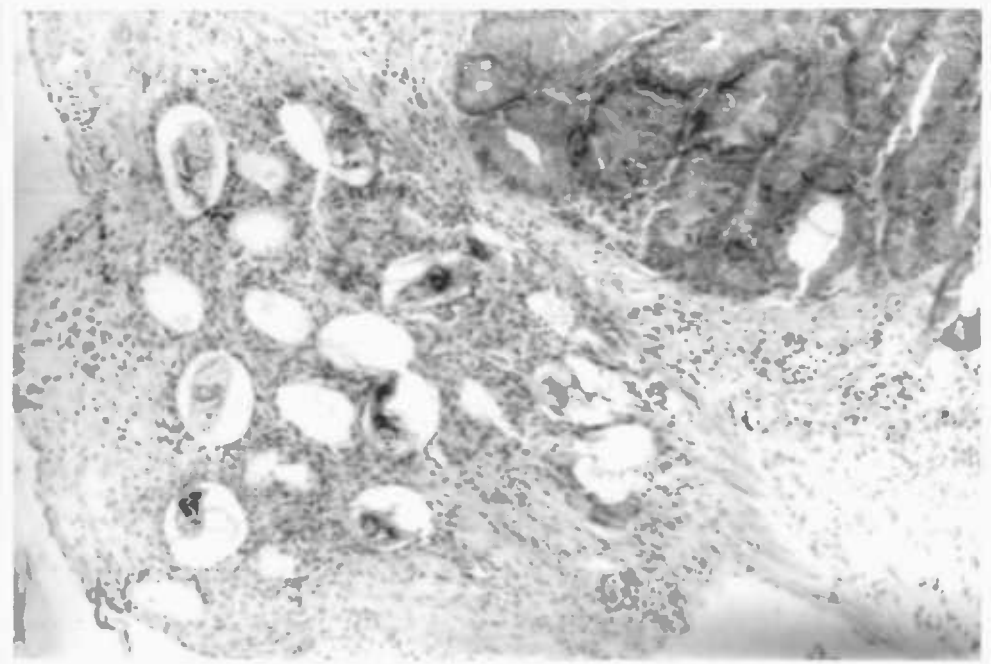
29.1 Gerbil, 50 days p.i., bisexual infection. Colon showing eggs deposited in the mucosa surrounded by inflammatory cells and fibrous tissue. The mucosa is completely distorted. (H & E). x160



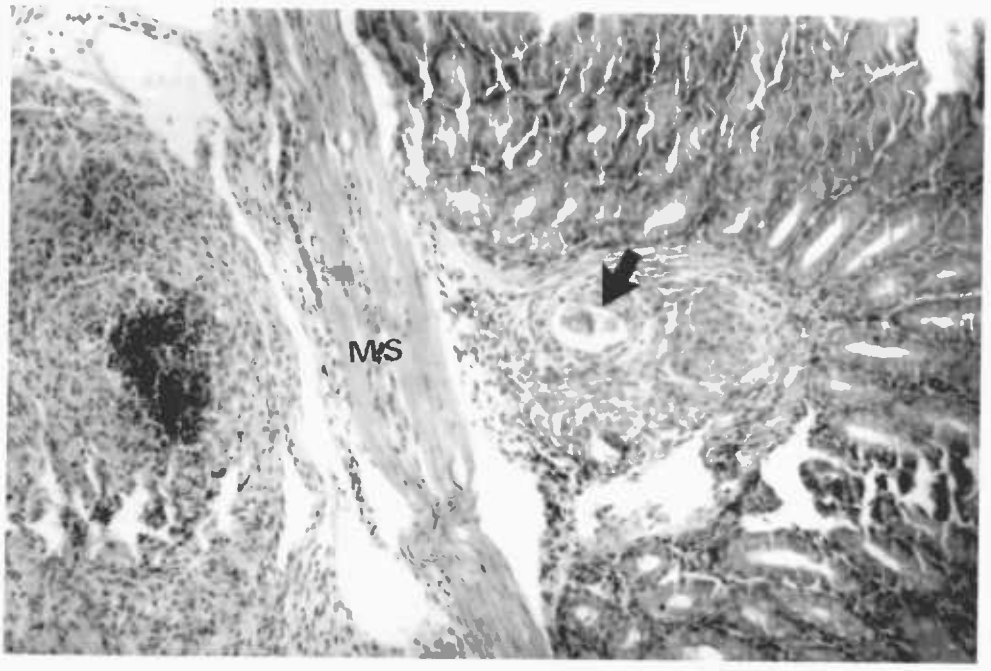
29.2 Gerbil, 60 days p.i., bisexual infection. Caecum showing severe disruption of the intestinal mucosa (M) and extensive damage of the intestinal lining. The lumen (L) is filled with blood clots, and there are eggs in the mucosa (arrows). (H & E). x160



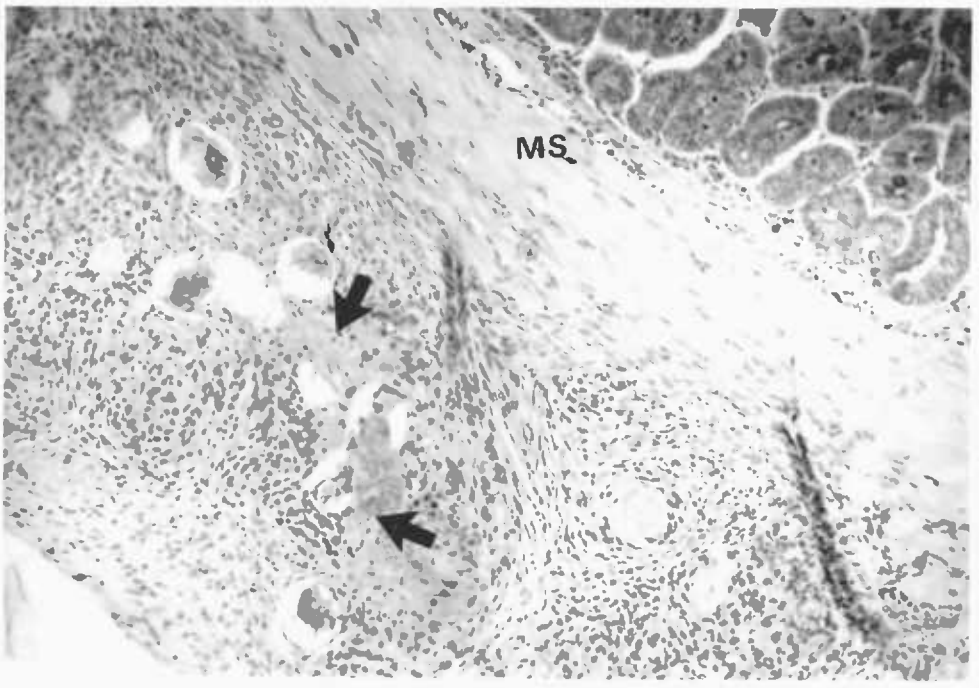
30.1 Hamster, 50 days p.i., bisexual infection. Colon showing distorted mucosa and eggs breaking through the intestinal lining into the lumen (arrows). (H & E). x160



30.2 Hamster, 60 days p.i., bisexual infection. Colon showing the involvement of the muscular layer and serosa, eggs at different stages of development and obliteration of the wall of the colon. (H & E). x160



31.1 Mouse, 60 days p.i., bisexual infection. Colon showing egg in submucosa (arrow) with fibrous tissue, and portion of serosal involvement with inflammatory infiltration in the muscularis (MS). (H & E). x160



31.2 Mouse, 70 days p.i., bisexual infection. Colon showing fibrosis of the serosa and obliteration of the wall of the colon and necrosis (arrows) around deposited eggs. (H & E). x160

5.3 DISCUSSION

The laboratory hosts, hamsters, mice and gerbils, used in this study were favourable for the development, maturation and survival of S. margrebowiei but showed different pathological reactions. The present observations have shown the histopathological changes in the tissues of these hosts associated with the presence of either male or female worms in unisexual infections, or both sexes in the mesenteric-portal system at early stages of bisexual infections, in the absence of deposited eggs. The histopathological changes associated with deposited eggs in various tissues in late bisexual infections has presented the general histopathology of S. margrebowiei infection in these laboratory definitive host species.

The distinctive histopathology produced by the hosts in this study appear to be the result of variable host response to a uniform stimulus. The varying host response to other schistosome species have already been reported by a number of workers (Hoeppli, 1932; Ito, 1955; Lurie & DeMeillon, 1956; Meleney et al., 1953; Cheever, 1965a, b; Warren, 1973; and Moore, Grove & Warren, 1977). The present study has shown that host response to worms did not vary in the different host species but that great variations occurred in the host response to deposited eggs in the tissues. The inflammatory response in relation

to the duration of unisexual infections in mice and hamsters, and early bisexual infections in the three host species seem to favour the view that living worms in the mesenteric-portal system elicit little or no inflammatory response (Meleney et al., 1952). The increased inflammatory reaction associated with worms in late bisexual infections may be due to toxic products from dead worms. This finally resulted in fibrosis of the portal vessels in mice but not in hamsters and gerbils. The absence of fibrosis in the liver in unisexual late infections in mice and hamsters (180 days p.i.) agrees with the observations by Meleney et al. (1952) of unisexual infections with S. mansoni. The presence of both sexes of the worm is required to produce fibrosis in the liver of mice as dead worms in unisexual long term infections in mice did not result in fibrosis of the portal vessels. The absence of fibrosis in unisexual infections might be due to the fact that worms in the portal venules only cause temporary obstruction as they could move about. This is opposed to the permanent obstruction which results from eggs deposited in bisexual infections.

Mature female worms produced eggs between 33 and 35 days p.i. in hamsters, mice and gerbils in bisexual infections. Eggs were found in the portal venules and the intestine of the three host species, but were also present in the lungs and spleen of mice with prolonged infections.

The deposited eggs were associated with inflammatory reaction and more typical lesions occurred in the liver of mice. The progress of egg granuloma in mice seems to follow the developmental process shown by Hsü et al., (1972) with S. japonicum and S. mansoni in mice. There was accumulation of predominantly mononuclear and polymorphonuclear leucocytes at the early stages followed by degeneration and formation of fibrous tissue around deposited eggs at later stages. The reaction in the liver of mice to S. margrebowiei eggs shows distinct stages of evolution and involution of lesions similar to those elicited by S. mansoni and S. japonicum in albino mice and rhesus monkeys. The granuloma in the liver of hamsters and gerbils were not as characteristic and the inflammatory responses were not as intense as those in mice. These histopathological differences in the liver of these hosts have further confirmed that egg lesions show host-species differences as was established by Hsü et al., (1972) with various hosts infected with S. mansoni and S. japonicum. These differences might be due to the varying susceptibility and immunological status of the host.

Worms and eggs were only observed in the lungs in prolonged bisexual infections in mice and they produced similar host responses as eggs deposited in the liver, indicating that pulmonary schistosomiasis resulted from S. margrebowiei infection in mice but not in hamsters

and gerbils. There has been much speculation as to the route or routes by which eggs and worms reach the lungs. Eggs delivered from mature female worms into the large vessels of the mesenteric-portal system are carried by the blood-stream into the liver. Some are apparently carried to the capillaries within the liver lobules. Two possible routes to the lungs are as follows :

1. from the liver, through large anastomoses between portal and hepatic venules and ultimately reaching the lungs;

and 2. from the mesenteric vein and through its anastomoses with the systemic.

The route through the liver seems more probable for eggs and is supported by the observations of Prinzmetal, Ornitz, Simkin & Bergman (1948) where glass beads of diameter 180 micra, injected into the portal vein were recovered from the lungs. This does not easily account for the passage of adult worms into the lungs. Krakower, Hoffman & Axtmayer (1943) described enlarged anastomotic veins, between the upper mesenteric vein and the vena cava and its branches, in guinea pigs infected with S. mansoni where massive egg deposition and dead worms in the liver had caused portal obstruction. They found worms in these anastomostic veins and recovered adult worms in the lungs. This might also account for the observation of worms in the lungs of mice with S. margrebowiei infection as worms and eggs were only found

in this host species. Mice showed severe portal obstruction as a result of large granuloma with fibrosis around deposited eggs and dead worms. It is likely that similar anastomotic veins developed through which eggs as well as worms were carried to the lungs in late S. margrebowiei infections in mice.

Diarrhoea and blood in faeces, which were noticeable from day 50 p.i. in hamsters and gerbils, resembles the acute toxic or pathogenic phase of the disease. Hamsters and gerbils seem to pass through a more severe pathogenic phase of S. margrebowiei infection than mice. Although most infected animals appeared ill from day 50 p.i., gerbils did not survive this phase and died of massive intestinal haemorrhage. Hamsters and mice which survived this period of the infection then maintained a low worm burden. A similar acute phase of S. margrebowiei infection in laboratory hosts have been reported by Pitchford (1975) and Southgate & Knowles (1977). The absence of this acute phase in unisexual infections supports the conclusion reached by other workers (DeWitt & Warren, 1959; Hsu & Hsu, 1961; von Lichtenberg, 1962; Moore et al., 1977) that it is the egg of the schistosome that is the main cause of the pathogenicity of mammalian schistosomiasis. Splenomegally which is another phase of human schistosomiasis was observed only in mice.

The histopathological changes at different intervals in hamsters, mice and gerbils infected with S. margrebowiei have been described and illustrated for the first time. Three distinct patterns of host response to S. margrebowiei are demonstrated by the hosts employed in this investigation. The pathological response of host species to schistosome infection, like most parasitic infections, is associated with defence mechanisms involving cellular infiltration and degeneration as shown by destruction of leucocytes.

S. margrebowiei infection in mice has proved them to be excellent hosts for the study of tissue changes and cellular reactions in schistosomiasis. The defence reaction in mice is demonstrated by the cellular proliferation in the spleen and the progressive development of the granuloma in the liver. The mouse therefore, would be a suitable host for prolonged and stable infections of S. margrebowiei. The high mortality rate and lack of progressive lesions observed in hamsters and gerbils make them poor hosts for the study of the histopathological changes associated with schistosome infections. However, hamsters and gerbils may be suitable hosts for the study of the acute phase of the disease.

6 *IN VITRO* CULTURE ATTEMPTS

Shortage of time and material only allowed preliminary culture experiments to be attempted.

6.1 TRANSFORMATION OF CERCARIAE TO SCHISTOSOMULA

The mechanical shaking of packed cercariae on a Rotamixer resulted in 100% tail loss in the cercarial population. The cercarial bodies obtained by this method were viable in saline but did not survive in water which shows that transformation of cercariae to schistosomula had occurred. Criteria used to identify schistosomula include : i) loss of tail; ii) viability of organism in saline but not in water; iii) pre-acetabular gland evacuation; and iv) ability to survive in culture.

The incubation of packed cercariae in Hanks' saline supplemented with varying concentrations of rabbit serum (RS) or new-born calf serum (NCS) did not result in total loss of cercarial tails in the population. Table 6.1 shows the mean percentage of cercarial tail loss from three cultures after incubation in Hanks' saline supplemented with varying concentrations of sera. Higher percentages of tail loss in cercarial population were recorded in Hanks' supplemented with 50% sera (63% RS and 31% NCS). Both cercarial bodies and cercariae with tails

intact after incubation in Hanks' containing serum were viable in saline and inviable in water, showing that transformation to schistosomula had occurred.

Schistosomula obtained by both methods were isolated from the tails, inoculated into culture tubes and their survival rates determined and compared. The results of survival in culture media NCTC 135 and RPMI 1640 are shown in Table 6.2. Schistosomula obtained by both methods showed high death rates in culture between 3 and 15 days post-inoculation, after which a low death rate was observed in schistosomula obtained by incubation in Hanks'. Schistosomula obtained by incubation survived for longer periods in culture (40 days) than mechanically derived ones (21 days).

6.2 DEVELOPMENT IN CULTURE

Morphological criteria used to describe the developmental stages *in vivo* (Section 3.2) were also used to assess the extent of development *in vitro*. As the gastric caecum was a distinct feature of the schistosomula when filled with dark brown pigment, indicating ingestion of red blood cells, its development was followed in culture. The caecum was easy to observe under the inverted microscope without removing the worms from the culture vessels. The five stages of development of the caecum observed are

similar to those described for S. mansoni (Faust, Jones & Hoffman, 1934). The stages are diagrammatically represented in Fig. 6.1 and are as follows :

Stage 1 - schistosomula presenting only a light stain which stands for the beginning of the caecum.

Stage 2 - a dark stain now bifurcating but not passing the acetabulum.

Stage 3 - the dark stain passes the acetabulum and its branches link up with each other later on.

Stage 4 - the dark bifurcated stain after linking grows to the posterior end of the worm but is not as long as the bifurcated caecum.

Stage 5 - the final linked caecum grows longer than the bifurcated section.

Table 6.3 shows the rate of development in media NCTC 135 and RPMI 1640 using either rabbit serum or new-born calf serum. The results show that the rate of development was faster in medium NCTC 135 with rabbit serum and rabbit red blood cells. The linking of the caecum (Stage 3), the closed-gut stage, was reached by day 24 post-inoculation. This was observed by day 26 post-inoculation in medium RPMI containing rabbit serum and rabbit red blood cells. Only worms in NCTC 135 containing RS and red blood cells reached Stage 5. Although worms remained in culture for 40 days, no differentation of sex organs were observed.

Table 6.1 The mean percentage of cercarial tail loss (range in brackets) after incubation in Hanks' saline supplemented with varying concentrations of rabbit serum (RS) or new-born calf serum (NCS).

Percentage of serum in Hanks' saline	Mean percentage of cercarial tail loss	
	RS	NCS
10	15 (12-18)	4 (3-6)
20	30 (28-33)	11 (8-15)
30	38 (32-45)	19 (12-24)
40	51 (44-56)	27 (25-34)
50	63 (58-67)	31 (28-36)

Table 6.2 Comparison of percentage survival of schistosomula obtained mechanically or by incubation, in culture from day 3 to 40 post-inoculation.

Days	Schistosomula obtained mechanically				Schistosomula obtained by incubation			
	NCTC + NCS	NCTC + RS	RPMI + NCS	RPMI + RS	NCTC + NCS	NCTC + RS	RPMI + NCS	RPMI + RS
3	60	65	50	51	60	95	55	80
6	23	63	32	42	32	82	23	60
9	9	24	6	30	15	43	12	15
12	4	15	0	12	7	22	4	10
15	1	10	0	8	2	20	0	5
18	0	8	0	0	0	16	0	2
21	0	4	0	0	0	12	0	2
24	0	0	0	0	0	11	0	2
27	0	0	0	0	0	11	0	2
30	0	0	0	0	0	10	0	2
33	0	0	0	0	0	10	0	0
36	0	0	0	0	0	8	0	0
39	0	0	0	0	0	8	0	0
40	0	0	0	0	0	2	0	0

Note : Media used - NCTC 135 and RPMI 1640; sera used - NCS, new-born calf serum and RS, rabbit serum.



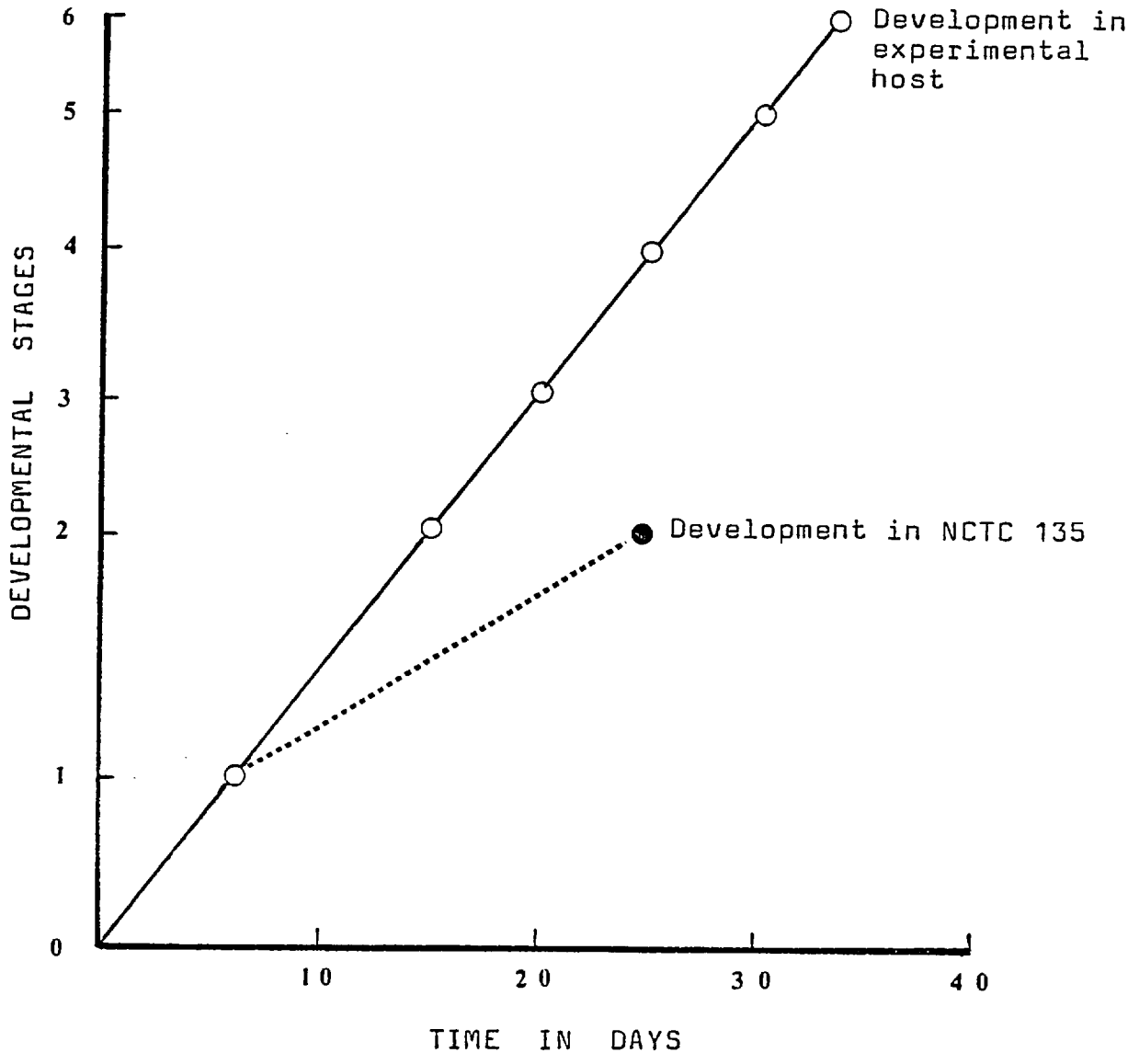
FIGURE 6.1 Diagrammatic representation of the development of the gastric caecum.

Table 6.3 shows days post-inoculation at which the developmental stages were observed in the culture.

Stages of caecum development	Days post-inoculation			
	NCTC + NCS	NCTC + RS	RPMI + NCS	RPMI + RS
Stage 1	5	5	5	5
2	14	12	14	14
3	-	24	-	26
4	-	30	-	-
5	-	33	-	-

Note : Media used - NCTC 135 and RPMI 1640; sera used - NCS, new-born calf serum and RS, rabbit serum.

The *in vitro* development of *S. margrebowiei* was observed to be slower than the development in the experimental hosts. Fig. 6.2 shows the development of *S. margrebowiei in vitro* compared with the development in the experimental hosts.



KEY

0 Cercaria
1 Lung form
2 Closed-gut form
3 Organogeny
4 Gametogeny
5 Egg-shell formation
6 Oviposition

FIGURE 6.2 Development of S. margrebowiei in vitro, compared with development in vivo.

6.3 DISCUSSION

S. margrebowiei cercariae can be transformed to schistosomula either mechanically or by incubation in Hanks' saline supplemented with serum. The schistosomula obtained by both methods were water intolerant and saline tolerant (criteria used for identifying schistosomula, Stirewalt, 1966). Although the mechanical shaking of cercariae on a Rotamixer resulted in 100% tail loss in the cercarial population and water sensitive organisms, their high mortality in culture is a great disadvantage to the method. The shaking might have caused some damage to the derived schistosomula. The simplicity of the operation of this method and the resulting large number of schistosomula are advantageous. Incubation of cercariae in Hanks' saline on the other hand resulted in a large number of cercariae with tails intact, though this did not affect their development in culture. The resulting cast-off tails in culture decomposed and resulted in contamination of the culture media.

S. margrebowiei can be cultured from in vitro derived schistosomula to the closed-gut stage in culture medium NCTC 135 containing 50% rabbit serum and 1% red blood cells. The rate of development in culture was observed to be slower (Fig. 6.2) than that observed in the experimental hosts employed in this study. The

development of the genital organs was not achieved in culture. This is the first attempt to culture S. margrebowiei through the stages of development which occur in the vertebrate host. Significant development in vitro, have been recorded for other species of schistosome (Clegg, 1965; Clegg & Smithers, 1972; Smith et al., 1976; Fu et al., 1976). The retarded development observed in vitro may be due to the fact that the culture media lack growth factors which are present in the host's tissue. The probable accumulation of excretory or metabolic products in culture during development, either by the schistosomula or by contamination organisms, may also have a retarding effect on the growth of schistosomula.

Wilson et al., (1978) suggested that the high nutrient level present in the hepatic portal blood but not in the peripheral blood probably initiates growth of S. mansoni in vivo. The closed-gut stage to which S. margrebowiei developed in culture, is similar to the early stages observed in vivo in the liver of experimental hosts. Major growth and development were observed in vivo after worms had reached the liver. It is then probable that the requirements of the schistosomula are not met by the culture media. An understanding of the worm's requirements in the liver and an increase in the nutrient content of the media might improve development in vitro.

Ottens & Dickerson (1969, 1972) showed that schistosomula in mice infected with bacteria, also became infected and that some but not all infecting bacteria were lethal to schistosomes. Although bacterial contamination of culture was not investigated in this study, contamination of culture by some organisms was a common feature. The contaminating organisms might have had retarding or lethal effects on schistosomula in culture. Eveland, Ludwikowska & Madden (1979) suggested that selection against contaminating organisms by varying the consitituents of the culture media may eliminate contamination in long term cultures.

Although this investigation has not successfully produced, *in vitro*, all the developmental stages of *S. margrebowiei* observed in the experimental hosts, it has shown that the parasite can develop to the early liver stage in culture medium NCTC 135 enriched with rabbit serum and rabbit red blood cells. An improvement of the medium might meet the nutritional requirements of the schistosomula to produce better development *in vitro*, but clearly physico-chemical factors may also play an important role in the growth and differentiation of this species.

7 GENERAL DISCUSSION AND CONCLUSIONS

The observations on the growth, development, ultrastructure, histopathology and *in vitro* culture of *S. margrebowiei* have already been discussed in detail in the preceding sections. In this section, highlights of some of the more significant findings, general conclusions and possible future areas of research are presented in order to give an overall description of the present study.

Exposure of hamsters, mice and gerbils to *S. margrebowiei* cercariae by the immersion technique and subsequent examination of these animals at intervals during the course of the infection, has allowed for the evaluation of the susceptibility of these commonly used laboratory hosts to the infection. The three hosts were favourable for the development, maturation and survival of the parasite. Hamsters and mice were more susceptible to the infection as they had higher tissue egg counts and higher worm burdens than the gerbils.

The general pattern of the infection was similar in the three hosts. The migration of the worms followed the already established course of infection (skin - lungs - liver - mesenteric vein). The reduction in worm burden, after the onset of egg production, is similar to that observed by Smithers and Terry in *S. mansoni* (1965b).

The mechanism of this spontaneous reduction in worm burden is still not understood and has been attributed to immunological and non-immunological mechanisms (Cioli & Dennert, 1976). If this period of reduction in worm burden was associated with the immune response related to the onset of egg production, the question arises - what was responsible for the slight worm reduction observed in male and female unisexual infections where there were no eggs produced? One possibility is that the worms also contribute to the immune response that results in worm reduction, but a greater contribution comes from the eggs. This argument can only be speculative since few animals were used in this investigation and no immunological studies were carried out. The mechanisms involved and the contribution by the worms and the eggs remain to be determined.

Scanning electron microscope studies have revealed differences between the tegument of immature worms and those of adult worms and thus shown changes which occur on the tegument during development. The SEM study has also demonstrated the remarkable difference between the male and female S. margrebowiei and also how this worm differs from already described schistosome species. The study has shown that the presence or absence of spines on some parts of the tegument is related to the age of the worm. The spines observed on the anterior parts of immature

worms but which were absent on adult worms have been associated with attachment during migration. The combination of information from both scanning and transmission electron microscopical techniques might help in the understanding of the development of these spines and of the general tegument of S. margrebowiei as has been reported for S. mansoni (Morris & Threadgold, 1967; Hockley, 1973; Hockley & McLaren, 1973).

The SEM has shown the microtopographical details of the S. margrebowiei egg and the difference between it and that of S. japonicum, which was reported by Le Roux (1933) to have a similar pattern.

The histopathological results in this study have shown great differences between unisexual and bisexual infections. From these observations, it could be concluded that most of the histopathological changes in the experimental hosts were produced by deposited eggs and dead worms forming localized lesions similar to those described for other species of schistosomes. The periportal infiltrations in the liver in unisexual and early bisexual infections could be interpreted as allergic reactions resulting from the presence of worms in the portal-mesenteric veins and appear to make very little contribution to the granuloma formation associated with schistosomiasis. The results also agree with previous

reports that the granulomatous response to schistosome eggs is stage specific and follows a distinct sequence of histological events (Hsü et al., 1961; Warren & Domingo, 1970). The various stages of the evolution and involution of granuloma formation observed in mice, corresponded with those described by Hsü et al., (1961, 1972). This study has also shown that the site of deposited eggs, the organs involved and the mammalian host species under investigation are very important factors that must be taken into consideration when interpreting the sequence of histopathological events.

The three disease syndromes associated with schistosomiasis (Warren, 1973) were apparent in Schistosoma margrebowiei infection in the laboratory hosts employed :

1. Dermatitis resulting from cercarial penetration of the skin. This is due to hypersensitivity reactions of immediate and delayed types. They tend to be more severe when the host is naturally resistant to the schistosome species. The variations in resistance might account for the difference in worm burden observed in the hosts employed.

2. Acute schistosomiasis which occurs in heavy initial infections after the onset of egg laying. This may be a form of serum or immune complex disease. The stage was more pronounced in hamsters and gerbils infected with S. margrebowiei; these hosts might be suitable for the

study of this phase of the disease.

3. Chronic schistosomiasis in which organ involvement is dependent on the habitat of adult worms. Most schistosomes are found in portal and mesenteric venules and the disease primarily involves the gut and the liver. In S. margrebowiei infection, the liver and colon were greatly involved and the major factor responsible was the egg. The granulomatous response to the eggs play an essential role in the pathogenesis of the disease. The occurrence of a significant degree of histopathology is related not only to the intensity of the infection, but also to the intensity of host response. In mice, the development of the hepatosplenic disease is associated with the formation of large granuloma around deposited eggs which results in obstruction of tissue and blood flow. The obstruction of blood vessels of the liver might account for the development of new vessels that are responsible for eggs being deposited in the lungs and spleen in late infection.

It is hoped, however, that the histopathological studies on this parasite will form an additional comparative study of egg lesions produced by schistosomes in experimental hosts. Like S. japonicum (Meleney et al., 1952; Hsü et al., 1972), the eggs of S. margrebowiei produced an acute reaction in mice.

The fast rate of growth and development in the experimental hosts together with the large number of eggs produced per day when compared to S. haematobium, S. mattheei, S. intercalatum and S. bovis (Wright and Bennett, 1967a & b; Wright & Knowles, 1972 and Southgate & Knowles, 1975) make the parasite an excellent species for the study of schistosomiasis in the laboratory.

The preliminary attempts on the in vitro culture of S. margrebowiei have shown that the parasite develops normally to the closed-gut stage in artificial media. The fact that the development was slower in culture than in the experimental hosts employed may be related to the fact that the liver is a nutritionally rich environment and that the media used have not been able to provide such an environment. The present investigation on in vitro culture, like the past in vitro culture reports on other schistosome species, has not resulted in the development of sexually mature worms capable of producing eggs.

Until the reports by Pitchford (1974), Southgate & Knowles (1977), very little detailed information was available on the biology of S. margrebowiei after it had been described by Le Roux (1933). It is hoped that the results in this study must have made some contribution to the knowledge of the biology of S. margrebowiei and will stimulate further research.

REFERENCES

ARMSTRONG, J.C. (1965). Mating behaviour and development of schistosomes in the mouse.
J. Parasit., 51 : 605-616.

BELL, E.J. and SMYTH, J.D. (1958). Cytological and histochemical criteria for evaluating development of trematodes and pseudophyllidean cestodes in vivo and in vitro.
Parasitology, 48 : 131-148.

BIRGI, E. and GRABER, M. (1969). Mollusques pulmones d'eau douse Bossomatophores vecteurs au Tchad d'affections parasitoires du betail leur elevage au laboratoire.
Revue d'elevage et de Med. Veterinaire des pays Tropicaux, 22 : 393-408.

BRUMPT, E. (1936). Action des hostes definitifs sur l'evolution et sur la section des sexes de certain helminthes heberges per cur. Experiences sur de schistosomes.
Annls Parasit., 14 : 514-551.

BURTON, P.R. (1964). The ultrastructure of integument of the frog bladder fluke, Gorgoderina sp.
J. Parasit., 52 : 926-934.

CHEEVER, A.W. (1965a). A comparative study of S. mansoni infections in mice, gerbils, multimammate rats and hamsters. I. The relation of portal hypertension to size of hepatic granuloma.
Am. J. trop. Med. Hyg., 14 : 211-226.

CHEEVER, A.W. (1965b). A comparative study of Schistosoma mansoni infections in mice, gerbils, multimammate rats and hamsters. II. Qualitative pathological differences.
Am. J. trop. Med. Hyg., 14 : 227-238.

CHEEVER, A.W. (1970). Relative resistance of human schistosomiasis to digestion in potassium hydroxide.
Bull. Wld. Hlth. Org., 43 : 601-603.

CHEEVER, A.W. and WELLER, T.H. (1958). Observation on growth and nutritional requirements of S. mansoni in vitro.
Am. J. Hyg., 68 : 322-329.

CHRISTENSEN, N.O. and FRANDSEN, F. (1977). Host finding capacity of schistosome cercariae : Comparative efficiency of methods of mouse infection and radioisotope assay system.
J. Helminth., 51 : 105-113.

CIOLI, D., BLUM, K. and RUPPEL, A. (1978). S. mansoni : Relationship between parasite age and time of spontaneous elimination from the rat.
Expl Parasit., 45 : 74-80.

CIOLI, D. and DENNERT, G. (1976). The course of S. mansoni infection in thymectomized rats.
J. Immun., 117 : 59-65.

CLEGG, J.A. (1959). Development of sperm by S. mansoni cultured in vitro.
Bull. Res. Council Israel, 8E : 1-6.

CLEGG, J.A. (1965). In vitro cultivation of S. mansoni.
Expl Parasit., 16 : 133-147.

CLEGG, J.A. and SMITHERS, S.R. (1968). Death of schistosome cercariae during penetration of skin. II : Penetration of mammalian skin by S. mansoni.
Parasitology, 58 : 111-128.

CLEGG, J.A. and SMITHERS, S.R. (1972). The effect of immune rhesus monkey serum on schistosomula of S. mansoni during cultivation in vitro.
Int. J. Parasit., 2 : 79-98.

CLEGG, J.A. and SMYTH, J.D. (1968). Growth, development and culture methods : Parasitic platyhelminths.
IN Chemical Zoology 2 : 395-466 ACADEMIC PRESS : LONDON, NEW YORK.

COLLEY, D.G. and WIKEL, S.K. (1974). S. mansoni : Simplified method for production of schistosomules.
Expl Parasit., 35 : 44-51.

CRAM, E.B. and BOZICEVICH, J. (1944). Experimental *S. mansoni* infection by intraperitoneal injection. *Trop. Med. News*, *1* : 16-17.

DeWITT, W.B. and WARREN, K.S. (1959). Hepato-splenic schistosomiasis in mice. *Am. J. trop. Med. Hyg.* *8* : 440-446.

EL MOFTY, A. (1962). Clinical aspects of Bilharziasis. IN : Ciba Foundation Symposium. BILHARZIASIS Edit. By G.E.W. Wolstenholme & M. O'Connor CHURCHILL LTD., LONDON.

ERASMUS, D.A. (1967). The host-parasite interface of *Cyathocotyle bushiensis* Khan, 1962 (Trematode : Strigeoidea). III : Electron microscope observation studies on the tegument. *J. Parasit.*, *53* : 703-714.

ERASMUS, D.A. (1969). Studies on host-parasite interface of strigeoid trematodes. IV : The ultrastructural observations of the lappets *Diplostomum phoxini*. *Z. Parasitkde*, *33* : 48-56.

ERASMUS, D.A. (1972). The biology of Trematodes. EDWARD ARNOLD, LONDON.

EVELAND, L.K. (1972). *S. mansoni* : Conversion of cercariae to schistosomula. *Expl Parasit.*, *32* : 261-264.

EVELAND, L.K., LUDWIKOWSKA, K.G. and MADDEN, K.G. (1979). *S. mansoni* : Long-term Culture of *in vitro* Derived Schistosomula. *Expl Parasit.*, *48* : 375-383.

EVELAND, L.K. and MORSE, S.I. (1975). *S. mansoni* : *In vitro* conversion of cercariae to schistosomula. *Parasitology*, *71* : 327-335.

FAIRLEY, N.H. (1920). A comparative study of experimental bilharziasis in monkeys contrasted with hitherto described lesions in man. *J. Pathol. Bact.*, *23* : 289-314.

FAUST, E.C., JONES, C.A. and HOFFMAN, W.A. (1934). Studies on S. mansoni in Puerto Rico. III: Biological studies. 2. The mammalian phase of the life cycle. Puerto Rico J. Pub. Hlth Trop.Med., 10 : 133.

FORD, J.W. and BLANKESPOOR, H.D. (1979). Scanning electron microscopy of the eggs of three human schistosomes. Int. J. Parasit., 9 : 141-145.

FU, H., CHOW, K. and CHIU, J. (1976). In vitro cultivation of S. japonicum. Int. J. Zoon., 3 : 105-113.

GAZZINELLI, G., De OLIVEIRA, C.C., FIGUEIREDO, E.A., PEREIRA, L.H., COELHO, P.M.Z. and PELLEGRINO, J. (1973). S. mansoni: Biochemical evidence for morphogenetic change from cercaria to schistosomule. Expl Parasit., 34 : 181-188.

GHANDOUR, A.M. (1978). The development of Schistosoma haematobium in the hamster. Ann. trop. Med. Parasit., 72 : 219-225.

GHANDOUR, A.M. and BABIKER, A. (1978). The in vitro development of Schistosoma bovis in the Nile rat. Ann. trop. Med. Parasit., 72 : 227-230.

GHANDOUR, A.M. and WEBBE, G. (1976). A comparative study of the death of schistosomula of Schistosoma haematobium and Schistosoma mansoni in the skin of mice and hamsters. J. Helminth., 50 : 39-43.

GILBERT, B., Da ROSA, M., BOROJEVIC, R. and PELLEGRINO, J. (1972). Schistosoma mansoni: In vitro transformation of cercariae into schistosomula. Parasitology, 64 : 333-339.

GONNERT, R. (1955). Schistosomiasis - Studien I. Beitrage zur anatomie und histologie van S. mansoni. Z. Tropenmed. Parasit., 6 : 18-33.

GRABER, M. (1969). Helminths parasites de certains animeaux domestiques et sauvages du Tchad. Bull. epizoot. Dis. Afr., 17 : 403-428.

GRABER, M. (1978). Schistosoma margrebowiei of Cobs in Chad.
J. Helminth., 52 : 72-74.

GUEVARA, POZO D., (1971). El cultivo in vitro de helmintos parasitos: Sus particularidades, problemas y fines.
Circular Farmaceutica, 232 : 197-220.

HICKS, R.M. and NEWMAN, J. (1977). The surface structure of the tegument of Schistosoma haematobium.
Cell Bio. Int. Report, 1 : 157-164.

HOCKLEY, D.J. (1968). Small spines on the egg shells of Schistosoma.
Parasitology, 58 : 367-370.

HOCKLEY, D.J. (1970). An ultrastructural study of the cuticle of Schistosoma mansoni Sambon, 1907.
Ph.D. Thesis, University of London.

HOCKLEY, D.J. (1973). Ultrastructure of the tegument of Schistosoma.
Adv. Parasit., 11 : 233-305.

HOCKLEY, D.J. and McLAREN, D.J. (1973). Schistosoma mansoni: Changes in the outer membrane of the tegument during development from cercaria to adult worm.
Int. J. Parasit., 3 : 13-25.

HOCKLEY, D.J. and McLAREN, D.J. (1977). Scanning electron microscopy of 8 species of Schistosoma.
Trans. R. Soc. trop. Med. Hyg., 71 : 292.

HOEPPLI, R. (1932). Histopathology observations in experimental schistosomiasis japonica.
Chin. Med. J., 46 : 1176-1186.

HOFFMAN, D.B. Jr. and WARREN, K.S. (1978). Schistosomiasis IV: Condensations of the selected literature 1963-1975 (2 volumes).
Hemisphere Publishing Cooperation, Washington, London.

HSÜ, H.F., DAVIS, J.R. and HSÜ, S.Y. LI (1969). Histopathological lesions of rhesus monkeys and chimpanzees infected with Schistosoma japonicum.
Z. Tropenmed. Parasit., 20 : 184-205.

HSÜ, H.F., DAVIS, J.R., HSÜ, S.Y. LI and LUST, G.L. (1970). Comparative studies on the pathogenicity of schistosome egg lesions. J. Parasit., 56 : 158-159.

HSÜ, S.Y. LI and HSÜ, H.F. (1961). New approach to immunization against Schistosoma japonicum. Science, 133 : 766.

HSÜ, S.Y. LI, HSÜ, H.F., DAVIS, J.R. and LUST, G.L. (1972). Comparative studies on the lesions caused by the eggs of Schistosoma japonicum and Schistosoma mansoni in liver of albino mice and rhesus monkeys. Ann. trop. Med. Parasit., 66 : 89-97.

HUMASON, G.L. (1972). Animal Tissue Technique. W.H. Freeman & Company.

HYMAN, L.H. (1951). The invertebrates: Platyhelminthes and Rhynchocoela. The Acoelomata Bilateria. McGraw-Hill, New York.

ITO, J. (1955). Studies on the host-parasite relationships of Schistosoma japonicum in common laboratory animals. Jap. J. Med. Sci. Biol. 8 : 42-63.

JAMES, E.R. and TAYLOR, M.G. (1976). Transformation of cercariae to schistosomula: A qualitative comparison of transformation techniques and infectivity by different injection routes of organisms produced. J. Helminth., 50 : 223-233.

JORDAN, P. and WEBBE, G. (1969). Human schistosomiasis. Heinemann Medical Books Ltd., LONDON.

JOHRI, L.N. and SMYTH, J.D. (1956). A histochemical approach to the study of helminth morphology. Parasitology, 46 : 107-116.

KUNTZ, R.E., DAVIDSON, D.L., HUANG, T.C. and TULLOCH, G.S. (1979). Scanning electron microscopy of the integumental surface of Schistosoma bovis. J. Helminth., 53 : 131-132.

KUNTZ, R.E., TULLOCH, G.S., DAVIDSON, D.L. and HUANG, T.C. (1976). Scanning electron microscopy of the integumental surfaces of Schistosoma haematobium. J. Parasit., 62 : 63-69.

KUNTZ, R.E., TULLOCH, G.S., HUANG, T.C. and DAVIDSON, D.L. (1977). Scanning electron microscopy of integumental surfaces of Schistosoma intercalatum. J. Parasit., 63 : 401-406.

KRAKOWER, C., HOFFMAN, W.A. and AXTMAYER, J.H. (1943). Portal systemic collateral veins in the guinea pig with schistosomal cirrhosis of the liver and a discussion of congestive splenomegaly. Arch. Path., Chicago, 36 : 39-50.

LAPIERRE, J. and HIEN, T.V. (1973). Un cas de triple infestation bilharzienne pas Schistosoma haematobium, Schistosoma mansoni et Rhodobilharzia margrebowiei. Annls Parasit. hum. comp., 46 : 301-306.

LAWSON, R. and DRASKAU, T. (1977). Development of schistosomulum of Schistosoma mansoni during its migration in the mammalian host. Trans. R. Soc. trop. Med. Hyg., 71 : 286.

LEE, D.L. (1966). The structure and composition of helminth cuticle. Adv. Parasit., 4 : 187-254.

LENNOX, R.W. and SCHILLER, E.L. (1972). Changes in dry weight and glycogen content as criteria for measuring the postcercarial growth and development of S. mansoni. J. Parasit., 58 : 489-494.

LE ROUX, P.L. (1933). A preliminary note on Bilharzia margrebowiei, a new parasite of ruminants and possibly of man in Northern Rhodesia. J. Helminth., 11 : 57-62.

LE ROUX, P.L. (1955). A new mammalian schistosome, (Schistosoma leiperi sp. nov.) from herbivores in Northern Rhodesia. Trans. R. Soc. trop. Med. Hyg., 49 : 293-294.

LE ROUX, P.L. (1961). Some problems in bilharziasis in Africa and the adjoining countries. J. Helminth., R.T. Leiper Supplement, 117-126.

LICHTENBERG, F. von (1962). Host response to eggs of Schistosoma mansoni. I: Granuloma formation in the unsensitized laboratory mouse.
Am. J. Path., 41 : 711-731.

LICHTENBERG, F. von, SADUN, E.H. and BRUCE, J.I. (1962). Tissue response and mechanism of resistance in schistosomiasis mansoni in abnormal hosts.
Am. J. trop. Med. Hyg., 11 : 347-356.

LINCICOME, D.R. (1962). Frontiers in research in parasitism. I: Cellular and hormonal reactions in experimental schistosomiasis.
Expl Parasit., 12 : 211-240.

LINCICOME, D.R., ENI, E.U. and STIREWALT, M.A. (1963). Dry weight and nitrogen content of Schistosoma mansoni from experimental infections.
AIBS BULL., 13 : 75-76.

LURIE, H.I. and DeMEILLON, B. (1956). Experimental bilharziasis in laboratory animals. III: A comparison of the pathogenicity of Schistosoma bovis, South African and Egyptain strains of S. mansoni and S. haematobium.
S. Afr. Med. J., 30 : 79-82.

MADDISON, S.E., NORMAN, L., GEIGER, S.J. and KAGAN, I.G. (1970). Schistosoma mansoni in the rat. I: Worm burden and serological response in infected, reinfected and antigen-sensitized animals.
J. Parasit., 56 : 1058-1065.

MALEK, E.A. (1962). Laboratory guide and notes for medical malacology.
Burgess Publishing Company.

MAO, C.P. and LYU, K.I. (1957). Studies on the cultivation of Schistosoma japonicum in vitro.
Med. Parazitol. Parazit., 26 : 166-172.

MELENEY, H.E., MOORE, D.V., MOST, H. and CARNEY, B.H. (1952). The histopathology of experimental schistosomiasis. I: The hepatic lesions in mice infected with S. mansoni, S. japonicum and

S. haematobium.
Am. J. trop. Med. Hyg., 2 : 883-913.

MICHALICK, M.S.M., GAZZINELLI, G., PELLEGRINO, J. (1979). Cultivation of S. mansoni cercarial bodies to adult worms.
Rev. Inst. Med. trop. Sao Paulo, 21 : 115-118.

MILLER, F.H., Jr., TULLOCH, G.S. and KUNTZ, R.E. (1972). Scanning electron microscopy of integumental surface of Schistosoma mansoni.
J. Parasit., 58 : 693-698.

MOORE, D.L., GROOVE, D.I. and WARREN, K.S. (1977). The Schistosoma mansoni egg granuloma : Quantitation of cell population.
J. Path., 121 : 41-50.

MOORE, D.V., YOLLES, T.K. and MELENEY, H.E. (1954). The relationship of male worms to the sexual development of female Schistosoma mansoni.
J. Parasit., 40 : 166-185.

MORRIS, G.P. and THREADGOLD, L.T. (1967). A presumed sensory structure associated with the tegument of Schistosoma mansoni.
J. Parasit., 53 : 537-539.

MORRIS, G.P. and THREADGOLD, L.T. (1968). Ultrastructure of the tegument of adult Schistosoma mansoni.
J. Parasit., 54 : 15-27.

OLIVIER, L. (1952). A comparison of infections in mice with three species of schistosomes, Schistosoma mansoni, Schistosoma japonicum and Schistosoma douthitti.
Am. J. Hyg., 55 : 22-35.

OTTENS, H. and DICKERSON, G. (1969). Bacterial invasion of schistosomes.
Nature (London), 223 : 506-507.

OTTENS, H. and DICKERSON, G. (1972). Studies on the effects of bacteria on experimental schistosome infections in animals.
Trans. R. Soc. trop. Med. Hyg., 66 : 85-107.

PAUL, J. (1970). Cell and Tissue Culture.
E & S Livingston, Edinburgh & London.

PEREZ, H., CLEGG, J.A. and SMITHERS, S.R. (1974). Acquired immunity to Schistosoma mansoni in the rat: Measurement of immunity by the lung recovery technique.
Parasitology, 69 : 349-359.

PETERS, P.A. and WARREN, K.S. (1969). A rapid method of injecting mice and other laboratory animals with Schistosoma mansoni: Subcutaneous injection.
J. Parasit., 55 : 558.

PITCHFORD, R.J. (1974). Some preliminary observations on schistosomes occurring in antelope in Central Southern Africa.
Rhodesia vet. J., 4 : 57-61.

PITCHFORD, R.J. (1975). Introduction of Schistosoma leiperi Le Roux, 1955 and Schistosoma margrebowiei Le Roux, 1933 to the laboratory.
Trans. R. Soc. trop. Med. Hyg., 69 : 362.

PITCHFORD, R.J. (1976). Preliminary observations on the distribution, definitive hosts and possible relation with other schistosomes of Schistosoma margrebowiei Le Roux, 1933 and Schistosoma leiperi Le Roux, 1955.
J. Helminth., 50 : 111-123.

PITCHFORD, R.J. (1977a). A check list of definitive hosts exhibiting evidence of the genus Schistosoma Weinland, 1858 acquired naturally in Africa and the Middle East.
J. Helminth., 51 : 229-252.

PITCHFORD, R.J. (1977b). Absence of schistosomes in potentially endemic areas in Africa.
In Medicine in a Tropical Environment ED. By J.H.S. Gear (Cape Town: Balkema), pp. 667-677.

PITCHFORD, R.J. and Du TOIT, J.F. (1976). The shedding pattern of three little known African schistosomes

under outdoor conditions.
Ann. trop. Med. Parasit., 70 : 181-187.

PITCHFORD, R.J. and WOLSTENHOLME, B. (1977). Further observations on the relationship and distribution of Schistosoma margrebowiei and Schistosoma leiperi in central southern Africa.
J. Helminth., 51 : 327-336.

PRINZMETAL, M., ORNITZ, M.E. Jr., SIMKIN, B. and BERGMAN, H.C. (1948). Arterio-venous anastomoses in liver, spleen and lungs.
Am. J. Physiol., 152 : 48-52.

RACE, G.J., MICHEALS, G.,MARTIN, J.H., LARSH, J.E.,Jr. and MATTHEWS, J.L. (1969). Schistosoma mansoni eggs: An electron microscopic study of shell pores and microbarbs.
Proc. Soc. Exp. Biol. Med., 130 : 990-992.

RACE, G.J., MARTIN, J.H., MOORE, D.V. and LARSH, J.E., Jr. (1971). Scanning and transmission electron microscopy of Schistosoma eggs, cercariae and adults.
Am. J. trop. Med. Hyg., 20 : 914-924.

RADKE, M.G., SCHNEIDER, M.D. and HOUGHTALING, D.G. (1957). Dry weight, nitrogen and phosphorous content of Schistosoma mansoni.
Expl Parasit., 6 : 202-207.

RAMALHO-PINTO, F.J., GAZZINELLI, G., HOWELLS, R.E., MOTA-SANTOS, T.A., FIGUEIREDO, E.A. and PELLEGRINO, J. (1974). Schistosoma mansoni: Defined system for stepwise transformation of cercaria to schistosomule in vitro.
Expl Parasit., 36 : 360-372.

RAU, M.E., BOURNS,T.K.R. and ELLIS, J.C. (1972). An improved method for collecting miracidia.
Int. J. Parasit., 2 : 279-280.

RITCHIE, L.S., GARSON, S. and KNIGHT, W.B. (1963). The biology of S. mansoni in laboratory rats.
J. Parasit., 49 : 571-577.

ROBINSON, D.L.H. (1957). Schistosoma mansoni in vitro. Trans. R. Soc. trop. Med. Hyg., 51 : 300.

ROBINSON, D.L.H. (1961). Phosphatases in Schistosoma mansoni. Nature (London), 191 : 473-474.

SAKAMOTO, K. and ISHII, Y. (1976). Fine structure of schistosome eggs as seen through the scanning electron microscope. Am. J. trop. Med. Hyg., 25 : 841-844.

SAKAMOTO, K. and ISHII, Y. (1977). Scanning electron microscope observations of adult Schistosoma japonicum. J. Parasit., 63 : 407-412.

SCHNITZER, B., SODEMAN, T., SODEMAN, W.A., DURKEE, J. and DURKEE,T. (1971). Microspines on Schistosoma japonicum and S. haematobium egg shells. Parasitology, 62 : 385-387.

SENFT, A.W., (1959). Electron microscope study of the integument, flame cells and gut of the human parasite, Schistosoma mansoni. Biol. Bull., 177 : 387.

SENFT, A.W. (1963). Observations on amino acid metabolism of Schistosoma mansoni in a chemically defined medium. Ann. N.Y. Acad. Sci., 113 : 272-288.

SENFT, A.W. (1969). Considerations of schistosome physiology in the search for antibilharziasis drugs. Ann. N.Y. Acad. Sci., 160 : 572-592.

SENFT, A.W. and WELLER, T.H. (1956). Growth and regeneration of Schistosoma mansoni in vitro. Proc. Soc. Exp. Biol. Med., 93 : 16-19.

SEVERINGHAS, A.E. (1928). Sex studies of Schistosoma japonicum. Quart. J. Microsc. Sci., 7 : 653-702.

SHAW, J.R. (1974). In vitro studies on the reproductive biology of S. mansoni. Proc. B. Soc. Parasit. In Parasit., 69 : XIV

SHER, A., MacKENZIE, P. and SMITHERS, S.R. (1974). Decreased recovery of invading parasites from the lungs as a parameter of acquired immunity to schistosomiasis in the mouse. J. infect. Dis., 130 : 626-633.

SHORT, R.B. (1952). Sex studies on Schistosoma douthitti Cort, Price, 1913. Trematode schistosomatidae. Am. J. Med., 47 : 1-54.

SILK, M.H. and SPENCE, I.M. (1969). Ultrastructure studies of the blood fluke - Schistosoma mansoni. III: The nerve tissue and sensory structures. S. Afr. J. Med. Sci., 34 : 93-104.

SILK, M.H., SPENCE, I.M. and BUCH, B. (1970). Observations on Schistosoma mansoni blood flukes in the scanning electron microscope. S. Afr. J. Med. Sci., 35 : 23-29.

SILVERMAN, P.H. (1965). In vitro cultivation procedures for parasitic helminths. Adv. Parasit., 3 : 159-222.

SILVERMAN, P.H. and HANSEN, E.L. (1971). In vitro cultivation procedures for parasitic helminths: Recent advances. Adv. Parasit., 9 : 227-258.

SMITH, M., CLEGG, J.A. and WEBBE, G. (1976). Culture of Schistosoma haematobium in vivo and in vitro. Ann. trop. Med. Parasit., 70 : 101-107.

SMITH, J.H., REYNOLDS, E.S. and von LICHTENBERG, F. (1969). The integument of Schistosoma mansoni. Am. J. trop. Med. Hyg., 18 : 28-49.

SMITHERS, S.R. and TERRY, R.J. (1965a). The infection of laboratory hosts with cercariae of Schistosoma mansoni and the recovery of adult worms. Parasitology, 55 : 695-700.

SMITHERS, S.R. and TERRY, R.J. (1965b). Acquired resistance of experimental infection of Schistosoma mansoni in the albino rat. Parasitology, 55 : 711-712.

SMITHERS, S.R., TERRY, R.J. and HOCKLEY, D.J. (1969). Host antigens in schistosomiasis. Proc. R. Soc. Biol., 171 : 483-494.

SMYTH, J.D. (1966). The Physiology of Trematodes. Oliver & Boyd, Edinburgh and London; W.H. Freeman, San Francisco.

SMYTH, J.D. (1976). Introduction to Animal Parasitology. (2nd. EDITION). Hodder & Stoughton, London Sydney, Auckland, Toronto.

SMYTH, J.D. (1980). In vitro culture as a 'Parasitological Tool'. Proc. Int. Con. Parasit., Warsaw, 1978. (IN PRESS).

SMYTH, J.D. and CLEGG, J.A. (1959). Egg-shell formation in trematodes and cestodes. Expl Parasit., 8 : 286-323.

SMYTH, J.D. and HOWKINS, A.B. (1966). An in vitro technique for the production of eggs of Echinococcus granulosus by maturation of partly developed strobila. Parasitology, 56 : 111-133.

SOUTHGATE, V.R. and KNOWLES, R.J. (1975). Observations on Schistosoma bovis Sonsino, 1876. J. nat. Hist., 9 : 273-314.

SOUTHGATE, V.R. and KNOWLES, R.J. (1977). On Schistosoma margrebowiei Le Roux, 1933: The morphology of the egg, miracidium and cercaria, the compatibility with species of Bulinus, and development in Mesocricetus auratus. Z. Parasitenk., 54 : 233-250.

STANDEN, O.D. (1949). Experimental schistosomiasis. II: Maintenance of Schistosoma mansoni in the laboratory, with some notes on experimental infection with Schistosoma haematobium. Ann. trop. Med. Parasit., 43 : 268-283.

STENGER, R.J., WARREN, K.S., and JOHNSON, E.A. (1967). An ultrastructural study on hepatic granulomas and schistosome egg shells in murine hepatosplenic schistosomiasis mansoni.
Expl molec. Path., 8 : 116-132.

STIREWALT, M.A. (1959). Chronological analysis, pattern and rate of migration of cercaria of Schistosoma mansoni in body, ear and tail skin of mice.
Ann. trop. Med. Parasit., 53 : 400-413.

STIREWALT, M.A. (1961). Schistosomule vs. cercaria.
J. Parasit., 47 (No. 4 Section II) : 47.

STIREWALT, M.A. (1963). Cercaria vs. schistosomule (Schistosoma mansoni). Absence of the precercarial envelope in vivo and the early physiological and histological metamorphosis of the parasite.
Expl Parasit., 13 : 395-406.

STIREWALT, M.A. (1974). Schistosoma mansoni - cercaria to schistosomule.
Adv. Parasit., 12 : 115-182.

STIREWALT, M.A. and KRUIDENIER, F.J. (1961). Activity of the acetabular secretory apparatus of cercariae of Schistosoma mansoni under experimental conditions.
Expl Parasit., 11 : 191-211.

STIREWALT, M.A., KUNTZ, R.E. and EVANS, A.S. (1951). The relative susceptibility of the commonly used laboratory mammals to infection by Schistosoma mansoni.
Ann. J. trop. Med. Hyg., 31 : 57-82.

STIREWALT, M.A., MINNICK, D.R. and FREGEAU, W.A. (1966). Definition and collection in quantity of schistosomules of Schistosoma mansoni.
Trans. R. Soc. trop. Med. Hyg., 60 : 353-360.

STIREWALT, M.A., UY, A. (1969). Schistosoma mansoni: Cercarial penetration and schistosomule collection in an in vitro system.
Expl Parasit., 26 : 17-28.

STOLL, N.R. (1929). Studies on the strogyloid nematode, Haemonchus contortus. I: Acquired resistance of host under natural reinfection conditions out-of-doors.
Am. J. Hyg., 10 : 384-418.

SULIMAN, S. (1974). The rate of development of S. japonicum in the golden hamster, Cricetus auratus.
MSc. Thesis, University of London.

TAYLOR, A.E.R. and BAKER, J.R. (1968). The Cultivation of Parasites in vitro.
Blackwell Scientific Publication, Oxford.

TAYLOR, A.E.R. and BAKER, J.R. (1978). Methods of Cultivating Parasites in vitro.
Academic Press, London, New York, San Francisco.

THREADGOLD, L.T. (1963). The tegument and associated structures of Fasciola hepatica.
Quar. J. Micr. Sci., 104 : 505-512.

TIBA, Y., HOLANDA, J.G., RAMALHO-PINTO, F.J., GAZZINELLI, G. and PELLEGRINO, J. (1974). Schistosomula (Schistosoma mansoni) obtained in vivo: viability in culture and infectivity for mice.
Trans. R. Soc. trop. Med. Hyg., 68 : 72.

TULLOCH, G.S., KUNTZ, R.E., DAVIDSON, L. and HUANG, T.C. (1977). Scanning electron microscopy of the integument of Schistosoma mattheei Veglia & Le Roux, 1929.
Trans. Amer. Micros. Soc., 96 : 41-47.

VAN DEN BERGHE, L. (1934). Les schistosomes humaine et animales au Katanga (Congo Belge).
Annls Soc. belge Med. trop., 14 : 313-371.

VOGE, M., PRICE, Z. and BRUCKNER, D.A.(1978). Changes in tegumental surface during development of Schistosoma mansoni.
J. Parasit., 64 : 585-592.

VOGE, M., PRICE, Z. and JANSMA, W.B. (1978). Observations on the surface of different strains of adult Schistosoma japonicum.
J. Parasit., 64 : 368-372.

WALKIERS, J. (1928). Five cases of schistosomiasis with spineless eggs from Upper Uele.
Annls Soc. belge Med. trop., 8 : 21-22.

WARREN, K.S. (1968). Pathophysiology and pathogenesis of hepatosplenic Schistosoma mansoni.
Bull. N.Y. Acad. Med., 44 : 280-294.

WARREN, K.S. (1973). The pathology of schistosome infections.
Helminthological Abstracts, 42 : 591-633.

WARREN, K.S. (1978). The pathology, pathobiology and pathogenesis of schistosomiasis.
Nature, 273 : 609-612.

WARREN, K.S. and DOMINGO, E.O. (1970). Granuloma formation around Schistosoma mansoni, S. haematobium and S. japonicum.
Am. J. trop. Med. Hyg., 19 : 292-304.

WARREN, K.S. and PETERS, P.A. (1967). Comparison of penetration and maturation of Schistosoma mansoni in Hamster, Mouse, Guinea Pig, Rabbit and Rat.
Am. J. trop. Med. Hyg., 16 : 718-722.

WARREN, K.S. and NEWILL, V.A. (1967). Schistosomiasis: A bibliography of the world's literature from 1852-1962. (2 vols.).
The Press of Western Reserve University, Cleveland, Ohio.

WATSON, J.M. and AZIM, M.A. (1948). Comparative efficiency of various methods of infecting mice with Schistosoma mansoni.
Ann. trop. Med. Parasit., 43 : 41-46.

WEINSTEIN, P.P. (1966). The in vitro cultivation of helminths with reference to morphogenesis.
IN: Biology of Parasites. ED. Soulsby, E.J.L. Academic Press, New York.

WHITFIELD, P.J. (1979). The Biology of Parasitism: An introduction to the study of associating organisms.
Edward Arnold, London.

WILKS, N.E. (1967). Lung-to-liver migration of schistosomes in the laboratory mouse.
Am. J. trop. Med. Hyg., 16 : 599-605.

WILSON, R.A. and BARNES, P.E. (1977). Current Ideas on the schistosome surface.
Trans. R. Soc. trop. Med. Hyg., 71 : 289-290.

WILSON, R.A., DRASKAU, T., MILLER, P. and LAWSON, J.R. (1978). Schistosoma mansoni: The activity and development of schistosomulum during migration from skin to the hepatic portal system.
Parasitology, 77 : 57-73.

WRIGHT, C.A. (1977). Flukes and snails.
George Allen and Unwin LTD.

WRIGHT, C.A. and BENNETT, M.S. (1967a). Studies on Schistosoma haematobium in the laboratory: I. A strain from Durban, Natal, South Africa.
Trans. R. Soc. trop. Med. Hyg., 61 : 221-227.

WRIGHT, C.A. and BENNETT, M.S. (1967b). Studies on Schistosoma haematobium in the laboratory: II. A strain from South Arabia.
Trans. R. Soc. trop. Med. Hyg., 61 : 228-232.

WRIGHT, C.A. and KNOWLES, R.J. (1972). Studies on Schistosoma haematobium in the laboratory: III. Strains from Iran, Mauritius and Ghana.
Trans. R. Soc. trop. Med. Hyg., 66 : 108-118.

WRIGHT, C.A., SOUTHGATE, V.R. and HOWARD, G.W. (1979). Observations on the life cycle of Schistosoma margrebowiei and its possible interactions with S. leiperi in Zambia.
J. Nat. Hist., 57 : 49-56.

YAMAGUTI, S. (1958). Systema helminthum. Vol. 1 Pt. I, II. The Digenetic Trematodes of Vertebrates.
Interscience Publishers Inc. New York.

YASURAOKA, K., HATA, H. and SAITO, K. (1972). In vitro cultivation of Schistosoma japonicum. Jap. J. Parasit., 21 (SUPPLEMENT) : 87.

YASURAOKA, K., IRIE, Y. and HATA, H. (1978). Conversion of cercariae to schistosomula in serum supplemented media, and subsequent culture in vitro. Jap. J. Expl Med., 48 : 53-60.

YOLLES, T.K., MOORE, D.V. and MELENEY, H.E. (1949). Post-cercarial development of Schistosoma mansoni in rabbit and hamster after intraperitoneal and percutaneous infection. J. Parasit., 35 : 276-294.

KOPPISCH, E. (1937). Studies on schistosomiasis mansoni Puerto Rico. IV. The pathological anatomy of experimental schistosomiasis mansoni in the rabbit and albino rat. Puerto Rico J. Pub. Hlth. Trop. Med., 13 : 1-54.