THE ROUTES OF MIGRATION AND DISPERSION OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA HAEMATOBIUM* SCHISTOSOMULA IN THE MOUSE

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The objection to most amateur sciences lies not in the foolishness of its experiments, but in the inability of the experimenters to be satisfied with negative results. Most laboratory experiments are failures, and even when an apparent success has been obtained the competent researcher at once tries to catch himself out. I am going to waste tomorrow on an experiment which I hope and trust will be a failure, for if it were a success it would not only be quite inexplicable, but would destroy the theoretical results of a year's work. Amateur scientists commonly fail because they set out to prove something rather than to arrive at the truth, whatever it may be. They do not realize that a good half of most research work consists in an attempt to prove yourself wrong. Intellectual honesty is discouraged by politics, religion and even courtesy. It is the hardest but the most essential of the habits which the scientist, whether professional or amateur, must form. And if he can spread the habit among his fellowmen it may prove to be a contribution to the good life compared to which the applications of science to engineering and medicine are comparatively unimportant.

J.B.S. HALDANE

ABSTRACT

THE ROUTES OF MIGRATION AND DISPERSION OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA HAEMATOBIUM* SCHISTOSOMULA IN THE MOUSE

by

MARGARETHA NILSSON

The routes of migration and dispersion of *Schistosoma mansoni* and *Schistosoma haematobium* schistosomula in the mouse were investigated. Factors such as larval growth, the host's response to the larvae and the changing dimensions of the larvae, which may affect dispersion, were also studied. Host exposure was percutaneous, in order to reproduce the natural mode of infection. The distribution of parasites was traced throughout the first 16 days after infection, between the point of vascular entry in the skin and the site of maturation, the liver. The results of the investigation were obtained by histological, histochemical and autoradiographic techniques. It was shown that after penetration of the skin the schistosomula take pathways in a random fashion. Larval use of the blood vascular route was demonstrated by the presence of schistosomula in the vessels of various organs and tissues. Passive transportation within the systemic circulation to all regions seems likely, including multiple circulation in the absence of larval growth. The patterns of lymphatic drainage were determined in order to be able to ascertain whether the lymphatic system facilitates schistosomular migration. Schistosomula were seen to utilize lymphatic pathways; lymph nodes did not act as barriers. Infection via the tail or hind limb resulted in schistosomula being found in the lung as early as days 2 or 3, which indicates that the route was entirely through the blood vascular system. On subsequent days the lung contained a mixed larval population comprising those which arrived solely by the venous system and those which entered the venous system through the thoracic and right lymphatic ducts. Extravascular routes may be possible when larvae abnormally retain the enzymatic mechanism used for penetration and which is usually lost during the initial phase of migration through the skin. However, few larvae adopt an extravascular route and it is doubtful whether any are able to reach the liver in this way. The author discusses her findings in detail and compares them with those of other workers. Further problems arising from the present work are outlined and discussed.

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CHAPTER 1

INTRODUCTION: GENERAL

1.1 INTRODUCTION

Schistosomiasis, (bilharziasis, snailfever) a parasitic disease of considerable importance to man in sub-tropical and tropical regions, has become, according to many, "the greatest unconquered parasitic disease afflicting humans and animals" (Weller, 1976). In 1947, Stoll estimated that 114 million people were infected; in 1959, the World Health Organization (W.H.O.) gave a figure of 150 million; Jordan in 1975 thought the total to be as high as 300 million. By 1979 the estimated number of infected individuals had risen to 350 million, with an additional 600 million at risk (Webbe, personal communication).

Schistosomiasis increasingly appears to be a by-product of progress. As such, it has been referred to as "the poor man's emphysema" (Brown, 1970). Like schistosomiasis, emphysema has a gradually debilitating effect; both diseases are brought about mainly through environmental conditions created by man. Notwithstanding the immense amount of theoretical and practical understanding now available, the incidence of schistosomiasis is increasing at a faster rate than any other parasitic disease.

1.2 DISTRIBUTION

According to W.H.O. (1965), schistosomiasis is second only

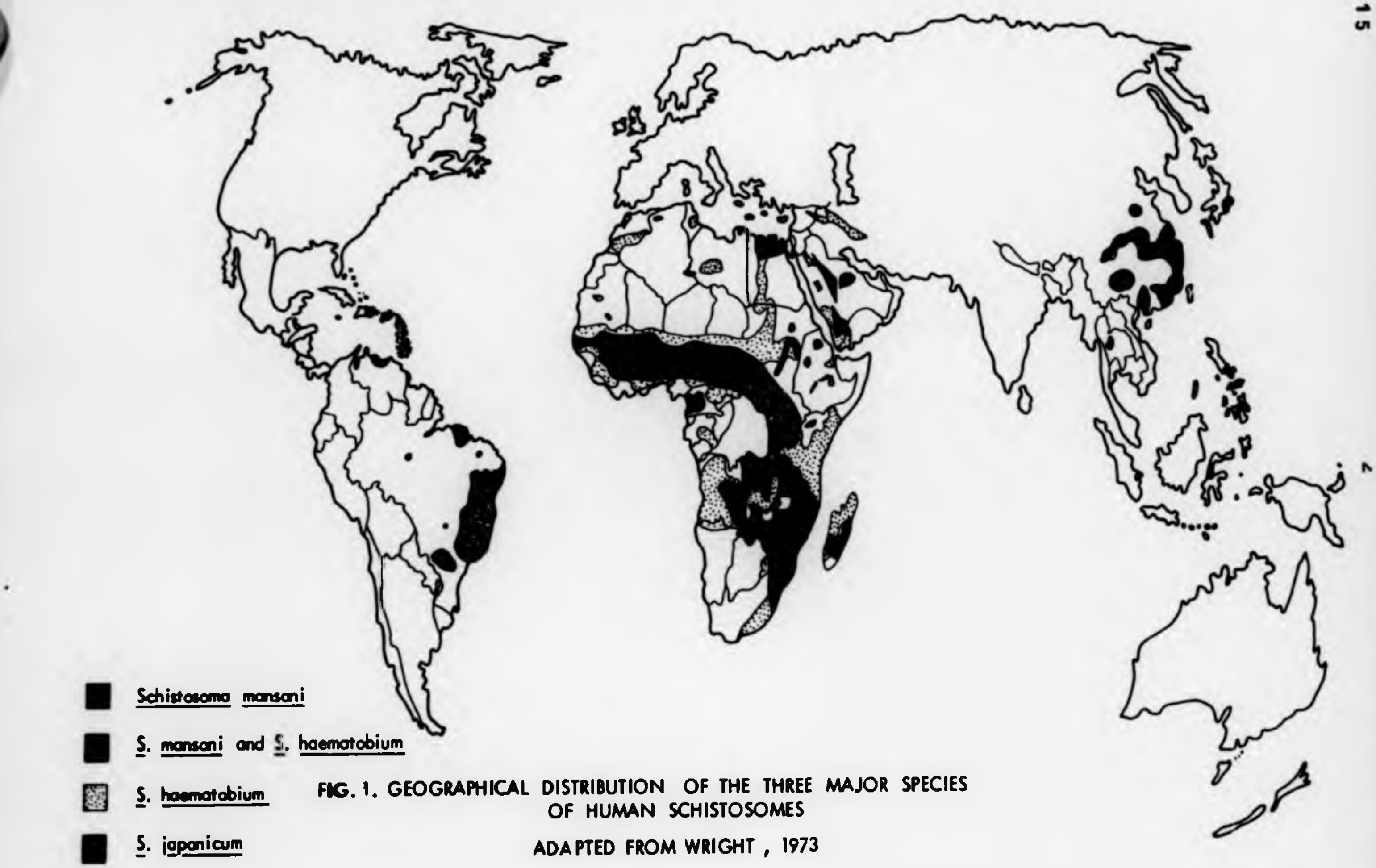

■ *Schistosoma mansoni*
■ *S. mansoni* and *S. haematobium*
▨ *S. haematobium*
■ *S. japonicum*

FIG. 1. GEOGRAPHICAL DISTRIBUTION OF THE THREE MAJOR SPECIES OF HUMAN SCHISTOSOMES

ADAPTED FROM WRIGHT, 1973

to malaria as the primary cause of morbidity and mortality in the tropics. The focal distribution is dependent on the availability of snails, the intermediate hosts, which are more widely disseminated than the disease. There are three major human schistosomes which differ in their epidemiological distribution. The principal geographical foci of the three species are shown in Figure 1, adapted from Wright (1973).

1.3 HISTORICAL BACKGROUND

There can be little doubt as to the great antiquity of schistosomiasis as a human disease. Whether or not Africa or the Middle East is considered the cradle of *S. mansoni* and *S. haematobium*, as argued by some (Wright, 1961; Nelson, Teesdale and Highton, 1962; Farooq, 1973), there is direct evidence of the disease in the calcified ova found in Egyptian mummies (Ruffer, 1910; Lewin, 1977), the oldest specimen dating from 1250 to 1000 B.C. Indirect evidence for the antiquity of the disease may be seen in the adaptation of the parasite to its human host; the complex mechanisms of this adaptation, which, in part, are the subject of the present work, and the predominance of the chronic form of the disease, would seem to argue in favour of this assumption.

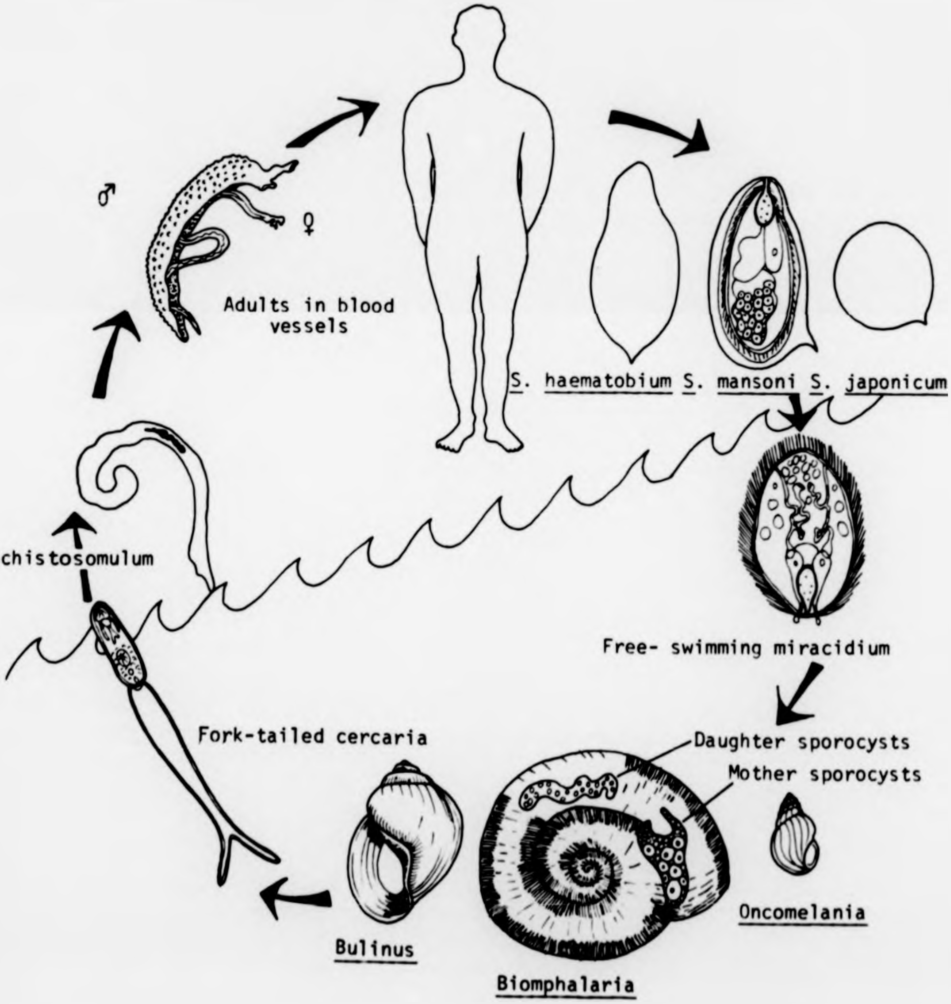
The disease may have been brought to Egypt by infected slaves and animals imported from Africa (Adamson, 1976). It is assumed that it then spread from Egypt throughout the Middle East. The further spread of schistosomiasis to the Western Hemisphere is thought to be a by-product of the African slave trade.

1.4 EPIDEMIOLOGY

Since the intermediate host is an essential link in the life cycle of all schistosomes, it goes without saying that the presence of the appropriate snail provides a potential breeding ground for the disease in all tropical and sub-tropical regions in which the snail occurs. Poverty, inadequate sanitation, nomadism, and the migration of large numbers of people, which play such a large role in these regions, contribute to the perpetuation of the disease where it is endemic or indeed its introduction into areas where it was previously non-existent. Portugal, for instance, where the snail host exists, might again become a focus of schistosomiasis, which could be introduced by people migrating or returning from the African colonies.

In many parts of the world, religious custom must be added to the factors that tend to increase the incidence of schistosomiasis. In modern times, vast irrigation projects and the opening up of virgin land, attracting

Fig. 2. Schematic life-cycle of Schistosoma mansoni, S. haematobium and S. japonicum


♂
♀
Adults in blood vessels
S. haematobium
S. mansoni
S. japonicum
Schistosomulum
Free- swimming miracidium
Fork-tailed cercaria
Daughter sporocysts
Mother sporocysts
Bulinus
Biomphalaria
Oncomelania

labour forces from endemic areas, have created a host of new foci. In this sense, schistosomiasis may be considered as a "by-product of progress" (see Introduction, page 14), though by no means an inevitable one.

1.5 LIFE CYCLE

The disease is of a chronic and insidious nature, caused by a blood fluke of the genus *Schistosoma*.

S. mansoni, *S. haematobium* and *S. japonicum* are the three principal species that complete their life cycle in man and are capable of causing systemic disease. They may infect hosts other than man, such as other primates, rodents and ungulates. The life cycles of the parasites are in essence similar (Figure 2). A sexual phase of reproduction takes place in man, the definitive host, with an asexual phase of reproduction in the snail, the intermediate host.

Man and other susceptible mammals acquire the infection from contact with fresh water which contains the free-swimming, infective larvae, the cercariae. After a transient period in the water, the cercariae enter the definitive host by actively penetrating the skin, at which time the larvae lose their tails and turn into the parasitic schistosomula. Many larvae are destroyed in the skin; those which survive eventually reach a vessel within the epidermis and migrate via the circulatory system to the right chambers of the heart and thence to the lungs. From the lungs the larval schistosomes find their way to the hepatic portal system. Here the larvae develop into male and female adult worms, mate and migrate against the blood flow to their final sites: *S. mansoni* to the smaller branches of the inferior mesenteric vein, *S. japonicum* to the branches of the superior mesenteric vein, and *S. haematobium* to the vesical, prostatic and uterine plexuses. The gravid female then leaves the male to lay her eggs in the smaller vessels. The ova, trapped in venules, pass through their walls to reach the lumen of the organ concerned, the rectum in the case of *S. mansoni* and *S. japonicum*, the bladder in the case of *S. haematobium*. The extrinsic phase of the life cycle of the parasite starts with the passage of viable eggs out of the body in urine or faeces. Upon contact with fresh water, the mature egg ruptures at once and a free-swimming ciliated larva, the miracidium, emerges. The survival of the parasite depends upon its finding the susceptible species of snails: the genus *Bulinus* for *S. haematobium*, *Biomphalaria* for *S. mansoni*, and *Oncomelania* for *S. japonicum*. The miracidia penetrate the soft tissues of the snail and change into mother sporocysts, which undergo further development and produce daughter sporocysts that migrate to the digestive gland, the liver of the snail (Pan, 1965). Here the daughter sporocyst gives rise to a large number of infective cercariae which break out of the snail into fresh water. The life cycle is completed when the cercariae enter a mammalian host.

1.6 PATHOGENESIS

Disease syndromes may follow pathological changes associated with each stage of the life cycle of the parasite in the definitive host - penetration of the skin by the cercaria, migration of the schistosomulum, the adult schistosome and the eggs.

The life span of the adult worms is estimated at between 3 and 8 years, though there are reports of parasite survival of 15 years and more (W.H.O., 1974). The females do not replicate in the mammalian host. Each female produces 300-3000 eggs per day, of which less than half are excreted in faeces or urine (Warren, 1975).

The disease syndromes associated with schistosomiasis vary according to the degree of susceptibility of the definitive host, the parasite species, the number of parasites and the frequency of reinfection. With low host susceptibility most of the parasites will die in the skin (Warren, 1973). Other factors to determine the type and degree of severity of pathological changes relate to the locality in which the parasite settles such as the portal venous system (*S. mansoni* and *S. japonicum*) or the vesical plexus (*S. haematobium*), the number of eggs produced as well as special characteristics of the eggs such as aggregation and calcification (Warren, 1973).

Apart from the chronic fibro-obstructive conditions two distinct clinical syndromes occur in schistosomiasis - schistosome dermatitis or "swimmer's itch" and acute schistosomiasis or Katayama fever (Warren, 1973). "Swimmer's itch" occurs in subjects of low susceptibility to avian or rodent schistosomes. Katayama fever is most commonly found in *S. japonicum* infections and is said to coincide with the onset of egg laying.

By far the more significant disease syndromes responsible for morbidity and mortality in schistosomiasis follow chronic fibro-obstructive changes succeeding host response evoked by those eggs which are not excreted but remain within the tissues of the host body.

Tissue damage is caused as a result of trapped eggs evoking a localized inflammatory response on the part of the host. A "pseudotubercule" or granuloma, representing an immunological reaction of the cell mediated type, is formed (Warren, Domingo and Cowan, 1967). At an early stage lymphocytes, macrophages and eosinophils infiltrate the area around the egg; at a later stage epithelioid cells and giant cells are formed. Cell proliferation leads to necrosis of the tissue alternating with repair and the formation of granulomatous tissue, which eventually results in fibrous tissue formation (Warren, 1968).

Eggs may be disseminated widely throughout the body to reach any organ. When they escape from the blood vessel into adjacent tissue they provoke the formation of granulomata, ultimately replaced by fibrous tissue.

It is the subsequent fibrosis which obstructs the flow of blood in infections with S. mansoni and S. japonicum and the flow of urine in infections with S. haematobium (Warren, 1975). These obstructive conditions eventually lead to hepatosplenomegaly and oesophageal varices (S. mansoni and S. japonicum) and to vesical and ureteric stricture (S. haematobium) with consequent functional impairment of the systems involved. Back pressure due to vesical and ureteric stricture in turn leads to hydronephrosis or pyonephrosis.

Polyposis and ulceration of the urinary bladder occur in infections with S. haematobium (Smith, Torky, Kelada and Farid, 1977). Cancer of the bladder associated with S. haematobium infections is recorded (Cheever, Kuntz, Moore, Bryan and Brown, 1976) but a causal relationship has not been established.

1.7 PURPOSE OF STUDY

A review of the literature shows that a fundamental aspect of the life cycle of the schistosome remains unclear, viz. the migration route(s) taken by the parasite within the mammalian host. Neither the lymphatic system, the blood vascular system, the extravascular route nor any combination of these pathways has been shown convincingly to be the definitive route of migration of the larva from its original point of entry, the skin, to the liver.

From the earliest descriptions up to the present, the majority of workers appear to favour the intravascular route as the one usually taken by the parasite. Others think that the normal route between lung and liver is extravascular: still others describe a dual route of migration, combining intra- and extravascular routes. The question, therefore, remains essentially unanswered.

The main aim of this study has been to elucidate the "normal" route(s) utilized by the schistosomula during their first 16 days after infection of the mammalian host.

Since the mouse has been shown to be a very good ("permissive") (Cioli, Knop, and Senft, 1977) host for *S. mansoni* but a poor ("non-permissive") one for *S. haematobium*, a study was carried out to establish the differences, if any, in migratory behaviour of the two species in the mouse. The mouse model was used extensively for *S. mansoni* but only at selected time intervals for *S. haematobium*. The investigation centered upon a histological study of the host organs, so as to ascertain the exact location of the parasites. To this effect, a detailed examination was made of the microscopic anatomy of the mouse organs most likely to harbour the parasites during their migration. This study was, moreover, essential for determining the sites where the parasitic larvae were most likely to be destroyed.

Among the many questions raised, the growth or transient arrest of growth of the larvae in relation to their paths of migration was considered to be of prime importance. This question was, therefore, dealt with in great detail, and constitutes a major part of the present study.

Another area of interest was the lymphatic system. An attempt was made to establish whether or not the lymph nodes act as a barrier to the progress of the parasite, constituting, as it were, a dead end where the larvae might be destroyed.

The elucidation of the normal pattern of migration in the mouse seemed important not only for its own sake, but also to provide a baseline for comparative studies in conditions other than the natural or normal. It was, therefore, thought to be particularly important to reproduce, in the study model, conditions of primary infection as closely comparable to the natural ones as possible. Inflammatory and immune host response may vary considerably in other conditions e.g., reinfection, multiple infections and artificially induced infections; this is an important factor to be taken into account in comparative investigations.

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CHAPTER 2

THE MIGRATING LARVA: THE SCHISTOSOMULUM

DEFINITION

LITERATURE: A SELECT REVIEW

The subject of this study is the schistosomular phase in the definitive host. During this phase, the parasitic schistosomulum, defined as the post-penetration larva, migrates from skin to liver.

The schistosomular stage is that which immediately succeeds the cercarial stage. Entry into the host by the cercaria is an active process (Stirewalt, 1966; 1971; Stirewalt and Dorsey, 1974) accompanied by decaudation. Exceptionally, the cercaria may enter the skin without shedding its tail. In the short time span between penetration by the cercaria and its transformation into a schistosomulum (about 20 minutes) the parasite undergoes remarkable changes of a morphological, biochemical and physiological nature. The literature abounds with work on these aspects (Hockley, McLaren, Ward and Nermut, 1975; McLaren and Hockley, 1977; for reviews see Coles, 1973; Stirewalt, 1974).

In the final larval stage of digenetic trematodes other than the schistosomes, the cercaria sheds its tail, becomes encysted, and is then referred to as a metacercaria. Metacercariae are passively transferred by one means or another to the definitive host. Thus the schistosome cercaria differs from the cercariae of other digenetic trematodes in two respects: (1) in its active mode of entry into the definitive host and (2) in that it does not become encysted.

In order to distinguish between the penetrating and non-encysting cercaria on the one hand, and the non-penetrating and encysting cercaria on the other, a new term had to be coined for the post-penetration stage of the former. A review of the literature reveals wide variations in the terminology used for the schistosome cercaria after skin penetration. The following terms have been used for this stage and can be found in present-day literature: schistosomulum, schistosomula, schistosomule, cercarial body, decaudated cercaria, post-penetration cercaria, agamodistome, cercaria and metacercaria. Leiper (1915) employs the terms "cercarial body" and "cercaria" for the post-penetration larva. Lampe (1927) uses the term "cercaria" throughout larval migration while Standen (1953) refers to the parasite in the skin and lymphatics as "cercaria". Griffiths (1953) and Kagan and Meranze (1955) call the parasite in the skin "cercaria"; Batten (1956) uses "cercaria" when referring to the larva in skin or venules. The same term is used as recently as 1975 by Brown in referring to the parasite after skin penetration.

The terms "cercarial body" and "decaudated cercaria", though descriptive of the post-penetration larva, are better avoided since the cercaria may for one reason or another lose its tail prior to entry.

Once the cercaria has gained entry through the skin it should no longer be referred to as a cercaria. This term immediately brings to mind the parasite in its free-swimming state - a state in which it does not exist following penetration of the host's skin.

Use of the term "metacercaria" might have been justified before the mode and portal of entry into the definitive host were established (Fujinami and Nakamura, 1909; Katsurada and Hasegawa, 1909; Matsuura, 1909; Tsuhciya, 1913 - as cited by Sasa, 1972). Since then, the term (see Faust, Jones and Hoffman, 1934; Koppisch, 1943*; Faust, Russell and Jung, 1970; Smith, Jones and Hunt, 1972) can no longer be justified and its use in respect of the schistosomular larva is considered inappropriate. Usage of the term metacercaria should be restricted to the encysted form of the digenetic trematodes.

Throughout this study the term used for the stage following penetration is "schistosomulum". The plural and adjectival forms are "schistosomula" and "schistosomular" respectively. The semantic aspect of this nomenclature has been dealt with in some detail (Li Hsü, Hybakken and Hsü, 1969).

Stirewalt (1959); Stirewalt, Minnick and Fregeau (1966); Stirewalt (1974) state that the term schistosomulum was used in 1924 (Faust and Meleney). It was, however, employed by Lutz as early as 1919. Lutz does not define it, nor does he comment on its origin. His use of the term suggests that [illegible] was already known by then and would be generally understood. A search of the literature has failed to disclose conclusively when and by whom the term schistosomulum was originally coined.

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* Previously Koppisch (1937) had referred to "schistosomulum".

Stirewalt (1959); Stirewalt *et al*. (1966); Stirewalt (1974) ascribe the use of the term schistosomulum for the "host skin" stage of the larva to Faust and Meleney (1924). Reference to the work of these authors (1924), however, shows that the term used was in fact agamodistome defined below but Faust and Meleney (1924) also used schistosomulum, without definition.

The following examples serve to illustrate the lack of unanimity as regards definition of the parasite's post-penetration stage and its end-point - the point at which the larva ceases to be a schistosomulum. Faust and Meleney (1924) state that upon penetration of the skin "the worm at this period ceases to be a cercaria and becomes an agamodistome". Pinto and Almeida (1945) use the term "schistosomulo" for the parasite after it has entered the skin but make no reference to an end-point for the post-penetration stage. Faust (1949) refers to the schistosomulum as the "immature stage of schistosomes or blood flukes, from the time of entry into the definitive host until the worm reaches sexual maturity". Stirewalt (1974) speaks of "schistosomule *in vivo* after it has penetrated and while it remains in skin". Muller (1975) adopted Faust's (1949) definition. Chatterjee (1976) describes the schistosomulum as the "immature or growing worm of schistosomes in the definitive host". Schmidt and Roberts (1977) refer to schistosomule, "a juvenile stage of a blood fluke, between cercaria and adult. It is a migrating form taking the place of a metacercaria in the life cycle". Ghandour (1978, personal communication) regards the end-point of the schistosomular stage as the beginning of feeding. Barbosa, Pellegrino, Coelho and Sampaio (1978) call the post-penetration larva in skin and lung "schistosomule".

Since there is no agreement among the authors quoted above on definition of the schistosomular stage, it is hardly surprising that the duration of this stage varies according to each author. The shortest period is given by Stirewalt (1974) from the moment of entry "and while it remains in skin", the longest, from the time of entry until the worm reaches sexual maturity, by Faust (1949) and Schmidt and Roberts (1977).

It is generally held that growth and maturation can only start after the migratory schistosomulum has reached the liver - the site where growth begins. In its initial migratory stage in the host, i.e. between skin and liver, the parasite is in an adaptive phase. After reaching the liver, it undergoes changes and enters an active phase, eventually growing to sexual maturity. Clearly there is a need to distinguish between the adaptive and the active phases in the parasite's development. No single term would serve to define or describe both these phases, and a separate designation is required for each of them. Chatterjee (1976) uses the terms "immature and growing" in defining the schistosomular stage, but in so doing he either ignores the adaptive, migratory stage of the schistosomulum, or implies that growth occurs during migration. Neither alternative is acceptable. This example underlines the need for clear definition of the schistosomular stage. Stirewalt *et al*. (1966) stress the importance of such a definition for experimental purposes; in 1974 Stirewalt comments on "the open ended" question - at what point the larva changes from a schistosomulum into a young worm. In this context it is appropriate to mention Cheever and Weller (1958) and Fu, Chow and Chiu (1976), conducting *in vitro* experiments, who speak of 17 day old parasites recovered from livers of animal hosts as "schistosomula".

Precise definition of the schistosomular stage requires stating the duration, beginning and end-point of this stage. While the start is unanimously considered to be the moment when the larva has gained entry into the host, the end-point is ill-defined. In the present study, the working definition of the schistosomulum is the post-penetration larva during its migratory phase between skin and liver so long as there is not evidence of growth as judged by the criteria employed in this investigation.

To avoid confusion it is important not only that one term be adopted for the post-penetration stage of the parasite, but that the end-point of this stage of the parasite be clearly defined in universally acceptable terms. At the conclusion of this study it is hoped that sufficient information will have been presented to indicate where this end-point should logically be placed.

CHAPTER 3

MATERIALS AND METHODS: GENERAL

3.1 PARASITES

Two species of schistosomes were used in this study, a Puerto Rican strain of *Schistosoma mansoni* (Sambon, 1907) and a Ghanaian strain of *Schistosoma haematobium* (Bilharz, 1852). The parasite *S. mansoni* was maintained at the London School of Hygiene and Tropical Medicine, Winches Farm Field Station, St. Albans, Herts.; it was derived from a laboratory colony maintained at the U.S.A. Army Tropical Research Medical Laboratory in San Juan, Puerto Rico. The Ghanaian strain was provided by Dr. K.Y. Chu, Accra, Ghana, as a prepatent infection in snails, infected from human urine.

3.2 INTERMEDIATE HOSTS

The snails used as intermediate hosts were *Biomphalaria glabrata* (Say), susceptible to *S. mansoni* and *Bulinus* (Bulinus) *truncatus rohlfsi* (Clessin), susceptible to *S. haematobium*.

3.3 MAINTENANCE OF SNAILS

The snails were kept in glass aquaria containing "conditioned water" which is tap water stored for a minimum of one week for the removal of chlorine by evaporation and of calcium by sedimentation. Each snail requires a certain volume of water which varies with the species; that used for *B. glabrata* was 200ml per snail, that for *B.B. truncatus rohlfsi* was 500ml per snail. Guppies (*Lebistes reticulatus*) were added to the water as scavengers. The minimum water-to-snail proportions were carefully observed because Chernin and Michelson (1957) showed that a snail population of high density gave a reduced rate of growth and a reduction in fecundity. According to Wright (1960), these effects are due not to overcrowding as such, but to products excreted by the snails. The water in the aquaria was oxygenated using "Hy-Flo" pumps to which airstones were attached. Temperatures were maintained between 25°-27°C. It has been shown that temperature plays a crucial role in the development of the larval schistosomes within molluscs. The aquaria were illuminated with warm-white fluorescent tubes for 10 hours a day. The food source was dried lettuce given three times a week. These conditions are suitable for maintenance and breeding of both snail species used.

3.4 INFECTION OF SNAILS

The eggs of *S. mansoni* were obtained from 6-8 weeks old mouse infections using the liver as source. The eggs were extracted from the liver by maceration and sedimentation in isotonic sodium chloride (Webbe and James, 1971) and hatched by incubation at 27°C. A light was provided within the incubator to ensure hatching (Standen, 1951). Snails measuring 4-5mm in diameter were individually infected with 6-8 miracidia by placing them in the wells of haemagglutination plates. Each well contained 1ml of "conditioned water" and the miracidia. The snails were left in the wells for five hours at 25°-27°C and then transferred to the aquaria.

The eggs of S. haematobium were collected from urine samples obtained from school children in the Upper Volta region. The eggs in the urine were concentrated by sedimentation. Distilled water was added to the deposit and the procedure described for S. mansoni (page 36) was then followed.

3.5 DEFINITIVE HOST

The definitive host used throughout this study was the white laboratory mouse, Mus musculus. The strain used was Theiler's Original (T.O.). Adult male mice, approximately 35 days old and weighing between 20-25g when infected, were used. The host animals were supplied by Tuck's Ltd., Rayleigh, Essex. They were fed ad lib. on diet 86E from Dixon and Sons Ltd., Ware, Herts. The mouse was chosen because its small size facilitates a comprehensive histological study of whole organs by serial sectioning. The male mouse was preferred as it has been shown to be more susceptible to schistosome infection than the female

(Purnell, 1966).

3.6 METHOD OF INFECTING THE DEFINITIVE HOST

The mammalian host was infected percutaneously with cercariae, using the tail as the initial site of penetration (Figure 3). Each animal was exposed to a 10ml cercarial suspension for a period of 1 hour. The tail-immersion method was preferred in this study to the more frequently used ring method (Smithers and Terry, 1965) for the following reasons:

1. The hosts remain unanaesthetized and partially restrained.
2. The elimination of anaesthesia prevents a fall in body temperature commonly associated with it.
3. Abnormal mode of entry through skin abrasion consequent upon shaving is eliminated by this technique.
4. The tail-immersion method has the further advantage of simplifying the tracing of the exact route(s) of migration after initial entry of the parasites. This is of importance in studying the subsequent dispersion of the schistosomes via the lymphatic and blood vascular systems.
5. The surface area of the tail exposed to cercariae is approximately 5 times as great as the abdominal surface area exposed in the ring method on mice

Figure 3

Two techniques for exposing mice to the cercariae of *S. mansoni* and *S. haematobium*:

a) the tail-immersion method, giving a skin exposure area of approximately 670mm^2.

b) the ring method, giving a skin exposure of approximately 132mm^2.

ae of

xposure

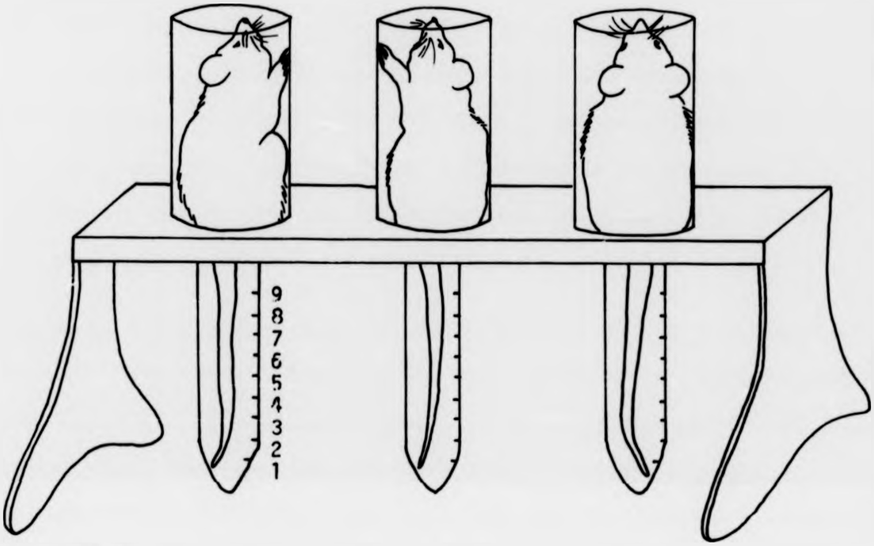


Fig. 3(a).

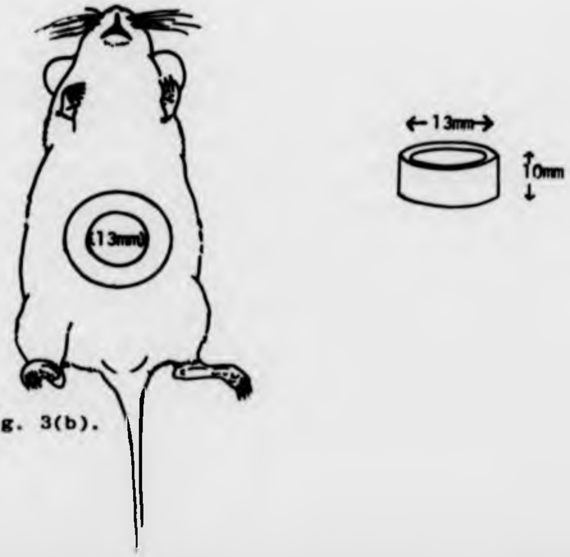

13mm
←13mm→
10mm

Fig. 3(b).

> (Figure 3). The larger skin surface area reduces the possibility of multiple invasion (more than one cercaria penetrating through the same puncture). Thus all the cercaria are likely to be similarly affected by difficulties in invasion, and those which penetrate may be expected to be in a similar physiological state after entry.

Cercariae were collected by shedding the snail intermediate hosts at 25°-27°C. The snails were placed in conditioned water under a strong artificial light. The time of exposure varied with the species of parasite: for S. mansoni approximately 4 hours. In addition to the usual technique, production of cercariae of S. haematobium can be stimulated by changing water at frequent intervals during shedding. The cercarial concentration was determined by taking a 0.1ml sample of the suspension with a precision micropipette. The sample was transferred to a squared glass plate. The cercariae were stained and killed with Lugol's iodine (1% aqueous solution) and counted. The mean count of 6 aliquot samples was obtained.

Mice were infected with approximately 2000 cercariae when only juvenile stages were required for study; if adult worms were required approximately 100 cercariae were given. A post-infection check on the suspension showed that approximately 94% of the cercariae had attached themselves to the host, although it was not known what proportion of these achieved a successful penetration. The experimental infections were much greater than would be expected under natural conditions. This was an advantage in the present work because the probability of finding the parasites in the tissues of the mouse was thereby increased. If the probability were too low it would be difficult to establish the dispersion pathways.

3.7 PHOTOGRAPHY

Black and white photographs were taken with a Zeiss II compound microscope using Ilford Pan F negative film rated at 50 A.S.A. The exposed film was developed in Ilford Perceptol developer diluted 1:1 for 19 minutes at 20°C with 5 seconds agitation every 60 seconds. The film was rinsed in water and fixed in Johnson's Fix-sol fixer, diluted 1:3, for 10 minutes at 20°C. After fixing, the film was washed for 30 minutes in running water, then rinsed in distilled water which contained Cascade wetting agent, and dried.

Prints were made of the negative using an Opemus III enlarger fitted with a 50mm Rokker lens. Exposures were made on Kodak Veribrom paper (size A4), glossy, grades 2 and 3, which was dish-developed using Johnson's Contrast developer, diluted 1:9. The prints were then rinsed in an acid stop bath and fixed in a fixer which contained sodium thiosulphate 500g and sodium metabisulphite 50g made up to 1000ml with distilled water, diluted 1:4 for 5 minutes. The prints were then rinsed in running water for 4 minutes and dried at room temperature. The Veribrom resin-coated paper has the advantage of drying flat and to a naturally glossy surface. The washing time is also reduced. Colour photographs were taken with a Zeiss photomicroscope II. Agfachrome L, rated at 50 A.S.A. and Kodak photomicrography, rated at 16 A.S.A. were used, and processed commercially.

CHAPTER 4

THE QUESTION OF GROWTH OF THE MIGRATING SCHISTOSOMULUM

4.1 INTRODUCTION

Whatever the main route(s) of schistosomular migration, the crucial question of larval growth between the time of penetration of the skin and arrival at the liver has not so far been settled. It has been established that schistosomular growth, i.e. cell division, takes place in the liver (Clegg, 1959; 1965; Smith, Clegg and Webbe, 1976). The problems to be resolved in this part of the study were, (a) whether growth occurs during migration, i.e. before the larva reaches the liver; (b) whether, during larval migration, there is a relationship between feeding and growth, and (c) the effect of this relationship, if any, on the pattern of migration.

In this study, growth is defined in terms of DNA synthesis and mitosis, which are separate but interdependent processes.

Previous investigations have been concerned primarily with gross changes, such as overall increases in size (Cort, 1921), development of the digestive tract (Faust, Jones and Hoffman, 1934; Yolles, Moore and Meleney, 1949; Clegg, 1959; 1965; Smith _et al_., 1976; Ghandour, 1978; Ghandour and Babiker, 1978) and development of the genital tract (El-Gindy, 1951). Prior to these morphological changes growth in terms of DNA synthesis and mitosis undoubtedly occurs.

The only study so far involving DNA synthesis in the schistosomal larva, though not concerned with the question of schistosomular growth, is that of Reid, Phillips and Roscinski (1977), who labelled infected snails in order to distinguish schistosomular sub-populations by means of cercarial uptake and retention of the label.

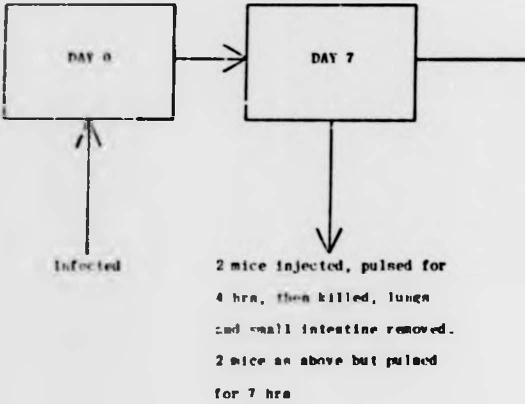
Inhibition of cell division by colchicine, in parasites removed from lung and liver tissue, has been studied in the mouse (Clegg, 1959; 1965) and in the hamster (Smith *et al*., 1976). Aceto-orcein squash preparations were used, a technique first employed by Smyth (1956). No evidence of mitosis was found in larvae of *S. mansoni* removed from the lung on day 7 (Clegg, 1965) or *S. haematobium* on day 8 after infection (Smith *et al*., 1976). Larvae removed from the liver, however, showed evidence of mitosis on day 15 (*S. mansoni*) and day 16 (*S. haematobium*).

In vitro experiments on transformed cercariae were carried out by Clegg (1965) and Clegg and Smithers (1972) (see page 85).

The present study involved the following two types of *in vivo* investigations, performed on mice infected with larval schistosomes. Intact organs from animals killed at selected time intervals were examined histologically with parasites *in situ*. In one set of experiments tritiated thymidine was used to label deoxyribonucleic acid (DNA); colchicine was used in the second set of experiments to arrest mitosis.

4.2 INCORPORATION OF TRITIATED THYMIDINE

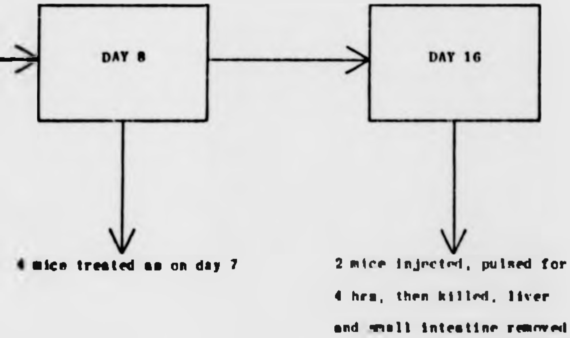
If DNA synthesis occurs, the cell nucleus may be labelled autoradiographically with specific radioactive precursors of DNA. The isotope is incorporated while the cell is actively engaged in DNA replication, during the S phase of the mitotic cycle, immediately before cell division. The labelled compound most often used as a tracer for cells that undergo DNA synthesis is (^{3}H) thymidine. This label is specific for DNA (Amano, Messier and Leblond, 1959). Tritiated thymidine injected into an animal appears either in DNA or tritiated water within 1 hour (Steel, 1962). THe tritium of labelled thymidine which is incorporated into DNA is referred to as "non-volatile tritium" whilst that found in tritiated water is called "volatile tritium" since it can be easily removed from tissues by fixation and washings (Steel and Lamerton, 1965). Microautoradiography was employed to investigate the non-volatile component i.e. the extent of thymidine incorporation into the schistosomulum. In this _in vivo_ procedure, the parasite is exposed to tritiated thymidine _in situ_. The parasitized tissue is then coated with a sensitized photographic film. If thymidine has been incorporated, dark grains are formed in the adjacent emulsion due to beta-emission from the tritiated thymidine. This method is sensitive to low levels of radioactivity and shows the location of the isotope within the cell; its specific advantage lies in the high resolution due to the short-range radiation and low energy of tritium.


DAY 0
DAY 7
Infected

2 mice injected, pulsed for 4 hrs, then killed, lungs and small intestine removed.

2 mice as above but pulsed for 7 hrs

Fig. 4


DAY 8
DAY 16
4 mice treated as on day 7
2 mice injected, pulsed for 4 hrs, then killed, liver and small intestine removed

4.2.1 MATERIALS AND METHODS: thymidine labelling

Infecting animals

Mice were infected with approximately 1000 *S. mansoni* or *S. haematobium* cercariae by the tail-immersion method (see page 38). Labelling with the nucleoside, (methyl-^{3}H) thymidine, sp. act. 17 Ci/mmol, (Radiochemical Centre, Amersham, U.K.) was carried out 7, 8 and 16 days (8 and 16 days in the case of *S. haematobium*) after infection in each of the respective groups. 2.5μCi of radioisotope per g of body weight was injected intraperitoneally in unanaesthetized animals. This dosage was used because Mendelsohn (1960) showed that at levels up to 2.5μCi per g of body weight the label had no detectable radiation effect. Isotonic sodium chloride was employed as a diluent. The animals were killed with ether either 4 or 7 hours after a single injection of nucleoside (4 or 7 hour "pulsing"), as shown in Figure 4. Lungs, small intestines and livers were removed immediately, and fixed as described later (see Preparation of tissues, page 50).

Days 7 and 8 were chosen because schistosomula in the lungs are then in the migratory phase and day 16 because by that time they have reached the liver and are considered to have terminated their migratory phase.

Evidence of successful labelling was obtained by examining the small intestine since it contains mitotically active cells.

Controls for the autoradiographic process were obtained at 7 and 8 days post-infection from the lungs and small intestines and at 16 days from the livers and small intestines of a group of unlabelled animals.

Preparation of tissues

Tissues were immersed in Carnoy's fixative at 4°C for 24 hours, passed through 2 changes of absolute ethyl alcohol at 4°C for a total of 24 hours, then cleared in 2 changes of Supercedrol (Gurr, U.K.) for a total of 24 hours and finally embedded in paraffin wax. 5μm sections were cut and mounted on "selected micro slides" (Chance Ltd., Birmingham, U.K.), which had been washed thoroughly in absolute ethyl alcohol. These slides were preferred as they gave a very low background count. After mounting, the sections were dried and the wax was removed with xylene and brought down to distilled water through a graded series of ethyl alcohol. The slides were then washed in at least 3 changes of distilled water over a period of 60 minutes before being coated with emulsion.

Preparation of autoradiographs: the stripping film technique (Appleton, 1972; Rogers, 1973)

The technique is carried out in a dark-room, at least 1m away from a safelight fitted with a dark red filter (Wratten Series No. 2, Kodak Ltd., U.K.) containing a 15 watt bulb.

Kodak AR 10 stripping film, floated out on a solution of sucrose-bromide (Stevens, 1974) at 25°C for 2-3 minutes to allow maximum expansion of the film, was applied to the slides. After coating, the slides were dried in a vertical position for 20-30 minutes and stored in light-proof boxes containing self-indicating silica gel, a drying agent. To prevent fogging, each box was further sealed with black adhesive tape and wrapped in black paper. The autoradiographs were exposed for 3 months at 4°C. The photographic process was as follows. All solutions were used at a temperature of 17°-18°C. Using Analar reagents and distilled water the autoradiographs were developed in freshly made Kodak D-19 developer for 6 minutes, rinsed in distilled water for 30 seconds and then fixed for 10 minutes in Fix-Sol (Johnson's, Hendon, U.K.) diluted 1:9 with distilled water. The slides were initially agitated for 10 seconds at each stage of treatment. The fixed slides were then washed in running water for 15 minutes and dried. Drying, at this stage, prevents the emulsion floating off the slide during subsequent treatment. The autoradiographs were stained in Harris's haematoxylin after drying was complete (approximately 24 hours).

The staining procedure was as follows:

1. the slides were soaked in distilled water for 30 minutes,
2. stained in Harris's haematoxylin diluted 1/1 with distilled water for 30 minutes,
3. rinsed in distilled water,

4. differentiated in 0.2% hydrochloric acid (aqueous) for 45 seconds,
5. counterstained in 0.2% eosin (aqueous) for 60 seconds,
6. rinsed in distilled water,
7. air dried.

After thorough drying, the excess emulsion was removed from the back of the slide by means of a scalpel.

Examination of autoradiographs

The distribution of the relative level of radioactivity in the preparations was determined by counting the number of silver grains over a source in standard areas of the autoradiographs. For this purpose, a Leitz De Luxe microscope, (X10 eyepiece and X100 oil immersion objective) was employed. The standard counting square of the eyepiece graticule at the magnification used corresponded to $225 \mu m^2$.

Each slide contained a number of sections, of which those containing parasites were counted. Five unit areas were counted within the parasite area and a further 5 within the tissue surrounding the parasite. Five unit areas were selected as being an adequate sample size following Simpson (1977) who reported that an increase in the sample size gave no greater accuracy. One hundred unit areas of the photographic emulsion only, covering each slide, were counted to provide a mean background density, i.e. natural radiation from sources other than those created by the experimental source

(Rogers, 1973). Autoradiographs can only be considered genuine when well above background (Pelc, 1972). The relative level of radioactivity for each parasite was expressed as the mean number of silver grains per unit area of emulsion ($225\mu m^2$). This was compared with the mean values for the unit areas over host tissue and over emulsion only.

The significance of the differences between mean counts over the various sources was tested by the "Student's t" test.

4.2.2 RESULTS

In agreement with published reports, (Hughes, Bond, Brecher, Cronkite, Painter, Quastler and Sherman, 1958) the incorporation of labelled thymidine was found to be confined exclusively to the cellular nuclei.

The background count was of the order of 0-4 silver grains per unit area. This was based on a count of 800 unit areas. The mean count of grains per unit area was of the order of 1.1-2.2. Parasites which showed a mean count above the highest background counts obtained i.e. above 4 silver grains per unit area, were considered to be labelled.

Control: tissues for the autoradiographic process

No significant difference in the distribution of silver grains over the emulsion surrounding the non-radioactive parasitic tissues and the emulsion surrounding the radioactive parasitic tissues was observed. It was, therefore, concluded that any uptake within the radioactive parasitic tissues was due to the effects of the precursor and not to extraneous background radiation as shown in the following:

Lung preparations: *S. mansoni* schistosomula *in situ* 7 and 8 days post-infection

Sections of 100 different parasites from each of 4 hosts were examined. There was no significant difference between relative levels of radioactivity found in the parasite, surrounding tissue or photographic emulsion ($P<0.20$). The figures for the grain counts from one slide (5 parasites) are shown in Table 1 (Appendix, page 308). The same results were obtained with both 4 hour and 7 hour pulses of labelling, and are representative of all the parasites examined in lung tissues.

These results demonstrate that no uptake by the schistosomula of labelled thymidine occurred in any of the lung preparations.

Control: lung tissue adjacent to parasite

Lung tissue containing parasites, from the same animals, was examined. As anticipated, the lung tissue adjacent to the parasites showed no incorporation of radioactive thymidine - Table 1 (Appendix, page 308).

Control: small intestine from the same animals

Samples from four host animals were counted. As expected, in view of the high cell turnover, the cellular nuclei of the small intestine showed high levels of incorporation (Plate 1A). It may thus be concluded that the non-incorporation recorded for schistosomula within the lungs (see above) which were obtained from the same four animals and treated in an identical fashion, is unlikely to be due to faulty technique.

Liver preparations: *S. mansoni* larvae *in situ* 16 days post-infection

Nineteen out of 25 parasites (76%) found within the liver 16 days after infection, (4 hour pulses) showed relative levels of radioactivity which were highly significant ($P<0.001$) - Figure 5 and 6. The figures for the grain counts from 25 parasites are shown in Tables 2-8 (Appendix, pp. 309-315). In the unlabelled parasites, control samples from the host small intestine showed the usual high level of relative radioactivity. Counts varied from area to area within the individual larva (Figure 7). There was also variation between labelled larvae (range of means 5.6-31.4 grains per parasite - 5 unit areas (Figure 5). An example of a labelled larva is shown in Plate 1B.

Control: liver cells from the same animals

As expected, the pattern for the uptake of radioactive

PLATE 1

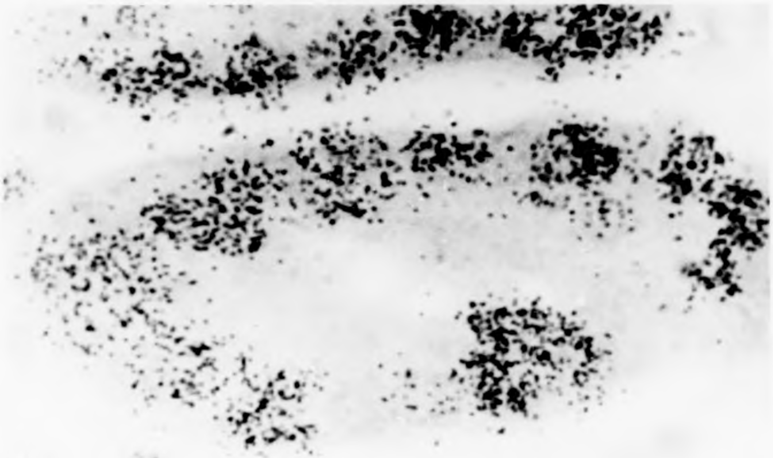
Autoradiographs of histological sections

A) Control: tritiated thymidine labelled nuclei of crypts of small intestine from the same mouse as in (B) 4μm Stained H. and E. (X1000 oil immersion)

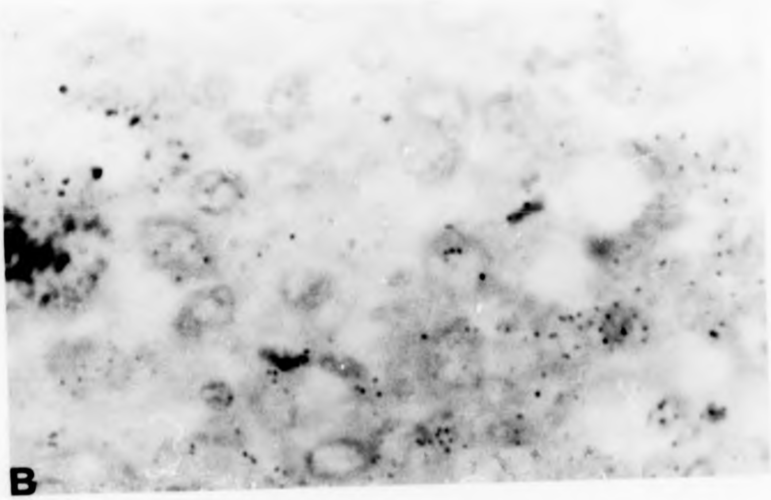
B) Tritiated thymidine labelled nuclei of larva (_S_. _mansoni_) 16 days post-infection in liver 4μm Stained H. and E. (X1000 oil immersion) ions

uclei of
mouse as
l immersion)

arva
iver 4μm

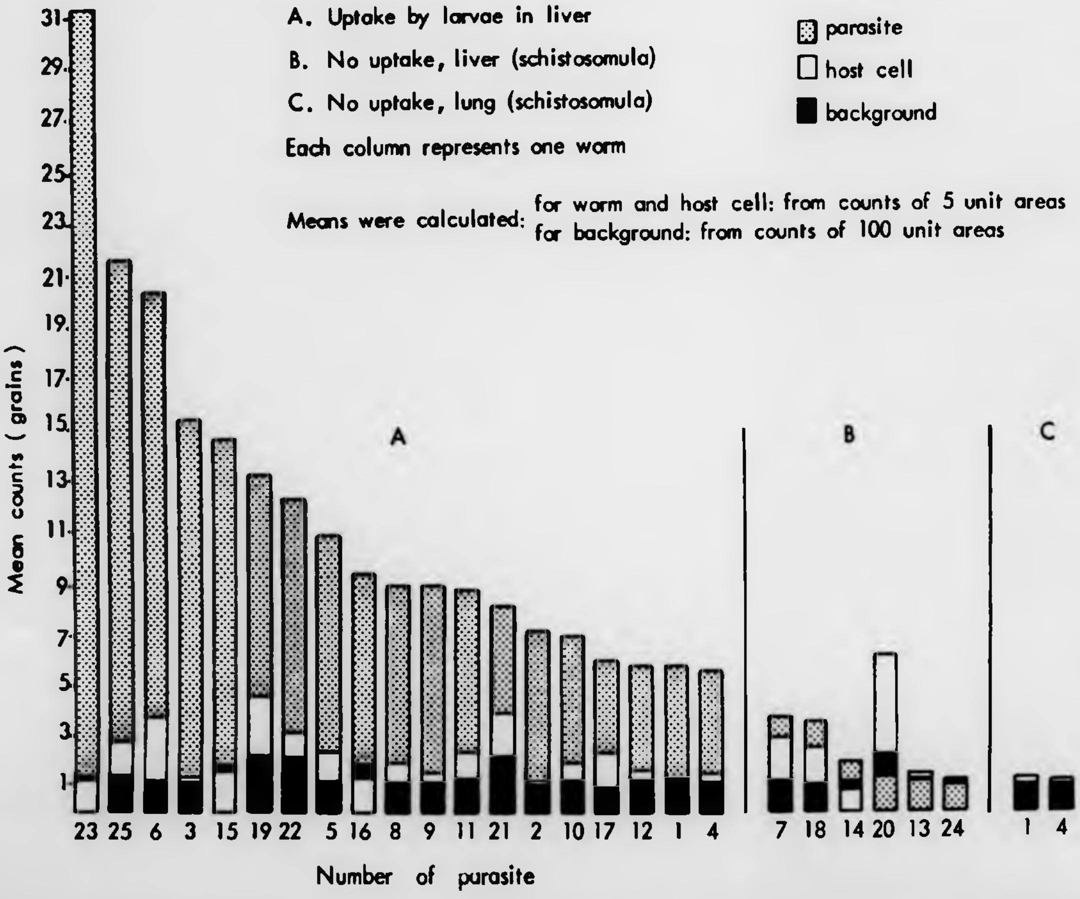


A



B

Fig. 5 Incorporation of tritiated thymidine by S. mansoni larvae. Larvae in situ in mouse liver and lung



A. Uptake by larvae in liver

B. No uptake, liver (schistosomula)

C. No uptake, lung (schistosomula)

Each column represents one worm

Means were calculated: for worm and host cell: from counts of 5 unit areas
for background: from counts of 100 unit areas

Fig. 6 Incorporation of tritiated thymidine by S. mansoni larvae. Larvae in situ in mouse liver and lung

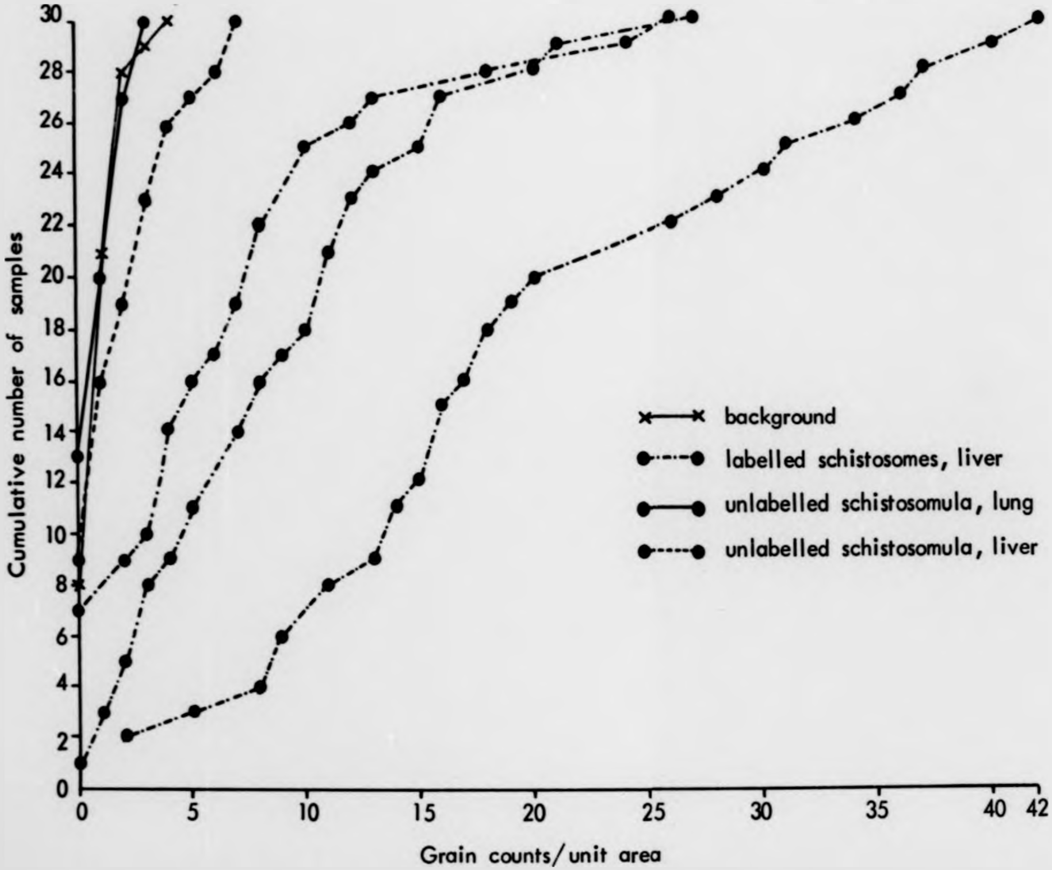
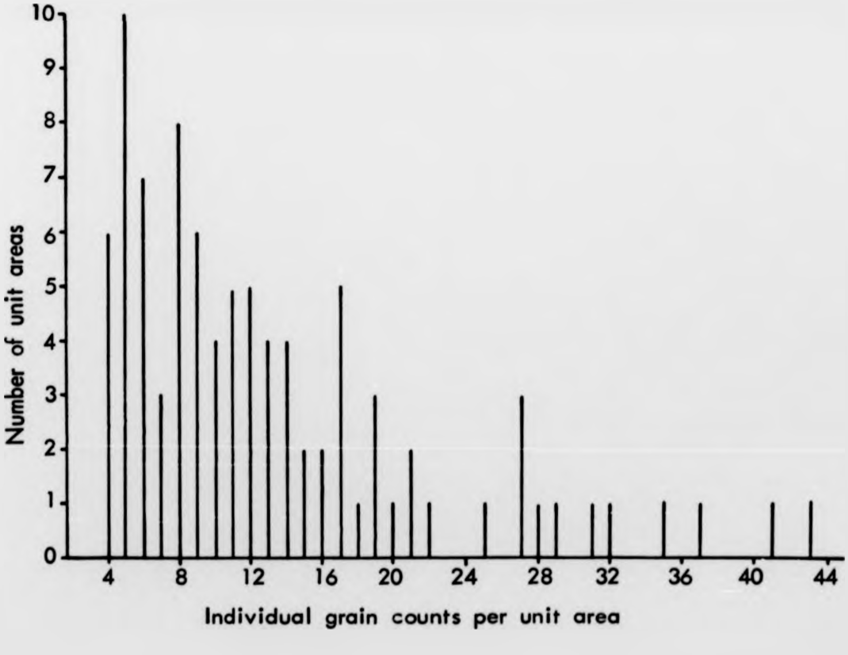


Fig. 7 Variation in grain counts between unit areas within labelled parasites



thymidine by the liver cells is typified by the small amount incorporated - Plate 2 and Tables 2-8 (Appendix, pp. 309-315).

S. haematobium in situ; lung forms day 8 and liver forms day 16 post-infection

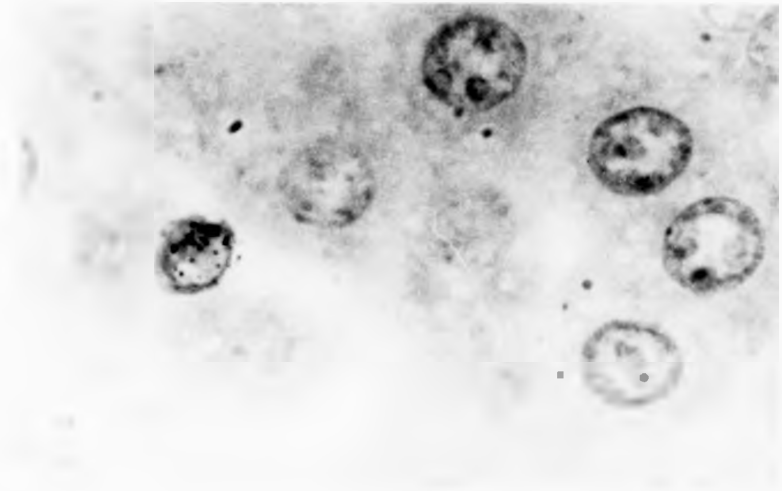
The larvae of *S. haematobium* also showed non-incorporation of radioactive thymidine at the lung stage - Table 9 (Appendix, page 316). Incorporation was observed at the liver stage only - Table 10 (Appendix page 317).

4.3 COLCHICINE ARREST

Cell division (mitosis) of the parasite can be demonstrated *in situ* by the *in vivo* introduction of a cytostatic agent such as colchicine ($C_{22}H_{25}O_6N$, an alkaloid of *Colchicum autumnale*). The effect of colchicine on cell division is direct, in that it arrests the process of mitosis at the metaphase stage by blocking spindle formation, causing the the accumulation of nuclear material at metaphase; thus, the chromosomes do not separate. Chromosomes appear shorter and thicker after treatment with colchicine and their arrangement resembles a mitotic figure, known as a colchicine metaphase. In a section the chromosomes of a dividing cell are cut at various angles and they appear as dark bodies of varying size and irregular shape. A cell in

PLATE 2

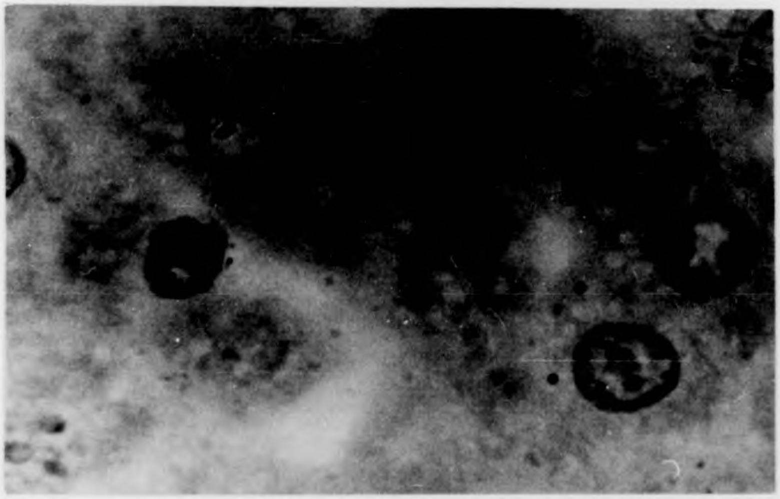
Autoradiograph of histological section



Control: tritiated thymidine labelled nuclei of hepatic cell from same mouse as in (1B) 4µm Stained H. and E. (X1000 oil immersion)

PLATE 2

Autoradiograph of histological section



Control: tritiated thymidine labelled nuclei of hepatic cell from same mouse as in (1B) 4μm Stained H. and E. (X1000 oil immersion) this state indicates that cellular growth is occurring, since division creates the condition for growth. Borisy and Taylor (1967) have demonstrated that colchicine acts specifically on the protein subunits of microtubules, including those of the spindle.

4.3.1 MATERIALS AND METHODS: colchicine arrest

Infecting animals

Mice were infected with approximately 1000 *S. mansoni* or *S. haematobium* cercariae by the tail-immersion method (see page 38). On days 6-16 (days 8 and 16 in the case of *S. haematobium*) after infection unanaesthetized animals were injected intraperitoneally, at a dosage of 4μg per g of body weight with colchicine (Hopkin and Williams, U.K.). Isotonic sodium chloride was employed as a diluent. The animals were killed with ether 4 hours after the colchicine injection. Lungs, livers and small intestines were removed.

Due to the presence of mitotically active cells, the small intestine provided a useful control for the efficacy of the colchicine treatment.

Preparation of tissues

The tissues were fixed in Carnoy's fixative or formol saline, dehydrated, cleared and embedded in paraffin wax. Sections were cut at 4 and 5µm and mounted on slides, dewaxed in xylene and brought down through a graded series of ethyl alcohol to water. The sections were stained using either Harris's haematoxylin, Heidenhain's iron haematoxylin or Bargmann's chrome haematoxylin (Pearse, 1968). When the latter staining technique was used the oxidation stage was omitted. The sections were then dehydrated, cleared and mounted.

4.3.2 RESULTS

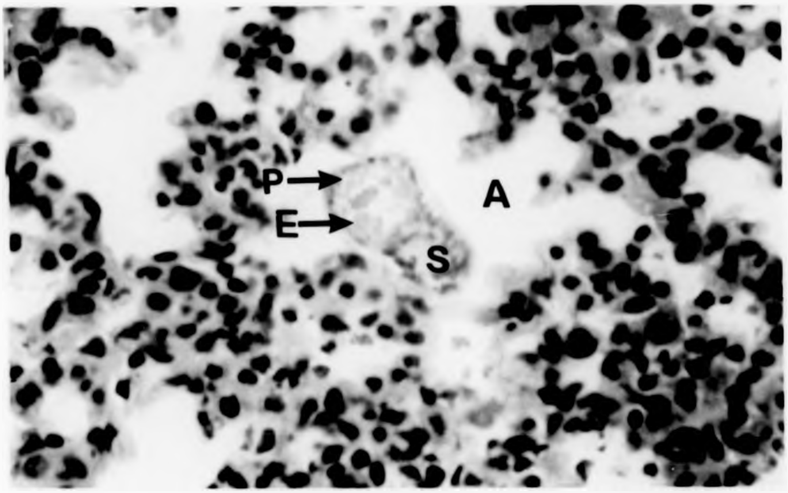
Lung preparations: *S. mansoni* schistosomula *in situ* 6-16 days post-infection

Four mice were killed on each of days 6 to 16 after infection. Sections of 100 parasites in lung tissue from this group of animals for each post-infection day were examined. No sign of mitosis or feeding was seen in any of the intravascular schistosomula examined. Neither was there any evidence of mitosis in intra-alveolar schistosomula containing yellow pigment and/or intact erythrocytes in the lumen of the gut (Plates 3 and 4). Plate 4 demonstrates the value of examining sequential serial sections.

Control: small intestine from the same animals

A large number of mitotically active cells were found in

PLATE 3



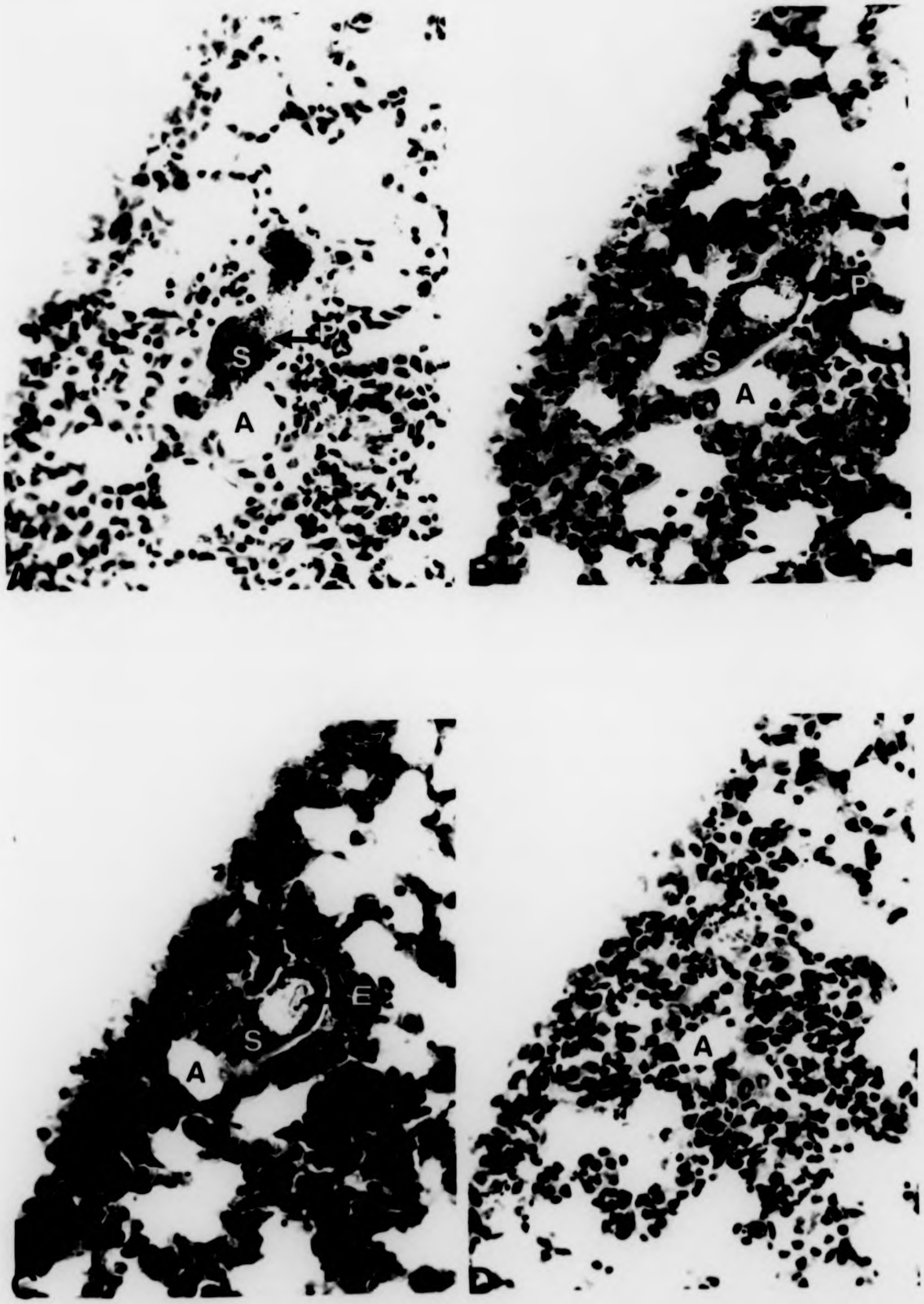
Colchicine treated schistosomulum (S. mansoni), in lung alveolus and containing yellow pigment and intact erythrocytes; no mitosis evident. 14 days post-infection 5µm Stained H. and E. (X400)

A - Alveolus
E - Erythrocyte
P - Pigment
S - Schistosomulum

PLATE 4

A, B, C and D) Serial sections of lung showing a colchicine treated schistosomulum (*S. mansoni*) in an alveolus. No mitosis is evident. (A and B) Pigment present in larval gut. (C) Erythrocyte contained in larval gut. (D) Schistosomulum no longer present. 14 days post-infection 5μm Stained H. and E. (X390)

A - Alveolus
E - Erythrocyte
P - Pigment
S - Schistosomulum


S
P
A
A
S
P
A
E
S
A
C
A
D

the sections of the small intestine from the respective host animal, showing the technique to be adequate (Plate 5A).

Liver preparations: *S. mansoni* larvae *in situ* 6-16 days post-infection

No sign of mitosis was observed in any of the sections examined (total 75) of parasites *in situ* in the liver, on days 6, 7 and 8 after infection. On day 9 and progressively thereafter until day 16, the majority of larvae showed mitotic figures; the number of mitoses increased with time after arrival in the liver (Plate 6 and 7). The mitotic figures were observed to occur indiscriminately throughout the larvae (Plate 8D). When mitosis occurred, heavy black pigment was observed in the lumen of the gut of the respective larvae. Mitosis was not seen in association with yellow pigment and/or erythrocytes in the lumen of the larval gut (Plate 9). In order to determine the presence (or absence) of pigment in the lumen of the gut of the larva it was important to examine serial sections (Plate 8A, B and C).

Control: small intestine from the same animals

The tissues showed mitotic figures. This was accepted as evidence of the effectiveness of the technique.

PLATE 5

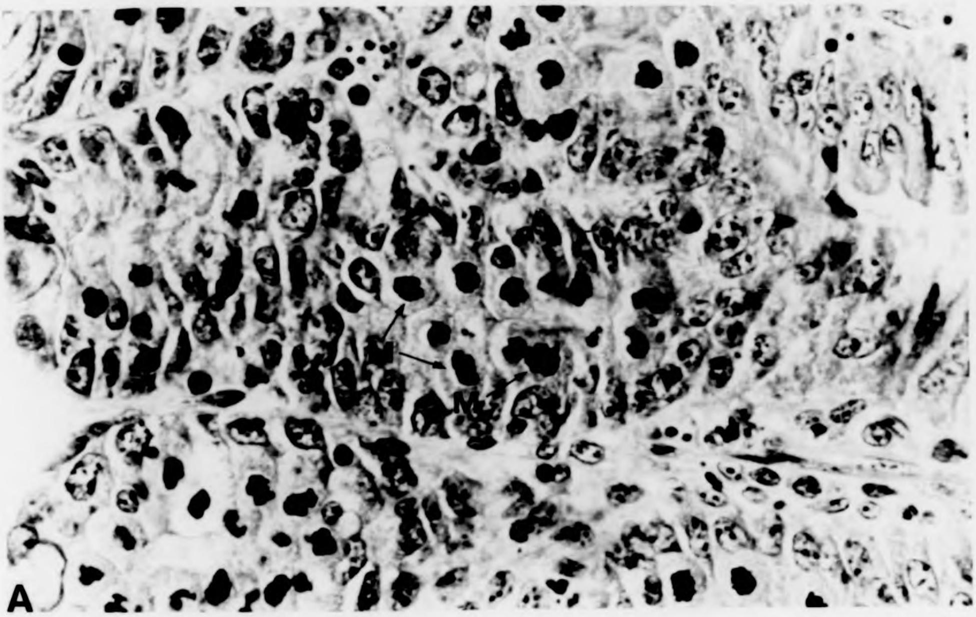
A) Control section of small intestine (colchicine treated) showing mitotic activity. 4µm Stained H. and E. (X1562 oil immersion)

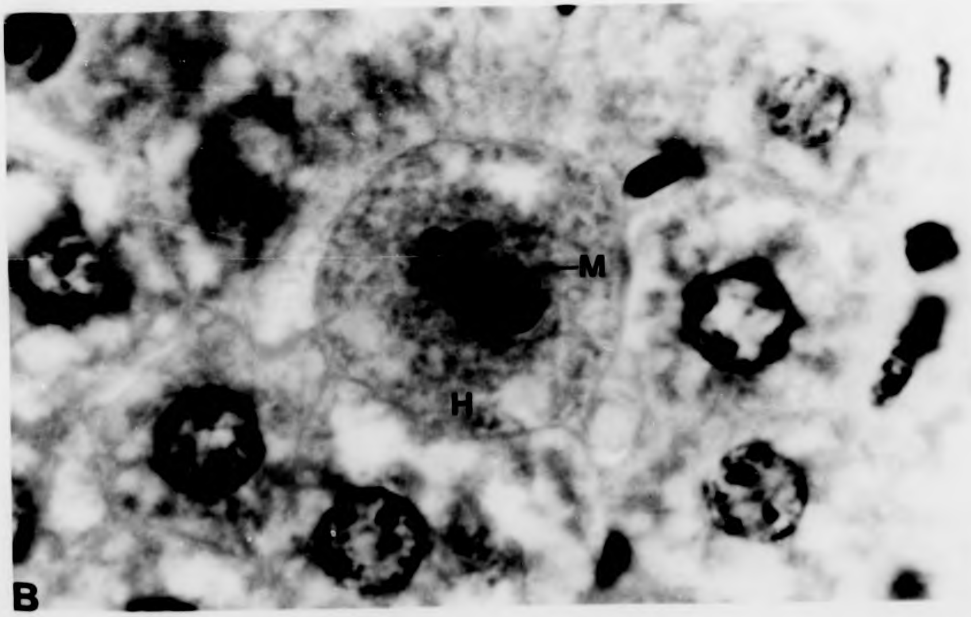
B) Control section of liver (colchicine treated) showing mitotic activity. 4µm Stained H. and E. (X1562 oil immersion)

H - Hepatic cell
M - Mitosis

treated)
E.

showing
562 oil





treated)
E.

showing
562 oil

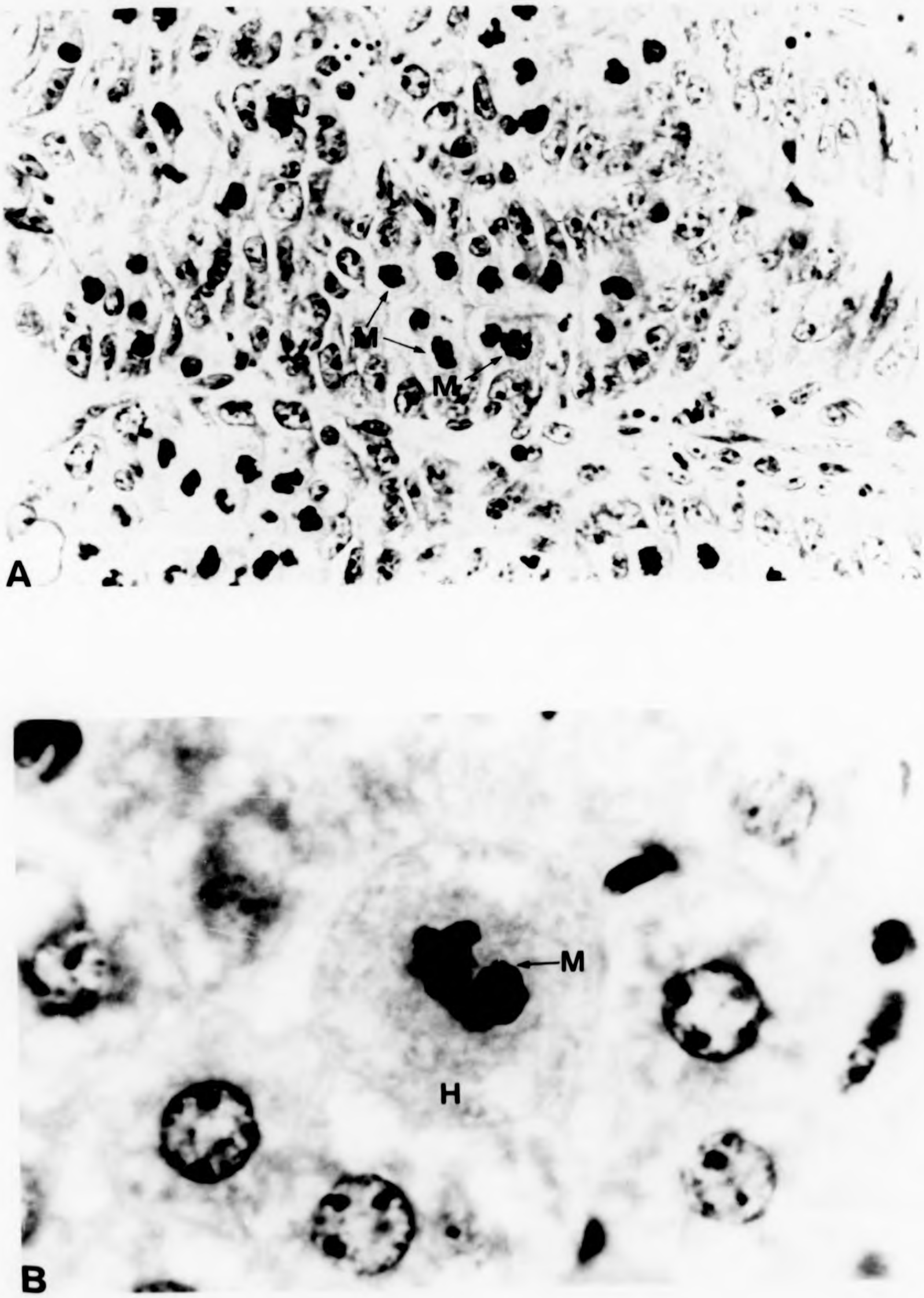


PLATE 6

A and B) Colchicine treated larva (*S. mansoni*) showing mitosis in a vein in the liver. 9 days post-infection 4µm Stained H. and E. (X625 and X1500 oil immersion)

C) Colchicine treated larva (*S. mansoni*) showing mitoses in a vein in the liver. 14 days post-infection 4µm Stained H. and E. (X625)

D) Colchicine treated larva (*S. mansoni*) showing mitoses in a branch of the hepatic portal vein. 14 days post-infection 4µm Stained H. and E. (X390)

M - Mitosis

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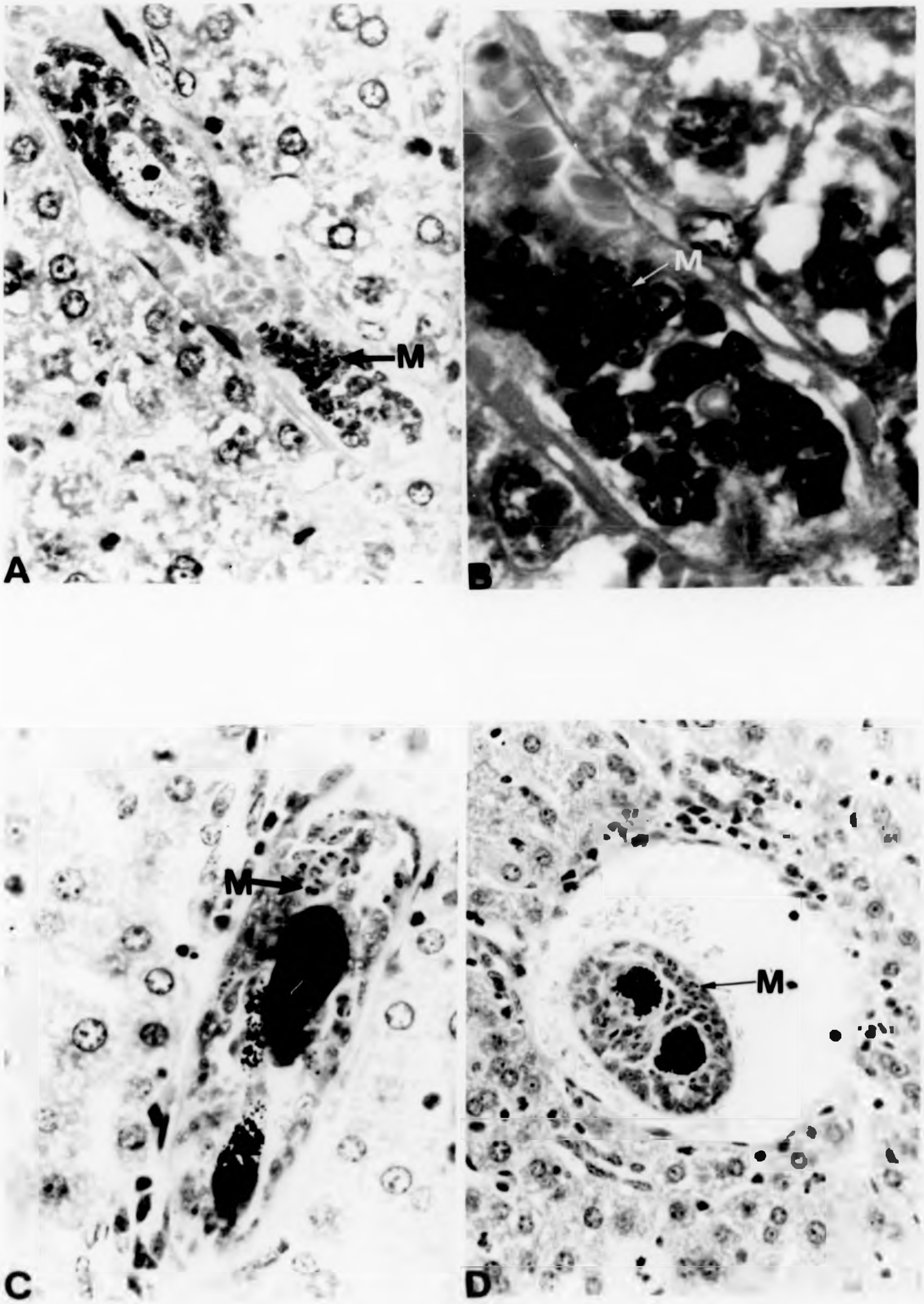

PLATE 7

A, B and C) Serial sections of a colchicine treated larva (_S_. _mansoni_) showing mitoses in a vein in the liver. (A) absence of pigment; subsequent sections (B and C) show presence of pigment. 11 days post-infection 4µm Stained H. and E. (X390)

D) Colchicine treated larva (_S_. _mansoni_) showing mitoses throughout in a vein in the liver. 16 days post-infection 4µm Stained H. and E.

L - Larva
M - Mitosis
P - Pigment

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4μm

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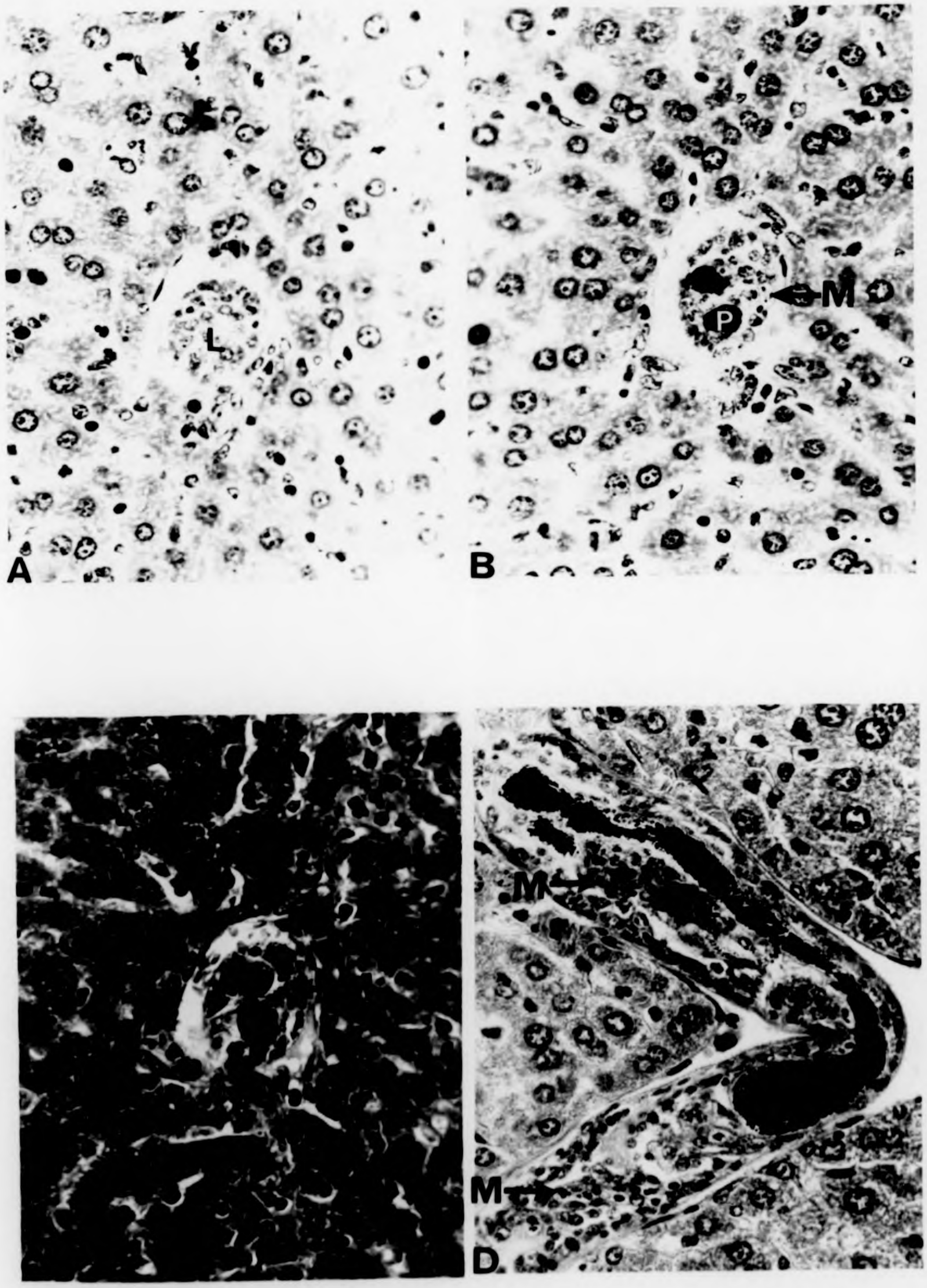


PLATE 8

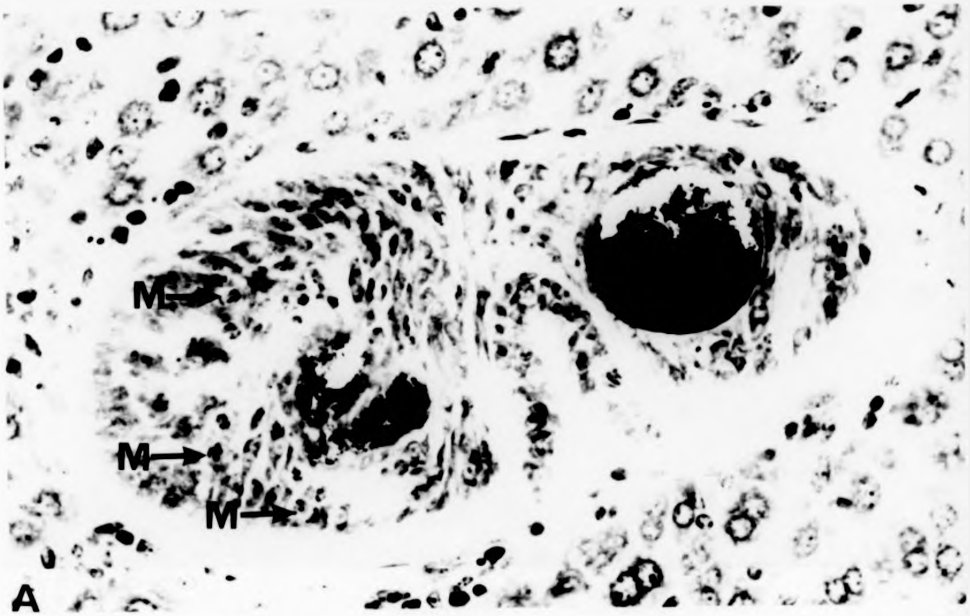
A) Colchicine treated larva (S. mansoni) showing mitoses in a vein in the liver. 16 days post-infcetion 4µm Stained H. and E. (X625)

B) Colchicine treated larva (S. mansoni) showing mitoses in a vein in the liver. 15 days post-infection 4µm Stained H. and E. (X625)

M - Mitosis

g mitoses
ion 4µm

g mitoses
ion 4µm



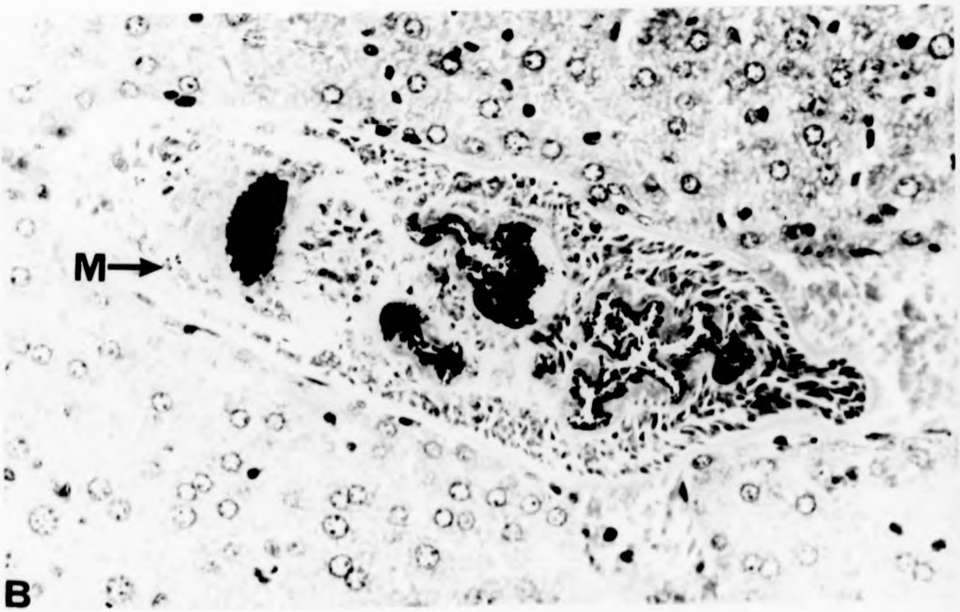
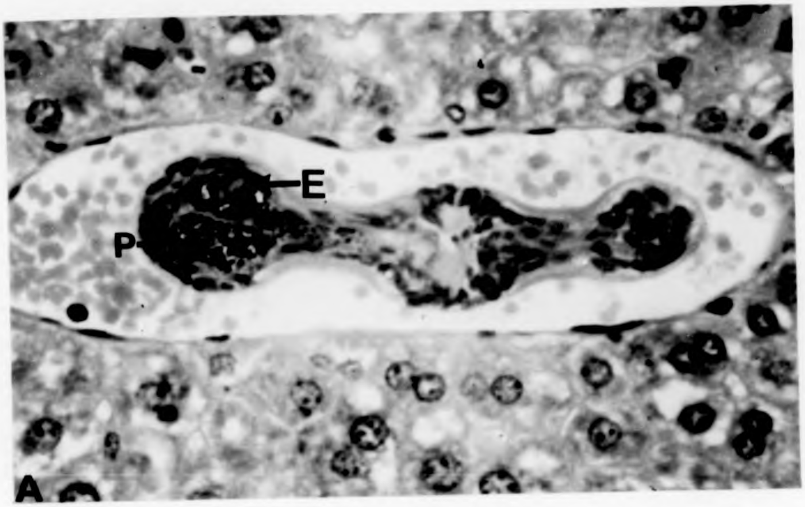


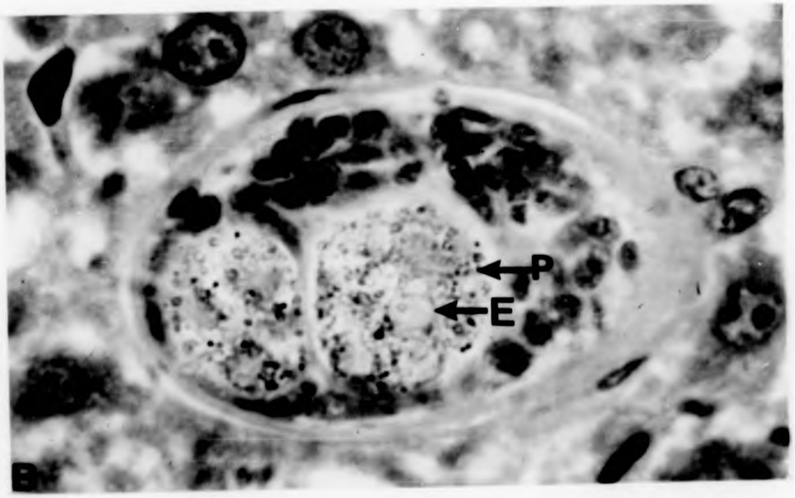
PLATE 9

A) Colchicine treated schistosomulum (S. *mansoni*) containing yellow pigment and intact erythrocytes in a vein in the liver; no mitosis evident. 9 days post-infection 4μm Stained H. and E. (X400)

B) Colchicine treated schistosomulum (S. *mansoni*) containing yellow pigment and intact erythrocytes in a vein in the liver; no mitosis evident. 11 days post-infection 4μm Stained H. and E. (X1000 oil immersion)

E - Erythrocyte
P - Pigment


E
P
A


P
E
B

Control: liver cells from the same animals

As expected the liver cells showed minimal cell division (Plate 5B).

S. haematobium in situ; lung forms day 8 and liver forms day 16 post-infection

The larva of *S. haematobium* showed no mitosis at the lung stage. Mitosis was observed at the liver stage only.

Since the results for *S. mansoni* and *S. haematobium* show similar patterns the discussion deals with larvae without reference to species.

4.4 DISCUSSION

Using DNA synthesis and mitosis as criteria of growth, this study shows that no growth takes place in the schistosomulum in the course of normal migration. Moreover, it is evident that even after arrival in the liver, no mitosis occurs in the larva for the first few days, although there is evidence of larval feeding within the liver before the start of cell division.

Neither DNA synthesis nor mitosis was seen in the pulmonary stage of schistosomular migration. This observation accords with those of Clegg (1959; 1965) and Smith *et al*. (1976) who found no mitosis in schistosomula removed from the lungs of mice and hamsters although 4% of S. mansoni and 60% of S. haematobium showed signs of feeding. In the present study also, the digestive tract of some schistosomula within the lung tissue was seen to contain intact red blood cells as well as haem. The pigment represents the breakdown products of host cell haemoglobin. However, the presence of pigment and/or intact red cells does not constitute evidence of mitosis*. Several workers have been concerned with the nature of the pigment, but their observations have been confined to its presence in the adult schistosome (Johnson, Hamilton and Gridley, 1954; Ostrow and Warren, 1965; Kloetzel and Lewert, 1966; Stenger, Warren and Johnson, 1967; Ramadan and Michael, 1969).

The contradiction in these observations may be more apparent than real. Schistosomula with pigment in the lumen of the gut may have started feeding in the liver; either before or just at the start of cell division, they may have been swept by the venous blood stream into the right chambers of the heart, and thence into the lung. Since pulmonary capillaries are narrower than liver sinusoids the schistosomula may act as emboli in the capillaries, causing haemorrhage and breakdown of the capillary wall, and so enter the lung alveoli.

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* The presence of unlabelled parasites or parasites showing no sign of mitosis cannot be attributed to technical error, as nuclei of the host tissue readily incorporated the isotope and nuclei of host tissue showed colchicine metaphase plates.

The hypothesis of recirculation of the schistosomulum after it has reached the liver would appear to be supported by the fact that schistosomula, with pigment in the lumen of the gut, were found in the alveoli, i.e. outside the pulmonary capillary network and thus in an ectopic site relative to schistosomular migration, by the blood vascular route. Certain investigators (Olivier, 1952; Wilks, 1967) have suggested that larvae, containing pigment in the lumen of the gut and found in the lung, had previously been feeding in the liver. Unfortunately it was not clear from these reports where the larvae were found, whether in capillaries, alveoli or bronchioli. In appearance the schistosomula in the lung alveoli resemble those found in the liver. Schistosomula on their initial passage are slender and flexible and are able to negotiate the capillary network; normally they do not act as emboli. Conversely, larvae that have passed through the liver have lost some of their ability to change shape (Wilson, Draskau, Miller and Lawson, 1978); accomodation in and passage through the capillaries thus becomes more difficult. In this condition they may act as emboli. The presence of such schistosomula in the alveoli would be supportive evidence for a second larval passage through the lungs. It is possible that a few schistosomula may make further journeys within the blood vascular system and may eventually end up in the liver or as emboli in other organs. In this study, schistosomula containing pigment were occasionally found in the kidney (see page 165). Clearly there is a limit to the number of journeys that any schistosomulum can make. Miller (1976) has also referred to the possibility of several "circuits" being made.

Schistosomula with pigment in the lumen of the gut were first found in alveoli, on day 9 after penetration. This lends support to the hypothesis of a second passage through the lung after feeding in the liver. The time elapsed is sufficient for the larva to re-enter the lung after passage through the liver, although there is no corroboration of this by direct observation. It should be noted that in the normal course of events the first appearance of the larva in the lung is on day 2, and in the liver on day 6 (see page 122); larvae in the liver were observed to undergo mitosis on day 9. It might be thought that schistosomula with pigment in the lumen of the gut, found in the lung on or after day 9, are larvae which started to migrate from the skin several days rather than hours after penetration, and fed en route or in the lung itself. However, schistosomula removed from the skin between days 4-16 are sluggish in motion and granular in appearance, suggesting that such larvae might find it difficult to migrate from the skin to reach either blood or lymphatic vessels. Thus it is unlikely that larvae containing pigment in the lumen of the gut and found in lung tissues, are late arrivals following a delayed start on migration after the initial infection of the host.

Since schistosomula within the lung have been shown to contain erythrocytes and pigment, several workers assumed that feeding had occurred in this site (Olivier, 1952; Clegg, 1965; Ghandour, personal communication). This assumption is not supported by the present investigation, however. It might be argued that larvae in the lungs, which contain erythrocytes and pigment, may have started to feed in the larger vessels before reaching the lung. The evidence available to date suggests that there is a relationship between environment and nutritional requirements on the one hand and growth on the other. Ectopy, i.e. presence of schistosomula in any but the maturation site, the liver, may significantly contribute to inhibition of larval growth. The factors that may play a part are relative proportions of carbon dioxide and oxygen in liver and lungs and differences in gaseous and nutrient content of blood in one or the other of these sites. A relationship was observed between variation in the colour of the pigment in the lumen of the gut of the larva and the microhabitat in which the parasite was found. Pigment in the lumen of the gut of the larvae found in the liver was yellow in the absence of mitosis and black after mitosis had started; larvae found in the lung contained yellow pigment in the lumen of the gut. Senft (1976) has observed a similar phenomenon in adult schistosomes located in sites other than the hepatic portal system. According to Morris and Threadgold (1968) "red blood cells are lysed almost immediately after ingestion" in adult schistosomes. Intact red blood cells were observed in the lumen of the gut of larvae found in liver and in ectopic sites.

The failure of the schistosomulum at the pulmonary stage of migration to incorporate radioactive thymidine may be due to one or more of several reasons. The schistosomulum could be independent of an external source of the nucleoside necessary for DNA synthesis; the isotope may not be able to pass through the membrane of the larva; again, the schistosomulum may lack the enzyme system necessary to phosphorylate thymidine. However, a reasonable explanation to account for the parasite not utilizing thymidine during its trans-pulmonary stage is that the schistosomulum is in a quiescent phase. Such a quiescent phase, whilst the schistosomulum is in the process of being transported to the site where growth will take place, would seem to provide a further logical explanation for the non-incorporation of the precursor. Thorell (1955) and Smellie (1955) have shown that synthesis of DNA does not occur when the cells are metabolically resting. It should also be noted that during transportation of the schistosomulum by the systemic circulation, when growth does not occur, the larva's requirements of an exogenous source of energy are minimal. It may be assumed that on arrival in the liver the schistosomulum would lack the immediate source(s) of energy necessary for DNA synthesis and cell division.

After reaching the liver, the schistosomulum starts to ingest red blood cells, and to grow. Growth, as evinced by cell division, does not start immediately. Some time elapses between the start of feeding, the appearance of pigment in the lumen of the larval gut, and mitosis. Previous claims (Clegg, 1965) that on reaching the mouse liver, the larva immediately starts feeding and undergoing mitosis are not supported by findings in the present study.

In vitro studies of schistosomula recovered from mouse skin and maintained in culture follow a different growth pattern in rate and nutritional requirements. It has been shown that in these circumstances growth occurs even in the absence of red blood cells (Clegg, 1965; Clegg and Smithers, 1972). Since the removal of schistosomula from the host involves a sudden change in environment for the larvae, results of experiments carried out in these conditions should not be extrapolated to represent *in vivo* conditions.

It has been observed that daughter sporocysts grow rapidly after reaching the liver of the snail intermediate host (Pan, 1965; Becker, 1970; Cheng and Bier, 1972), although the time span between arrival at the liver and start of growth is not mentioned.

Reid *et al*. (1977) infected snails with *S*. *mansoni* miracidia. Forty two days later, he exposed the snails to radioactive thymidine and reported on the uptake of the isotope by the cercariae and its retention by the derived schistosomula.

No retention of the isotope was detected at the schistosomular stage using the liquid scintillation method. On the other hand, using autoradiography low level of retention was detected. Possibly Reid _et al_. (1977) might have found a higher level of retention had they used snails more recently exposed to the isotope, when the daughter sporocysts were in a period of intense growth; this in turn might have increased the level of retention. According to Cheng and Bier (1972) who have described developmental stages of cercariae in the snail, rapid growth takes place during a period of 22-32 days after infection.

At first sight experimental data of Reid _et al_. (1977) might appear to contradict the observation that no growth takes place during the migratory phase of the schistosomulum. Reid _et al_. (1977), however, labelled "intramolluscan schistosomes"; whatever isotope retention was observed bears little relation to the growth of the migrating schistosomulum in the definitive host.

The two criteria for growth adopted in this investigation were DNA synthesis and mitosis. The results show that parasites within the liver incorporated exogenous thymidine to a variable degree, as evinced by scanty to heavy labelling of the larval nuclei. A small proportion of larvae showed no sign of incorporation. Similarly with regard to mitosis, varying degrees of mitotic activity were observed within the parasite. The variation shown in both instances, incorporation of thymidine and cell division, confirmed earlier microscopical observations on the range of migration lag, i.e. the time interval between skin penetration and arrival in the liver. There was a marked and progressive increase in cell division of the larval schistosomes from day 9 on. Cell division and DNA synthesis were not restricted to any particular area within the parasite. The relative level of radioactivity was not uniform from area to area within the parasite. This would be expected as the larval tissues were not homogeneous. On the other hand the emulsion was a homogeneous substrate and consequently exhibited uniform relative levels of radioactivity distributed uniformly throughout all areas observed.

Incorporation of thymidine into the different tissues of the host or the schistosome larva is an indication of the rate of cell production. Successful incorporation of thymidine into the nuclei is related to the ability to synthesize DNA. Within the cell thymidine is phosphorylated by the enzyme thymidine kinase prior to entering the pathway leading to DNA synthesis. This process requires a pool of the four nucleoside phosphates, ATP and specific enzymes. From the results obtained it is evident that ingestion and digestion of the host's red cells by the larvae are prerequisites for DNA synthesis and for mitosis to take place. Zussman, Bauman and Petruska (1970) concluded that adult worms utilize host haemoglobin nutritionally. Schistosome larvae in the growth phase, which showed both thymidine uptake and mitotic activity, invariably contained black pigment in the lumen of the gut.

It has thus been shown that growth, the first sign of development, occurs only after the parasite reaches the liver. The presence or absence of both DNA synthesis and mitosis are shown to be adequate indicators that growth has or has not taken place and each technique - autoradiography and colchicine arrest of cell division - corroborates the results obtained by the other.

It can be concluded from this investigation that both the behaviour of the parasite and its normal migratory route will be modified when irradiated cercariae and schistosomula are used. If the irradiation dosage is high enough (Standen and Fuller, 1959; von Lichtenberg and Sadun, 1963; Radke and Sadun, 1963), DNA synthesis will ultimately be impaired and growth inhibited. This will lead to the inability of the parasite to establish itself in the liver. The results of this study indicate, moreover, that non-growing parasites carried in the circulation end up as emboli.

CHAPTER 5

THE CHANGING SHAPE OF THE MIGRATING SCHISTOSOMULUM

5.1 INTRODUCTION

Establishment of schistosomal infection depends in part on successful larval penetration of the skin barrier and entry of schistosomula into the blood or lymphatic circulation by way of respective vessels. As the migration proceeds along the lumen of these vessels, capillary beds may have to be traversed before the larvae reach the liver.

It is generally accepted that approximately 40% of S. mansoni cercarial penetrants will eventually become schistosomes in the mouse, the loss of the remaining parasites taking place at various points of migration. With regard to S. haematobium Ghandour and Webbe (1976) found that 8% of cercarial penetrants reach adulthood in the mouse. Some of the larvae perish within the epidermis even in highly susceptible hosts (Clegg and Smithers, 1968). According to Ghandour and Webbe (1973) increased age either of cercariae or host or both is a factor adversely affecting the survival of the parasite during entry. Smithers (1976) and Ghandour and Ibrahim (1978) consider death of the parasite to be related to exhaustion of its energy supply.

In this study it is shown that intravascular routes, both haematic (see Chapter 6, page 174) and lymphatic (see Chapter 7, page 251) are used by the schistosomula. Rhodin (1968) states that the calibre of blood capillaries varies with different organs, tissues and species, but that generally they are larger than the erythrocytes which pass through them. In rabbit, Rhodin (1968) found capillaries to have a maximum diameter of 8µm at their venous ends and the narrowest dermal blood capillary observed by him in the rabbit had a diameter of 4µm. These findings (Rhodin, 1968) were based on light microscopical and electron microscopical techniques. The present author has been able to find only scanty published information on the size of blood vessels in the mouse (Zweifach and Kossman, 1937). These authors observed variations in diameter of 3-10µm in blood capillaries of the ear, mesentery and intestinal wall of the living animal. However, it is known that the erythrocyte in the mouse has a mean diameter of the order of 6µm (Altman and Dittmer, 1971). In the present study histological sections showed microvessels containing erythrocytes in tandem, which suggests that capillaries exist of the order of 6µm in the mouse. The microcirculation in the mouse does not appear to present an obstacle to all migrating schistosomula. The narrowest capillaries found in the mouse might present an obstacle, unless the schistosomulum is able to adapt its shape to this extreme limit.

Transient changes of shape probably constitute an adaptive phase of schistosomular migration; any apparent increase in size e.g. increased length, during this migratory process is not indicative of growth (see Chapter 4, page 79).

Once a parasite reaches a vessel larger than a capillary, it is probably flushed along its path by the flow of blood or lymph. In such vessels changes of shape are less likely to be necessary, until the schistosomulum reaches a microvascular network (e.g., in the lung).

The experiments to be described were designed to illustrate the extent of changes in shape and dimensions (increased length and concomitant decreased body width of the schistosomulum). The experiments were also designed to demonstrate that changes in shape, particularly elongation, are not related to schistosomular growth.

5.2 MATERIALS AND METHODS

Techniques were carried out on both fixed and living material.

Fixed material

Various tissues were sectioned and stained for larvae *in situ* (see Chapters 6, page 121 and 7, page 214).

Living material

Mice were exposed to an estimated 500 *S. mansoni* or *S. haematobium* cercariae using the tail-immersion method as described earlier (see page 38). The mice were killed with ether at different time intervals and schistosomula recovered from skin, lymph nodes and lungs after excision of the tissues as follows:

a) skin - the skin of the tail was slit longitudinally and stripped off;

b) lymph nodes - the left and right sciatic lymph nodes which are located just above the root of the tail, dorsal to the pelvis (see Diagram, page 212), were removed;

c) lungs (after method of Clegg, 1965) - the thoracic cavity was exposed by dissection. The lungs were perfused by injection of 10ml of Hank's balanced salt solution (HBSS), to reduce the population of red blood cells in the specimen.

Each tissue was placed in a 20ml Universal tapered container (Sterilin) and minced with a pair of fine scissors. 10ml of HBSS was added to the minced tissue and the container was tightly capped and incubated in a water bath at 37°C for 4 hours. After incubation the suspension was filtered through a 250µm mesh stainless steel gauze and the filtrate was centrifuged for 3 minutes at 1000rpm. The supernatant fluid was pipetted off, leaving 1ml which was resuspended in 9ml of HBSS and centrifuged again. The supernatant fluid was again removed and a residual 1ml transferred to a Sedgewick Rafter counting cell (Gallenkamp 1ml capacity) for examination.

Sequential photographs of living schistosomula were taken through a Zeiss II compound photomicroscope in order to record larval changes in shape and dimensions. At least 4 different photographs were taken of each schistosomulum and the following procedures carried out:-

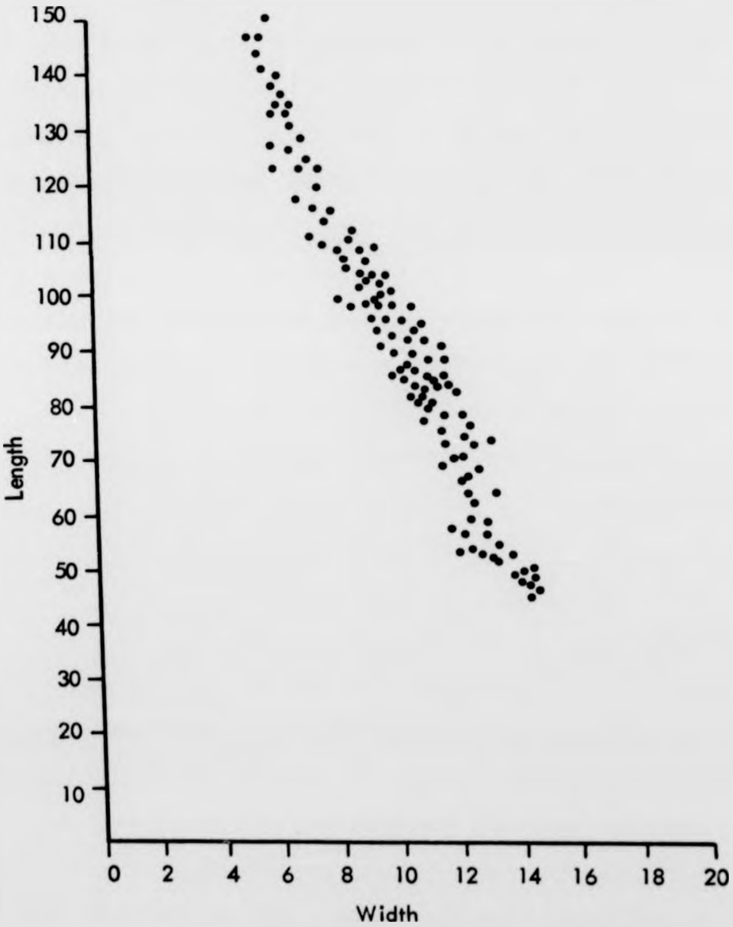
a) the length and average width of the photographic image of each larva were measured directly by means of a scale, prepared by photographing a slide micrometer at the same magnification used for photographing the larva. Additionally, photographic enlargements of larvae were traced on to graph paper for measurement;

b) outlines of the photographic images of each larva were drawn on paper measured with a planimeter and cut-out to be weighed. Identical planimeter measurements or identical weight measurements of the 4 different shapes of each larva indicate identical projected surface areas.

5.3 RESULTS

Samples of 10 schistosomula each were obtained from dermis (days 1 and 3), lymph nodes (days 3 and 6) and lungs (days 3 and 6) making a total of 60 samples. Each of the parasites was photographed in 4 differing shapes giving a total of 40 aspects for each sample group. The worms were then measured as described. The consolidated results are

Fig. 8 Lengths and average widths of 4 different conformations in respect of each of 5 worms from skin (days 1 and 3), lungs (days 3 and 6) and lymph nodes (days 3 and 6)



plotted in Figure 8. The scatter-plot indicates a high degree of negative correlation between changes of length and width.

Measurements of length and average width of 4 different forms assumed by a worm recovered from skin and a worm from a lymph node, both on day 3 and the same measurements of 6 different forms assumed by a worm recovered from lungs, also on day 3, are individually plotted in Figure 9, showing a high degree of negative correlation between changes of length and width. The conformations of the schistosomulum from lungs and those of a schistosomulum from a lymph node are shown in Plates 10 and 11.

The schistosomulum exhibits a potential for elongation and contraction and is pliable. The larva was seen to have its width reduced to at least 10µm in parts of its length and larvae were also seen with a width reduced to approximately 16µm almost uniformally throughout their length. Various degrees of elongation, contraction and angulation are shown by individual larvae (see Plates 12 and 13).

Living schistosomula were investigated with regard to surface area. Schistosomula used for this purpose were 1 and 3 day-old parasites from skin, 3 and 6 day-old parasites from lymph nodes and lungs. Measurements of projected surface area and cut-out weight of each of 4 different forms assumed by each parasite gave the same results: diverse conformations of the same worm in

Fig. 9 Lengths and average widths of 4 different conformations in respect of single worms recovered on day 3 from skin, lymph nodes and lungs respectively

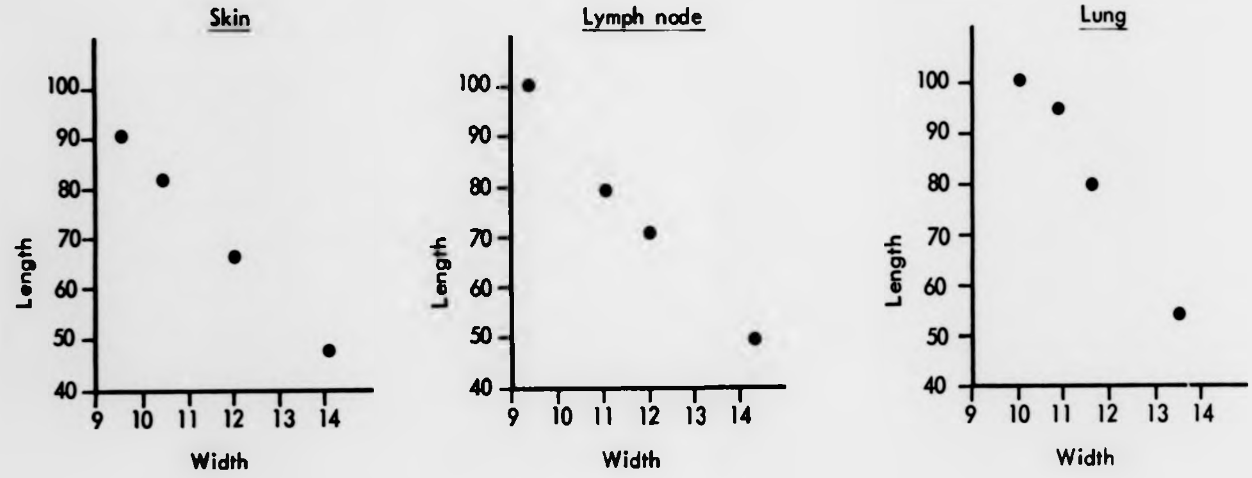


PLATE 10

A, B, C, D, E and F)	A schistosomulum (*S. mansoni*) recovered from lungs 3 days post-infection in 6 different conformations

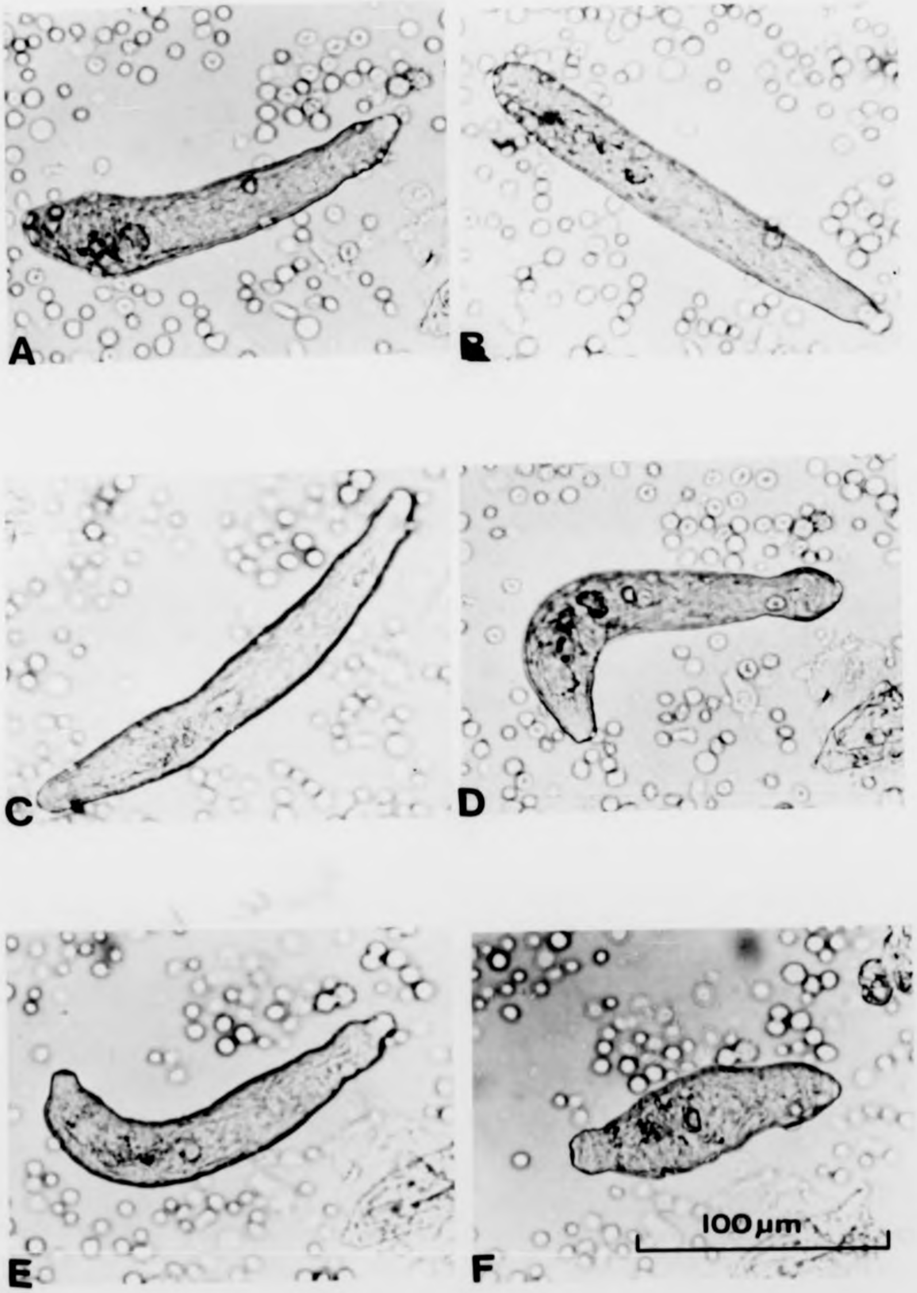

A
B
C
D
E
F
100 µm

PLATE 11

A, B, C and D)	A schistosomulum (*S. mansoni*) recovered from sciatic lymph nodes 3 days post-infection in 4 different conformations

recovered
post-
rmations

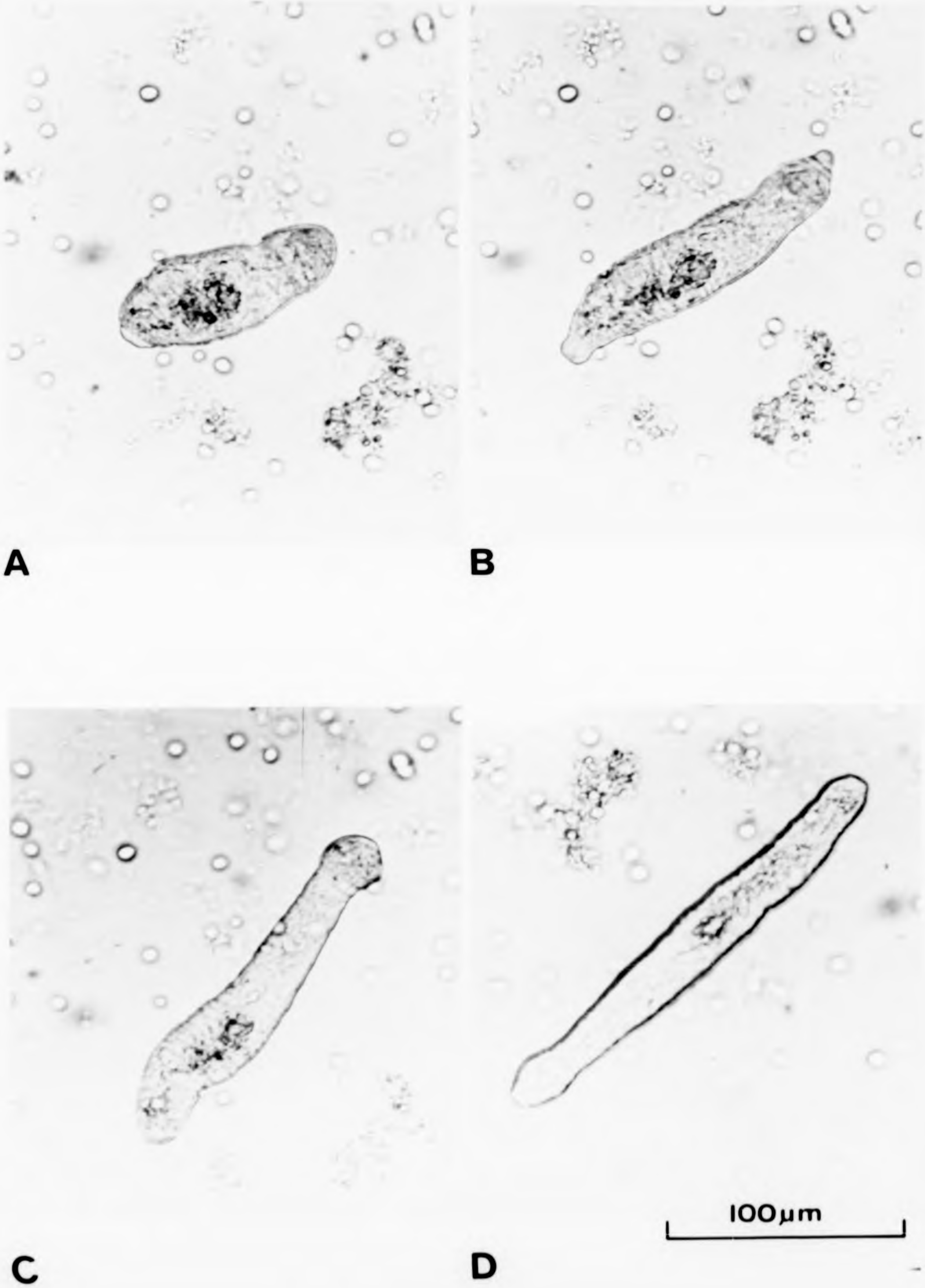


PLATE 12

A, B, C and D) A schistosomulum (S. *mansoni*) 6 days post-infection showing various degrees of elongation

days post-
es of

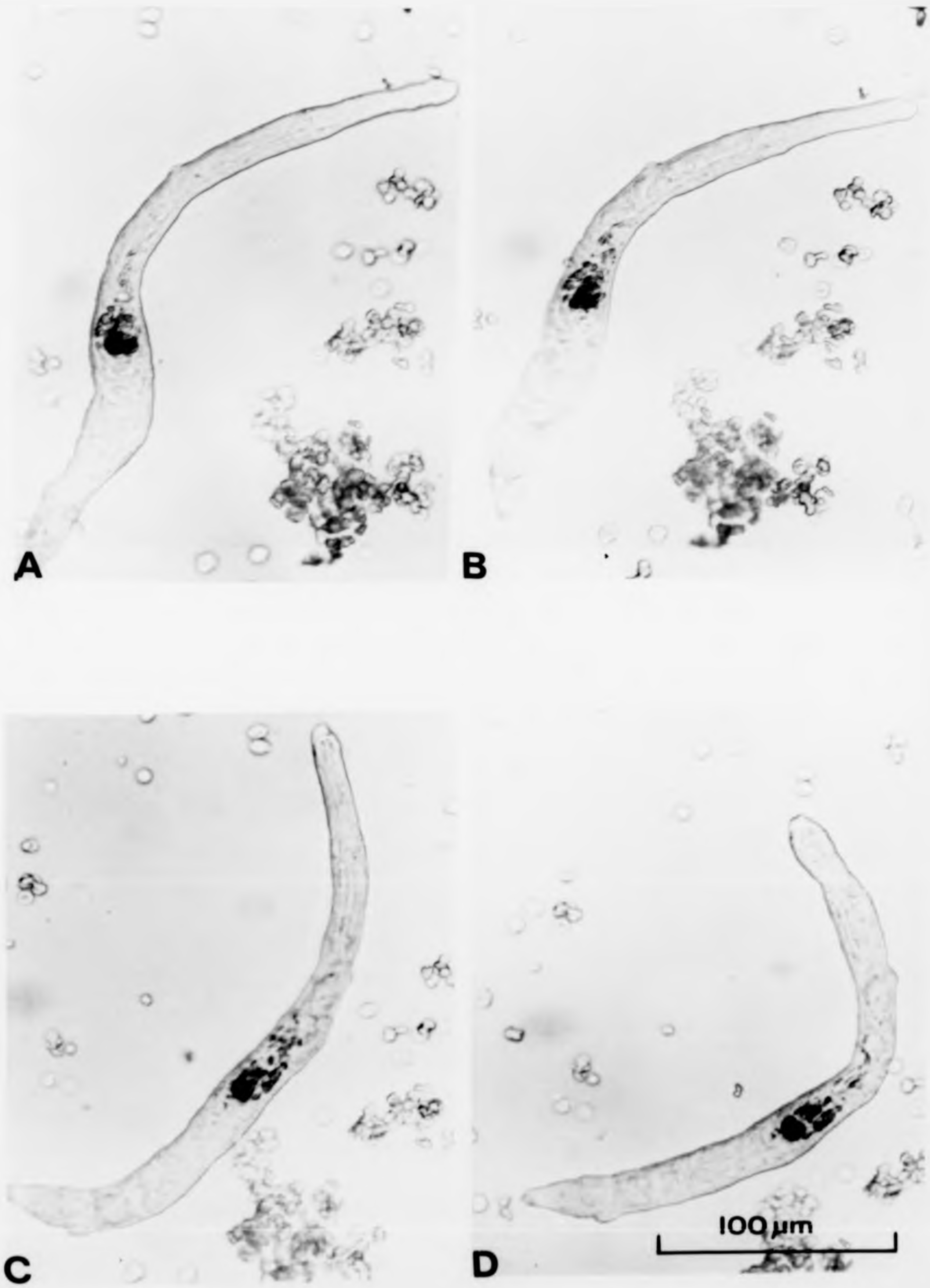
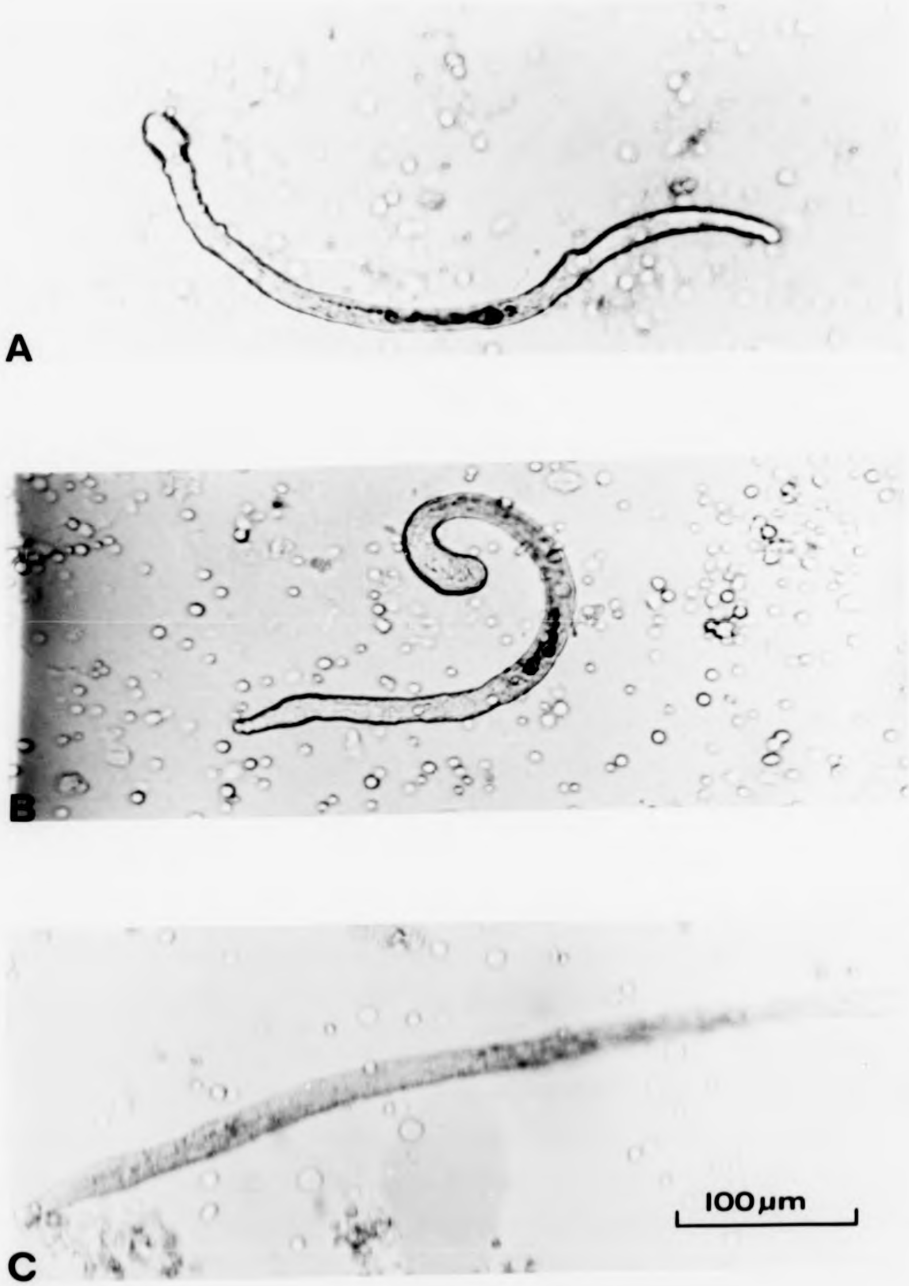


PLATE 13

A) Schistosomulum (S. *mansoni*) 6 days post-infection exhibiting angulation

B) Schistosomulum (S. *mansoni*) 6 days post-infection exhibiting angulation

C) Schistosomulum (S. *haematobium*) 6 days post-infection exhibiting elongation with an almost uniform width throughout its length


A
B
C
100µm

contraction having a smaller projected area than when elongated.

Histological sections of skin show larvae, within microvessels in the dermis, elongated to an extent comparable with those seen in microvessels of the lung, myocardium, brain (see Plate 14) and other organs. Larvae located outside microvessels in the dermis were seen to be elongated to a lesser extent.

Experiments similar to those described for *S. mansoni* were also carried out with larvae of *S. haematobium* which were found to behave in an identical manner.

5.4 DISCUSSION

After leaving the epidermis the schistosomulum may immediately encounter a blood or lymphatic vessel; more probably, however, it will eventually chance upon one of these vessels. The actual penetration of a haematic or lymphatic vessel within the living dermis by a schistosomulum has not been observed, though histologically, entry into a lymphatic vessel (Standen, 1952) and similarly, entry into blood vessels are recorded (Stirewalt, 1959; Wheater and Wilson, 1979). The mode of penetration of dermal vessels is not known. It may be assumed to occur either by mechanical probing and thrust with the larva's anterior end, requiring expenditure of energy, or by means of enzymatic action, or

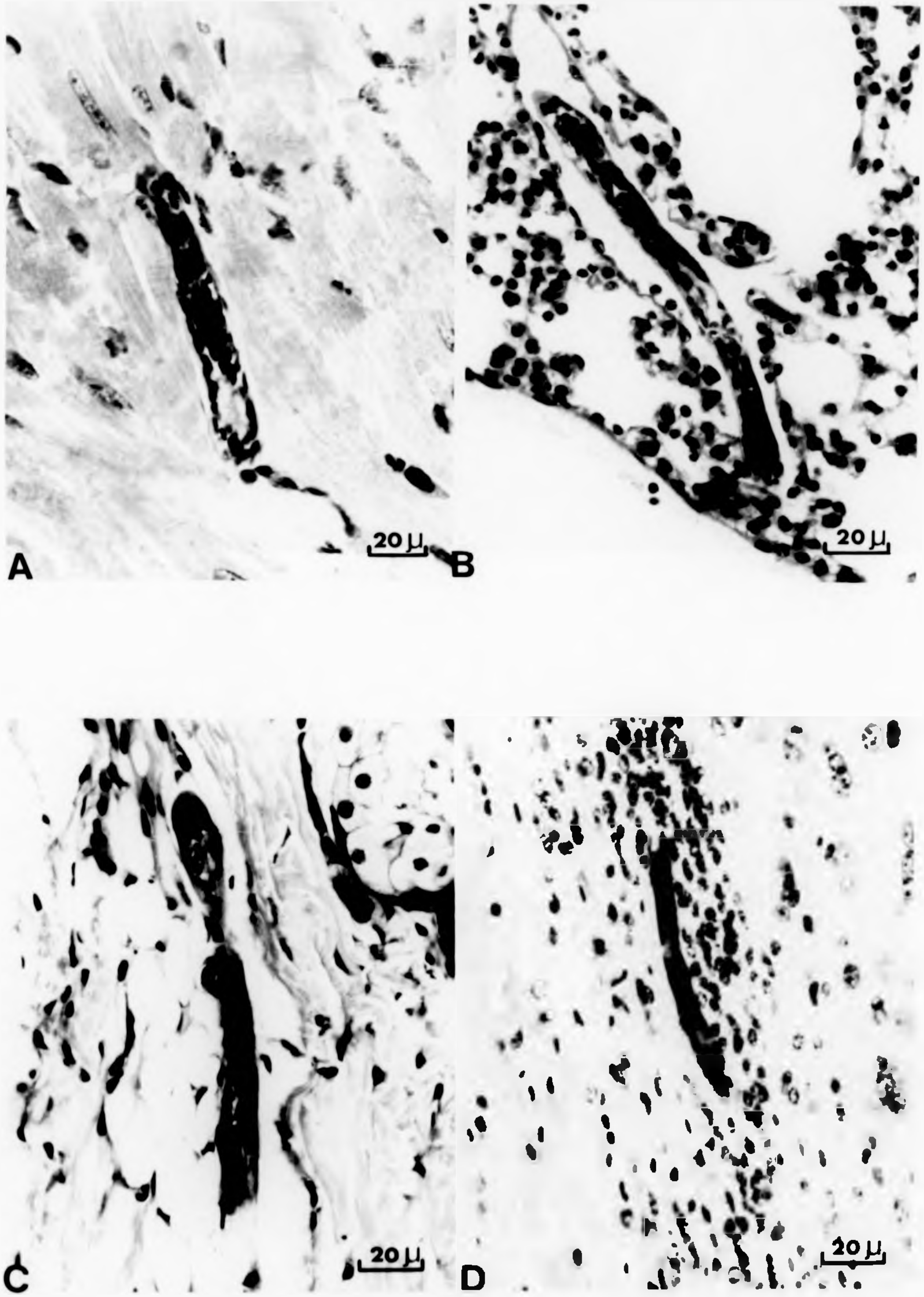
PLATE 14

A) Schistosomulum (S. mansoni) in a blood capillary of the myocardium 16 days post-infection 6µm Stained H. and E. (X625)

B) Schistosomulum (S. mansoni) in a pulmonary vessel of the lung 7 days post-infection 6µm Stained H. and E. (X390)

C) Schistosomulum (S. mansoni) in a capillary of the dermis 3 days post-infection 6µm Stained H. and E. (X390)

D) Schistosomulum (S. mansoni) in a blood capillary of the cerebellar cortex 15 days post-infection 6µm Stained H. and E. (X390)


20 µ
A
20 µ
B
20 µ
C
20 µ
D

both.

For its passage along vascular channels, apart from being pliable, the parasite must have the potential to change its shape in order to be able to fit the narrow luminal space of small-calibre vessels which it is likely to encounter in the course of migration.

Histological observations have shown schistosomula lying extravascularly (see Chapter 6, page 128 and Chapter 8, page 273), which might suggest that their containment in certain vessels has not been possible. It is also shown histologically in the present study, as evinced by observation of schistosomula within vessels, that the larva can accommodate in shape and dimensions to the confinement of the microvessels it enters. While, from evidence obtained in the present study it is not possible to state whether the parasite is able to deform sufficiently to permit passage through all capillary beds, the inability to do so might account to some extent for the loss of parasites which is known to occur during the migration. The experiments carried out in this study showed that in relatively short periods of time during which the possibility of growth can be discounted, a schistosomulum is capable of undergoing substantial deformation. Transient changes of shape result from the parasite elongating or contracting, as well as from angulation both in horizontal and vertical planes. This is based on observations of living schistosomula recovered from various organs of the host.

It should be noted that in the following experiments the larvae were not studied within vessels. The parasites examined _in vitro_ were recovered from hosts by the technique of mincing and incubation. This work has shown that the parasites have a considerable potential for elongation: increases in length by up to more than 3 times (Figure 9) were seen in individuals from one set of larvae recovered from hosts. From another set of larvae, a 6 day post-infection lung schistosomulum was observed to elongate to approximately 500µm. In one series of observations Miller (1976) found a comparable schistosomulum elongated to 264µm. Wilson _et al_. (1978) studying larvae obtained by the same technique, observed a fourfold increase in elongation. However, the environmental conditions within the host are not likely to be reproducible in studies conducted _in vitro_ and the results should be regarded with caution. From an accurate appreciation of the parasite's capacity for deformation it would be necessary to study the behaviour of the worm within the host or at least in environmental conditions which simulate those in the host. A variety of factors such as restricted movement within a microvessel, the chemical and physical properties of the surrounding fluid (e.g. viscosity, oxygen and carbon dioxide tensions), separately or in association, might be critical in influencing deformation.

Within limits of its potential the extent of elongation (and concomitant narrowing) of the parasite is likely to be determined by the vascular boundaries in which it is enclosed. For instance, the schistosomulum probably needs to assume a filiform shape in passing through the terminal arterioles and metarterioles, the true bottle-necks of the blood circulatory system (Cliff, 1976), where even erythrocytes are substantially deformed - "parachute cells" as described by Bond, Derrick and Guest, 1964). Deformation to such an extent might not be required for passage through lymphatic capillaries with their larger calibre (Hudack and McMaster, 1932; Godart, 1968) and less rigid walls (Leak and Burke, 1966; 1968). Some live worms, recovered from lungs on day 6 after infection, measured less than 10µm in width, but in histological sections of parasites within pulmonary and other capillaries the narrowest width observed was less than that seen in live worms. It is realized that in processing histological material some shrinkage is inevitable.

So far, there is no conclusive evidence that the larva passes through arterio-venous anastomoses, bypassing capillary networks in various organs but this should not exclude the possibility that a certain number of parasites might randomly be transported through these where they exist.

Certain workers (Wilson *et al*., 1978) have drawn a distinction between the extended form of the larva in the pulmonary stage of migration and a shorter form taken from the skin*. The present author supports the view of Wilson _et al_. (1978) that the elongated form of the worm in the lungs is probably adaptive in order to facilitate its passage through the pulmonary capillary network to the left side of the heart. On the assumption that the elongated form results from an adaptive shape change to facilitate intravascular migration it is reasonable to expect the short form to predominate in the dermis. Following cercarial penetration of the epidermis short form larvae which have not entered microvessels may be expected to accumulate in the dermis. This has been observed by the present author. Elongated forms which have also been identified histologically in the microvasculature of the dermis in this study suggest that such deformation occurs in this site in order to facilitate the larva's passage through dermal microvessels. Moreover, elongated forms were seen histologically, in the present study, in microvessels of many organs supplied by the systemic circulation - e.g. brain, kidney and myocardium, further suggesting that elongation takes place in order to permit passage through microvascular channels.

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* von Lichtenberg, Sher and McIntyre (1977) state that schistosomula in their lung model are "somewhat larger than those in the skin"; without definition, the term "larger" is too vague for purposes of comparison. It could refer to increase in mass, overall increase in size, greater width and length, or increased length without decrease in width.

A high negative correlation between elongation and width was established in the present study. Changes have also been observed in the projected surface area of individual schistosomula. It is interesting to speculate whether a change in projected surface area indicates a change in volume or is the result of change in orientation in the field of view. In the measurements taken, for example, no allowance is made for re-entrants of the surface of the schistosomulum which are beyond the limits of optical resolution. However, random sampling in random directions of an axially symmetrical organism would show zero correlation between apparent length and width. The conclusion is that a functional relationship between length and width probably exists but the evidence is not sufficient to decide whether this is connected with changes of shape or volume, or both.

The schistosomulum is capable of elongation to a remarkable degree, but increase in length in itself is not necessarily an indication of growth. In an organism with skeletal structure, increased length is relatively easy to measure. In the invertebrate schistosomulum undergoing rapid and frequent alterations in shape, permanent changes due to growth must be distinguished from transient changes in length. According to Lawson and Draskau (1977), changes in shape are not in themselves an indication of growth. Their criterion for growth is based on increased wet weight. Conversely, from in vitro culture studies on survival of schistosomula taken from infected hosts, Senft and Weller (1956) and Cheever and Weller (1958) considered that, because of increased length, growth had taken place. The present author considers DNA synthesis and mitosis as incontrovertible criteria for growth irrespective of changes in length or shape during schistosomular migration. Unless these criteria are met it cannot be asserted that growth has occurred; increase in length by itself is certainly not sufficient basis for such claims at the schistosomular stage. This is of importance in studies on schistosomular growth when deformation in the form of elongation might be considered as indicative of growth. As shown in Chapter 4, page 79 growth does not occur during schistosomular migration and only begins to take place after the parasite reaches the liver. Smyth (1966) in discussing the pulmonary phase of schistosomular migration states that "it is remarkable to find that at this stage, larvae do not undergo mitosis so that no growth in size is taking place in the lungs". Suspension of growth till the liver is reached would seem to be an obvious advantage to the schistosomulum in intravascular migration. Growth and increase in size of the worm before it reaches the liver would impede the parasite's passage through the narrow vessels of the microcirculatory blood systems.

For intravascular schistosomular migration to succeed it is essential for the parasite to accommodate itself within the vascular lumen so as to pass along the interior of the vessel either by active or passive movement. The possibility of the larva being contained within microvessels will depend on the ability of the parasite to undergo sufficient deformation and/or on some degree of vascular distention. In general blood vascular capillaries are rigid, though various chemicals can cause dilatation in these vessels (Burton, 1966). It is possible that larval entry into a dermal vessel may stimulate the release of histamine leading to vasodilatation. With regard to passage of erythrocytes through microvessels it is known that they are subjected to such pressure as to undergo substantial deformation - "parachute cells" - when forced through the vessels. It is the rigidity of capillaries which ensures sustained maintenance of pressure within them. In this connection the optimum shape for an object to pass through the lumen of a capillary is considered to be cylindrical with hemispherical ends - "hot dog" shaped (Canham and Burton, 1968). While an erythrocyte does not fulfill these requirements the schistosomulum appears to approximate to that shape. Although movement through intravascular routes is probably passive, analogous to that of the erythrocyte, the possibility of active movement by the schistosomulum merits consideration.

In addition to its ability to undergo changes in shape, the parasite is equipped both with an oral and a ventral sucker. It seems conceivable that the suckers are used alternately to anchor the parasite, synchronized with alternate contractile and elongatory shape changes producing a net forward progression.

CHAPTER 6

SCHISTOSOMULAR ROUTES: THE BLOOD VASCULAR SYSTEM

6.1 INTRODUCTION

This chapter deals with the blood vascular system as a route of migration of the schistosomulum from skin to lung and from lung to liver. Schistosomular migration from the skin via the lymphatics is dealt with in Chapter 7 (page 198) and the possibility of an extravascular route of migration is discussed in Chapter 8 (page 266).

On leaving the skin, two courses to the lung are open to the larva, one a direct route and the other an indirect route:-

1. direct by way of blood vessels to the right side of the heart and thence to the lung;
2. indirect, through vessels of the lymphatic system whose thoracic and right lymphatic ducts join the blood vascular system by draining into the left and right subclavian veins; thence to the right side of the heart and to the lungs.

Migration of larvae between skin and lung has been assumed rather than investigated. It is generally agreed that 30% to 40% of invading cercariae perish in the skin leaving not more than 60% to 70% to migrate to the lungs (Clegg and Smithers, 1968; Ghandour and Webbe, 1976). Irrespective of the site of infection the greater part of the time spent in migration from skin to lung will be in the skin and in the microcirculatory blood system. From the skin the parasite proceeds either to blood or lymph vessels; occasionally it is found in lymph nodes. Once the parasite reaches the larger blood vessels, either directly or via the lymphatic ducts, it's passage there will be brief - probably not more than half the circulation time. It is only from the larger blood vessels that samples of blood can be taken for evidence of circulating parasites. The experimental demonstration of migration from the skin to lung exclusively by the blood circulatory system would require the blocking of access to the blood vascular system via the lymphatics. The likelihood of finding direct evidence of schistosomula in the blood circulatory system is remote even after heavy cercarial infection.

Considerable doubt still surrounds the routes of migration of the schistosomulum between lung and liver, for which two possible courses have been postulated. One is dispersal by way of the blood circulatory system; the other is extra-vascular, by active penetration through the tissue structures intervening between lung and liver. The majority of workers (Miyagawa and Takemoto, 1921; Faust and Meleney, 1924; Faust, Jones and Hoffman, 1934; Koppisch, 1937; Tang, Tang and Tang, 1973; Miller, 1976; Wheater and Wilson, 1979; Miller and Wilson, 1980) consider schistosomular migration from lung to liver to be either exclusively or principally by way of the blood vascular route. There are some workers, however, (Goto, 1932; Sadun, Lin and Williams, 1958) who favour two routes of migration - blood vascular as well as extravascular.

With regard to migration by the blood vascular system from the lungs via the left chambers of the heart two routes have been suggested. The more commonly held view is that the parasites migrate from the lungs through the pulmonary venous system to the left side of the heart to be dispersed with the arterial stream throughout the body; the liver is reached directly through the hepatic artery and indirectly by way of the hepatic portal circulation. On the other hand, it has been suggested by Kruger, Heitman, van Wyk and McCulley (1969) that the blood vascular migration proceeds against the blood flow, from the lung back into the posterior vena cava, and through the hepatic vein into the hepatic portal system by the way of the liver sinusoids (see page 189).

In the present investigation an attempt has been made to trace the blood vascular pathways and the fate of the schistosomulum in its migration from the skin to the liver. Serial histological sections of various tissues were examined for the presence of schistosomula which might be contained within blood vessels in the tissues as well as evidence of host response to the parasite.

6.2 MATERIALS AND METHODS

Separate batches of mice were infected with approximately

2000 *S. mansoni* and *S. haematobium* cercariae respectively by the tail-immersion method (see page 38) for a period of 60 minutes. Individual mice were killed with ether at 10-minute intervals over a period of 120 minutes after infection with *S. mansoni* and at 24-hour intervals from days 1-16 following infection with *S. mansoni* and *S. haematobium*. After killing the mice, the tails were amputated, cut into 4 parts and fixed in 10% formol saline for a period of at least 48 hours. The tails were decalcified by immersion for 72 hours in 10% formic acid. The volume used was at least one hundred times that of the tissue. The solution was changed twice daily. The tissues were then rinsed in running tap water for 24 hours and again fixed in 10% formol saline for 24 hours. It should be noted that following decalcification, staining with haematoxylin was less intense whilst with eosin staining was enhanced.

From days 1-16, heart, lungs, diaphragm, kidneys, spleen, brain, pancreas and liver were removed daily from the mice described above. Heart and lungs were excised together as were diaphragm and liver. In other animals diaphragm and liver were excised separately. The diaphragm was flattened between filter papers before fixation, to facilitate parallel sectioning. The organs were fixed intact in Carnoy's fluid or 10% formol saline. The remaining procedures, - dehydration, clearing and impregnation, were carried out in an automatic tissue processor. After embedding in paraffin wax, serial sections of the whole

TABLE 11 Presence of parasites (*S. mansoni*) in blood vessels of various organs and tissues by days after infection

	Days after infection															
Organ/Tissue	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Tail skin	∅	+	+	+	+	+	∅	∅	+	∅	∅	∅	∅	∅	∅	∅
Tail bone	∅	∅	∅	∅	∅	+	∅	∅	∅	∅	+	∅	∅	∅	∅	∅
Heart	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+
Lungs	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain	O	O	O	O	-	+	+	+	+	+	+	+	+	+	+	+
Diaphragm	O	O	O	O	-	+	+	+	+	+	+	+	+	+	+	+
Kidney	O	O	O	O	-	+	+	+	+	+	+	+	+	+	+	+
Pancreas	O	O	O	O	O	-	-	-	-	-	-	-	-	-	-	+
Spleen	O	O	O	O	O	-	-	-	-	-	-	-	-	-	-	-
Liver	O	O	O	O	-	+	+	+	+	+	+	+	+	+	+	+

+ presence of parasite
O section not cut over period indicated
- parasite not detected
∅ organ cut and examined in part (the amount of tissue cut and examined was never less than 50%). Parasites not detected.

organs were cut at 5µm or 6µm and mounted. After dewaxing they were hydrated and then stained. Staining methods include the following:- Ehrlich's alum haematoxylin and eosin; Lendrum's method for eosinophils (Lendrum, 1944); Verhoeff's method for elastic tissue (Verhoeff, 1908); Periodic Acid Schiff (Lillie, 1965) and Metachromatic staining of sodium bisulphite derivatives of glycogen (Malinin, 1970).

6.3 RESULTS

Table 11 shows a summary of appearance by days after infection of parasites (S. mansoni) in blood vessels of various organs and tissues, determined histologically.

Histological findings for individual organs:

Tail

In serial sections of tails, schistosomula were seen on days 2 to 6 and on day 9 after infection in the lumen of vessels of the haematic microcirculatory system of the dermis. Parasites were not associated with cellular infiltrate. Plate 15 shows schistosomula within dermal blood vessels. Schistosomula were also found within blood vessels of tail-bones on day 6 and day 11 after infection (see Plate 16).

PLATE 15

A) Schistosomulum (S. *mansoni*) in a dermal blood vessel 4 days post-infection via the tail 6μm Stained H. and E. (X390)

B) Schistosomulum (S. *mansoni*) in a dermal blood vessel 3 days post-infection via the tail 6μm Stained H. and E. (X390)

S - Schistosomulum

d vessel
ined H.

d vessel
ined H.

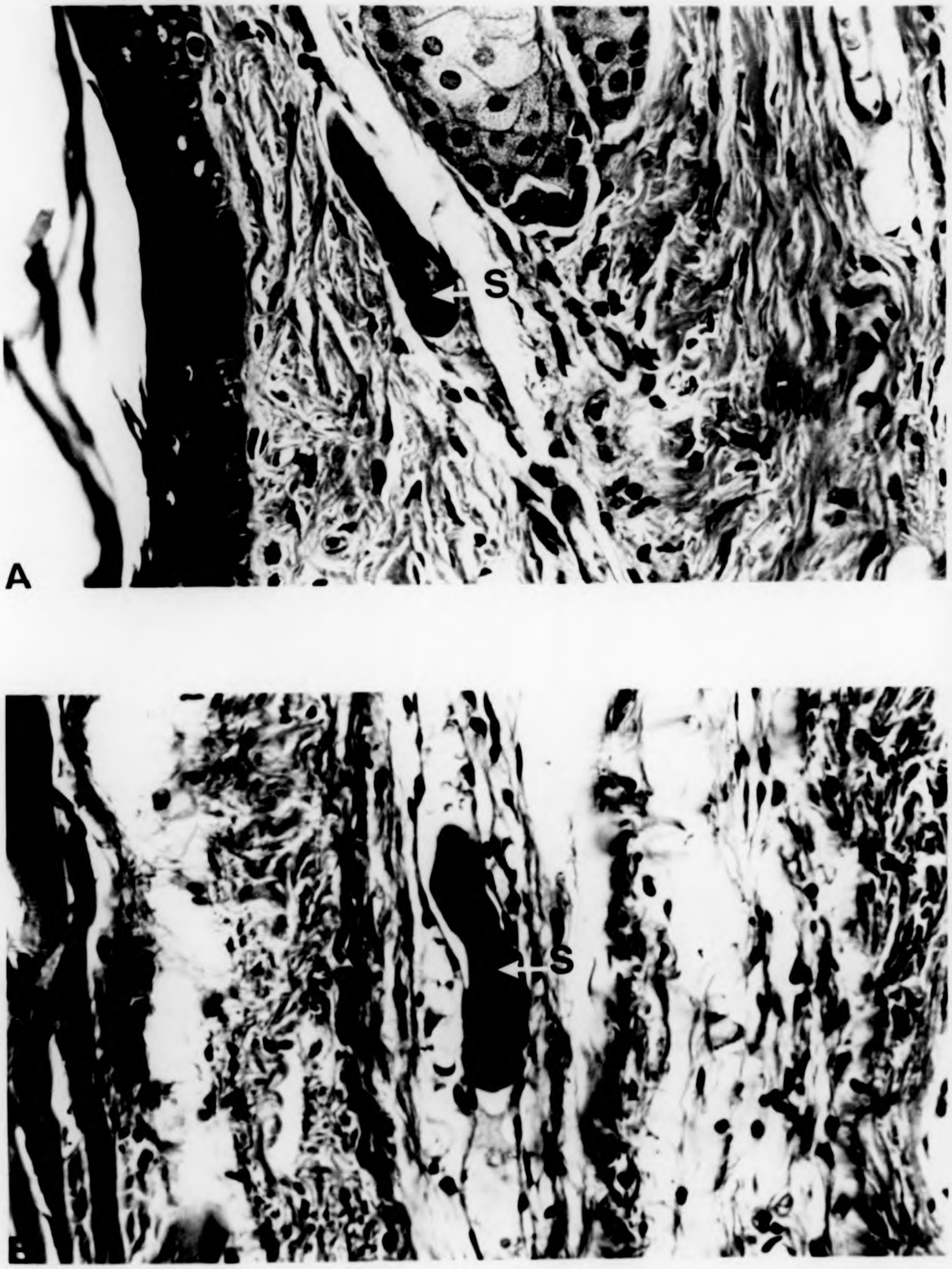
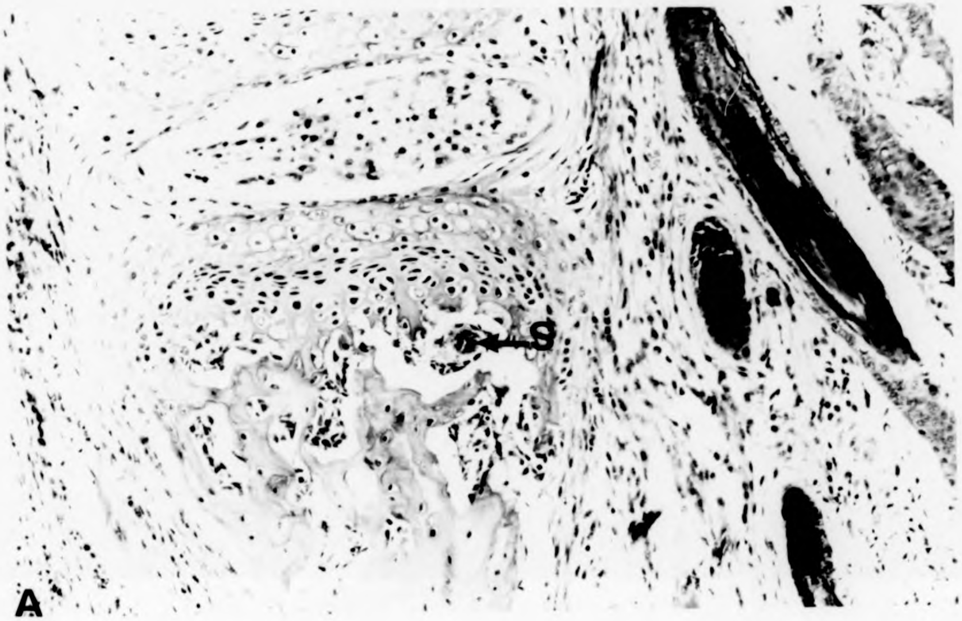


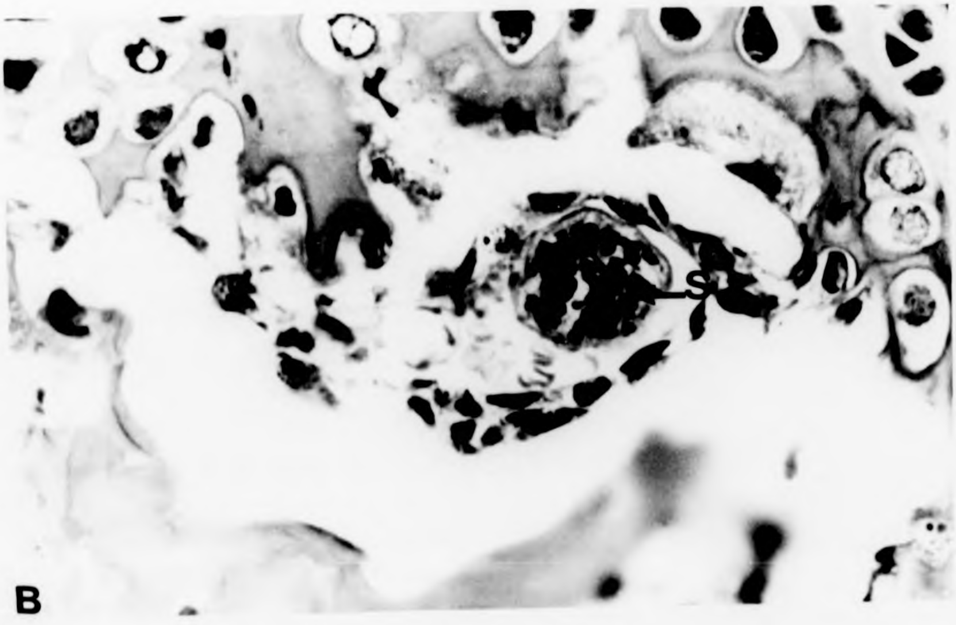
PLATE 16

A) Schistosomulum (S. mansoni) in a blood vessel of the tail-bone 6 days post-infection 6μm Stained H. and E. (X125)

B) Higher magnification of the same parasite shown in (A) (X625)

S - Schistosomula


S
A


S
B

Heart

Rarely, in serial sections of whole hearts, schistosomula were seen lying freely in the blood contained in the four chambers of the heart and/or in capillaries of the myocardium. On one occasion, a coronary vessel in subepicardial connective tissue was seen to contain a larva. A parasite was seen in one or other cardiac situation on days 7, 9, 12 and 16 after infection. No inflammatory reaction was observed. Plate 17 shows schistosomula within myocardial capillaries.

Lungs

Schistosomula were seen in the haematic macro- and microvasculature, both arterial and venous, of serially sectioned lungs from 2-16 days after infection. Parasites were occasionally seen lying outside vessels (see Chapter 8 - Schistosomular migration: Extravascular routes page 273). When within vessels parasites were generally not associated with evidence of inflammatory reaction though after day 9 occasionally a minimal degree of a cellular infiltrate comprised of neutrophil and eosinophil polymorphonuclear leucocytes was observed. Plates 18-20 show schistosomula within lumen of pulmonary vessels*.

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* Cardiac muscle fibres in the walls of the pulmonary veins and their branches is a normal feature of the mouse (Hummel, Richardson and Fekete, 1966).

PLATE 17

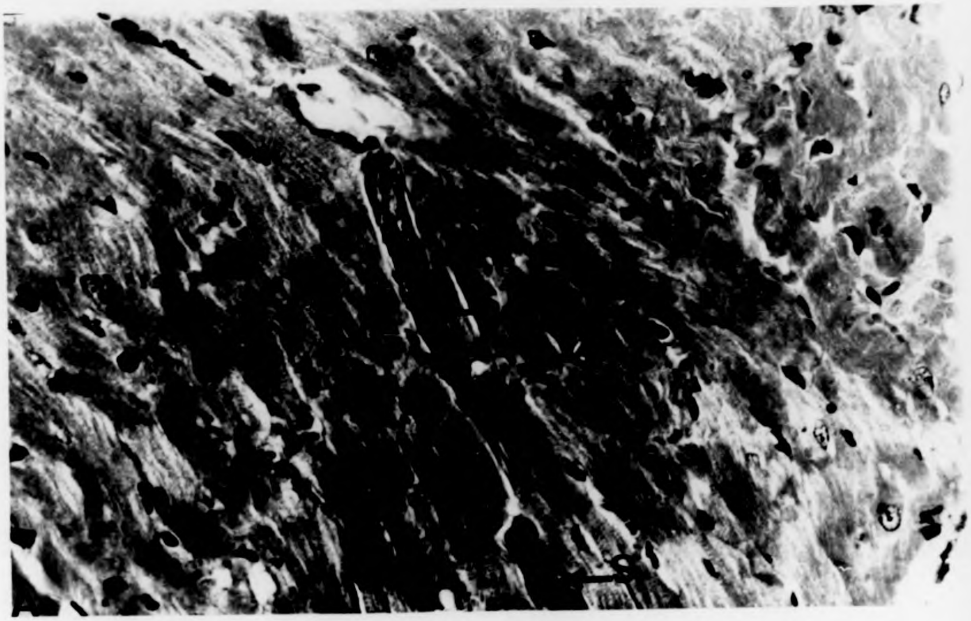
A) A schistosomulum (S. *mansoni*) within a myocardial capillary 16 days post-infection 6µm Stained H. and E. (X390)

B) A schistosomulum (S. *mansoni*) within a myocardial capillary 12 days post-infection 6µm Stained H. and E. (X390)

S - Schistosomulum

yocardial
Stained

yocardial
Stained



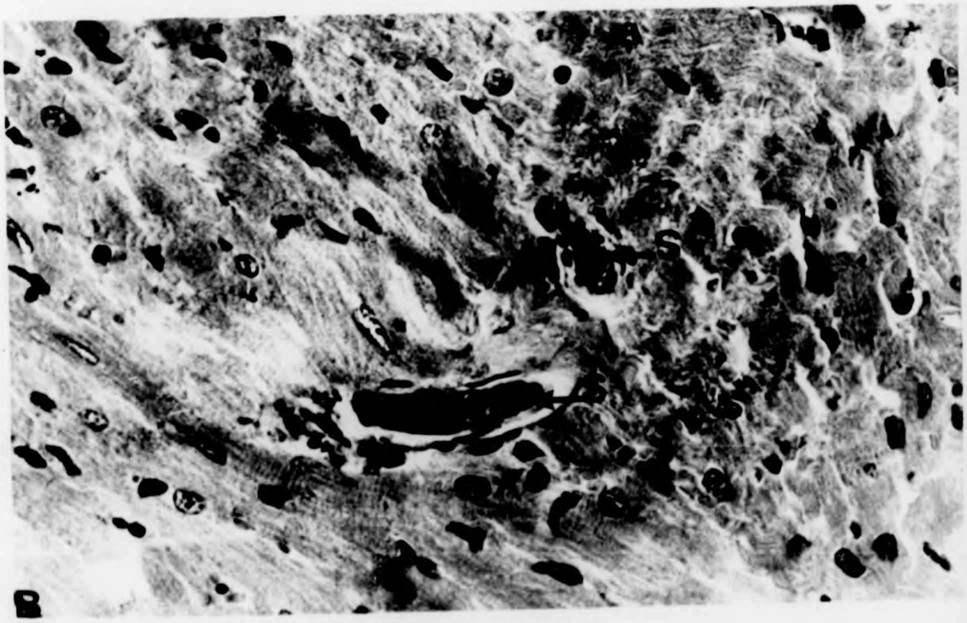


PLATE 18

Invasion of lung by schistosomula (S. mansoni)

A) Schistosomulum in pulmonary microvessel and schistosomulum in branch of pulmonary vein 7 days post-infection 6μm Stained H. and E. (X390)

B) The same schistosomulum as above in branch of pulmonary vein at a higher magnification (X625)

P - Pulmonary vein
S - Schistosomulum

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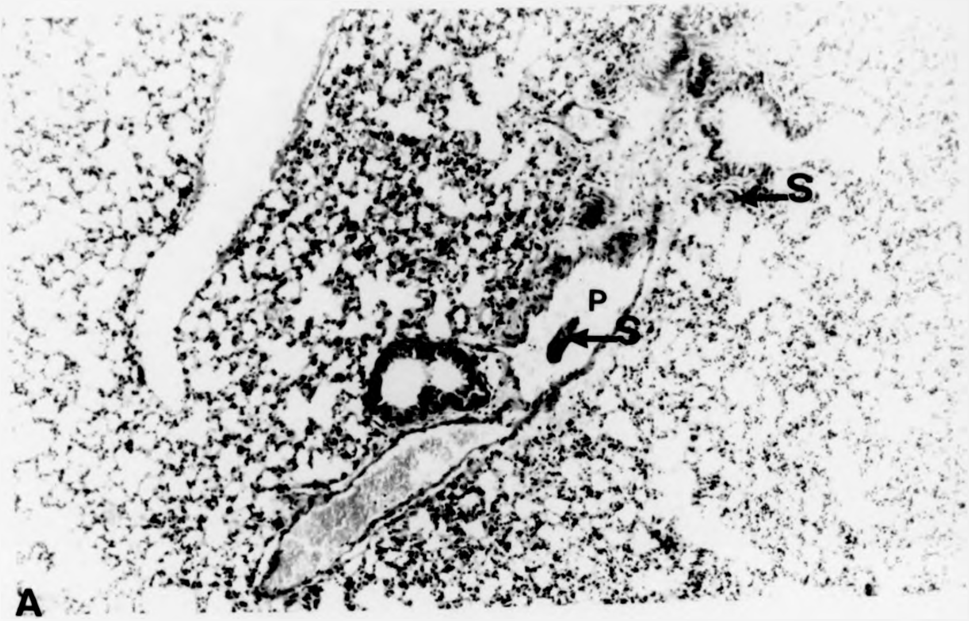
n 7 days

X390)

h of

(X625)



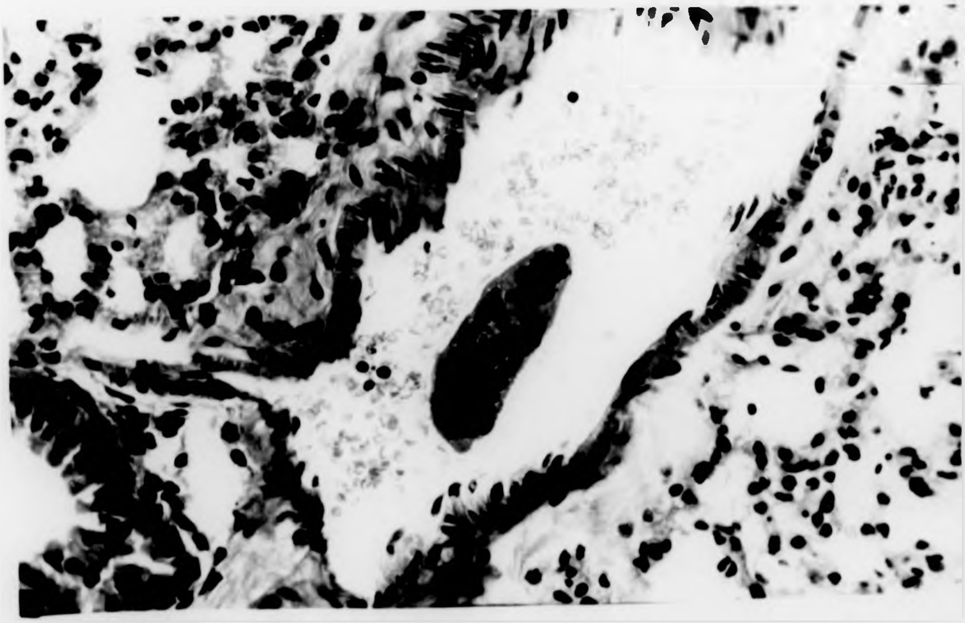


PLATE 19

A, B and C) Serial sections of lung showing a schistosomulum (S. *mansoni*) partly within a branch of the pulmonary vein and partly within a tributary venule. 7 days post-infection 6µm Stained H. and E. (X625)

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and partly
s post-
(X625)

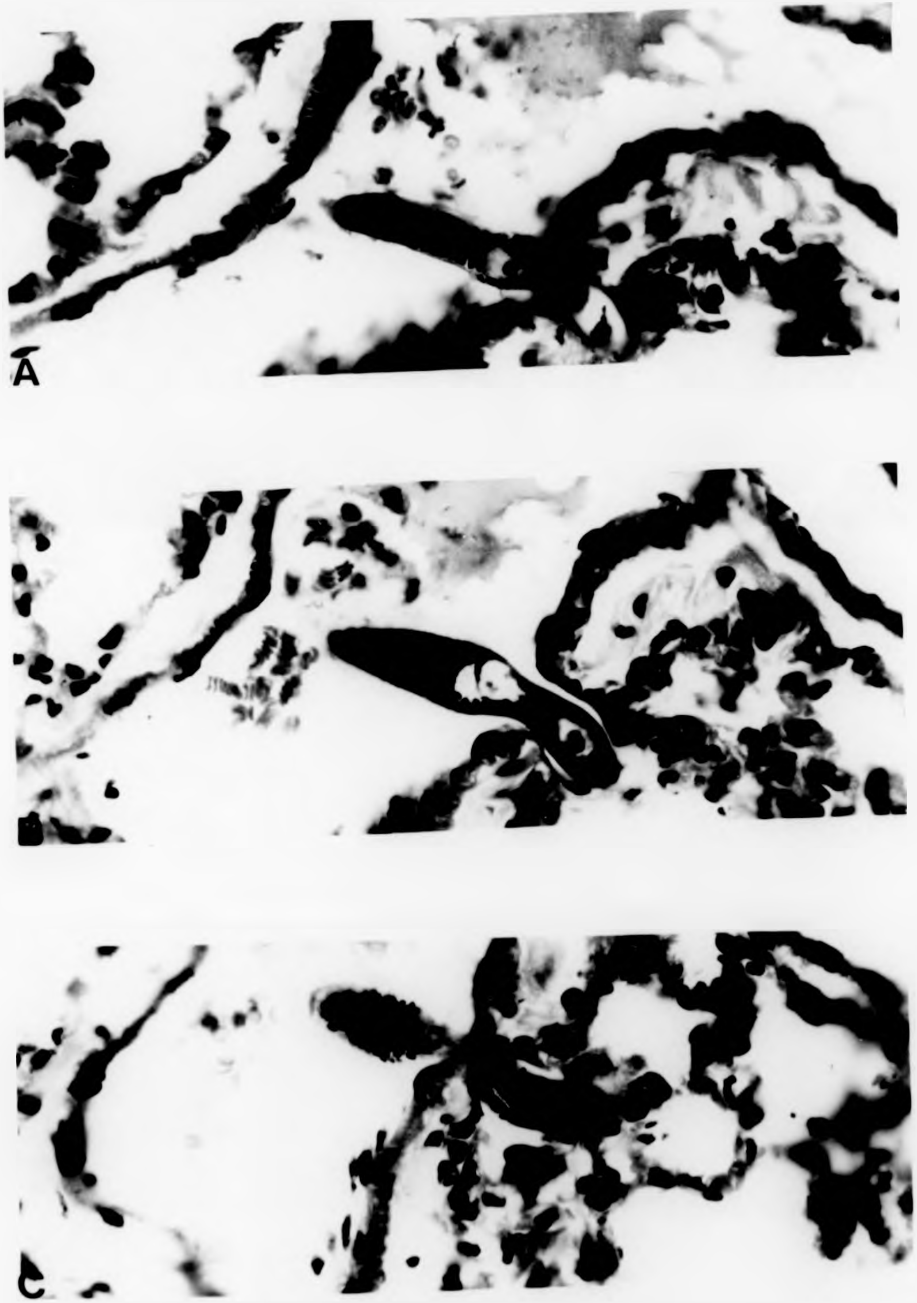
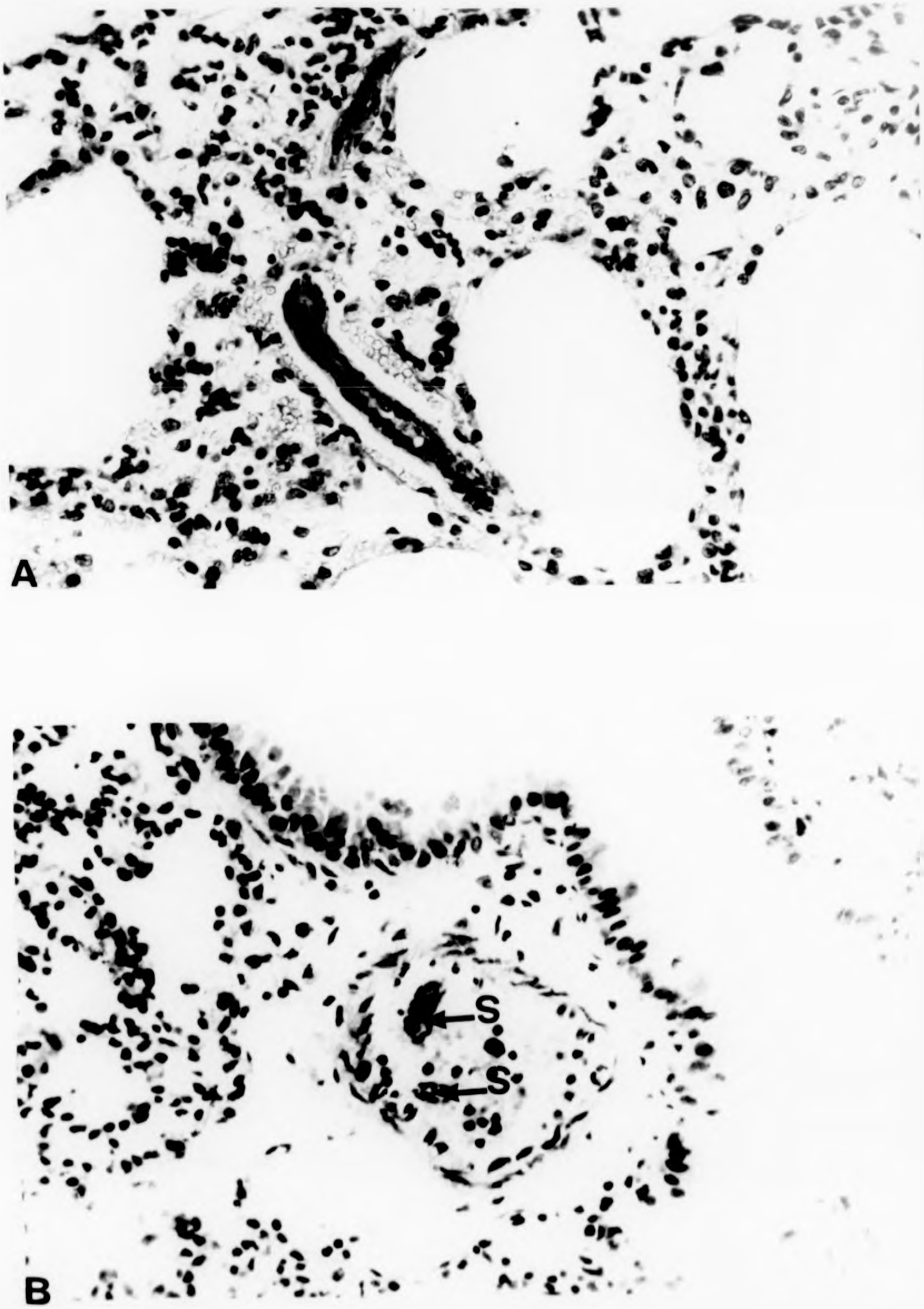


PLATE 20

A) Schistosomulum (S. *mansoni*) in a pulmonary blood vessel 14 days post-infection 6μm Stained P.A.S. (X390)

B) Schistosomulum (S. *mansoni*) in a pulmonary blood vessel showing minimal cellular infiltrate. 16 days post-infection 6μm Stained Verhoeff's and van Gieson (X390)

S - Schistosomulum


A
S
S
B

Brain

In serial sections of whole brains from days 6 to 16 after infection schistosomula were found scattered throughout the cerebrum, cerebellum and brainstem. Parasites were seen both intravascularly and perivascularly; those seen lying perivascularly were associated with perivascular cuffing. Diffuse or focal changes seen, included haemorrhage, necrosis and microglial proliferation. All the aforementioned observations are illustrated in Plates 21-27.

Diaphragm

Schistosomula when found in serially sectioned whole diaphragms from days 6 to 16 were almost invariably observed within blood vessels. Occasionally a parasite was seen within or immediately adjacent to what appeared to be a damaged blood vessel and in such cases there was an associated focal neutrophil and eosinophil polymorphonuclear leucocyte and lymphocyte infiltrate. More peripherally, small numbers of histiocytes were present. Schistosomula located within diaphragms are shown in Plates 28-30.

Kidney

Schistosomula were present in the haematic microcirculation of renal cortex and medulla from days 6 to 16 after infection. They were seen in both arterial and venous channels.

PLATE 21

A, B, C, D, and E)	Serial sections of cerebellar cortex showing a schistosomulum (*S. mansoni*) in a blood vessel in the granular layer. No cellular reaction present. 15 days post-infection 5µm Stained H. and E.
A, B, C, D, and F)	X390
E)	X625
E and D)	the same parasite

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Stained

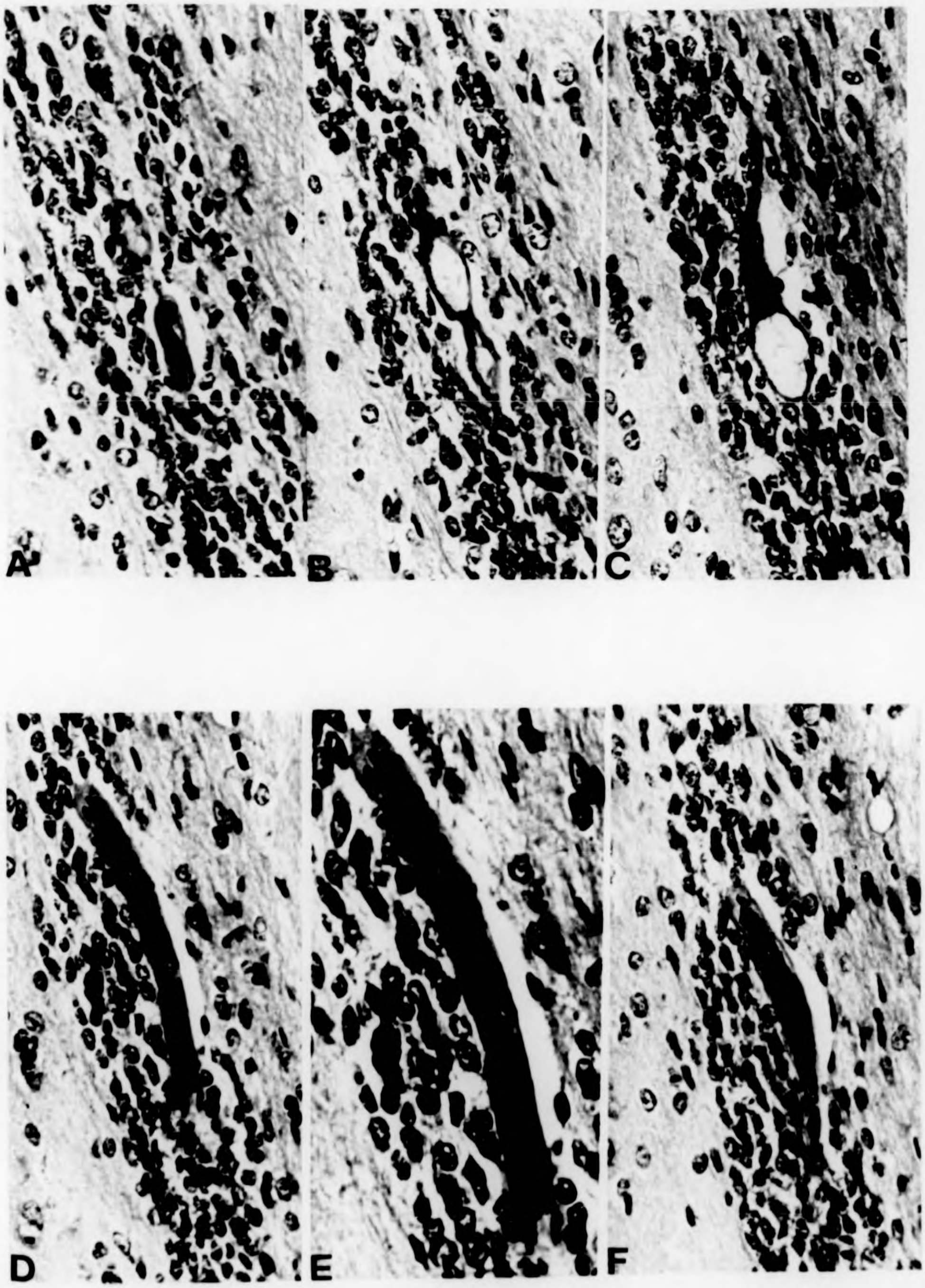

PLATE 22

A, B, C, D, and E)	Serial sections of cerebrum showing a schistosomulum (*S. mansoni*) lying perivascularly. Perivascular cuffing is present. There is an adjacent area of necrosis, haemorrhage and proliferation of microglia. 14 days post-infection 5µm Stained H. and E.	
A)	X98	and
C)	X390	the same section
B)	X98	and
D)	X390	the same section
E)	X390	

H - Haemorrhage
N - Necrosis
P - Perivascular cuffing
S - Schistosomulum

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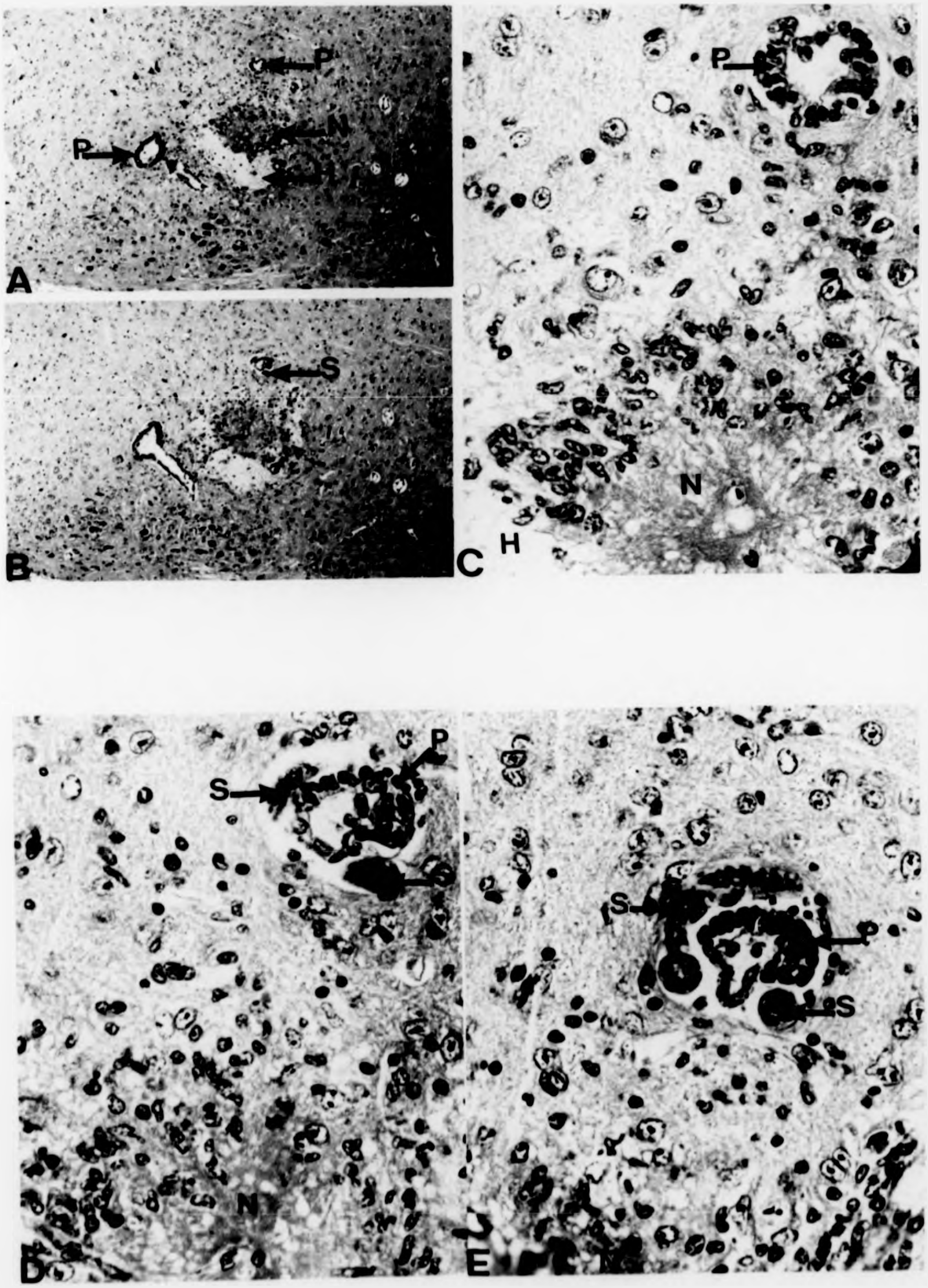
and
14 days
H. and E.


P
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H
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S
E

PLATE 23

A) Schistosomulum (S. mansoni) in a blood vessel with adjacent haemorrhagic area beneath the ependymal lining of a cerebral lateral ventricle in the cerebral hemisphere. No cellular reaction present. 15 days post-infection 5µm Stained H. and E. (X625)

B) Schistosomulum (S. mansoni) in a blood vessel in the pia of the cerebellum. 10 days post-infection 5µm Stained H. and E. (X625)

E - Ependymal cells
H - Haemorrhage
S - Schistosomulum

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15 days
5)

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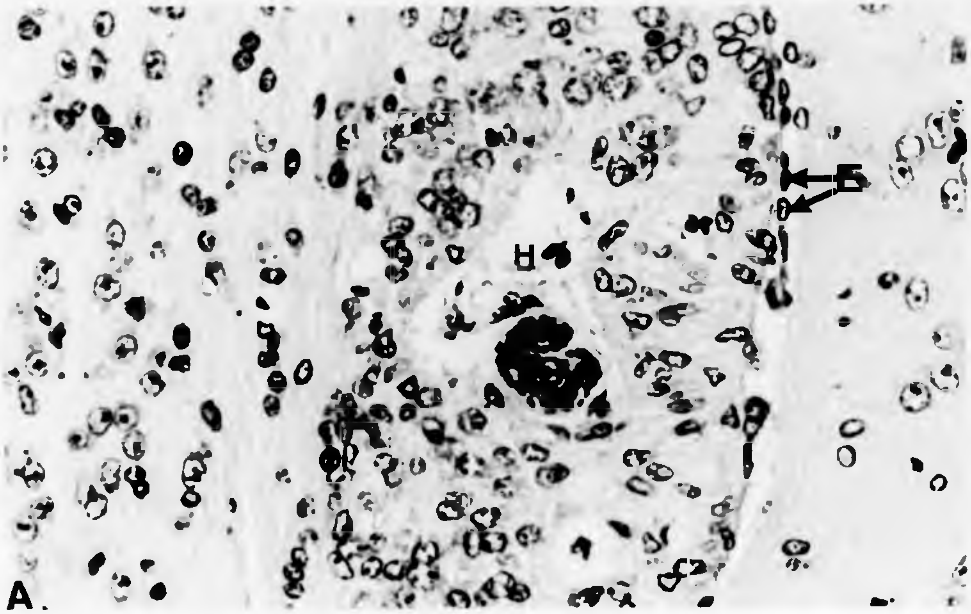

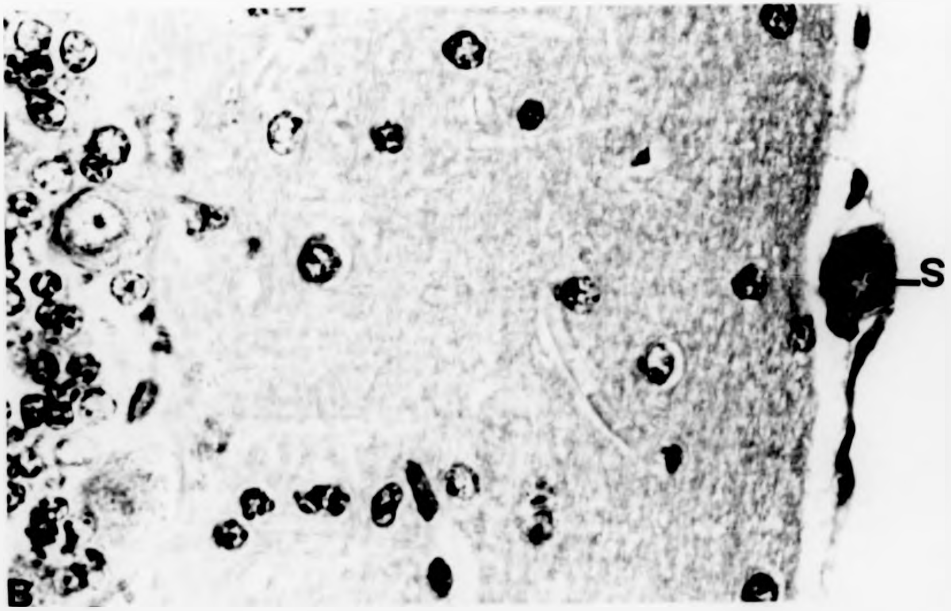

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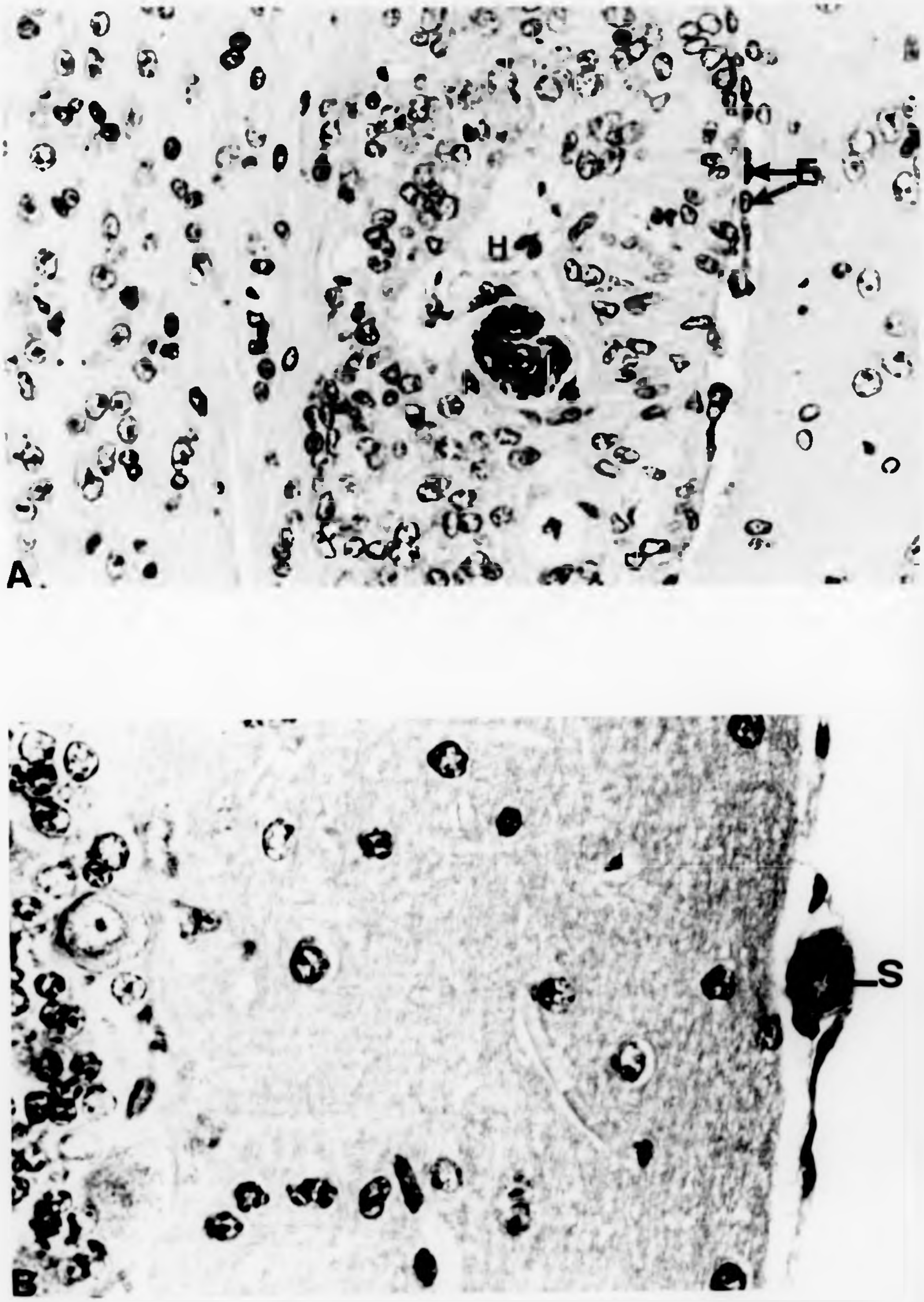

PLATE 24

A, B, C, and D)	Serial sections of cerebellar cortex showing a schistosomulum (*S. mansoni*) immediately beneath the granular layer. 16 days post-infection 5µm Stained H. and E. (X625)

G - Granular layer
P - Purkinje cells
S - Schistosomulum
W - White matter

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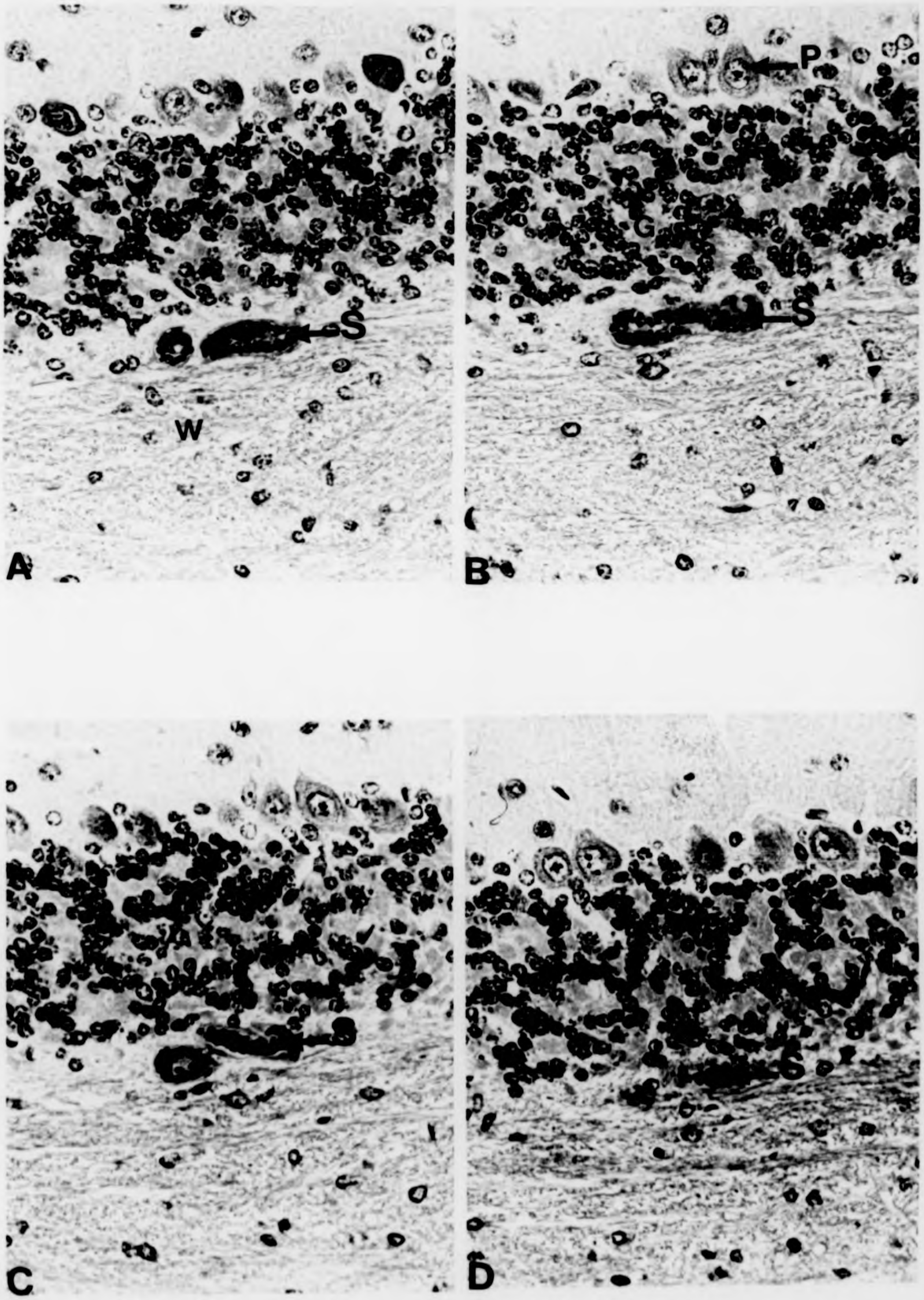

PLATE 25

A, B, C, and D) Serial sections of cerebrum showing a schistosomulum (S. mansoni) in the deep cortex Adjecent proliferation of microglia 16 days post-infection 5µm Stained H. and E. (X625)

S - Schistosomulum

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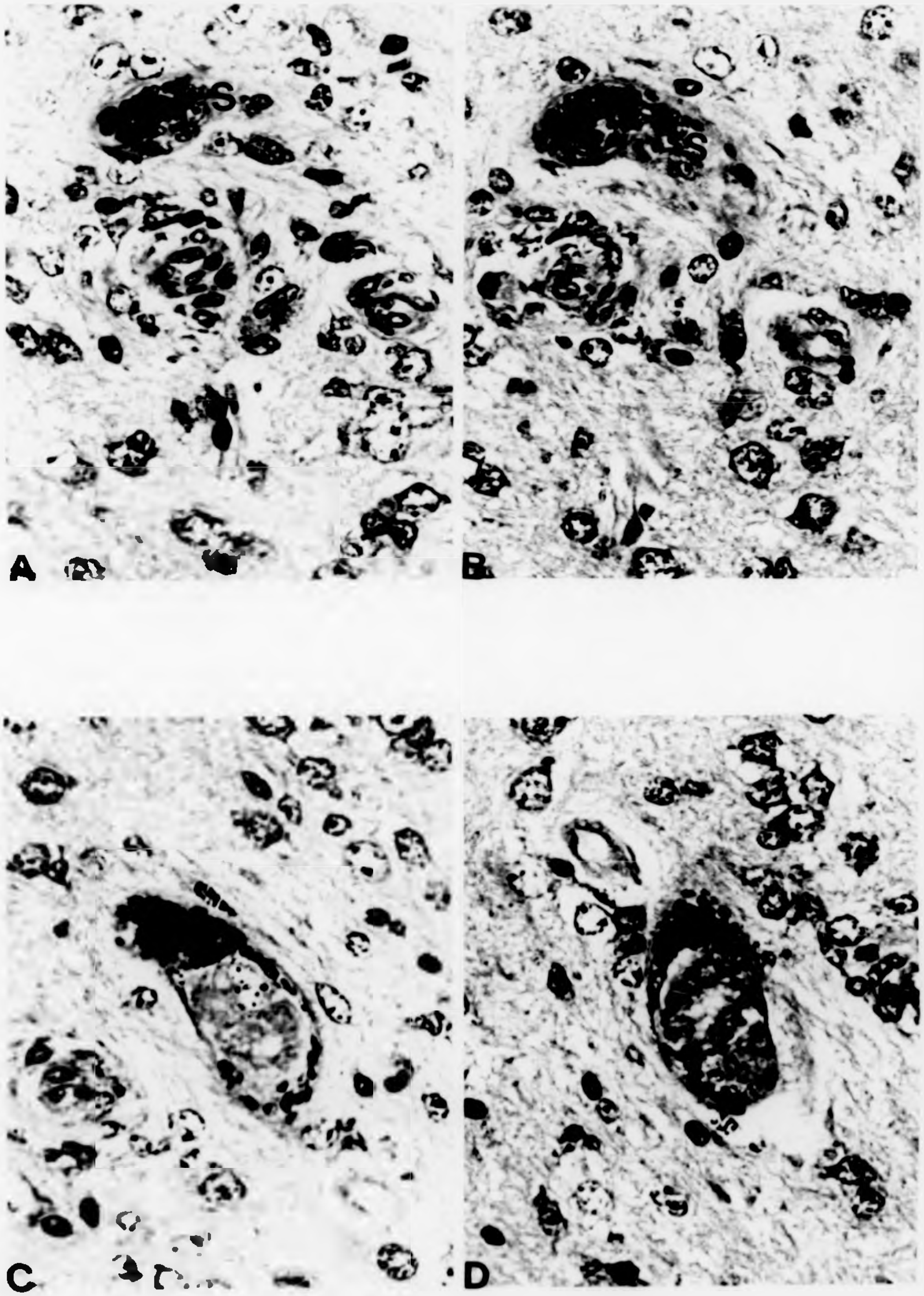

PLATE 26

A) Schistosomulum (S. mansoni) in the choroid plexus of a lateral ventricle in a cerebral hemisphere 15 days post-infection 5μm Stained H. and E. (X390)

B) Schistosomulum (S. mansoni) in a haemorrhagic area in the cerebrum. 16 days post-infection 5μm Stained H. and E. (X390)

C - Choroid plexus
S - Schistosomulum

PLATE 26

A) Schistosomulum (*S. mansoni*) in the choroid plexus of a lateral ventricle in a cerebral hemisphere 15 days post-infection 5μm Stained H. and E. (X390)

B) Schistosomulum (*S. mansoni*) in a haemorrhagic area in the cerebrum. 16 days post-infection 5μm Stained H. and E. (X390)

C - Choroid plexus

S - Schistosomulum

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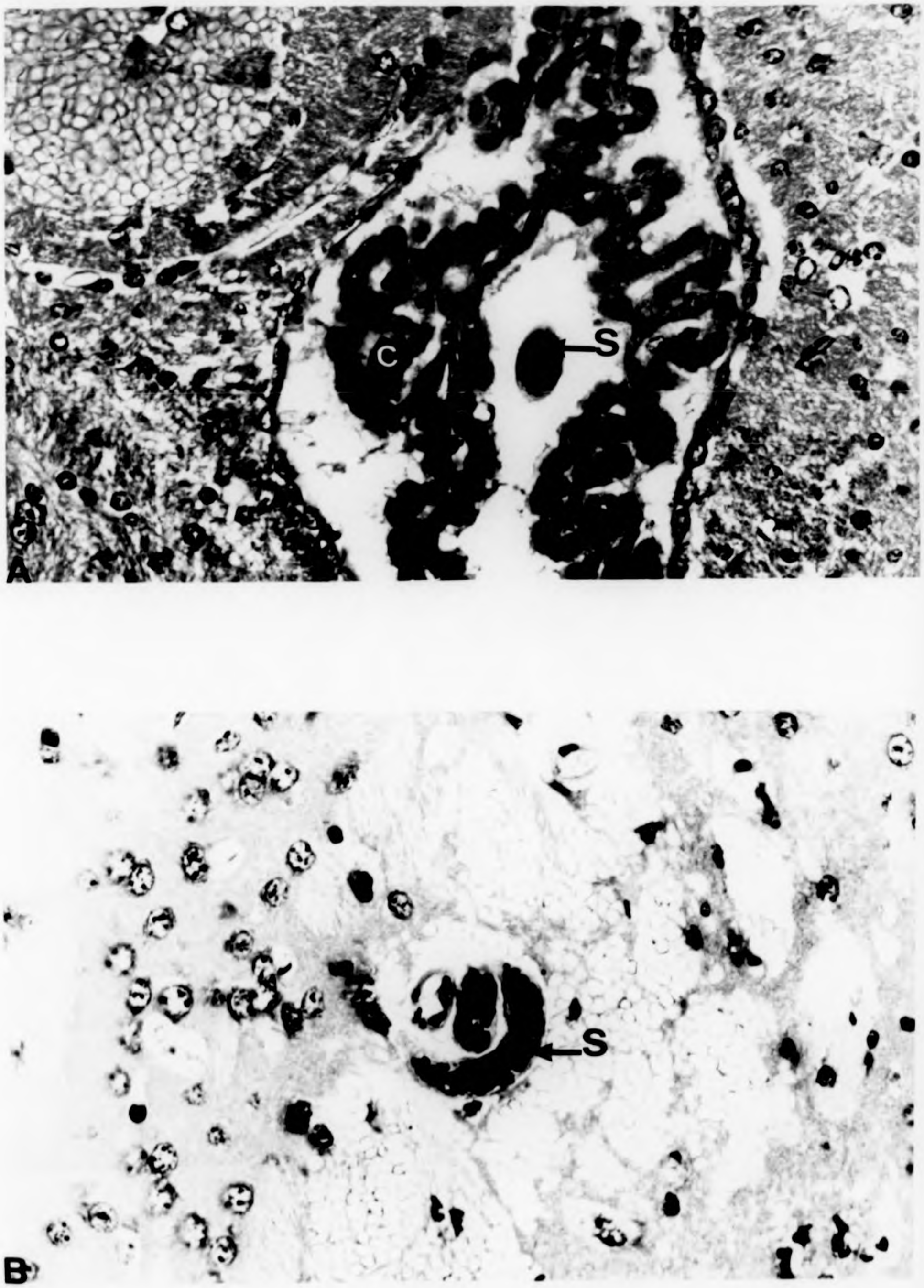

PLATE 27

A, B, C, and D)	Serial sections of cerebrum showing a schistosomulum (*S. mansoni*) partially within an area of necrosis and haemorrhage. Diffuse cellular reaction is evident. 13 days post-infection 5μm Stained H. and E.
A)	X98
B)	X390 and
C)	X625 the same section
D)	X390

H - Haemorrhage
N - Necrosis
S - Schistosomulum

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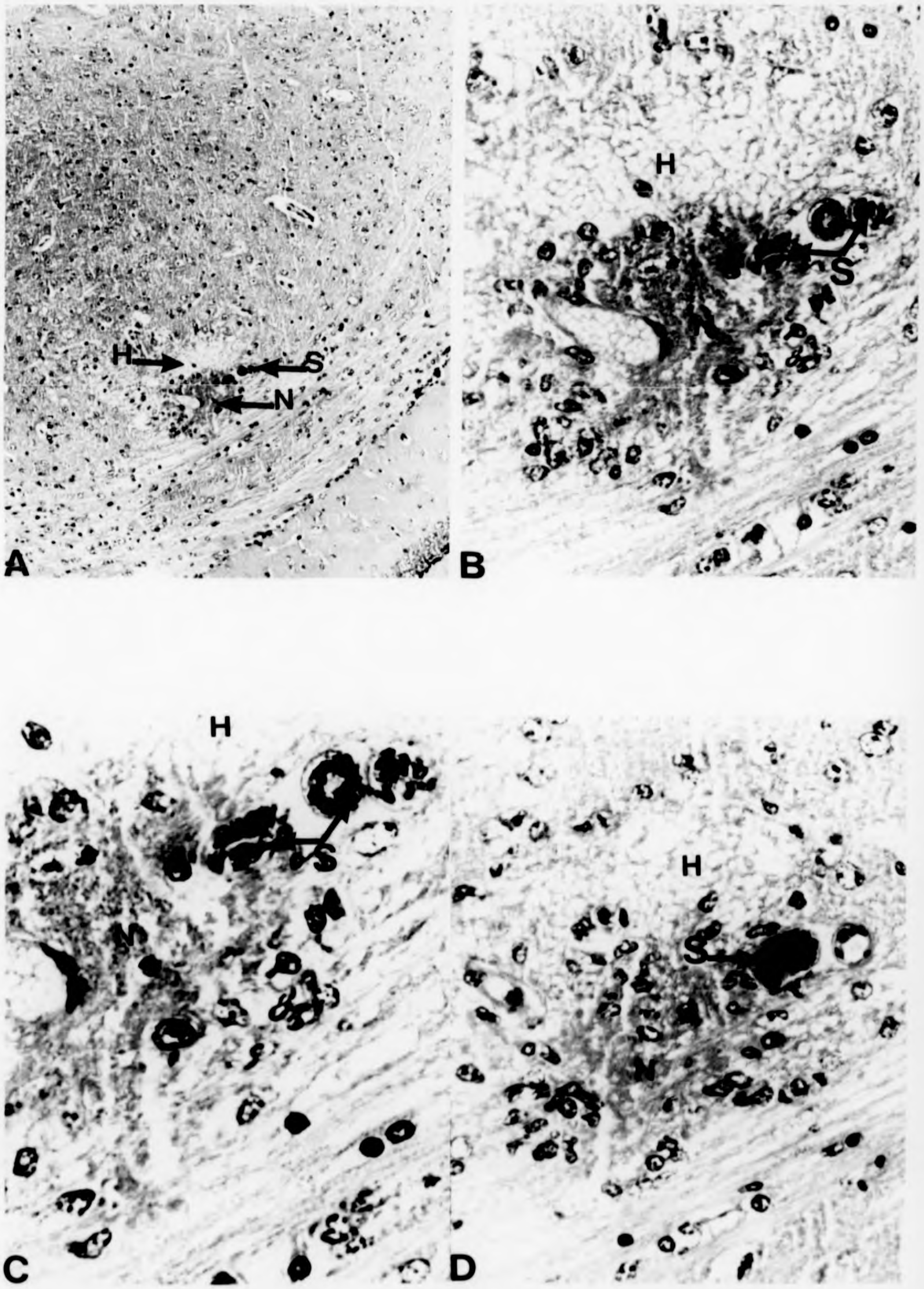
H
S
N
A
H
S
B
H
S
N
C
H
S
N
D

PLATE 28

Serial sections A to D show a diaphragmatic blood vessel containing a schistosomulum (*S. mansoni*) at different levels. 11 days post-infection 6μm Stained H. and E. (X390)

S - Schistosomulum

c blood
soni) at
6μm

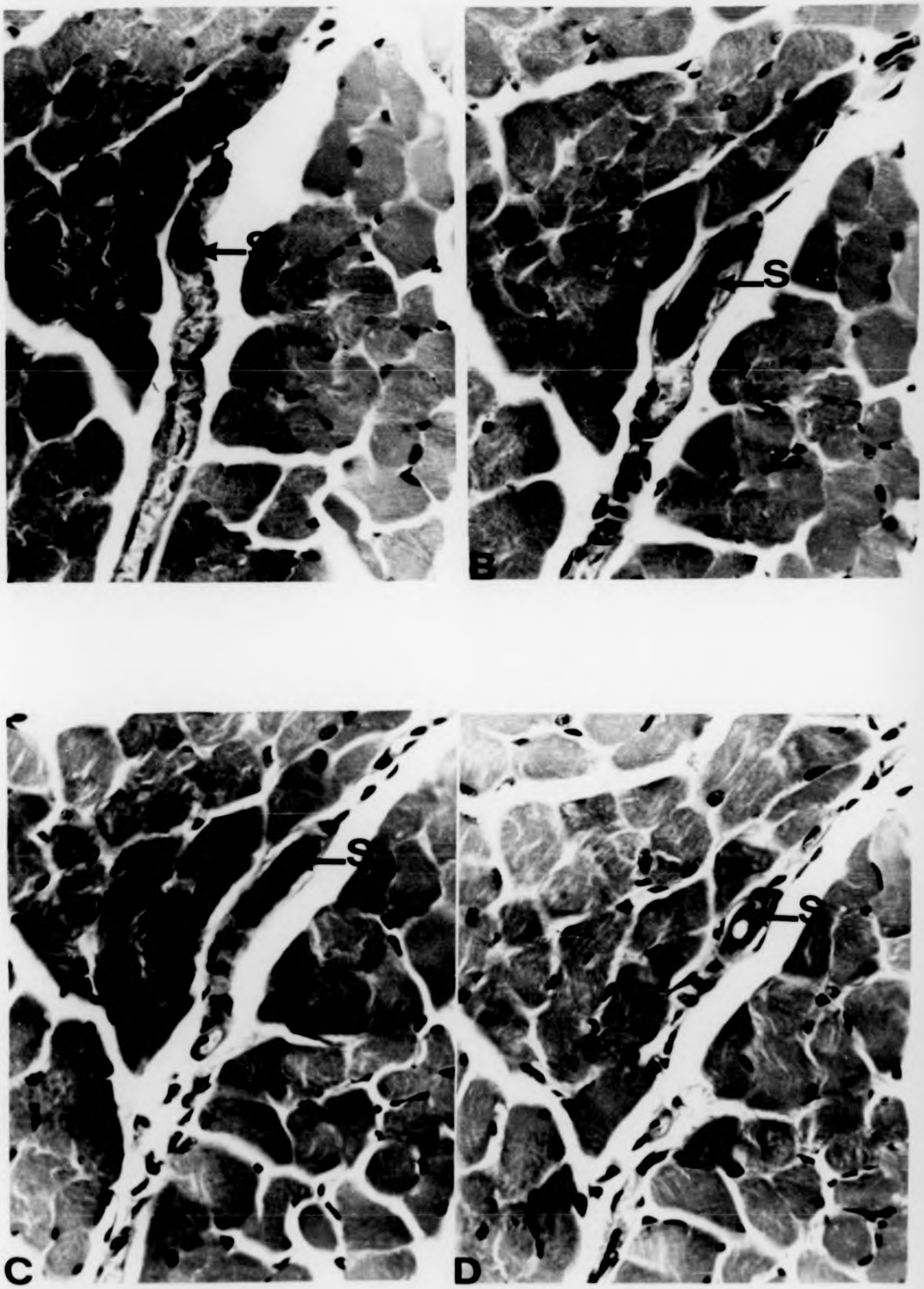

PLATE 29

A) A schistosomulum (S. mansoni) in a blood vessel of the diaphragm 13 days post-infection 6μm Stained H. and E. (X625)

B) A schistosomulum (S. mansoni) in a blood vessel of the diaphragm 11 days post-infection 6μm Stained H. and E. (X625)

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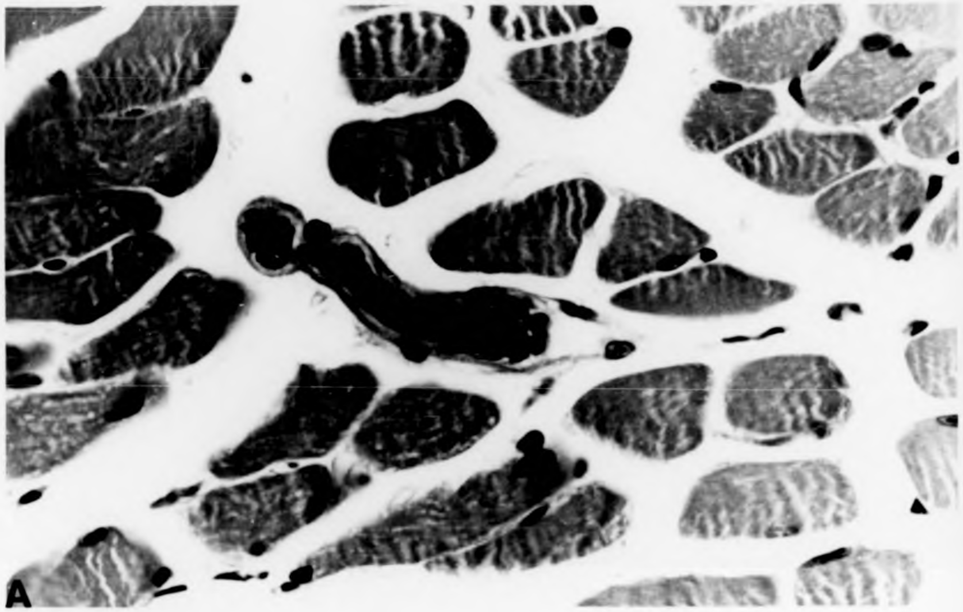

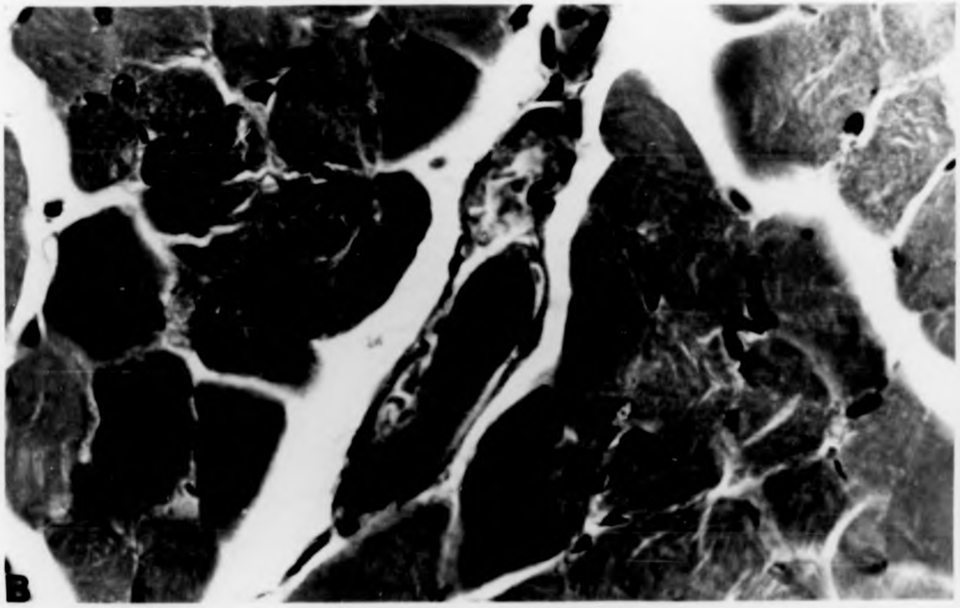

PLATE 30

A and B) Serial sections of diaphragm showing in (A) a schistosomulum (S. mansoni) in a blood vessel with associated cellular infiltrate and in (B) – the succeeding section – showing only infiltrate. 11 days post-infection 6μm Stained H. and E. (X390)

C and D) Serial sections of diaphragm showing two views of a schistosomulum in a blood vessel with associated diffuse cellular infiltrate. 13 days post-infection 6μm Stained H. and E. (X390)

S – Schistosomulum

(A) a
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views
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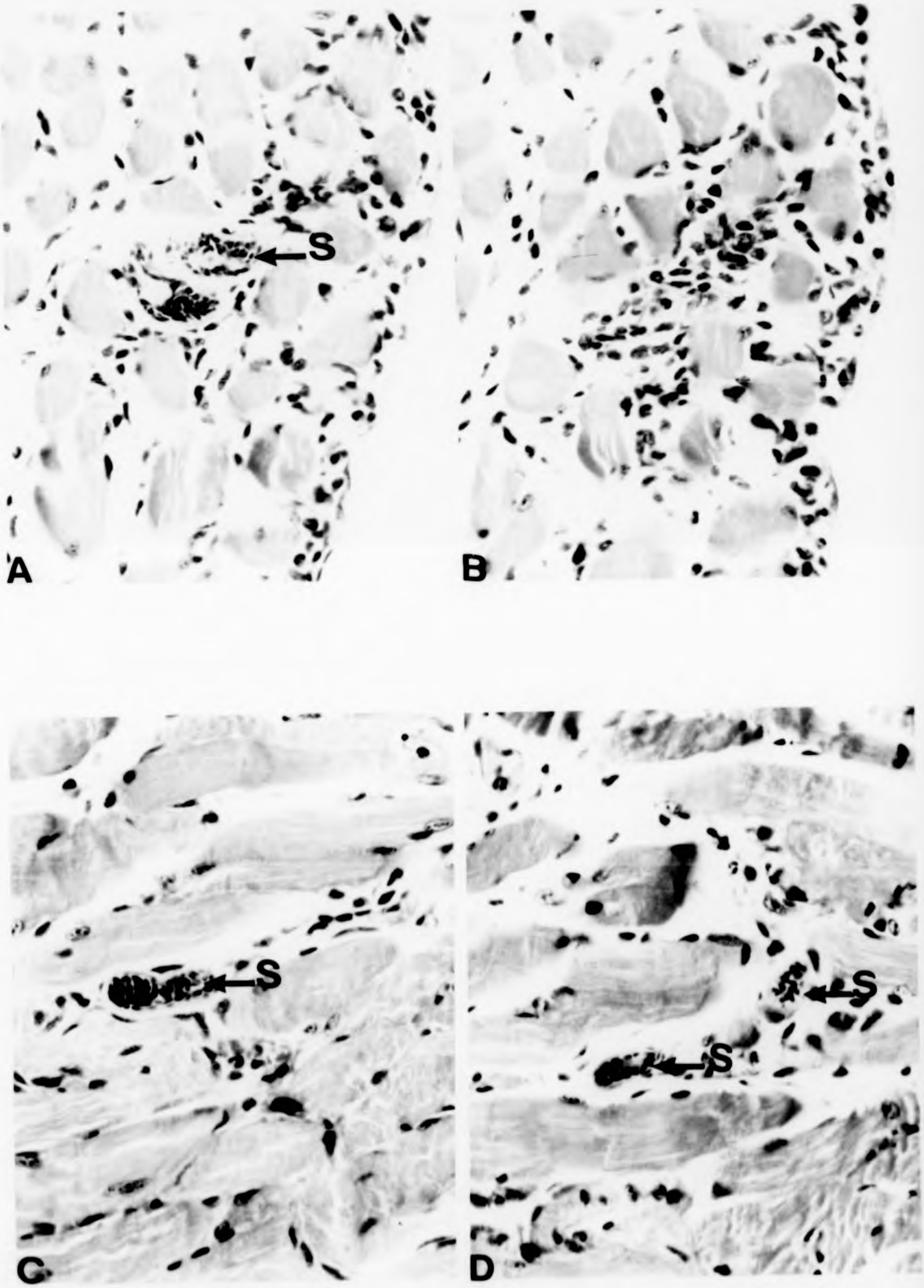
13 days
(X390)



Schistosomula were also seen lying outside vessels of the microcirculation. Parasites were present interstitially and some contained pigment in the lumen of the gut. Apparently damaged glomeruli* and tubules were observed**. Hyaline casts were seen both in collecting and convoluted tubules of some kidneys. While schistosomula located within apparently intact renal vasculature were seen to be devoid of any cellular reaction, others lying partially within or wholly outside blood vessels were seen to be associated with varying degrees of cellular infiltrate even to the extent of granuloma formation. Granuloma comprised both neutrophil and eosinophil polymorphonuclear leucocytes, histiocytes, occasional lymphocytes and giant cells. A granuloma containing a schistosomulum was found as early as 8 days after infection. Schistosomula within kidney are demonstrated in Plates 31-34.

Spleen

No schistosomula were found in the spleen in serially cut

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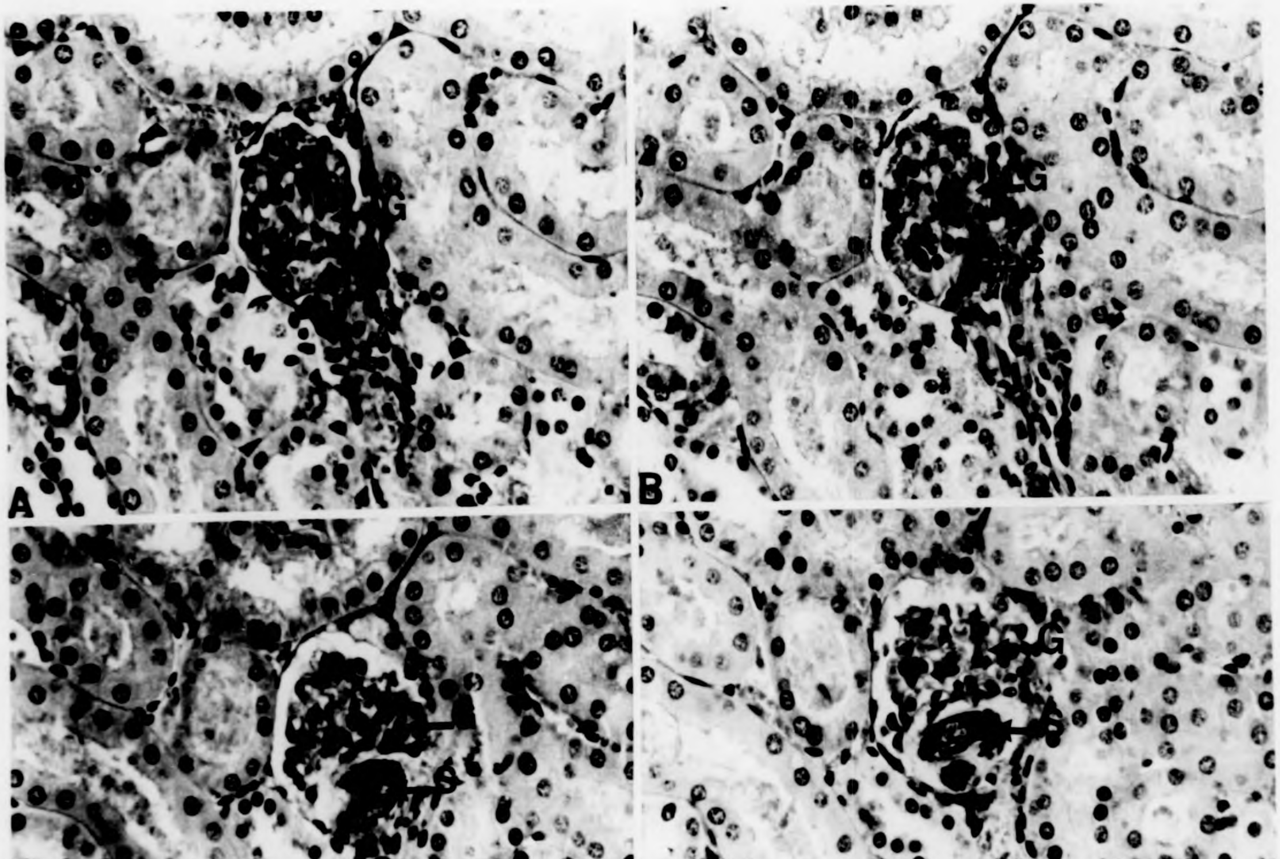
* It is to be noted that the presence of cuboidal cells in the parietal layer of the Bowman's capsule in the glomerulus is not necessarily an indication of pathological lesions. Crabtree (1940) has demonstrated that this is a characteristic of the kidney in the mature male mouse (Plate 33A)

** Chloroform for anaesthetizing or killing mice is to be avoided. Even low concentrations of chloroform inhaled by the male mouse have been known to provoke pathological changes in the kidney including nephrosis (Jacobsen, Krag Andersen and Thorborg, 1964).

PLATES 31 and 32

Serial sections A to H show different levels of a renal glomerulus containing a schistosomulum (*S. mansoni*) within a capillary tuft. No cellular infiltrate is evident. 16 days post-infection 6μm Stained Lendrum's method (X390)

G - Glomerulus
S - Schistosomulum


G
A
G
S
B
S
C
G
S
D

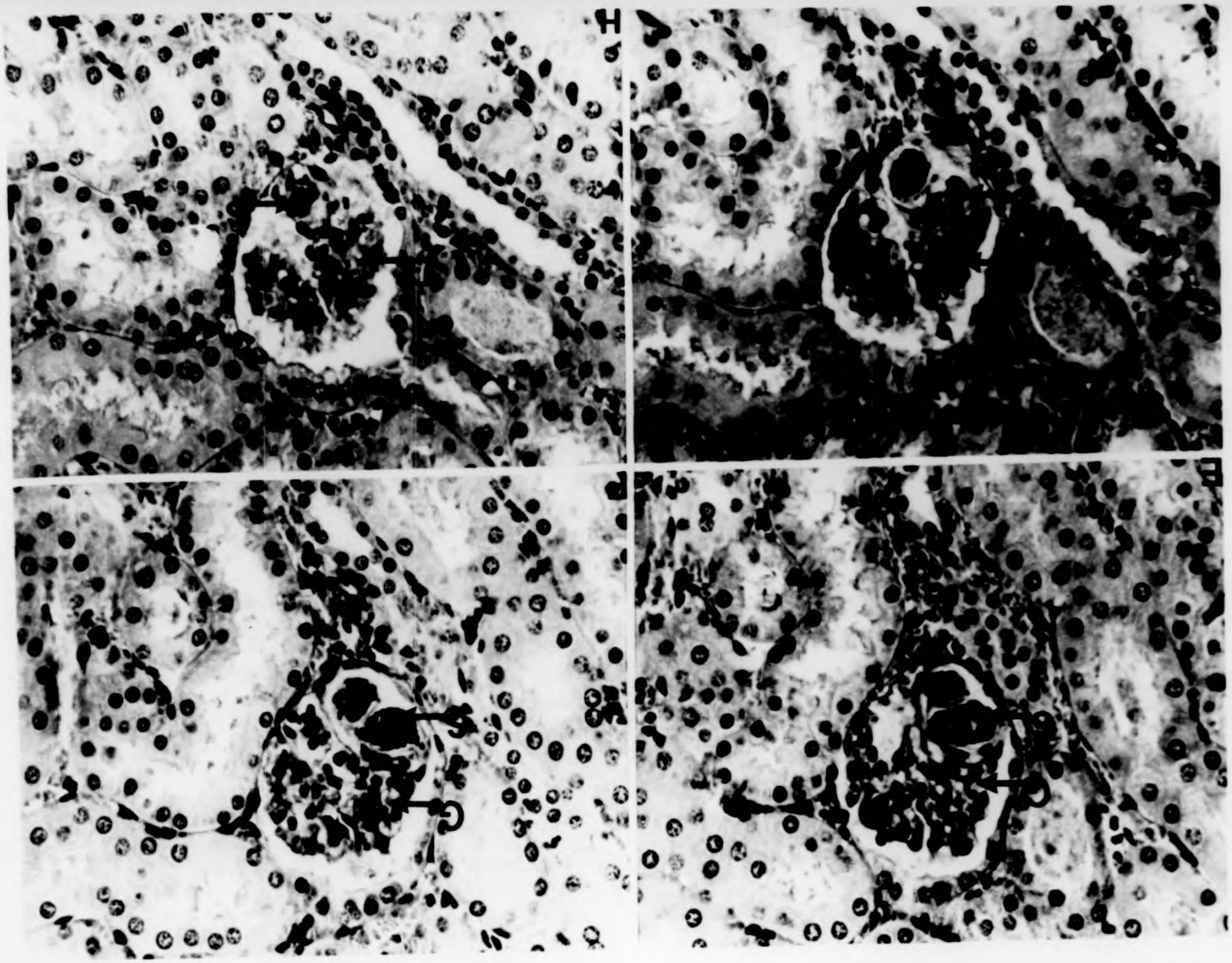

H
E
J
G
J
G

PLATE 33

A) Schistosomulum (*S. mansoni*) within a capillary tuft of a renal glomerulus showing no evidence of cellular reaction. Note cubiodal epithelium of the parietal layer of Bowman's capsule which is characteristic of the glomerulus of the male mouse. 15 days post-infection 6μm Stained H. and E. (X390)

B) Schistosomulum (*S. mansoni*) in a blood vessel between convoluted tubules in the cortex of the kidney; no cellular reaction is evident. 16 days post-infection 6μm Stained Lendrum's method (X390)

C) Schistosomulum (*S. mansoni*) situated interstitially in the renal cortex with cellular infiltrate adjacent to convoluted tubules. 16 days post-infection 6μm Stained H. and E. (X390)

D) Schistosomulum (*S. mansoni*) within a granuloma with early giant cell formation in the renal cortex 8 days post-infection 6μm Stained H. and E. (X390)

C - Cuboidal epithelium
G - Giant cell
R - Renal Glomerulus
S - Schistosomulum
V - Vessel

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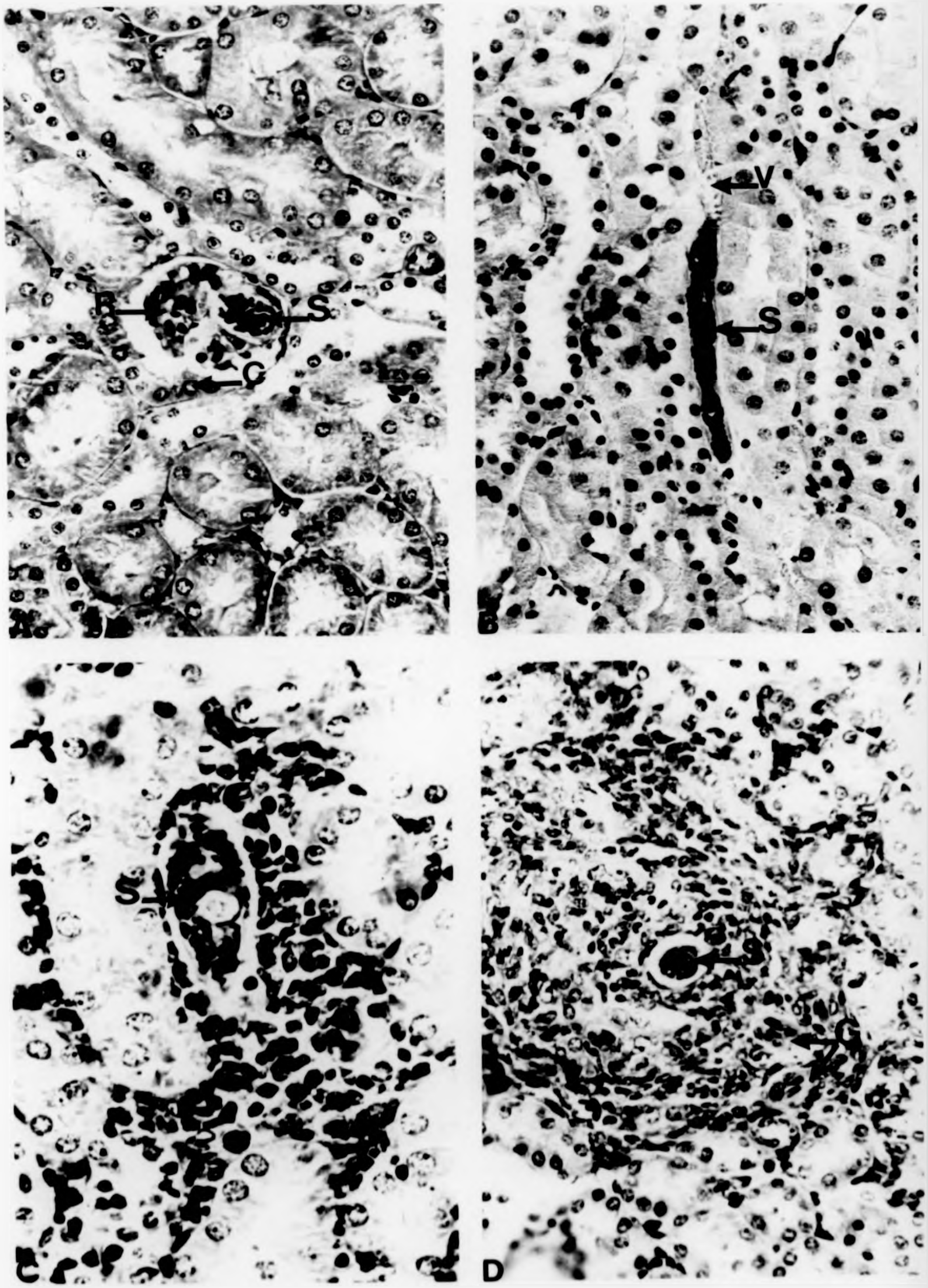
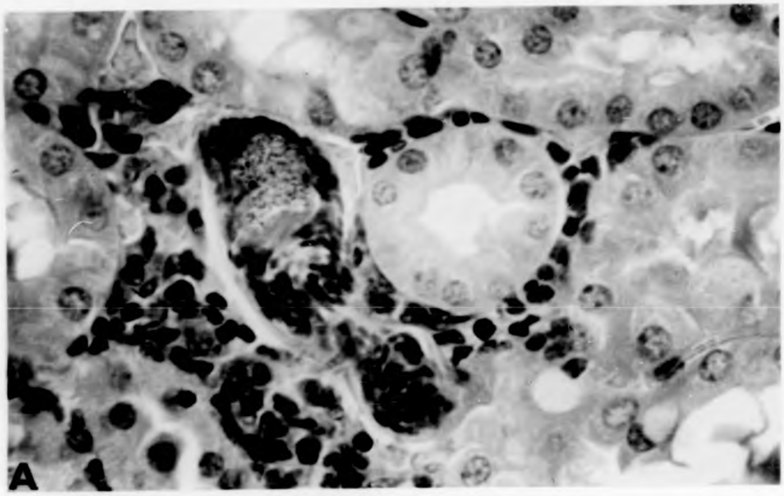
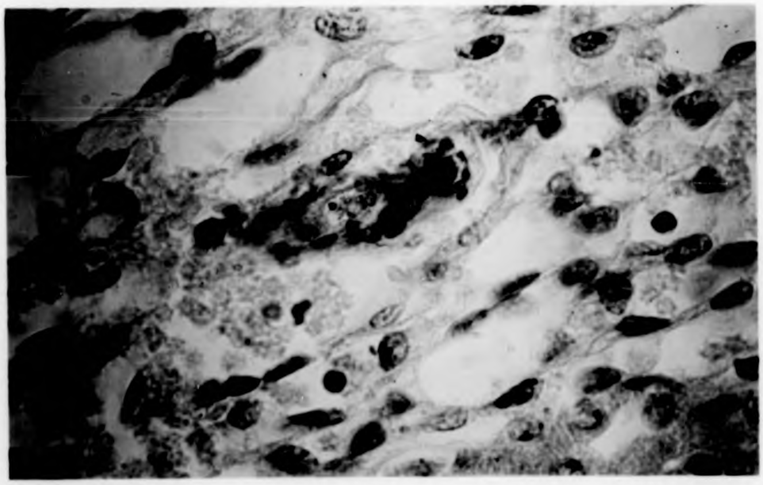

R
S
C
A
V
S
B
S
C
S
G
D

PLATE 34

A) Schistosomulum (S. mansoni) lying interstitially between convoluted tubules in the renal cortex and showing minimal focal cellular infiltrate. 16 days post-infection 6µm Stained H. and E. (X1000 oil immersion)

B) Schistosomulum (S. mansoni) in the renal medulla 9 days post-infection 6µm Stained H. and E. (X1000 oil immersion)


A



sections covering days 6 to 16 after infection.

Pancreas

In serial sections of pancreas from days 6 to 16 after infection a larva was found in sections relating to day 16 only. The larva was contained within a blood vessel. Haem was seen in the lumen of the larval gut (see Plate 35).

Liver

Larvae were present in serial sections of liver from days 6 to 16 after infection. The parasites were seen within branches of the hepatic portal vein, hepatic sinusoids and branches of the hepatic vein. No parasites were found in branches of the hepatic artery. Larvae were not seen to be associated with any cellular infiltrate. Plates 36-38 show parasites within afferent and efferent vessels of the hepatic blood circulatory system. See also Chapter 4 - Plates 6-9 pages 71-78.

S. haematobium infections

Serial sections were cut only on days 4, 8 and 15 following infection. Sections of skin on day 4 revealed schistosomula within dermal blood vessels. In sections of lung, brain, diaphragm, kidney and liver, schistosomula, located intravascularly, were seen both on day 8 and day 15. Parasites were not found in sections of spleen and pancreas.

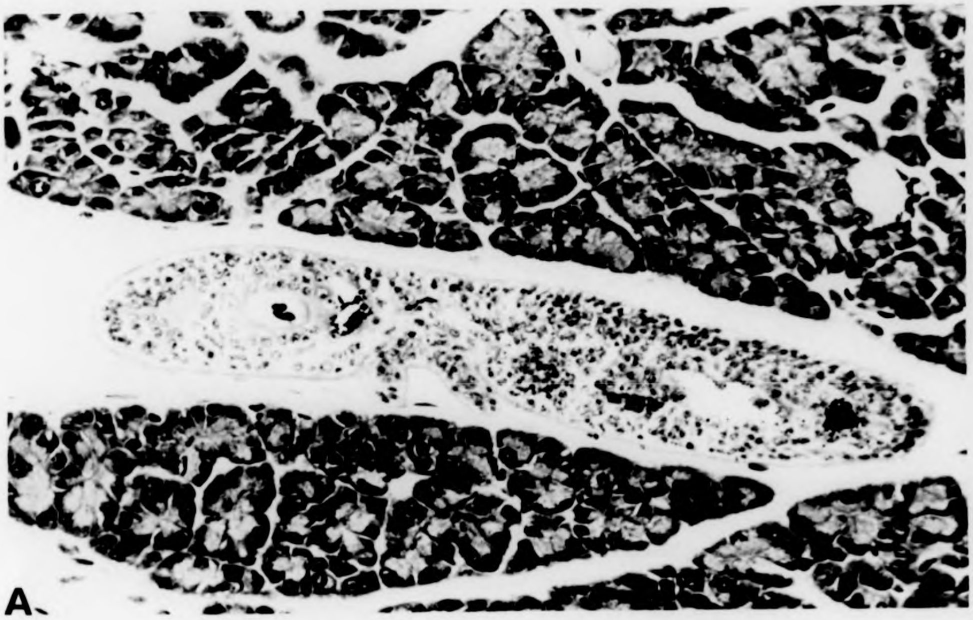
PLATE 35

A) A larva (S. mansoni) in a pancreatic blood vessel 16 days post-infection 6µm Stained H. and E. (X625)

B) The same larva as in (A) at a higher magnification (X1500 oil immersion)

essel
E. (X625)

cation



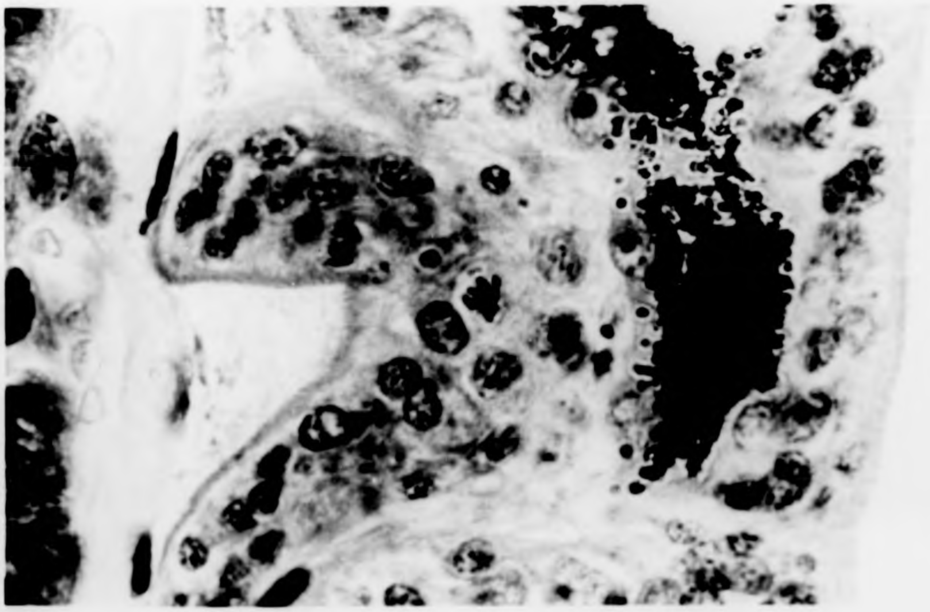


PLATE 36

A, B, C and D) Serial sections of liver showing a larva in a vein. 9 days post-infection 5μm Stained H. and E. (X390)

larva
5μm

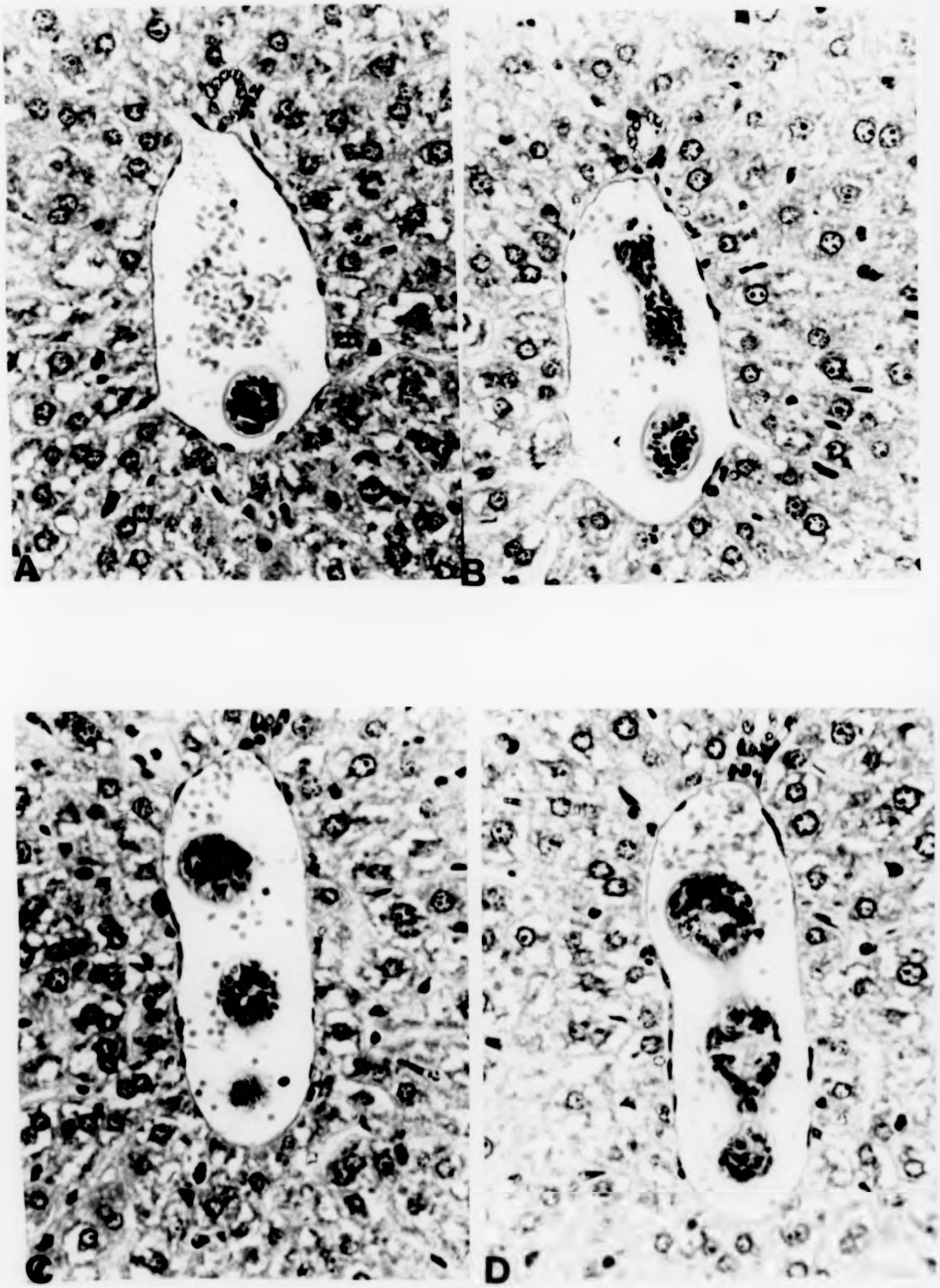
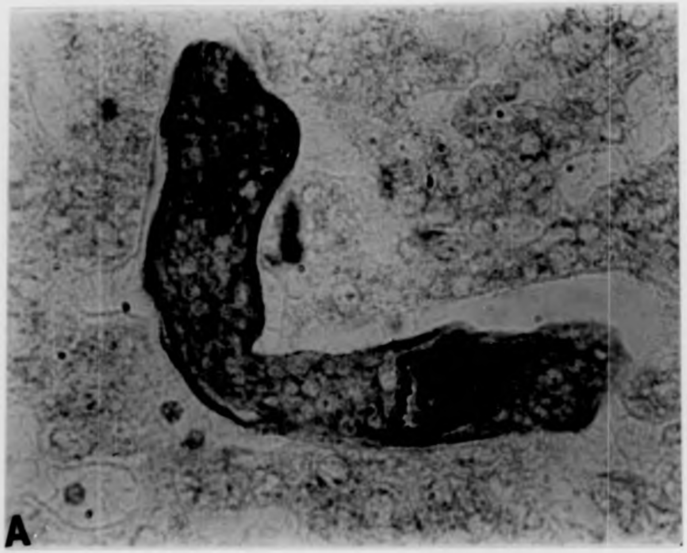


PLATE 37

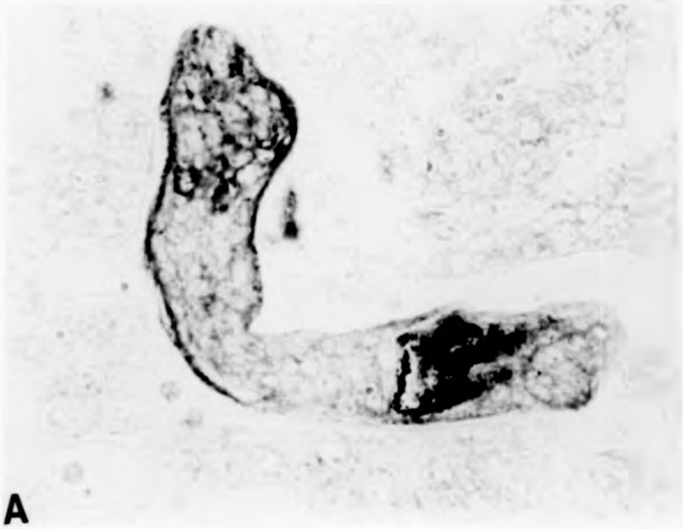
A, B, C and D) Serial sections of a larva (S. mansoni) in hepatic sinusoid. 16 days post-infection 5µm Stained metachromatically after Malinin (1970)



Section A reproduced in colour in addition to black and white

PLATE 37

A, B, C and D) Serial sections of a larva (S. mansoni) in hepatic sinusoid. 16 days post-infection 5μm Stained metachromatically after Malinin (1970)



Section A reproduced in colour in addition to black and white

Section A reproduced in colour in addition to black and white

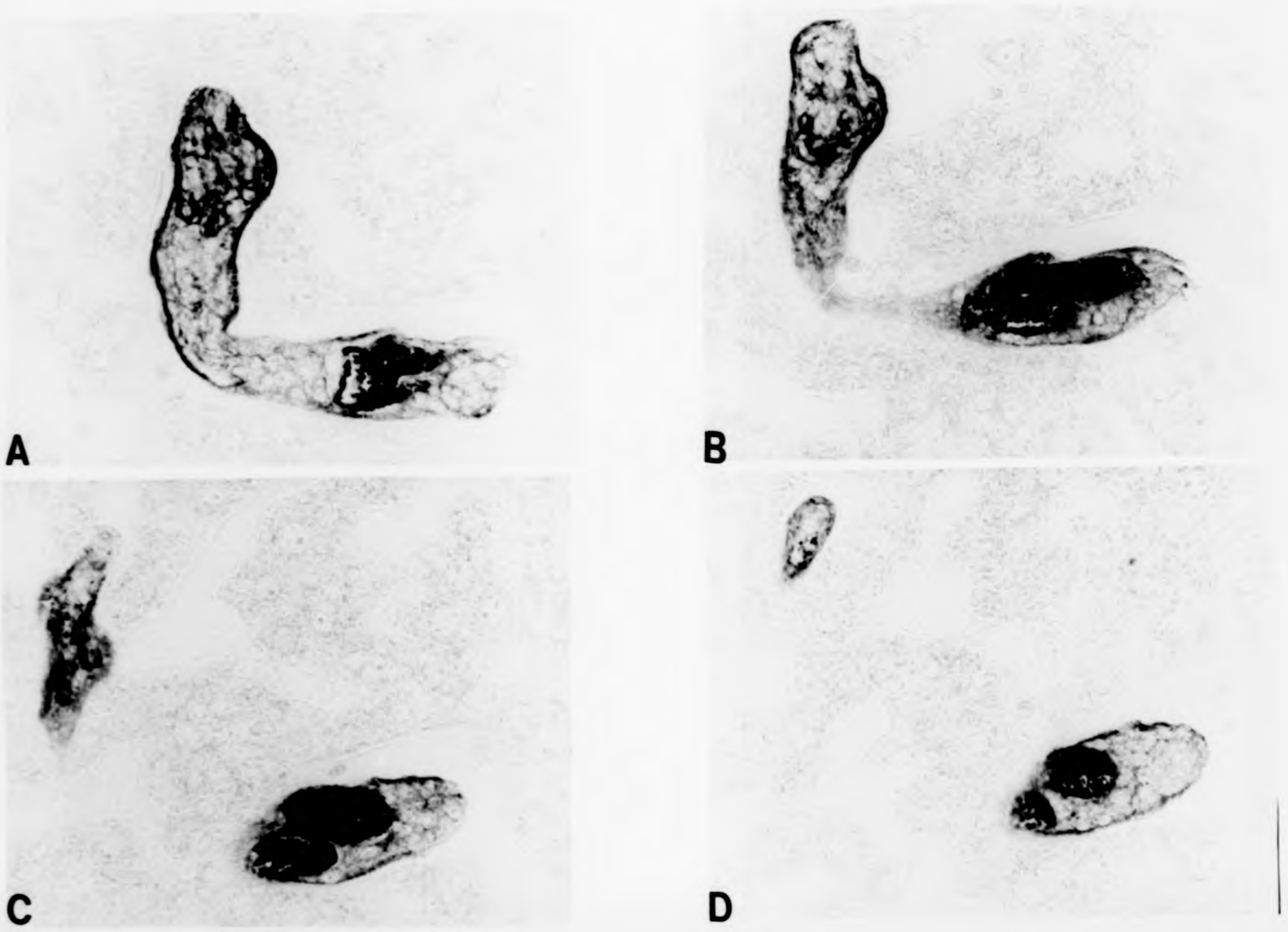
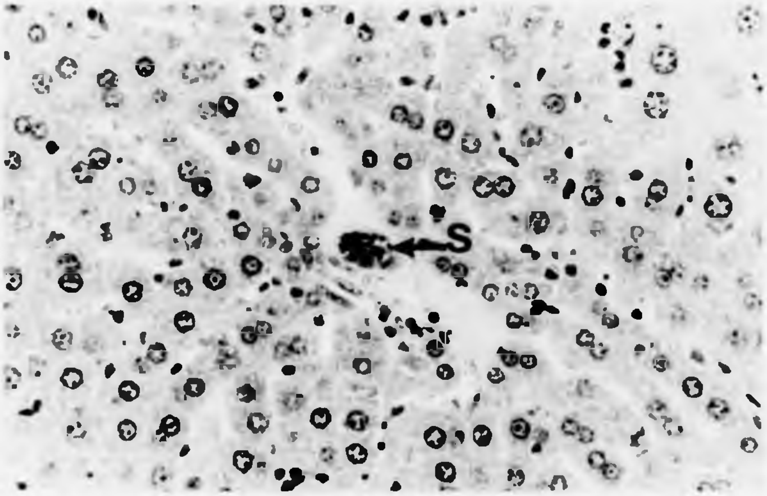


PLATE 38



A schistosomulum (S. mansoni) in a liver sinusoid 8 days post-infection 5μm Stained H. and E. (X390)

S - Schistosomulum

6.4 DISCUSSION

The mouse, a good host for *S. mansoni* and a poor one for *S. haematobium*, was used in the present study of experimental infections. There can be no doubt that the schistosomulum of either species proceeds with the flow of blood and utilizes the blood circulatory system as a route of migration from the skin via the lung to the liver. This conclusion has been reached on the basis of evidence provided by histological serial sections revealing the presence of schistosomula in both macro- and microvessels in different tissues and organs. Moreover, there is evidence that in the course of migration to the liver a proportion of schistosomula are trapped in various organs and tissues. There is also indirect evidence suggesting that the parasite may enter the pulmonary and systemic circulations more than once.

While no quantitative analysis of distribution was attempted in this histological study, schistosomula were found more often in some organs than in others; they were least difficult to locate in smaller and more vascular organs.

Migration from the skin by the blood circulatory route in addition to exit by the lymphatic route (see Chapter 7) has been established in this study. As early as day 2 after percutaneous infections via the tail schistosomula were seen in dermal blood vessels, larvae were also seen in dermal lymphatics on day 2. Stirewalt (1959), employing percutaneous tail exposure, found that parasites did not appear in the dermal blood vessels till 5 days after infection. Infecting through the abdominal skin the same worker obtained histological evidence of parasites in dermal blood vessels within 1 hour of infection. The difference in time of appearance is attributed by Stirewalt (1959) to larval delay in tail skin occasioned by its different texture as compared with abdominal skin. On the other hand Wheater and Wilson (1979) infecting through abdominal skin first observed exit from the skin by a dermal blood vessel on day 3 post-infection. The disparity in these observations does not appear to be related to the level of infection but can possibly be explained in part by differences in the numbers of sections cut and the numbers of observations made. In addition it is suggested that chance may play a role both as regards parasites entering dermal blood vessels and cutting sections which contain a parasite. In the present study schistosomula were found on day 6 to be widely dispersed throughout the body by the systemic circulation (Table 11). By the same token parasites might simultaneously be dispersed to the skin. From day 6 onwards, the blood vasculature of the skin will probably contain a mixed population made up of schistosomula leaving the skin and parasites returned there by the systemic circulation. Finding a schistosomulum in a subcutaneous caudal artery on day 9 after infection

Stirewalt (1959) considered it to be a parasite which had forced its way out of a venule into an artery, rather than that it might be a recirculating schistosomulum arriving back at the skin. The author of this study suggests that the converse is the likelier alternative.

Schistosomula, which have entered the dermal blood vessels leaving the skin, after passing through the right atrium, begin the pulmonary phase of migration by being pumped with the blood from the right ventricle into the pulmonary artery. In this study serial sections of whole heart and lung revealed the presence from time to time of parasites lying free in the blood contained in the right atrium and right ventricle as well as in branches of the pulmonary artery within the lungs. After passing through the pulmonary artery the transpulmonary phase begins and the schistosomula are dispersed through the pulmonary blood vasculature. Histological sections of lung show schistosomula lying for the most part within blood vessels. Occasionally they were seen lying extravascularly within alveoli (see Chapter 8, page 273). On the basis of the histological studies undertaken in this investigation it is not possible to state with any accuracy how long the parasite remains in the pulmonary circulation. Since parasites were found in the lungs no earlier than 2 days after infection, and the earliest they were found in organs supplied by the systemic circulation was 6 days after infection, it might be inferred that schistosomula were delayed for 4 days in the pulmonary circulation. However, considering the time normally spent in the skin prior to migration the appearance of parasites in the lungs at day 2 post-infection is both unusually early and is representative of only a small proportion of the original infecting cohort. The chances of finding any of these dispersed to organs by the systemic circulation would be small. They would be more likely to be dispersed to the carcass having regard to the greater share of cardiac output which it receives (Wetterlin, Aronsen, Björkman and Ahlgren, 1977). The number of parasites in the lung peaks over the period 4-6 days after infection (Sher, Mackenzie and Smithers, 1974) and the finding of parasites 6 days after infection in organs supplied by the systemic circulation accords well with such a time scale. At a certain stage after initial infection - probably from day 6 there is likely to be a mixed population in the pulmonary blood circulatory system. This is made up of parasites delayed in the initial exodus from the skin and parasites which left the skin early, returning to the lung through the right side of the heart after having been previously dispersed through the systemic circulation. In addition to the pulmonary circulation which is concerned exclusively with respiratory gaseous exchanges, the systemic circulation provides blood for nutrient purposes to the bronchi and their branches, lung tissue and pleura. It is, therefore, possible for parasites which have entered the systemic circulation to be dispersed to microvessels of the bronchial tree, lung parenchyma or pleura. Terminal pulmonary arteries may communicate directly with pulmonary venules through anastomotic channels, thus bypassing the pulmonary capillary circulation. Such a situation exists in the rat (Sirsi and Bucher, 1953) but it is not known whether it exists in the mouse. If arteriovenous anastomoses also exist in the mouse lung, they would facilitate the pulmonary passage of parasites.

In the presence of an incompletely closed foramen ovale it would theoretically be possible for parasites arriving in the right atrium from the skin to pass directly to the left side of the heart and be dispersed through the systemic circulation, thus by-passing the pulmonary phase. Conversely parasites arriving in the left side of the heart from the lung might pass to the right side of the heart and be returned to the lungs. Incomplete anatomical closure of the interatrial septum is said to exist in approximately 20% of adult human subjects. It is not known what the incidence is in the laboratory mouse.

After passing through the pulmonary circulation parasites are returned to the left atrium by the pulmonary vein. In this study parasites have been identified in serial sections of pulmonary vein. From the left atrium parasites pass to the left ventricle whence they are dispersed with the blood throughout the body by the systemic circulation.

In the present study sections of heart showed the presence of schistosomula in the left ventricle indicating that parasites are distributed by the systemic circulation. On day 6 after infection schistosomula were seen in the blood vasculature of a variety of organs - kidney, liver and diaphragm, including such widely separated organs and tissues as brain and tail-bone, indicating their arrival by the systemic circulation. They continued to be seen subsequently in these organs and tissues up to day 16 after infection. Furthermore, schistosomula were found in capillaries of the myocardium, an observation which lends additional support to the view of their dispersal through the systemic circulation.

For purposes of this discussion, and as a result of finding schistosomula in organs and tissues supplied by the systemic circulation it is reasonable to assume that the dispersal of schistosomula within the host from the left ventricle through the systemic circulation is related to the distribution of cardiac output to various organs and tissues.

Published work on the distribution of cardiac output in the mouse is scanty, and the only two reports dealing with this subject are those of Wetterlin *et al*. (1977) and Quintana Raczka and Bonacoorsi (1979). Both groups of workers have used radioactive microspheres; Wetterlin *et al*. (1977) have also used radioactive rubidium. There is considerable divergence and poor replicability in the two sets of results as determined by the microsphere technique, whereas Wetterlin _et al_. (1977) claim that the results obtained with labelled rubidium are reproducible; they were, therefore, used in this study. Wetterlin _et al_. (1977) indicate that approximately 42% of the cardiac output will reach the liver, approximately 8% directly through the hepatic artery and approximately 34% indirectly, via the hepatic portal system. Roughly 37% of the output is distributed to the carcass, 5.7% to heart and lungs, 14,6% to the kidneys and 0.2% to the brain. In part the difficulties in finding schistosomula in individual organs can be explained by the high percentage (37%) of the cardiac output going to the carcass. With activity (i.e. in unanaesthetized animals) the proportion going to the carcass will increase, thus making it still more difficult to find parasites in individual organs. With regard to the brain, Wetterlin _et al_. (1977) state that rubidium fails to pass the blood-brain barrier; only minimal radioactivity was observed in the brain in their experiments. However, the comparative frequency with which parasites were observed in brain sections in the present study suggests that the actual percentage distribution of cardiac output is higher than the figure of 0.2% obtained by Wetterlin _et al_. (1977). The finding of parasites in the brain may be due partly to the fact that, with such a small organ, it is relatively easy to cut sections of whole brain for examination. On the assumption that the pattern of distribution of schistosomula dispersed through the systemic circulation will approach the pattern of distribution of cardiac output as calculated by Wetterlin et al. (1977) using labelled rubidium, approximately 42% of the parasites pumped from the left ventricle might be expected to reach the liver and the remaining 58% to be distributed to tissues and organs other than the liver.

Apart from the proportion of the cardiac output received, there are, of course, considerable structural and other differences in the vasculature of the various tissues and organs. Moreover,vessels are not isolated but are intimately related to their surrounding tissues, and undergo changes in accordance with physiological requirements.

Within the closed blood circulatory system consisting of vessels of varying calibre, migration of schistosomula from lung to liver may be direct, through the hepatic artery, or indirect, through the hepatic portal circulation from any one of the splenic, gastric, cystic or mesenteric veins. In addition, parasites which may have been dispersed to organs and tissues whose blood supply is not associated with that of the liver, may eventually reach the liver by a more circuitous route.

Schistosomula which, on first leaving the lungs, fail to reach the liver by the direct or indirect route, may be trapped and irretrievably lost. There is a disparity between the number of infecting cercariae and the number of recoverable adults, Quite apart from the numbers which are known to perish both in the skin and in the pulmonary phase of migration, it is evident as shown histologically in this study that a number of larvae succumb in the brain and kidney and other organs supplied by the systemic circulation. The histological evidence suggests that the parasite has been impeded during migration through the microvasculature of these organs. This is supported by finding larvae situated perivascularly in the brain with focal and sometimes diffuse areas of haemorrhage, necrosis and cellular infiltrate suggesting that the parasite's continued containment within the microvessel has not proved possible. In addition, in lung and kidney, schistosomula surrounded by granulomata have been seen. It is a matter for speculation as to whether the parasite has lost some of its potential for deformation or whether the microvessels in question are of smaller calibre or whether combinations of these or other factors are involved

Parasites were never found in the spleen. Possible reasons for this are considered to be due to chance and/or the specialized type of circulation, with only few and scattered capillary networks, which exist in the mouse spleen. The spleen is described as having an "open" circulation (Parpart, Whipple and Chang, 1955), which permits considerable freedom of movement of blood. In these conditions it is considered unlikely that larvae would be halted in the vasculature. Wheater and Wilson (1979) conducting a histological study failed to find larvae in the spleen.

Only on day 16 was a parasite found in the pancreas. In appearance this parasite had developed beyond the schistosomular stage and it is considered likely that this particular parasite had passed from the liver through the portal vein into the splenic vein and so reached the pancreas.

As an alternative to being obstructed in the microcirculation some parasites may be successfully recirculated to enter a second pulmonary phase and be distributed once more from the left side of the heart.

Recirculation of larvae has been suggested by several workers (Faust, Jones and Hoffman, 1934; Koppisch, 1937; Olivier, 1952; Miller, 1976; Miller and Wilson, 1980). Recent work by Miller and Wilson (1980) indicates that schistosomula introduced into the left ventricle of the hamster heart are able to pass through systemic capillary beds to return to the lungs. These workers have not mentioned the possibility of some parasites introduced into the left ventricle being pumped directly through the bronchial artery. This means that in the recovery of parasites from the lungs by mincing and incubation the possibility exists of including parasites which have not traversed capillaries. In addition, arterio-venous anastomoses in organs and tissues should be taken into account. A criticism of the work by Miller and Wilson (1980) is that they base their findings on results in only two experimental animals and over a period of only 48 hours. From the experimental work completed in the present study there are several reasons for considering the possibility that a proportion of larvae may enter the pulmonary phase on a second occasion. In histological sections of kidneys, organs supplied by the renal arteries which spring directly from the aorta, schistosomula were observed in venules eventually draining into the posterior vena cava, and so evidently being returned to the right side of the heart. Sections of lung have revealed parasites in pulmonary vessels as late as 16 days after infection; at the same time schistosomula have been observed in the cavities of both left and right ventricles. It seems possible that parasites found in the right ventricle and lung so long after infection have returned there from the systemic circulation. Various explanations for the presence of schistosomula in the lung 16 days after infection might be advanced, such as delayed exit from the skin, delay in migration from the lung, recirculation or a combination of these. On the other hand, schistosomula containing haem in the larval gut were observed in sections of lung suggesting that they had previously reached the liver and subsequently drained out as indicated by the presence of schistosomula in branches.

of the hepatic veins. In considering the haematic route from skin to lung and from lung to dispersal through the systemic circulation, it is not possible from histological studies to produce conclusive evidence for or against recirculation to the pulmonary phase. By routing donor schistosomula through the left ventricle of the hamster Miller and Wilson (1980) have excluded the skin-to-lung phase of the migratory route, obviating the uncertainties surrounding the delayed presence of parasites in lungs due to late exit from the skin or lung or both. These experiments lend some support to the present author's conclusion that a proportion of schistosomula are at times capable of traversing the systemic capillary vasculature. Clearly there is a limit to the number of times the parasite can recirculate. From the generally accepted proportion of recoverable adults it is evident that a number of schistosomula fail to find their way to the liver.

In the present study histological sections have shown parasites within branches of the hepatic portal vein and within sinusoids and branches of the hepatic veins. They were not seen in the branches of the hepatic artery. In a comparable histological study Wheater and Wilson (1979) found parasites in the branches of the hepatic portal vein and branches of the hepatic artery. They found no parasite in the sinusoids or branches of the hepatic veins. The proportion of cardiac output going through the hepatic portal vein is greater than that going through the hepatic artery and, therefore, the likelihood of finding a parasite in the former is greater. In addition, pressure in the hepatic artery is considerably greater than that in the portal vein (approximately 9 times greater in the human subject). Parasites seen within sinusoids may have arrived either with the systemic circulation through branches of the hepatic artery as well as through the hepatic portal circulation. It was noted that of the parasites seen within sinusoids, some contained haem in the larval gut while others did not. It is suggested that those containing haem probably arrived via the hepatic portal circulation where the surrounding blood is rich in nutrient, the flow is slow and opportunity to feed is probably optimal. While arterial portal anastomoses between the lesser branches of the hepatic artery and branches of the portal vein exist in several animals (Bloch, 1955) no evidence of these was found in the mouse by McCusky (1966) who described the hepatic arterial vessels in that animal as entering directly into the hepatic sinusoids.

In the mouse (Lee, Elias and Davidsohn, 1960) and rat (Gershbein and Elias, 1954; Elias and Popper, 1955; Lee et al., 1960) sinusoids drain into branches of the hepatic veins at all levels unlike sinusoidal drainage in man and dog (Elias and Popper, 1955; Lee et al., 1960) where it drains into the central veins. The outflow of blood from sinusoids in the mouse (and rat), being direct and even, ensures that mouse (and rat) livers are well drained as compared with those of man and dog. The possibility, therefore, exists for parasites to flow from sinusoids and drain out of the liver via the left and right hepatic veins. These veins enter the posterior vena cava at a point closely adjacent to its entry to the right atrium. Therefore, parasites, which have been feeding in the liver and which according to Wilson _et al_., 1979 have lost their potential for deformation, once having drained out of sinusoids, could be transported through the large vessels, with no intervening capillary bed, to the right side of the heart and so reach the lungs. This could explain the presence of parasites, with haem in the gut, in lung alveoli. The possibility that a small number of parasites delivered by the hepatic portal circulation may subsequently leave the sinusoids is borne out in the hamster, at least, as a result of work done by Miller and Wilson (1980) who injected schistosomula into the mesenteric veins and subsequently recovered them from lungs.

Exceptionally, adults and eggs are recorded as occurring ectopically in various organs. So far as adults are concerned, they might pass with the venous flow from the mesenteric vessels through the hepatic portal system and into the posterior vena cava to reach the right side of the heart and pulmonary circulation. It is unlikely that, due to their size and loss of deformative potential, they would be able to pass through lung capillaries. They might, however, pass through arterio-venous anastomoses to reach the left side of the heart and be dispersed in the systemic circulation. The eggs would be borne along the same route.

It is generally agreed that parasites entering the hepatic portal system tend to be sequestered there. This phenomenon has been attributed to a possible trigger mechanism related to feeding which halts further migration. The author of the present study considers that the comparative stagnation within the portal system may be a contributory factor in the sequestration of parasites in the liver. Stagnation reduces the likelihood of parasites being carried out through the blood vascular system, at the same time providing the opportunity to feed. Miller and Wilson (1980), in considering a reason for the sequestration of parasites in the hepatic portal system, suggest a physical limiting factor in the size of the sinusoids and in support of this invoke the findings of Madden, Paparo and Schwartz (1968) who worked with labelled microspheres to investigate vascular diameters in the rat. Miller and Wilson (1980) cite Madden _et al_. (1968) as showing that "liver sinusoids were respectively 14, 17 and 23 per cent narrower than muscle, lung and kidney capillaries" which is in fact a percentage derivation on the findings of Madden _et al_. (1968). What Miller and Wilson (1980) did not state is what Madden _et al_. (1968) actually found and recorded viz. "that the mean limiting vascular diameters by the method of microspheres in the rat are as follows: kidney 23.0μm, lung 21.2μm, muscle 20.6μm and liver 17.7μm". These diameters are all adequate for the passage of schistosomula. Furthermore, Madden _et al_. (1968) go on to point out that the diameters of liver sinusoids may well be larger than inferred from the microsphere technique. Owing to the low pressure in the portal vein the larger spheres would be more readily arrested than at capillaries where pressure is higher. The capillary diameters as reflected by the microsphere technique are large as compared with generally accepted values. Madden _et al_. (1968) go on to point out that the diameters as determined by rigid microshperes "cannot be interpreted as the absolute resting diameters of capillaries."

Sinusoids are distinguished from capillaries by wider and more irregular size and shape. The basement membrane which is characteristic of capillaries is absent in sinusoids. Exceptionally the basement membrane is present in sinusoids of ruminants (Grubb and Jones, 1971). The diameters of sinusoids is given by Rappaport (1980) as being 7-15μm though "when necessary the diameter can increase to 180μm".

A blood circulatory migration route against the blood flow has been postulated. Working with sheep percutaneously infected with _S_. _mattheei_ cercariae, Kruger _et al_. (1969) isolated blood vessels in the host using strategically placed haemostatic clamps after exsanguination of the animal by severing the "large blood vessels" in the neck. They recovered schistosomula from pulmonary arteries, right side of the heart, posterior vena cava and hepatic veins both in perfusates and washings as well as from the walls of these vessels and the walls of the right atrium and ventricle. No parasites were found in the pulmonary veins, left side of the heart or aorta. From these findings they conclude that contrary to other schistosomula in other hosts, S. mattheei schistosomula migrate from lung to liver against the blood flow, in a reverse direction, viz. through the pulmonary arteries, right ventricles and right atrium, posterior vena cava and hepatic veins.

The details of the experimental work carried out by Kruger et al. (1969) are meagre and not entirely clear, making it difficult to accept or reject the conclusion they reach. The author of the present study has some reservations e.g. on account of the possible disturbance in distribution of parasites in the blood circulatory system following exsanguination of the host by severing the "large blood vessels" in the neck. However, on the assumption that there are no major flaws in their experimental techniques the observations of Kruger et al. (1969) raise points of interest .

The observations of Kruger et al. (1969) that all parasites recovered from the right side of the heart, pulmonary arteries and posterior vena cava were similar in morphology to lung forms and that all of them contained "haematin" in the larval gut raises the question as to why there are no mixed populations in these locations. It may be assumed that some schistosomula at least would leave the skin late and others return early from the lungs giving rise to mixed populations. Several workers (Olivier, 1952; Wilks, 1967) including the present author (see Chapter 4, page 81) consider that the haem in the lumen of the larval gut is acquired in the liver. It can only be assumed that schistosomula observed by Kruger *et al*. (1969) had started to feed immediately on entering blood vessels leaving the skin or in the lung prior to returning to the right side of the heart against the flow by the pulmonary artery.

Unlike *S*. *mansoni* and *S*. *haematobium*, which are considered to reach the liver by chance after being generally dispersed in the systemic circulation, the blood vascular route against the flow taken by *S*. *mattheei* as described by Kruger *et al*. (1969) occurs entirely within deoxygenated blood. Furthermore, the same condition prevails in migration from skin to lung. Thus the entire blood vascular migration from skin to liver of *S*. *mattheei* in the experimental work of Kruger *et al*. (1969) would take place in venous blood. A carbon dioxide gradient possibly acts as a "signpost" for *S*. *mattheei*. In following the migration route through the right side of the heart and posterior vena cava to the hepatic veins and into the liver, Kruger *et al*. (1969) appear to attribute unique selectivity to the schistosomulum and to disregard the possibility of it entering any one of the veins draining into the vena cava.

A further point requiring clarification is the mechanism of return migration once the parasites have reached the pulmonary capillary beds. While recognizing the pliability and deformability of the schistosomulum (see Chapter 5, page 96) a U turn within the confinement of the pulmonary capillary seems improbable. A possibility would be for the schistosomulum to return rear end first till it reached a larger vessel where it could turn about. Another possibility would be for the schistosomulum to break out of the capillary and enter another capillary "head first" to return against the blood flow.

Kruger _et al_. (1969) though not claiming the ability of schistosomula to swim found them capable of movement and also observed them on the walls of vessels and chambers of the right heart. In the present study serial histological sections have revealed _S_. _mansoni_ lying free in all the chambers of the heart. Given the mobility ascribed by Kruger _et al_. (1969) to _S_. _mattheei_ together with the laminar flow in blood vessels, where movement of the blood in contact with vessel walls is absent or minimal, it is conceivable that the parasite could progress in a direction opposite to the blood flow. After entering the right ventricle it is astonishing that the parasite should be able to make for the ventriculo-atrial opening and negotiate the tricuspid valves guarding the opening. This in turn raises the question of energy supply available to the parasite to satisfy what must be considerable demands for the activity involved.

Kruger *et al*. (1969) failed to find parasites in the aorta and left chambers of the heart. This, is not necessarily conclusive evidence that the schistosomula have not been dispersed with the blood flow through the systemic route. In the present study schistosomula were seen only intermittently, over a period of 16 days, lying free in the chambers of the left side of the heart. As pointed out earlier (see page 119) the likelihood of finding larvae in a major blood vessel is remote. Parasites were also found in branches of the hepatic veins. This latter finding is possibly a result of parasites escaping from liver sinusoids and being collected with the blood flow to be recirculated. With regard to larvae recovered from the posterior vena cava on day 11 after infection Kruger *et al*. (1969) appear to have overlooked the possibility that they may have escaped from the liver rather than coming directly from the lung through the right side of the heart to the posterior vena cava since by that time most of the parasites which survived the intial stage of skin-to-lung migration could be expected to have reached the liver. Furthermore, Kruger *et al*. (1969) describe the schistosomula recovered from the posterior vena cava as containing "haematin" in the larval gut. This would suggest that the parasites found in the posterior vena cava had escaped from the liver where they had been feeding.

In the work with *S*. *mansoni* and *S*. *haematobium* in the present study there is no evidence to support a reverse migration against the blood flow. Kruger *et al*. (1969) concede that in its reverse migration from lung to liver against the blood flow *S. mattheei* may be unique amongst the schistosomes. The concept of reverse migration by *S. mattheei* against the blood flow is one of interest but requires further investigation so that the hypothesis of a retrograde route can be either refuted or supported; it is suspect as it stands. Although the mouse is a permissive host to *S. mattheei* (Bickle, personal communication) its small size does not readily lend itself to the various procedures described by Kruger *et al*. (1969). On that account it should probably be discarded as a possible model. It is worth recalling that the final stage of migration of *S. mansoni* and *S. haematobium* from the liver to their final habitats is retrograde. However, at this stage of migration the situation is different. The larvae have become adults which have fed in the liver and their environment is rich in nutrients providing energy requirements for a reverse migration to the mesenteric veins.

A computer model of schistosomular (*S. mansoni*) migration in mice was advanced by Miller (1976) on the assumption that schistosomula are confined intravascularly. The author states that a successful model requires a full understanding of "the complexities exhibited by the real situation". In Miller's model the predicted time of arrival of parasites in the hepatic portal circulation agrees well with the observed time. Thereafter, however, with regard to numbers and rate of arrival of worms in the hepatic portal circulation the predictions do not fit the observed data and it is clearly necessary that we must await further refinement of the model. In such a model it is desirable that only one animal species be used throughout and that sample size should be adequate. Sample sizes of 1, 2 and 3 animals are considered to be inadequate. It is surprising that Miller (1976) states that the model is a simulation of schistosomular migration in "mice only", since the model incorporates data from observations in hamsters and rats. If only one animal species and far greater sample sizes were used for the model it is conceivable that predictions of numbers and rate of arrival of worms in the hepatic portal system would accord more closely with observed data.

In calculating "the probability of finding a schistosomulum in unit volume of tissue", Miller and Wilson (1980) used published data from various sources. With regard to proportions of cardiac output distributed to various organs they used data on the rat (Blahitka and Rakusan, 1977). At the same time, the data they quoted on "relative vascular volumes µl/g" relate to the mouse (Altman and Dittmer, 1971). In the compilation of "relative vascular volumes" for various organs, the figure for skin was obtained from yet another source, and arrived at by a different technique. Since data for percentage cardiac output in the mouse (Wetterlin et al., 1977) and data for "total blood volume µl/g tissue" relating to the rat (Altman and Dittmer, 1971) are available, it is not clear why Miller and Wilson (1980) used data referring to different species rather than to one or other animal.

CHAPTER 7

SCHISTOSOMULAR ROUTES: THE LYMPHATIC SYSTEM

7.1 INTRODUCTION

To be able to reach the liver, where it will feed and grow, the schistosomulum must gain access to the blood circulatory system at some point before the blood reaches the right side of the heart. There is a large measure of agreement on the blood circulatory system as an initial pathway of schistosomular migration. After penetration of the skin, a proportion of larvae find their way into blood capillaries and start migrating towards the heart (see page 118).

There has long been speculation and disagreement, as to the role of the lymphatic system in the migration of the schistosomular larva from the skin to the blood circulatory system.

In considering the lymphatic pathways as a possible initial route for the migrating parasite, several questions need to be answered. Do the lymph nodes constitute a barrier to migration? Would such a barrier be of a purely mechanical nature in view of the physical complexity of the nodal filter or might it be of a biological nature e.g., an immune response or phagocytic activity as in the case of invasion by micro-organisms?

A review of the literature reveals little direct evidence concerning the schistosomulum's ability to traverse the lymph nodes.

Miyagawa (1912) working with *S. japonicum* and using dogs, found no parasites in the inguinal lymph nodes. Nonetheless, he continued to consider the possibility of schistosomular migration through the lymphatics.

In a further study Miyagawa (1913) again used *S. japonicum* and dogs. In addition to the histological examination of inguinal lymph nodes he punctured the thoracic duct to withdraw lymph, and succeeded in recovering parasites. On this evidence he concluded that at least some migration took place through the lymphatics. Unfortunately neither of these two studies gives any information on the time elapsed between infection and retrieval of parasites.

In histological studies on mice infected with *S. japonicum* Miyagawa and Takemoto (1921) found parasites in the lymphatic spaces of the skin 1½ hours after infection. On day 3, parasites were still seen in the lymphatic spaces of the skin, and others were found in lymph nodes (unspecified). These workers claimed that some of the parasites were trapped in the lymph nodes and were undergoing lethal atrophic changes. Other larvae had succeeded in passing unharmed through the lymph nodes to gain entry to the blood circulatory system by way of the thoracic duct.

Faust and Meleney (1923), in a study of mice, rabbits and dogs experimentally infected with *S. japonicum*, reported that the parasites lodged temporarily in inguinal and popliteal lymph nodes. It is not recorded whether the histological or direct method was used, nor is it mentioned at what times the parasites were found.

Faust and Meleney (1924) examined the popliteal lymph nodes of rabbits and inguinal lymph nodes of mice infected with S. japonicum. Nodes were examined daily from day 1 to 6 after infection. The nodes were minced for examination. In rabbit popliteal nodes, parasites were observed from day 1 to 4. In the case of mice, parasites were seen in the inguinal nodes from day 2 to 5. On day 5 one of the larvae was seen to be undergoing degeneration.

Faust, Jones and Hoffman (1934) studied the cervical, axillary, popliteal and inguinal lymph nodes of rats experimentally infected with S. mansoni. Lymph nodes were examined 22, 44, 64 and 70 hours after infection, both macroscopically and by mincing. Macroscopic appearances included enlargement, injection and petechiae. Microscopy of minced tissues revealed a single larva from a popliteal lymph node 64 hours after infection. On the basis of the macroscopic appearances and the almost total absence of parasites in minced node concentrates, these workers concluded that larvae, after lodging briefly in the lymph nodes, had continued to pass through them on days 2, 3 and 4. This report did not include any histological examination of lymph nodes.

Watarai (1936) working with S. japonicum in rats found parasities in the lymphatic spaces in the dermis.

Koppisch (1937) undertook histological investigations in rats and rabbits infected with _S_. _mansoni_. He observed parasites in the dermal lymphatics and in the axillary, inguinal and popliteal lymph nodes. He occasionally found parasites in blood capillaries of the skin, and concluded that migration from the skin took place principally by way of the lymphatics. Because he rarely saw disintegrated schistosomula, he inferred that the parasites were able to pass through lymph nodes.

Standen (1953) carried out histological investigations of skin in mice infected with _S_. _mansoni_. Using two infected mice - one killed 8 minutes and the other 20 minutes after infection - he observed a parasite in process of entry into a lymphatic vessel and a schistosomulum actually within the lumen of a lymphatic vessel. On the basis of these observations he thought entry into the "circulatory system" was most probably by way of the lymphatics. A further factor influencing Standen (1953) in favour of the "lymphatic theory" was the time elapsed before the parasite could be found in the lung. He observed that whereas the parasites had the opportunity to enter a vessel of one kind or another within 1 hour after infection, none was seen in the lungs for at least 4 days and even then large numbers only appeared on days 9 and 10 after infection. This suggested to Standen (1953) that the nodal filter delayed the passage of the parasites. While recording that Koppisch (1937) had on two occasions found parasites "within capillaries that probably represented blood channels" Standen (1953) omitted to mention in his publication that Koppisch (1937) had also recorded the presence of parasites within the lymphatic vessels in the dermis.

Griffiths (1953) regarded the observation made by Standen (1952) concerning penetration of "cercariae" into lymphatic vessels as "an important observation not previously recorded in the literature". However, the studies of Watarai (1936) page 200 and Koppisch (1937) page 201 covered the same ground and should have been mentioned.

Stirewalt (1959), working with S. mansoni, infected mice through circumscribed areas of skin of abdomen, ear and tail. The study involved serial sections of skin taken at successive time intervals up to 11 days after exposure to infection. Exit from the skin included the lymphatics.

Miller and Wilson (1978) using mice, hamsters and rats experimentally infected with S. mansoni, carried out investigations to determine the extent of migration from the skin through the lymphatic system. Following infection at the abdominal skin site, schistosomula were recovered from minced tissue of "axial" lymph nodes of the animal on day 2 after infection, (the period 0-24 hours after infection is referred to by Miller and Wilson (1978) as day 0 and the following 24 hour periods as day 1, day 2 and so on). Surgical removal of "axial" lymph nodes 24 hours after exposure to infection had no apparent effect on actual numbers of parasites arriving in the lung, although the maximum recovery was postponed by one day. Viable schistosomula recovered from mouse lymph nodes were injected into the tail vein of uninfected mice. The rate of recovery of these schistosomula at adulthood (approximately 30%) suggested to these authors that the larvae had suffered no adverse effect while in the lymphatic system.

In a quantitative histological study of schistosomular migration Wheater and Wilson (1979) found parasites in "all the lymphatic channels of the lymph node including the afferent lymphatics and the hilar efferent lymphatics". On this evidence they conclude that schistosomula migrate via the lymphatic system. They are at variance with the hypothesis that schistosomula enter the blood vascular system within lymph nodes, a hypothesis which they attribute to Koppisch (1937). However, their assertion is not confirmed by reference to Koppisch (1937). Also Weather and Wilson (1979) cite Miyagawa and Takemoto (1921) on the one hand as claiming that schistosomula are unable to pass through lymph nodes and Standen (1953) on the other as describing schistosomula within lymph nodes. In fact Miyagawa and Takemoto (1921) conclude that lymph nodes do not constitute a complete barrier and the work of Standen (1953) was not concerned with lymph nodes.

Because of inadequate criteria applied in certain of the investigations referred to, the validity of some of the results and conclusions of such studies are open to question. In several of these investigations the number of animals used is insufficient; sometimes the actual number used is omitted altogether (Miyagawa, 1912; 1913; Miyagawa and Takemoto, 1921). Time intervals between observations or procedures in some investigations are not always stated (Miyagawa, 1913; Faust and Meleney, 1923). "Paddling"* as a method for infecting animals was used (Miyagawa, 1912; 1913; Miyagawa and Takemoto, 1921; Faust and Meleney, 1924; Faust, Jones and Hoffman, 1934; Koppisch, 1937).

In order to answer the questions raised at the beginning of this chapter (see page 198), a detailed knowledge of the lymphatic pathways is essential.

Reference to the literature provides little information concerning the pattern of lymphatic drainage in the mouse, though detailed mapping of the lymphatic vessels and lymph nodes is available for the hamster (Miotti, 1961), the rat (Tilney, 1971) and the Mongolian jird (Ah and Thompson, 1973). In the mouse, some of the pathways from the tail have been traced (Engeset and Tjötta, 1960) and subsequently lymphatic routes from the abdominal skin were defined by Miller and Wilson (1978). The latter work, however, contains errors (see page 207).

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* "Paddling" is the immersion of torso and limbs as a method of presenting maximum skin surface for penetration by cercariae. It is impossible by this method to determine the exact lymphatic route(s) of migration taken by parasites.

In the present study wherever possible the nomenclature for the important lymph nodes is that used by Dunn (1954).

7.2 PRELIMINARY INVESTIGATIONS

The lymphatic channels available are numerous; it is the site of penetration that determines the particular route taken by a parasite. An investigation of those sections of the lymphatic system relevant to the sites of infection employed in this study was undertaken to define the pathways from the tail, hindfoot and forefoot. After previous colouring with Evans blue for the purpose of identification, lymph vessels and nodes draining the tail, hindfoot and forefoot in the T.O. mouse were exposed by dissection. Twenty five uninfected mice were used for each site.

Evans blue, 1% was injected subcutaneously into interdigital webs, the dorsum and the sole of one foot, fore or hind, under light ether anaesthesia. In the case of the tail the injections were made on the dorsal, ventral and lateral surfaces. In order to stimulate lymphatic drainage and uptake of the dye, the limbs were moved actively until animals recovered from anaesthesia. After 30 minutes, the animals were killed with ether, and dissected. In most cases the lymph nodes were stained intensely blue and could be readily located. In doubtful

TABLE 12

Injection of Evans blue into left/right hindfoot, in 2 groups of 25 mice each

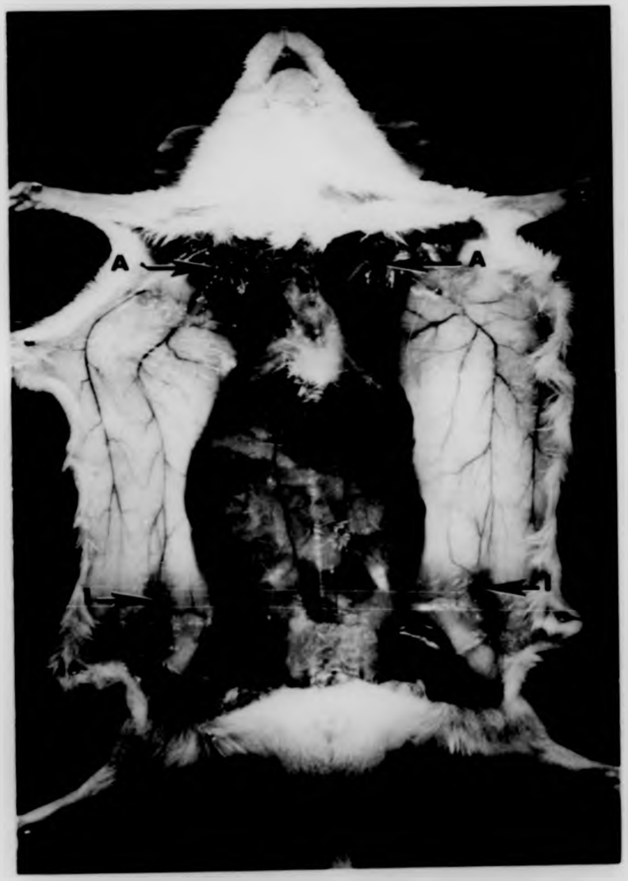
	NUMBER OF MICE 25	NUMBER OF MICE 25
NODES	LEFT	RIGHT
POPLITEAL	25	25
SCIATIC	25	25
LUMBAR	25 (3)	25
RENAL	25 (3)	25 (2)
INGUINAL	16	14
AXILLARY	16	14

The numbers between brackets represent animals in which staining was observed in contralateral lymph nodes cases the findings were checked microscopically. The number and topographical arrangement of lymph nodes were constant. Occasionally subsidiary intercommunicating lymphatic vessels between nodes were observed (Table 12).

A diagrammatic representation of the pattern of the lymphatic system draining the tail, hindfoot and forefoot, exposed by dissection, is given in Figure 10 and 11. The position of the inguinal lymph nodes as published by Miller and Wilson (1978) is at variance with the findings in this study on dissection of the mouse and with Dunn's (1954) diagrammatic representation. The inguinal lymph nodes lie adherent to the subcutaneous tissue of the flank (Plate 39) and not in the groin as shown by Miller and Wilson (1978). A search in the literature has failed to disclose the existence of the "subilical" node, nor has anatomical exploration revealed such a node in the position indicated by Miller and Wilson (1978). Moreover, the term "axial" used by these authors to describe the lymph node in the axillary fossa would appear to be a misnomer.

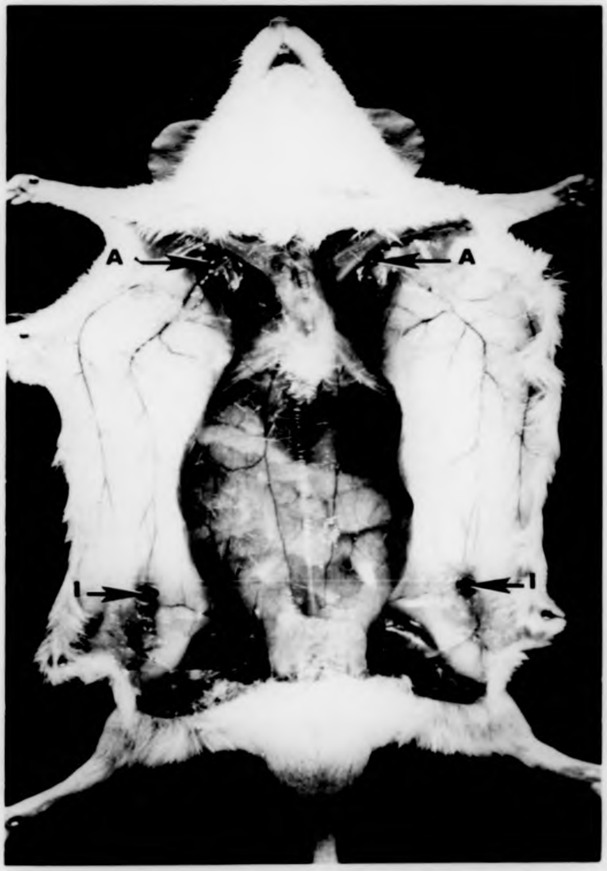
Having determined the lymphatic pathways by this method, related lymph nodes were then studied by direct examination and histologically at fixed intervals after infection. Direct examination was used to reveal the presence or absence of parasites, and histological examination to show the exact position of any parasites present.

PLATE 39



Dissection of T.O. mouse illustrating lymph nodes (A) left and right axillary and (I) left and right inguinal.

PLATE 39



Dissection of T.O. mouse illustrating lymph nodes (A) left and right axillary and (I) left and right inguinal.

FIGURE 10

Diagrammatic representation of lymphatic pathways and the principal lymph nodes in the T.O. mouse.

● lymph nodes, ventral aspect
◉ lymph nodes, dorsal aspect

Paths of migration after infection at the following sites:

———————— tail
-------- right hindfoot
•••••••• left hindfoot
—•—•—•—•— right forefoot
-•-•-•-•- left forefoot

Fig. 10 T.O. mouse ventral aspect

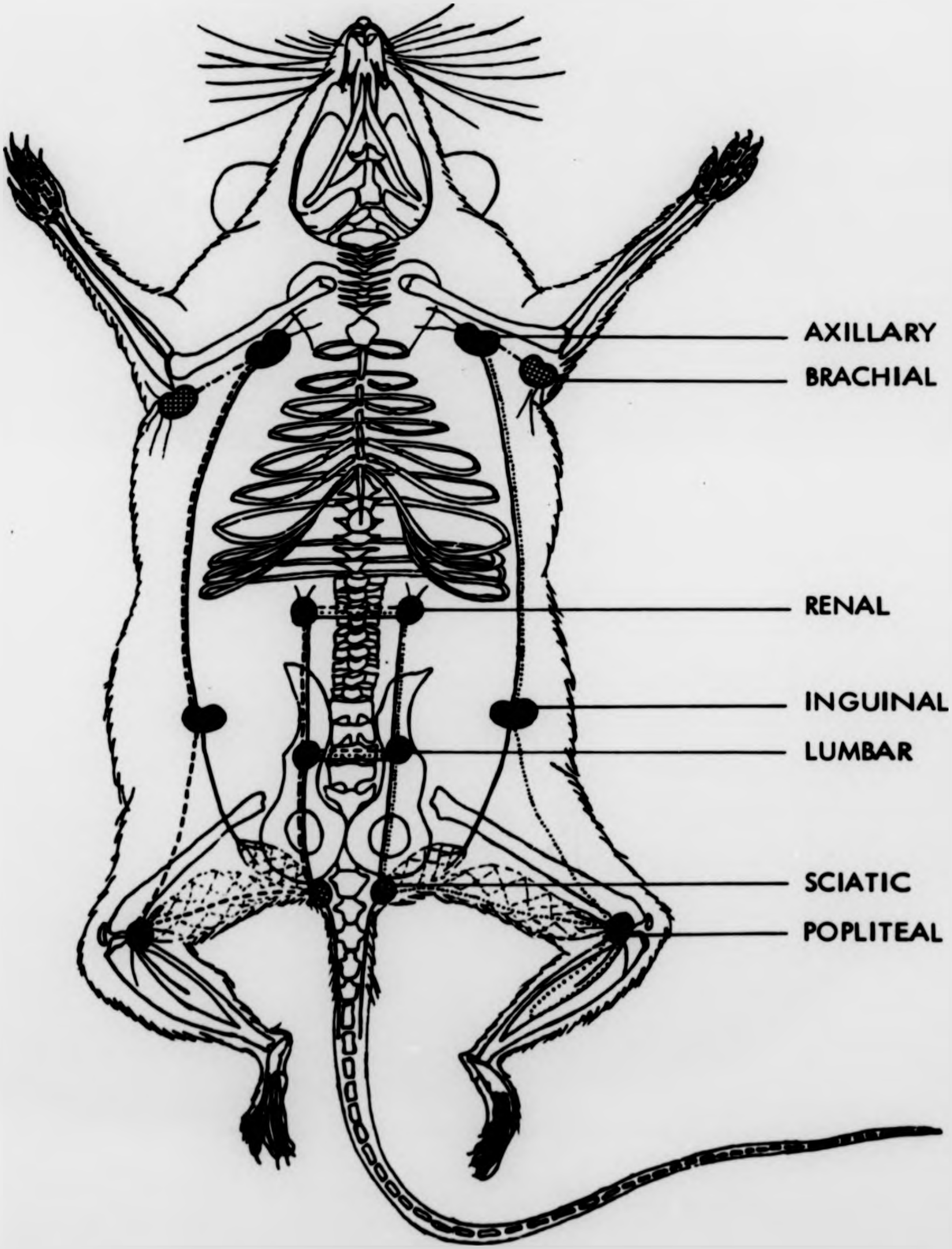

AXILLARY
BRACHIAL
RENAL
INGUINAL
LUMBAR
SCIATIC
POPLITEAL

FIGURE 11

Diagrammatic representation of the principal lymph nodes in the T.O. mouse

● lymph nodes, dorsal aspect
⊕ lymph nodes, ventral aspect

FIGURE 11

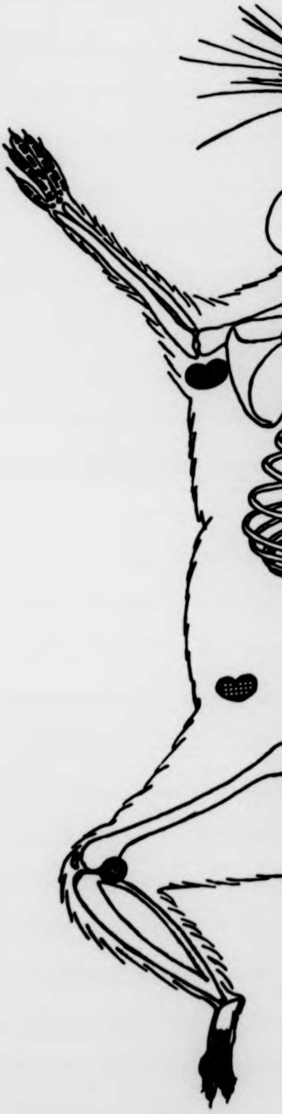
Diagrammatic representation of the principal lymph nodes in the T.O. mouse

● lymph nodes, dorsal aspect
◍ lymph nodes, ventral aspect

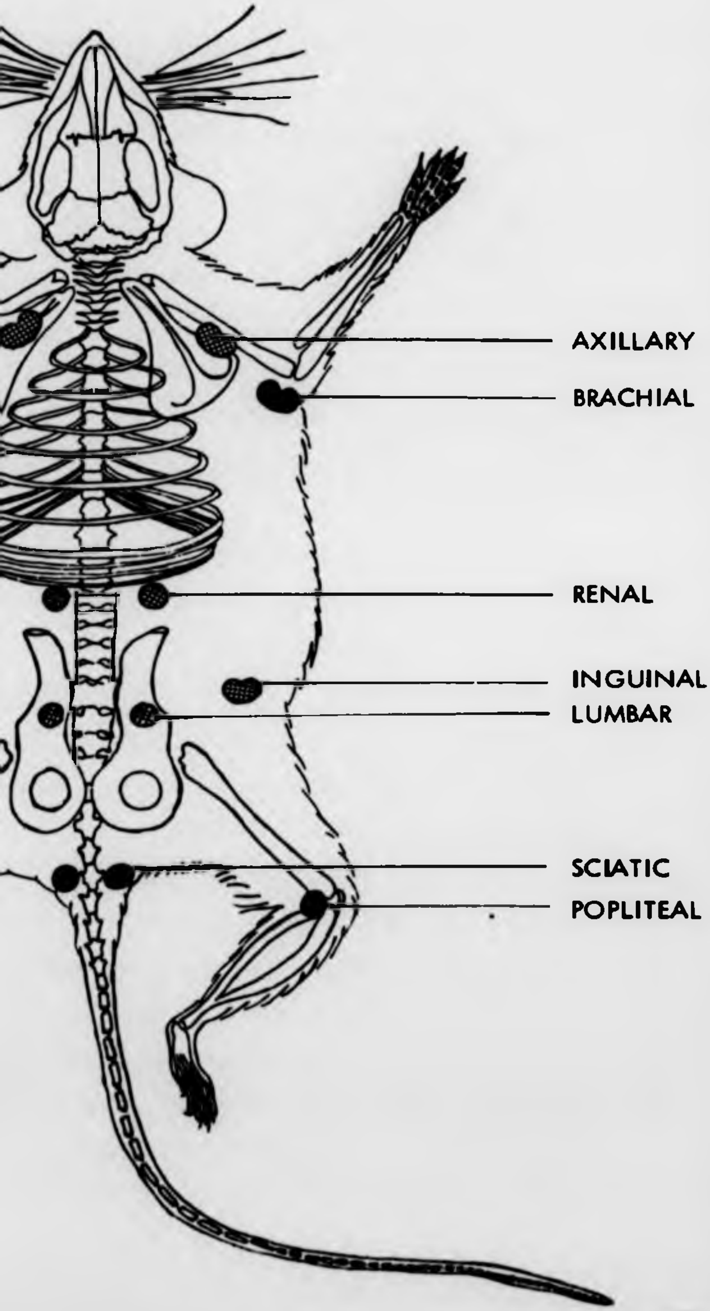
mph nodes

ct
ect

Fig. 11



T.O. mouse dorsal aspect


AXILLARY
BRACHIAL
RENAL
INGUINAL
LUMBAR
SCIATIC
POPLITEAL

7.3 MATERIALS AND METHODS

Individual mice were infected with S. mansoni cercariae. Separate sites of infection by immersion were used - the tail (approximately 2000 cercariae to this site) and left and right forefoot (approximately 500 cercariae to each of these sites). A separate group of mice was infected with S. haematobium via the tail (approximately 2000 cercariae). A description of the tail-immersion method is given on page 38. For exposure to infection via the feet, mice were anaesthetized with 1ml pentobarbital sodium (Nembutal 60mg/ml, Abbott Laboratories) diluted in 9ml of isotonic sodium chloride. A dose of 0.01ml per g body weight was administered intraperitoneally. The anaesthetized mouse, ventral surface downwards, was supported on a grid with the foot to be exposed to infection hanging through the grid and immersed in the cercarial suspension contained in a cut-off Bijou bottle. The duration of exposure was 30 minutes. At 24 hour intervals following exposure the animals were killed with ether, and dissected. Tracings with Evans blue to demonstrate the positions of lymph nodes and drainage areas were not necessary, as these had been established earlier (see page 207). The lymph nodes serving each particular drainage area were excised. Lymph nodes intended for histological examination were removed with special care to include surrounding tissue, when possible, in order to avoid stimulating the node to further activity. Excised lymph nodes were placed either in Earle's medium with lactalbumin hydrolysate (Gibco Bio-cult) or a fixative for subsequent examinations by direct and histological methods.

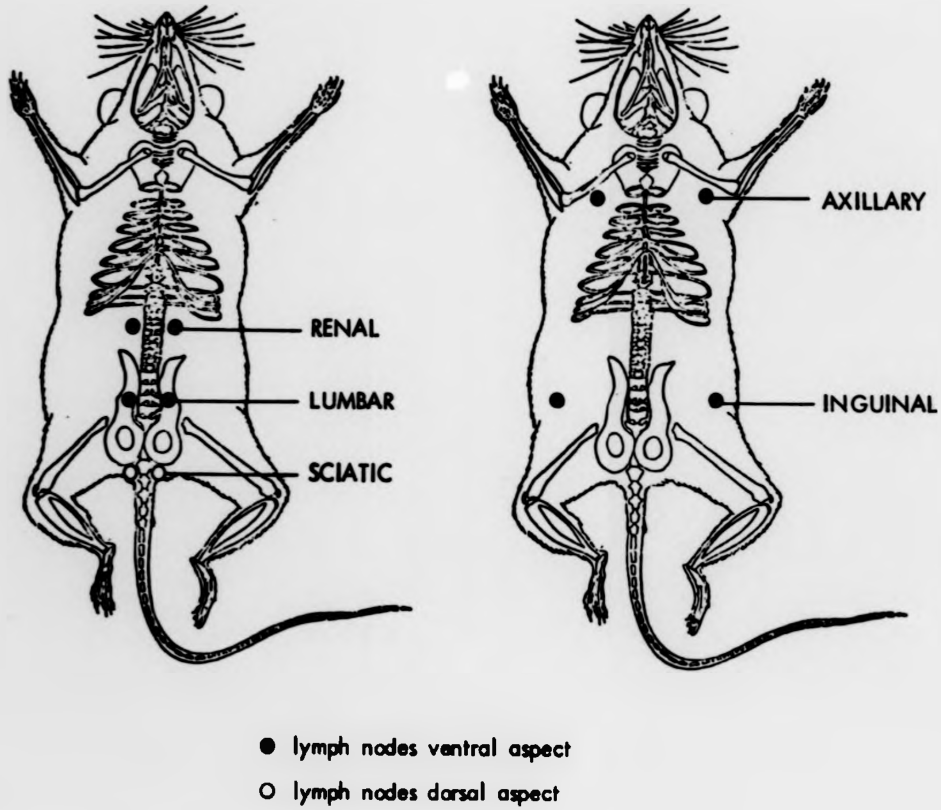
Direct examination

The lymph nodes were placed on a slide with a drop of Earle's medium covered with a coverslip, and gently squashed. The preparation was then examined under a compound microscope (magnifications X100 and X250) to determine the presence (or absence) of parasites.

Histological examination

Lymph nodes were fixed in Carnoy's fluid (2 hours), Bouin's fluid (24 hours) or 10% formol saline (24 hours). Subsequent treatment for dehydration varied according to fixative used - 3 changes of absolute ethyl alcohol over a period of 3 hours following the use of Carnoy's; 1 wash of 70% ethyl alcohol and 2 changes in absolute ethyl alcohol over a period of 3 hours following fixation with Bouin's or 10% formol saline. Irrespective of fixative used, Supercedrol (2 changes) was used for clearing, followed by impregnation and embedding in paraffin wax. Sections of entire nodes were cut at 6 μm, mounted, and after removal of paraffin wax and bringing down to water, were stained. Stains used were: Harris's haematoxylin and eosin; Gordon and Sweets's silver impregnation method for reticulin fibres

Fig. 12 Lymphatic routes available to the parasite when percutaneously infected via the tail


RENAL
LUMBAR
SCIATIC
AXILLARY
INGUINAL
● lymph nodes ventral aspect
○ lymph nodes dorsal aspect

(Gordon and Sweets, 1936); Lendrum's method to show up eosinophils in tissue (Lendrum, 1944); or methyl-green pyronin to show up plasma cells (Kurnick, 1955).

7.4 RESULTS

Direct examination of lymph nodes involved in drainage following infection via the tail.

In a series of experiments 288 mice were infected with *S. mansoni* cercariae. From day 1 to 12 days after infection 24 mice were killed and examined each day.

The parasite, migrating via the lymphatics, may take one of several routes after entering the tail skin, depending on which lymphatic vessel it first encounters. The relevant routes with associated lymph nodes are shown schematically in Figure 12. Tables 13-17 in Appendix, pp. 318-322, show presence (or absence) of parasites in the deep and superficial lymph nodes involved in drainage from the tail. The following summary describes the lymphatic pathways available to schistosomula and those that were taken:

1) The sciatic nodes, situated one on each side of the midline, deep to the gluteal muscle and close to the emergence of the sciatic nerve. Parasites were present in these nodes from days 2 to 12 after infection in a pro-

● lymph nodes ventral aspect
○ lymph nodes dorsal aspect

portion of the nodes varying from 33%* to 100%.

Efferent lymphatic vessels from the sciatic nodes drain into the lumbar nodes. The lumbar nodes lie in the abdomen, one on each side of the point of bifurcation of the aorta. Schistosomula first appeared in these nodes on day 3 (1 day after first appearing in the sciatic nodes), and continued to be found in lumbar nodes throughout to day 12 in a proportion of the nodes varying from 15% to 92%.

In turn, efferent vessels pass from the lumbar nodes to the renal nodes which lie between kidney and aorta. Schistosomula were seen in these nodes from day 4 (1 day after first appearing in the lumbar nodes), and continued to be found in renal nodes throughout to day 12 in a proportion of the nodes varying from 8% to 79%. Both renal nodes drain finally into the thoracic duct.

2) The inguinal nodes lying superficially in the flanks in subcutaneous fat and in close proximity to the superficial epigastric vein. Schistosomula first appeared in these nodes on day 2 and continued to be found throughout to day 11 in a proportion of the nodes varying from 4% to 77%.

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* Daily percentage variations of infected nodes in this section are shown in Figures 13-17 and all percentages are approximations to the nearest whole number.

Fig. 13

Direct examination of lymph nodes for presence of schistosomula following infection via the tail. Nodes containing parasites are given as a percentage of the daily total of the respective lymph nodes. Maximum possible : 48 nodes showing parasites = 100%

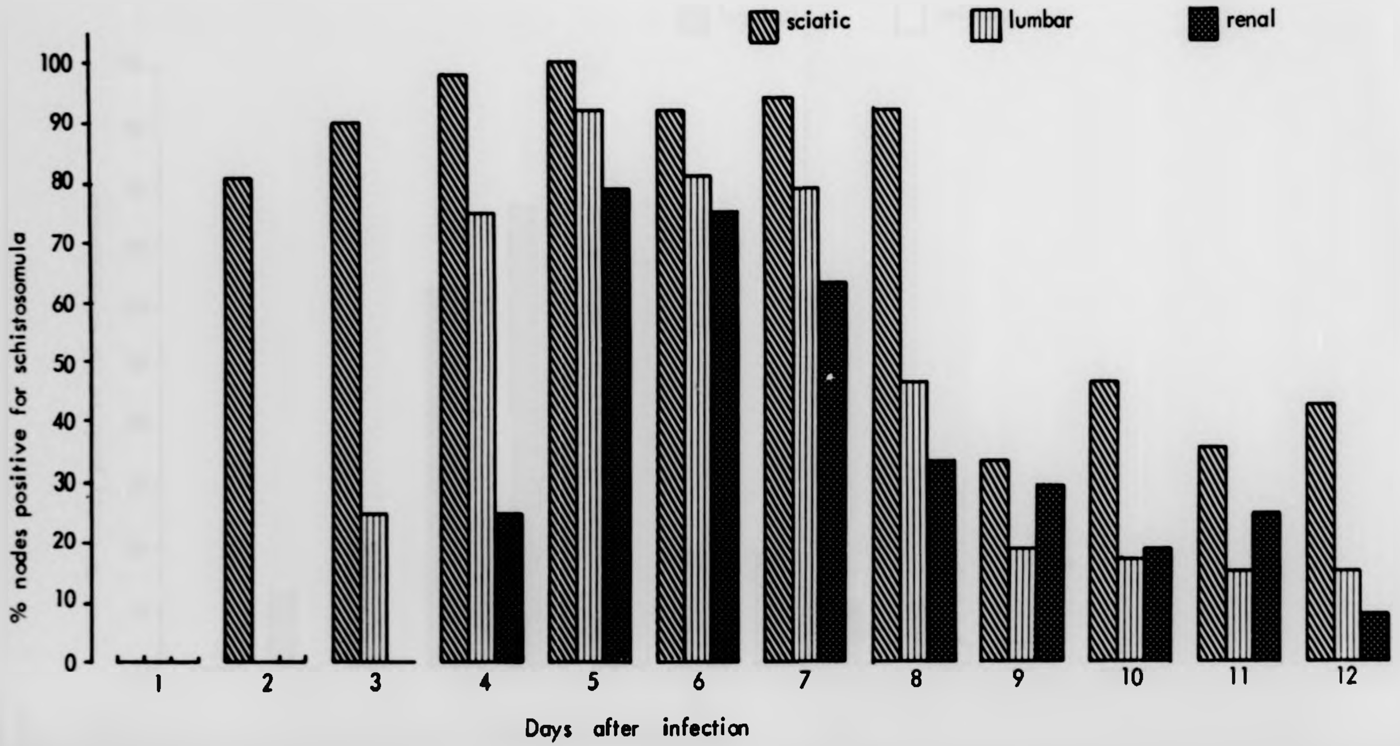


Fig. 14

Direct examination of lymph nodes for presence of schistosomula following infection via the tail. Nodes containing parasites are given as a percentage of the daily total of the respective lymph nodes. Maximum possible : 48 nodes showing parasites = 100%

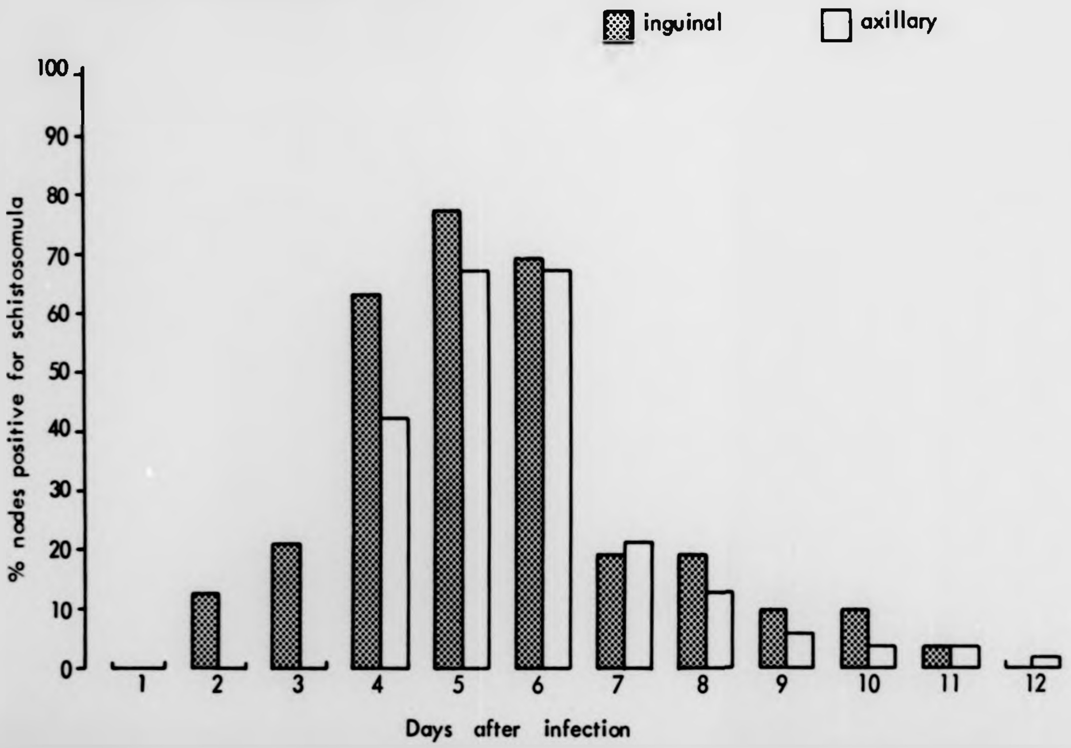


Fig.15

Direct examination of lymph nodes for presence of schistosomula following infection via the left hindfoot. Nodes containing parasites are given as a percentage of the daily total of the respective lymph nodes. Maximum possible : 24 nodes showing parasites = 100%

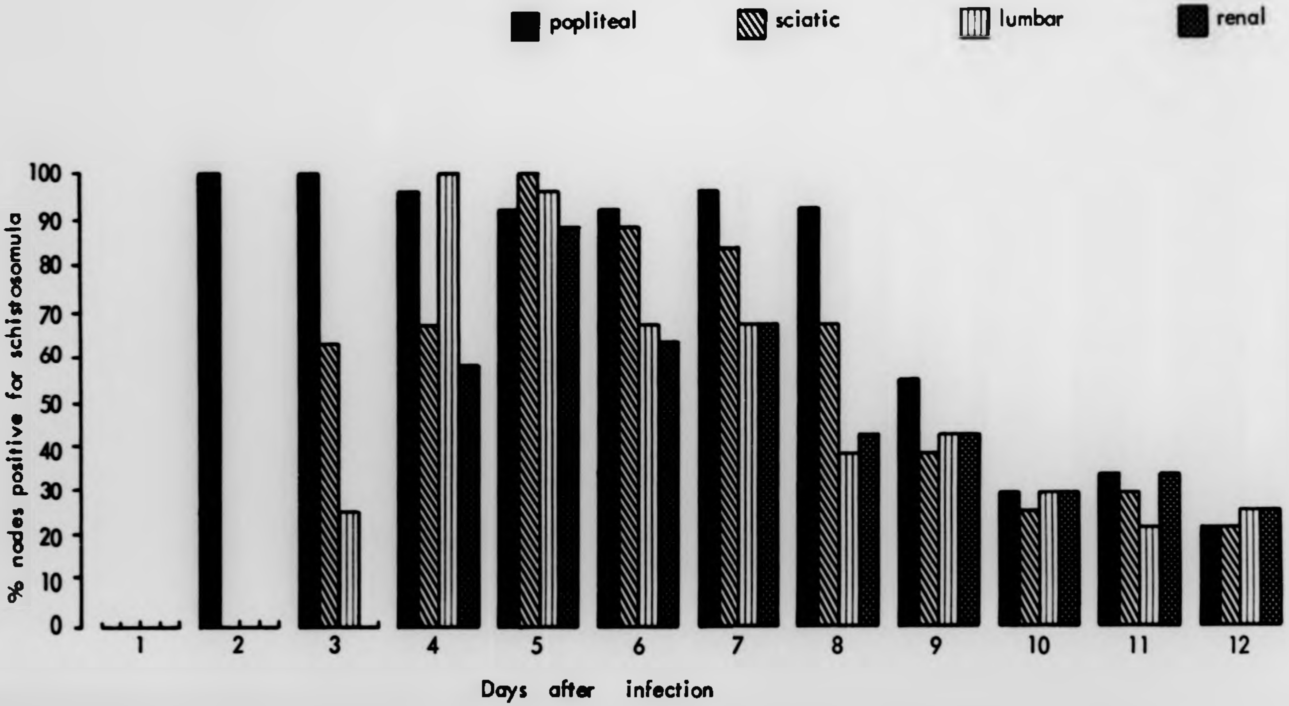


Fig. 16

Direct examination of lymph nodes for presence of schistosomula following infection via the left hindfoot. Nodes containing parasites are given as a percentage of the daily total of the respective lymph nodes. Maximum possible : 24 nodes showing parasites = 100%

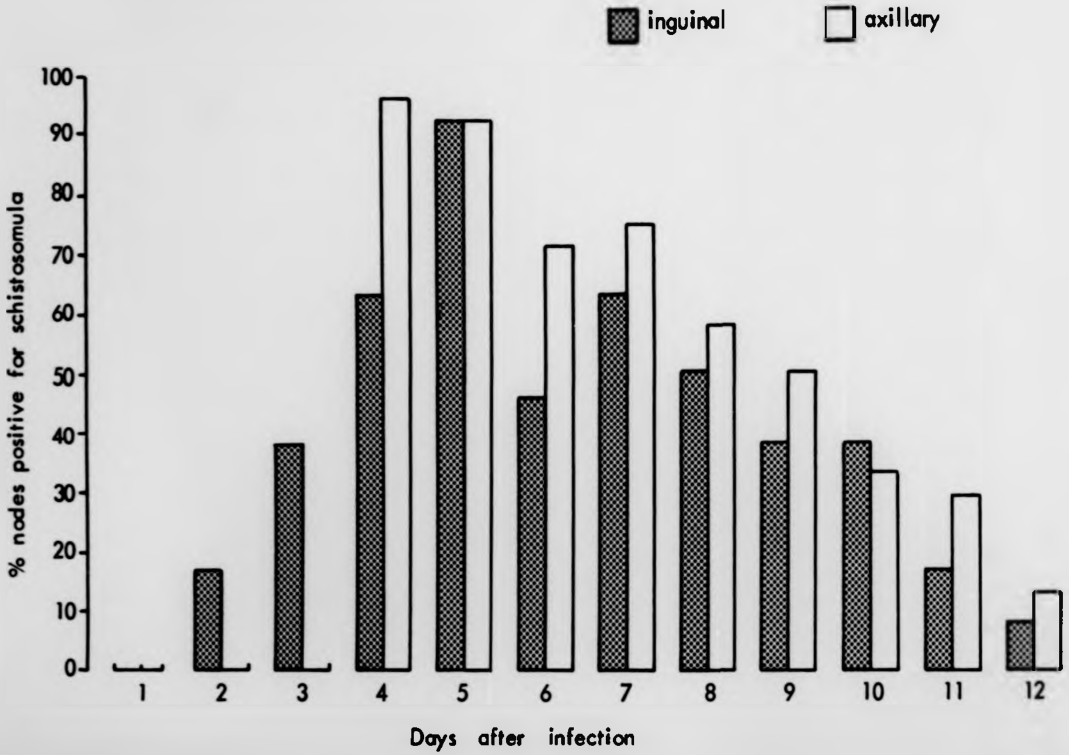
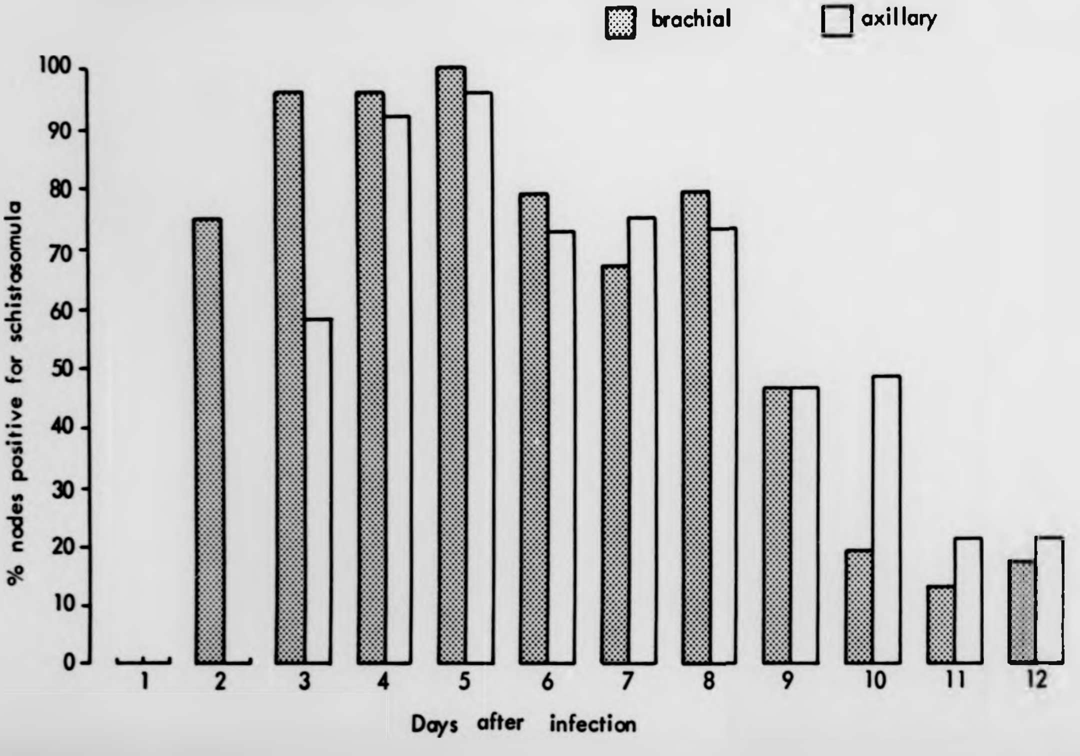


Fig. 17

Direct examination of lymph nodes for presence of schistosomula following infection via the left and right forefoot. Nodes containing parasites are given as a percentage of the daily total of the respective lymph nodes. Maximum possible : 24 nodes showing parasites = 100%



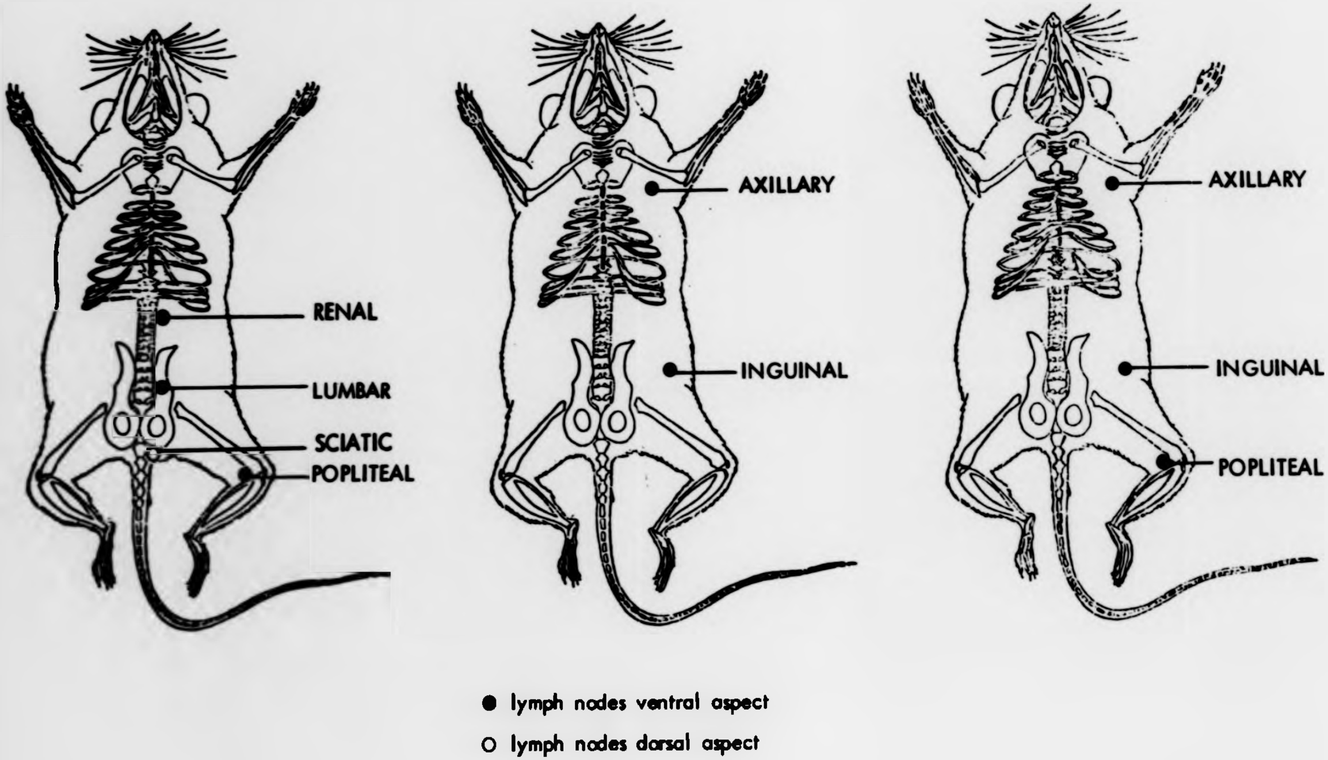
Efferent vessels from the inguinal nodes proceed craniad along the nipple line to reach the axillary nodes situated superficially in the axilla. Schistosomula were found in these nodes from day 4 (2 days after first appearing in the inguinal nodes), throughout to day 12 in a proportion of the nodes varying from 2% to 67%. The axillary nodes drain into the subclavian ducts and finally into the subclavian veins.

Rarely, parasites were found in left and right popliteal nodes. This leads to speculation as to whether there may occassionally be an anomalous pathway between tail and popliteal node. No confirmation of the existence of such a route was obtained by the dye injection method. Alternatively it is possible that the parasites gained entry to the popliteal nodes via the blood circulatory system after passing through the lung. In this context it is of interest to note that Tilney (1970; 1971), working on rats, found that the popliteal node "acts as a minor secondary site for the tail". If the popliteal node has a similar role in the mouse, the pathway from the tail is probably too narrow to be identified using ordinary dye injection methods.

Direct examination of lymph nodes involved in drainage following infection of the left hindfoot

In a series of experiments, 288 mice were infected with *S. mansoni* cercariae. From 1 to 12 days after infection

Fig. 18 Lymphatic routes available to the parasite when percutaneously infected via the left hindfoot


RENAL
LUMBAR
SCIATIC
POPLITEAL
AXILLARY
INGUINAL
AXILLARY
INGUINAL
POPLITEAL
● lymph nodes ventral aspect
○ lymph nodes dorsal aspect

24 mice were killed and examined each day.

Following infection, one of the two routes may be taken from the left hindfoot. The relevant drainage patterns are shown schematically in Figure 18. Tables 18-23 in Appendix, pp. 323-328, show presence (or absence) of parasites in the deep and superficial lymph nodes involved in drainage from the left hindfoot.

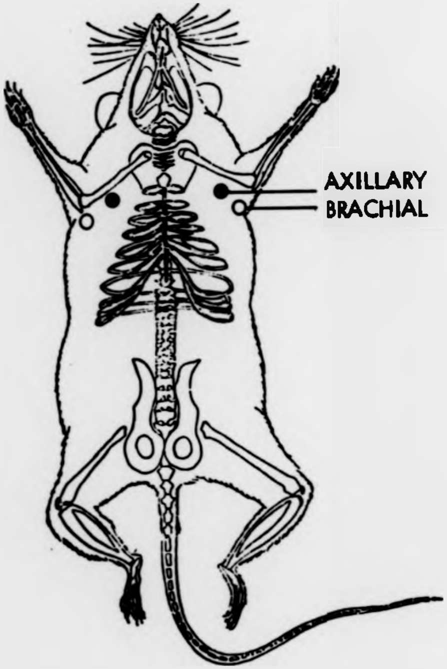
The alternative routes are:

1) To the popliteal node and thence in sequence to the sciatic, lumbar, and renal nodes which latter drain into the thoracic duct. Parasites were found in each of nodes. They first appeared in the popliteal nodes on day 2, in the sciatic and lumbar nodes on day 3 and on day 4 in the renal nodes. The proportion of nodes showing schistosomula from days 2 to 12 varied from 21% to 100% for the popliteal, sciatic, and lumbar nodes, and from 25% to 88% for the renal nodes.

2) To the inguinal node, then to the axillary node which latter drains into the thoracic duct. Parasites were found in the inguinal node from day 2 and in the axillary node from day 4. Schistosomula were present in inguinal nodes in a proportion of the nodes varying from 8% to 92% and in axillary nodes in a proportion varying from 13% to 96%.

3) To the popliteal node, and then sequentially to the inguinal and axillary node which latter drains into the thoracic duct.

Fig. 19 The lymphatic route available to the parasite when percutaneously infected via the left and right forefoot



AXILLARY
BRACHIAL

● lymph nodes ventral aspect

○ lymph nodes dorsal aspect

Direct examination of lymph nodes involved in drainage following infection of the left and right forefoot

In a series of experiments, 144 mice were infected with *S. mansoni* cercariae. From 1 to 12 days after infection 12 mice were killed and examined each day.

Following infection, only one route can be taken from either left or right forefoot. The drainage and lymph nodes involved are shown schematically in Figure 19. Tables 24 and 25 in Appendix, pp. 329 and 330, show presence (or absence) of parasites in the superficial lymph nodes involved in drainage from the left and right forefoot.

Parasites migrating via the lymphatics after penetration of the skin of a forefoot, first reach the brachial nodes situated subcutaneously in connective tissue next to the belly of the biceps muscle. Parasites were present in these nodes from days 2 to 12 in a proportion of the nodes varying from 13% to 100%.

Efferent lymphatic vessels from the brachial nodes drain into the axillary nodes. Schistosomula first appeared in the axillary nodes on day 3 (1 day after first appearing in the brachial nodes). Parasites continued to be found in axillary nodes throughout to day 12 and were present in a proportion of the nodes varying from 21% to 96%. Left and right axillary nodes drain respectively into the left and right subclavian duct and finally into the left or right subclavian vein.

Figures 13-17 - show the order of appearance of schistosomula in various relevant lymph nodes following infection at three sites - tail, hindfoot and forefoot.

Irrespective of the site of infection, the parasite is found on day 2 in the nearest lymph node of the relevant lymphatic pathway.

Figure 13 (following infection via the tail) shows that there are parasites on day 2 in the sciatic nodes and there after, at 1-day intervals, in the lumbar and renal nodes.

Figure 15 (following infection via the left hindfoot) illustrates presence of parasites in the popliteal node on day 2, and subsequently 1 day later (day 3) in both sciatic and lumbar nodes and after a further day (day 4) in the renal nodes.

Following infection via the tail or the left hindfoot, Figures 14 and 16 show that there is a 2-day delay between the first appearance of parasites in inguinal nodes and their subsequent presence in axillary nodes.

The highest proportion of lymph nodes infected in respect of the various pathways investigated occurred between days 4 and 7 after infection.

Histological examination: infection with *S. mansoni* cercariae

Skin

Schistosomula were seen on days 2 to 6 in lymphatics of the dermis following infection via the tail. No cellular response around the schistosomulum was seen. (Plate 40).

Lymph nodes

Table 26 show the presence (or absence) of schistosomula in lymph nodes involved in the migration of the parasites, from day 1 for various periods up to day 9 after infection via the tail, left hindfoot or forefoot. Serial sections show that a similar pattern of dispersal of schistosomula occurs in all lymph nodes involved in the migration. Schistosomula were found in marginal, cortical and medullary sinuses. No cellular reaction to the schistosomulum was apparent. No marked infiltration by lymphocytes, histiocytes, plasma cells or eosinophils was seen in the lymph nodes (Plates 41-48).

Histological examination: infection with S. *haematobium* cercariae

Skin

Schistosomula were seen on days 2 to 6 in lymphatics of the dermis following infection via the tail. No cellular response around the parasites was seen (Plate 49).

Lymph nodes

On day 2 after infection via the tail, *S. haematobium* larvae were found in the sciatic lymph nodes. By day 5 all relevant lymph nodes on the route of migration were

PLATE 40

A) Schistosomulum (S. mansoni) in lymphatic of dermis 5 days post-infection via the tail 6μm Stained H. and E. (X625)

B) Schistosomulum (S. mansoni) in lymphatic of dermis 4 days post-infection via the tail 6μm Stained H. and E. (X625)

C) Schistosomulum (S. mansoni) in lymphatic of dermis 3 days post-infection via the tail 6μm Stianed H. and E. (X625)

D) Schistosomulum (S. mansoni) in lymphatic of dermis 2 days post-infection via the tail 6μm Stained H. and E. (X390)

f dermis
tained H.

f dermis
tained H.

f dermis
tianed H.

f dermis
tained H.

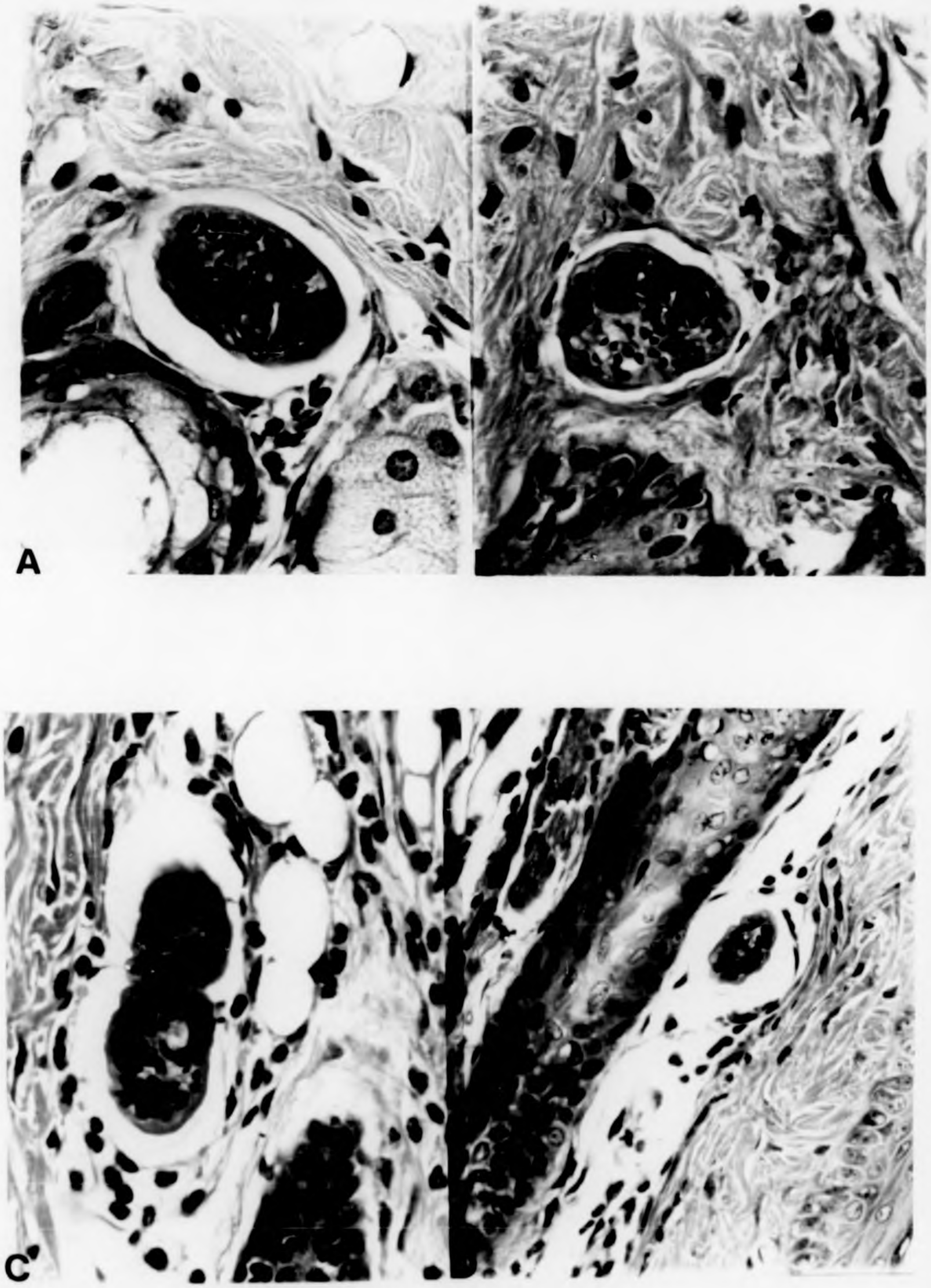


TABLE 26 Histological examination for presence of schistosomula in lymph nodes involved in drainage of the tail (A), the left hindfood (B) and the forefoot (C).

	NODES									
DAY	SCIATIC LEFT	SCIATIC RIGHT	LUMBAR LEFT	LUMBAR RIGHT	RENAL LEFT	RENAL RIGHT	INGUINAL LEFT	INGUINAL RIGHT	AXILLARY LEFT	AXILLARY RIGHT
1	– –	– –	– –	– –	–	–	– –	– –	– –	– –
2	– +	– +	– –	– –	–	–	+		– –	– –
3	+		+		– –	– –	+	+	+	–
4	+		+		– –	+ –	–	+	–	+
5	+		+	+	+		+	+	+	+
6	+		–	+	+		–	+	+	+
7	–	+	–	+	+		+	–	–	–
8	+		+		+		–	–	–	+
9	–	+	–	+	–	+	–	+	–	–

A

	NODES											
DAY	POPLITEAL		SCIATIC		LUMBAR		RENAL		INGUINAL		AXILLARY	
1	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	–	–	–	–	–	–	–	–	–	–
3	+		+		+		–	–	–	+	–	–
4	+		+		+		–	+	+		+	
5	+		+		+		+		+		+	

B

	NODES			
DAY	BRACHIAL		AXILLARY	
1	–	–	–	–
2	+		–	–
3	+		+	
4	+		+	
5	+		+	
6	+		+	

C

PLATE 41

Multiple invasion of a sciatic lymph node by
schistosomula (S. mansoni)
following infection via the tail.

A) Schistosomulum in the marginal sinus. 5 days post-infection 5µm Stained H. and E. (X390)

B) Schistosomula in cortical sinuses. 5 days post-infection 5µm Stained H. and E. (X98)

C and D) The same schistosomula as in (B) at a higher magnification X390

C - Cortical sinus
Ma - Marginal sinus
S - Schistosomulum

days
(X390)

ays post-
)

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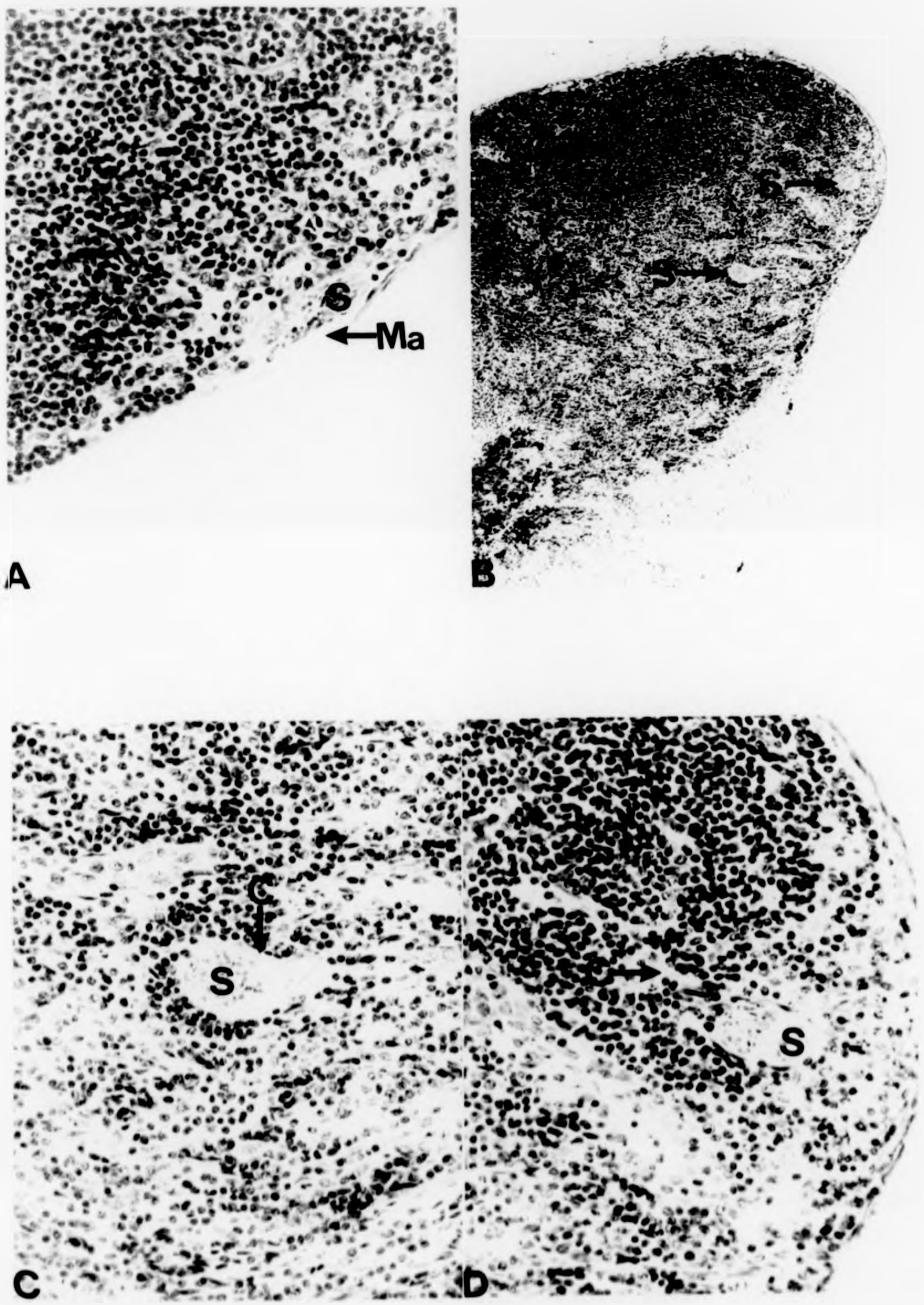
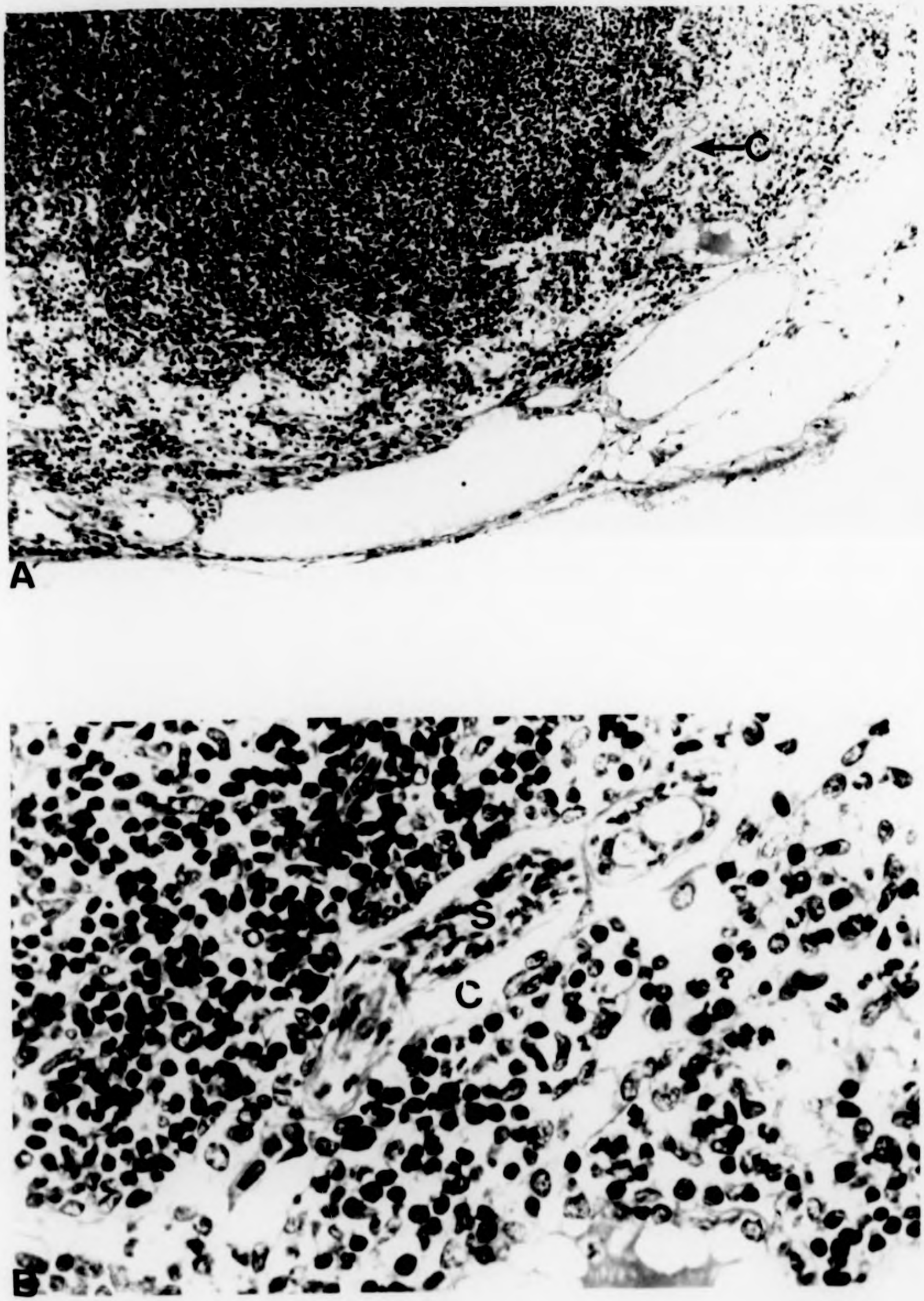


PLATE 42

A and B) A schistosomulum (S. mansoni) in cortical sinus in a lumbar lymph node. 5 days post-infection via the tail. 5µm Stained H. and E. (A) - low power X98; (B) - high power X390

C - Cortical sinus
S - Schistosomulum

al sinus
fection
(A) -



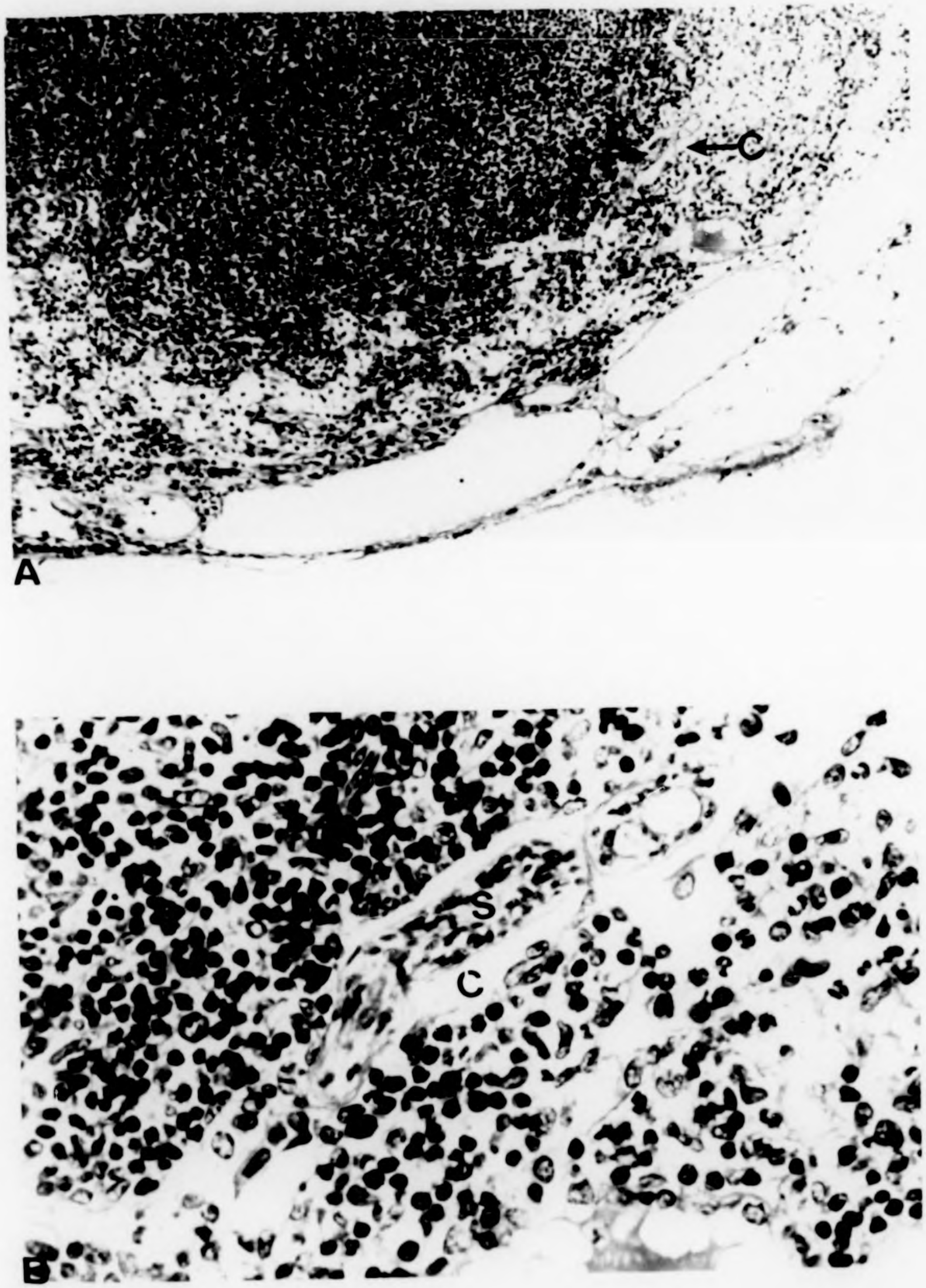

C
A
S
C
B

PLATE 43

A) Schistosomulum (S. mansoni) in the marginal sinus of a renal lymph node. 5 days post-infection via the tail. 5µm Stained H. and E. (X390)

B) Schistosomulum (S. mansoni) in medullary sinus of a renal lymph node. 5 days post-infection via the tail. 5µm Stained H. and E. (X390)

C) Schistosomulum (S. mansoni) in the marginal sinus of a renal lymph node. 5 days post-infection via the hindfoot 5µm Stained H. and E. (X390)

D) Schistosomulum (S. mansoni) in medullary sinus of a renal lymph node 5 days post-infection via the hindfoot 5µm Stained H. and E. (X390)

Ma - Marginal sinus
Me - Medullary sinus
S - Schistosomulum

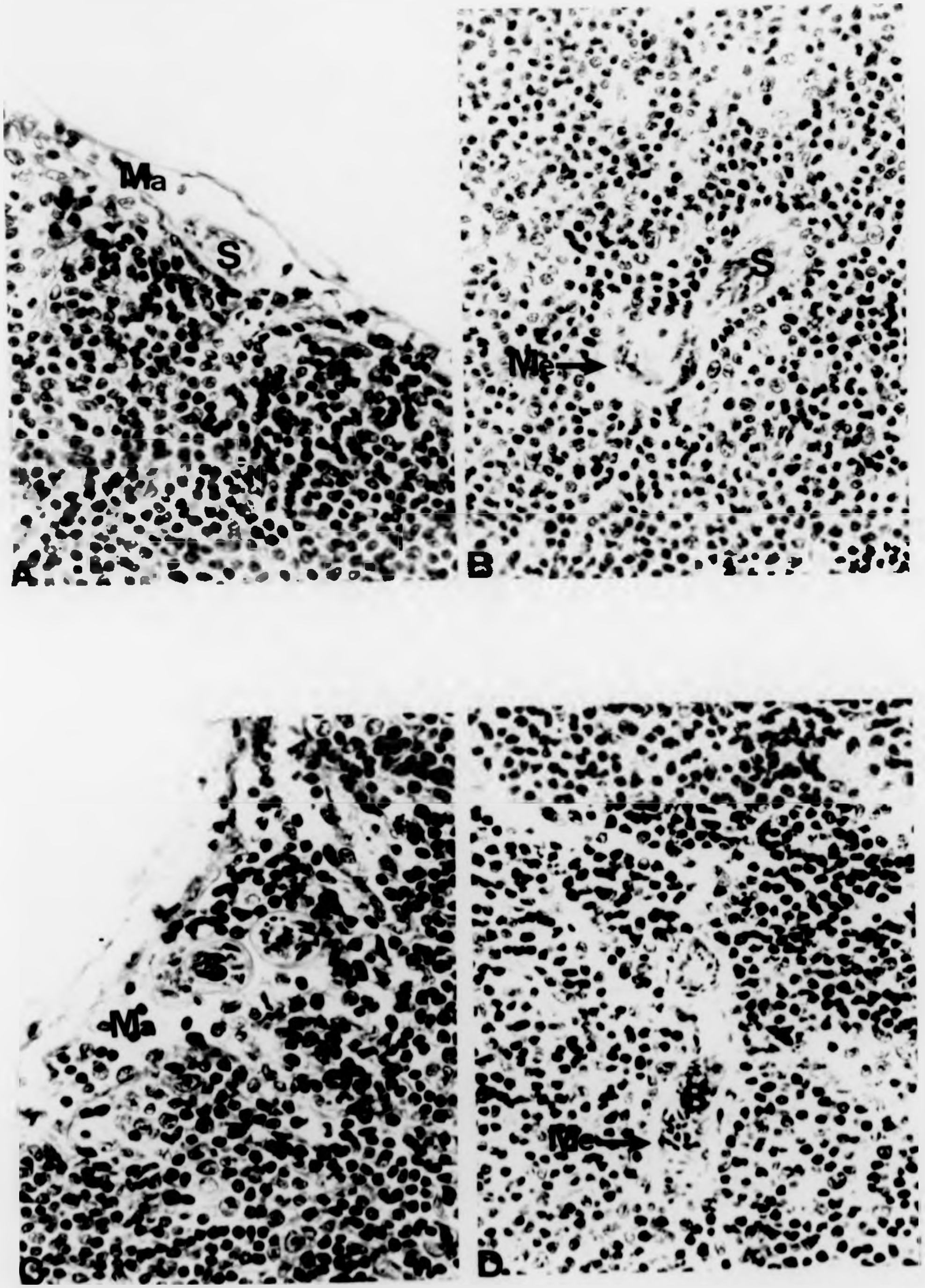

Ma
S
A
S
Me
B
Ma
C
Me
D

PLATE 44

A, B, C and D) Serial sections of an inguinal node showing a schistosomulum (S. mansoni) in medullary sinus. 5 days post-infection via the tail 5µm Stained H. and E. (A and C) - low power X98; (B and D) - high power X390. The lymphatic sinuses though not readily identifiable in section (A and B) is clearly seen in a later section (C and D) illustrating the value of examining serial sections.

Me - Medullary sinus
S - Schistosomulum

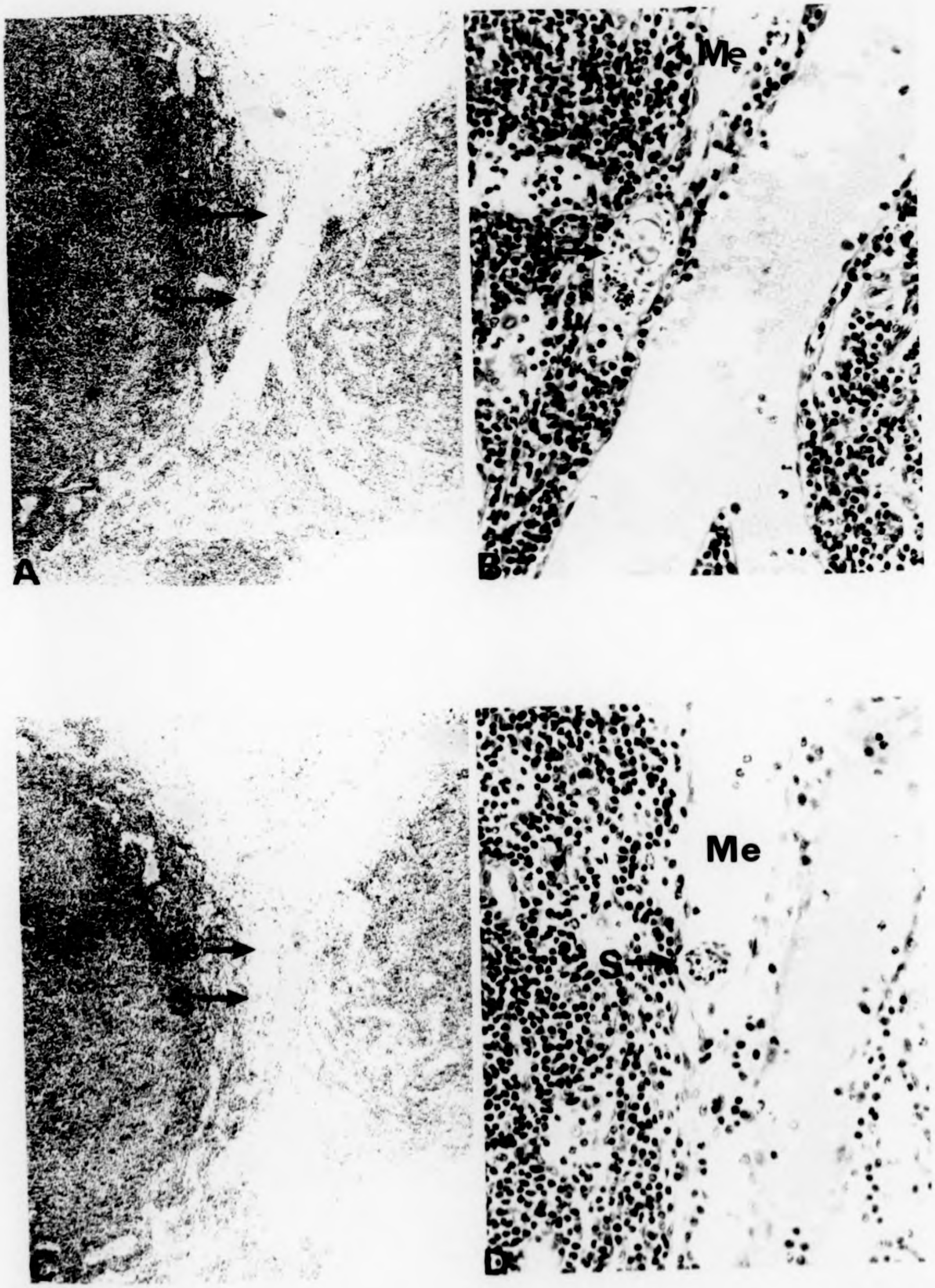

Me
A
B
Me
S
C
D

PLATE 45

A and B) A schistosomulum (*S. mansoni*) in the marginal sinus of an inguinal lymph node. 2 days post-infection via the tail 5µm Stained H. and E. (A) - low power X98; (B) - high power X390

C) Schistosomulum (*S. mansoni*) in the marginal sinus of an inguinal lymph node. 3 days post-infection via the tail 5µm Stained H. and E. (X390)

D) Schistosomulum (*S. mansoni*) in the marginal sinus of an axillary lymph node. 3 days post-infection via the tail 5µm Stained H. and E. (X390)

Ma - Marginal sinus
S - Schistosomulum

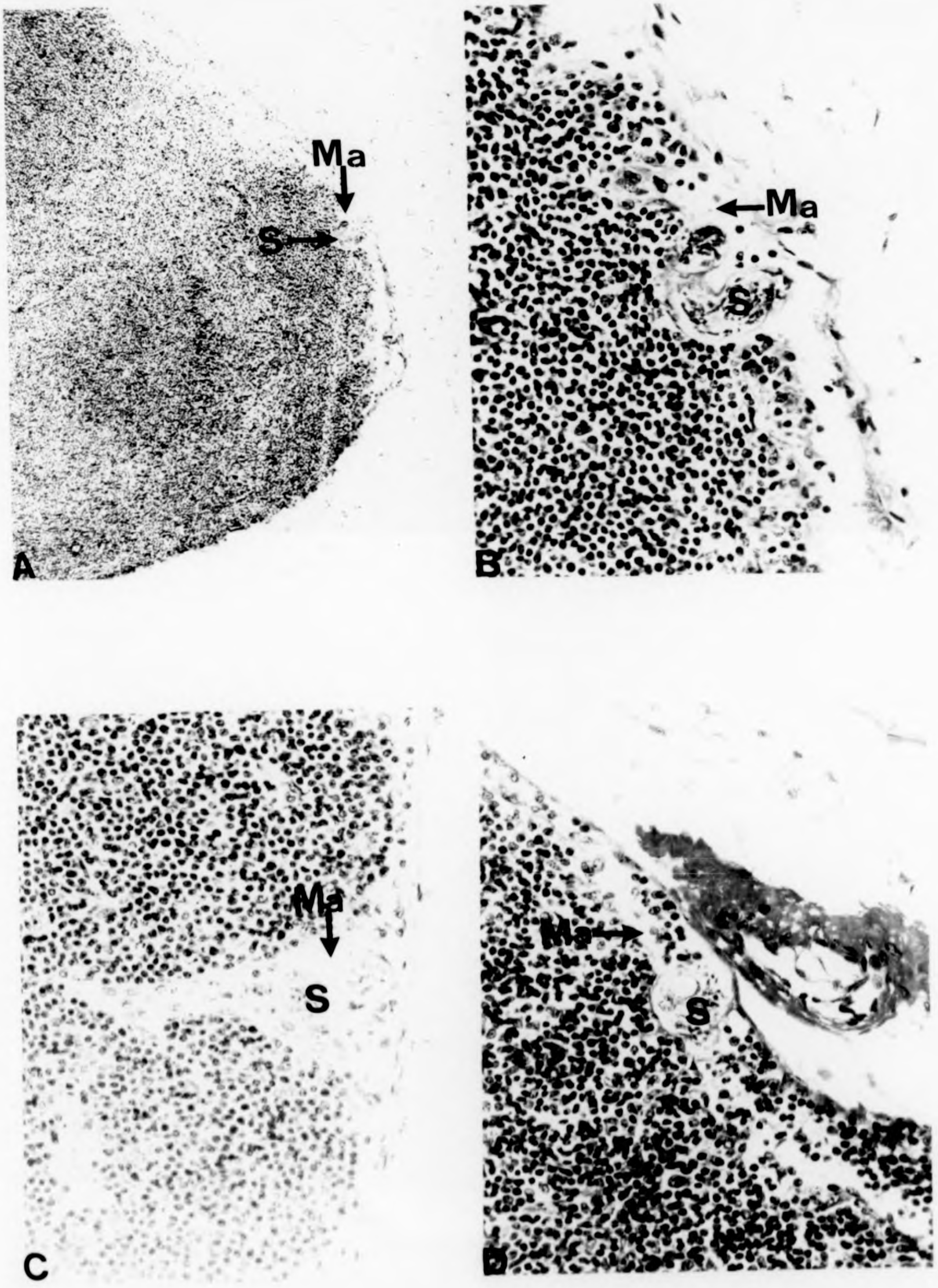

Ma
S
A
Ma
S
B
Ma
S
C
Ma
S
D

PLATE 46

Schistosomula (S. mansoni) in the marginal and medullary sinuses of a popliteal lymph node. 3 days post-infection via the hindfoot 5µm Stained H. and E. (X98) See also Plate 47

Ma - Marginal sinus
Me - Medullary sinus
S - Schistosomulum

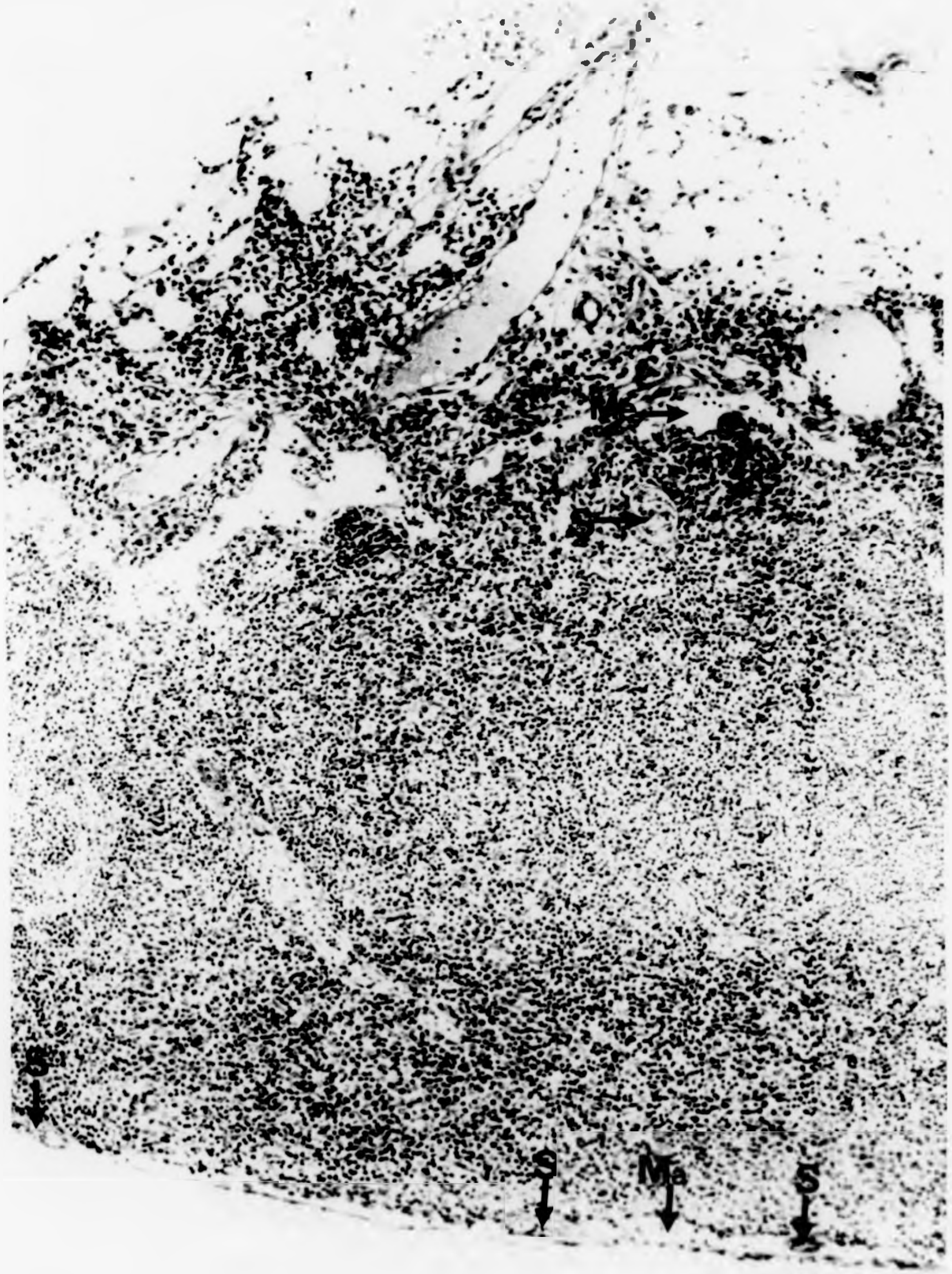

Ma
S
S
Ma
S

PLATE 47

A high power magnification of areas of Plate 46. Schistosomula (S. mansoni) in a popliteal lymph node 3 days post-infection via the hindfoot 5μm Stained H. and E. (X390)

A) in medullary sinus

B) in the marginal sinus

Ma - Marginal sinus
Me - Medullary sinus
S - Schistosomulum

isto-
post-
X390)

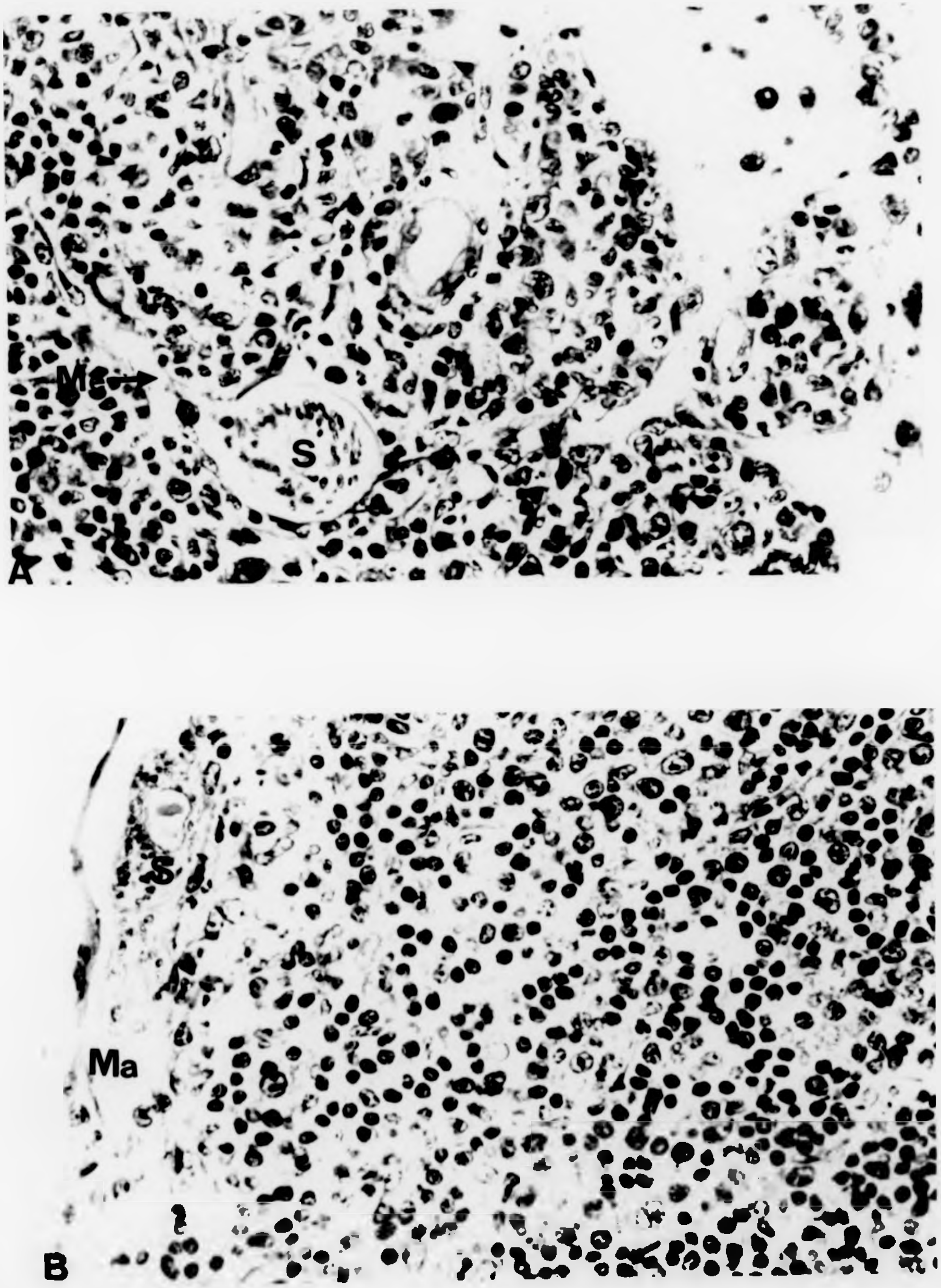


PLATE 48

A) Schistosomulum (S. mansoni) in the marginal sinus of an inguinal lymph node. 4 days post-infection via the hindfoot 5µm Stained H. and E. (X390)

B) Schistosomulum (S. mansoni) in the marginal sinus of a brachial lymph node. 6 days post-infection via the forefoot 5µm Stained H. and E. (X390)

Ma - Marginal sinus
S - Schistosomulum

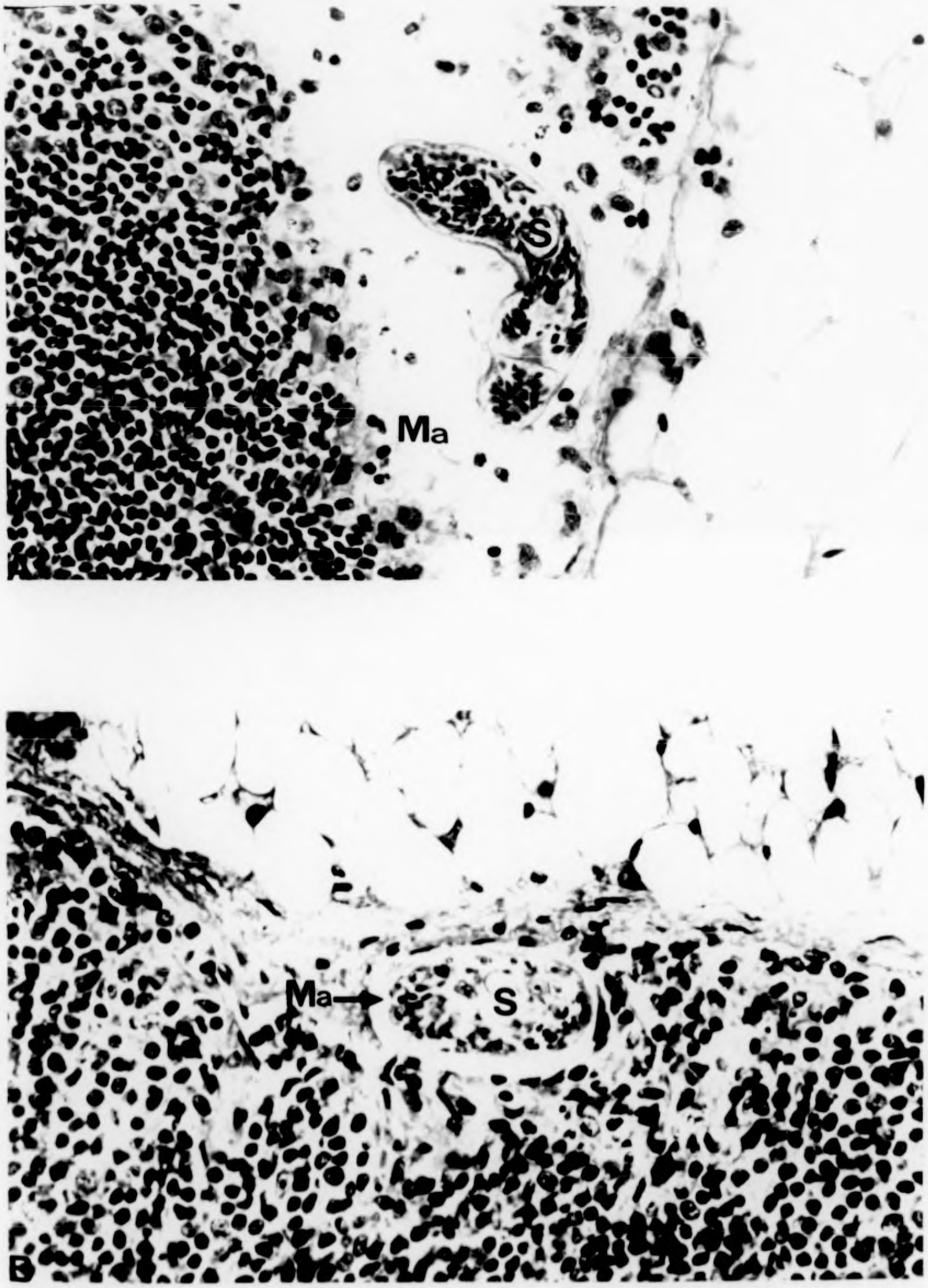

S
Ma
A
Ma
S
B

PLATE 49

A) Schistosomulum (S. haematobium) in lymphatic of dermis. 1 day post-infection via the tail. 6µm Stained H. and E. (X390)

B) Schistosomulum (S. haematobium) in lymphatic of dermis showing marked dermal cellular infiltration. 4 days post-infection via the tail 6µm Stained H. and E. (X390)

C) Schistosomulum (S. haematobium) in lymphatic of dermis showing marked dermal cellular infiltration. 3 days post-infection via the tail 6µm Stained H. and E. (X390)

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ed H.

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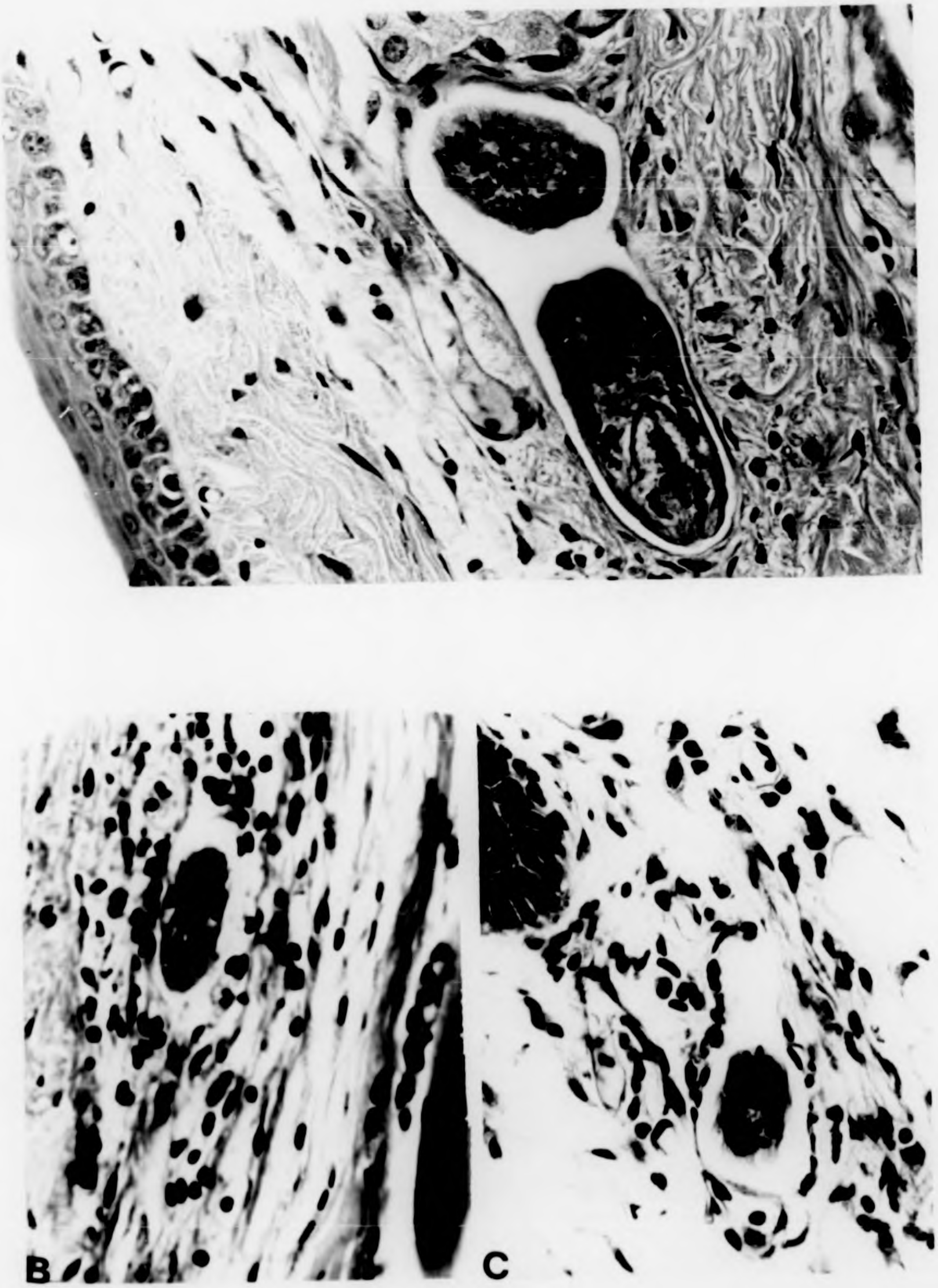
days
d E.

dermis
days
nd E.



positive. *S. haematobium* schistosomula were dispersed through lymph nodes in the same manner as were *S. mansoni* schistosomula. There was no visible cellular reaction to the parasite. Lymph node sinuses were markedly dilated and markedly infiltrated by histiocytes. Post-capillary venules were surrounded by lymphocytes (Plates 50-51).

7.5 DISCUSSION

A necessary preliminary to the investigation of the role the lymphatic system plays in the migration of the schistosomulum between skin and lung in the T.O. mouse, was the identification of such pathways as relevant to sites of infection employed in this study.

There can be no doubt that the schistosomulum utilizes the lymphatic system on leaving the skin. Both *S. japonicum* and *S. mansoni* larvae have been found in the lymphatics of the dermis by other workers (Miyagawa and Takemoto 1921; Watarai, 1936; Koppisch, 1937; Standen, 1953; Stirewalt, 1959). The author, working with *S. mansoni* and *S. haematobium*, found larvae both in lymphatics and blood capillaries in the skin. *S. haematobium* in dermal lymphatics had not been previously recorded. Standen (1953) found parasites, *S. mansoni*, only in the lymphatics. However, his observations were limited to minutes as compared with prolonged observations totalling days by this author. This could

PLATE 50

A and B) A schistosomulum (S. haematobium) in cortical sinus of a sciatic lymph node with marked infiltration with histiocytes. 4 days post-infection via the tail 5μm Stained H. and E. (A) - high power X390; (B) low power X98

C and D) Serial sections of a sciatic lymph node showing a schistosomulum (S. haematobium) in medullary sinus with marked infiltration with histiocytes. 4 days post-infection via the tail 5μm Stained H. and E. (X390)

C - Cortical sinus
H - Histiocyte
Me - Medullary sinus
S - Schistosomulum

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post-
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X98

showing
dullary
tiocytes.
n Stained

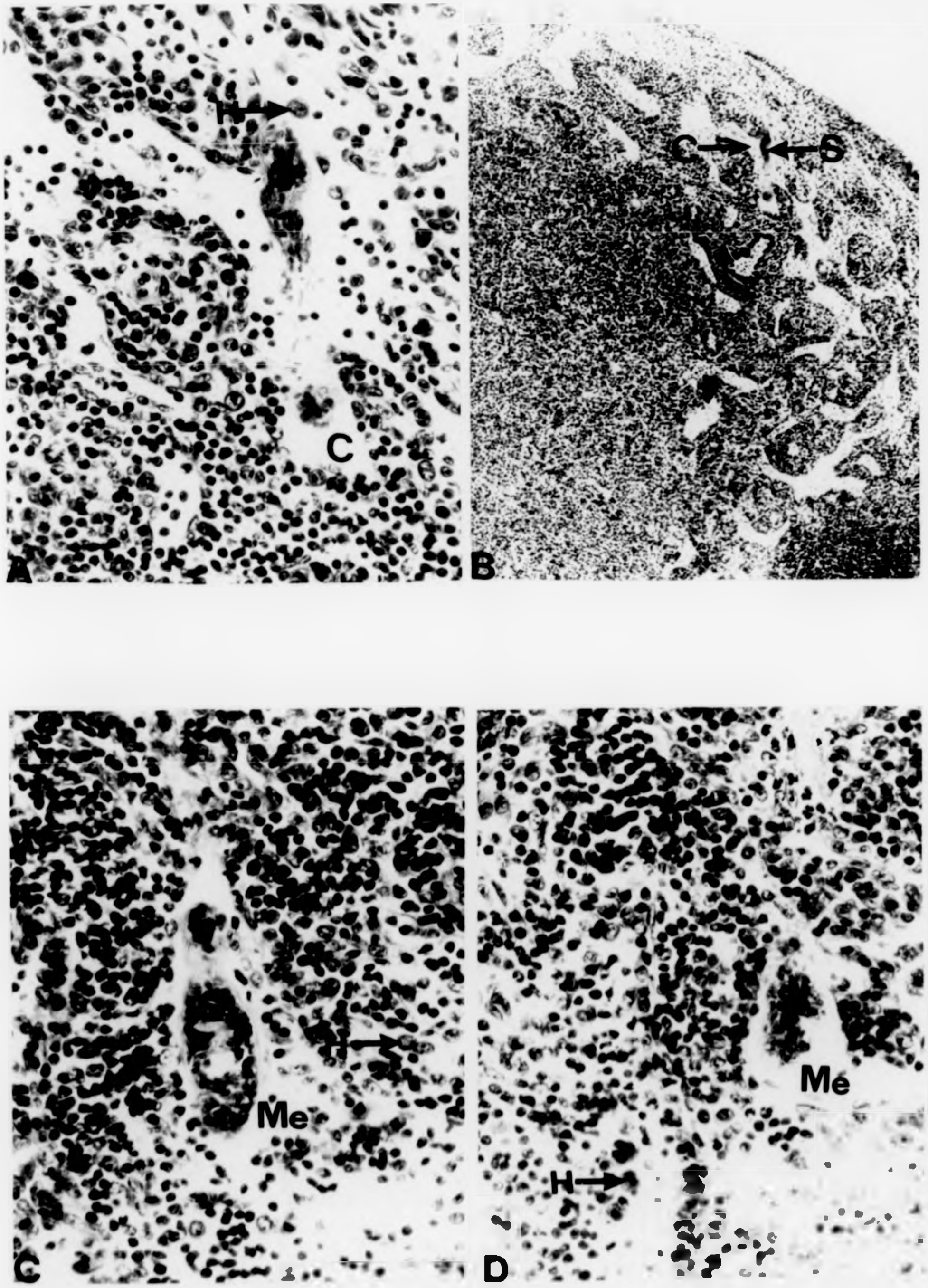
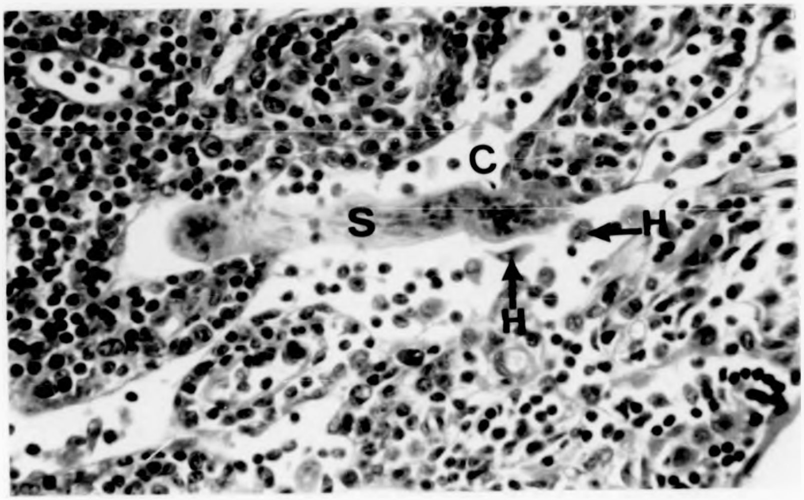

H
C
A
C
S
B
Me
C
Me
H
D

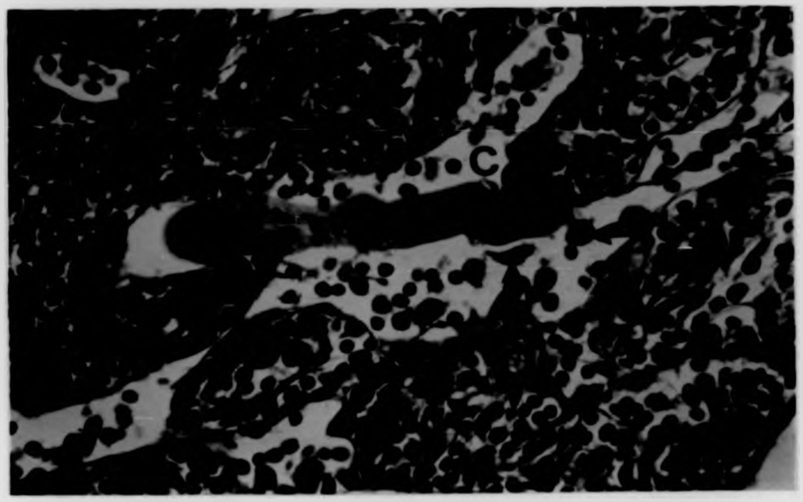
PLATE 51



Schistosomulum (S. haematobium) in cortical sinus of a sciatic lymph node showing marked increase of histiocytes. 4 days post-infection via the tail 5μm Stained H. and E. (X390)

C - Cortical sinus
H - Histiocytes
S - Schistosomulum

PLATE 51



Schistosomulum (S. haematobium) in cortical sinus of a sciatic lymph node showing marked increase of histiocytes. 4 days post-infection via the tail 5µm Stained H. and E. (X390)

C - Cortical sinus
H - Histiocytes
S - Schistosomulum

account for the differences in the two sets of findings.

In taking this route the schistosomulum is not unique. The larva of Ancylostoma duodenale migrates unharmed through the lymphatics after entering via the skin (Looss, 1911) and so does Nippostrongylus brasiliensis (Gharib, 1961). In the case of the latter it has been shown that an immune response is evoked around the parasite within the lymph node only when a non-specific host is invaded (Gharib, 1961).

The present study shows that schistosomular transportation via the lymphatics is not limited to those of the skin but proceeds further along the lymphatic route. Schistosomula pass through successive lymph nodes; the environment of the nodes does not appear to be hostile to them. No histological evidence has been found either of successful phagocytic activity against the schistosomulum within a lymph node or of an immune reaction around the parasite. A difference was observed, however, between S. mansoni and S. haematobium schistosomula in respect of the cellular response of the lymph node. S. haematobium produced marked infiltration by histiocytes in the lymph sinuses, which were dilated. The post-capillary venules were surrounded by lymphocytes.

Schistosomula were found in all the lymph nodes of the various groups draining the different sites of infection. It seems reasonable to conclude that they passed from one node to the next in any group en route to the thoracic or right lymphatic duct. The number of lymph nodes traversed depends on the site of initial infection and varies from 2 to 10 or possibly more.

These conclusions were reached by monitoring nodes both by histological and direct examination 9 and 12 days respectively, following infection at 3 different sites. A similar pattern emerged for all 3 routes.

A false positive result by the direct method could be produced by the presence of a schistosomulum, not in a lymph node sinus, but in a lymph node post-capillary venule; concomitant histological examination determined whether or not the larvae were present in the sinuses of lymph nodes.

Lymph nodes were monitored only for the presence or absence of parasites. On practical grounds it is impossible to determine actual numbers of schistosomula present in lymph nodes by assay of the nodes; hence the percentage of parasites which migrate via the lymphatics following infection by a known number of cercariae cannot be established. In the present study, all examinations were made at 24 hourly intervals, therefore the time of arrival of parasites in nodes within a 24 hour period could not be stated precisely. It could only be said that a given number of nodes were or were not infected at the end of a particular 24 hour period. In the light of this reservation, the conclusion of Miller and Wilson (1978), who examined lymph nodes at 24 hour intervals by the technique of mincing and incubation, and claim that the recovered parasites in lymph nodes represent 4% of the cercariae applied, is open to question. They concede that only a fraction of the parasites in lymph nodes may have been recovered. Moreover, not all lymph nodes draining the abdominal skin area were examined by these authors.

It is demonstrated in the present study that the first appearance of a schistosomulum in the nearest proximal lymph node after leaving the skin is between 24 and 48 hours following infection. This accords with the results of Miller and Wilson (1978). Movement of schistosomula out of the skin, up to their first appearance in lymph nodes 48 hours after infection, is irrespective of texture of skin at the site of infection, whether tail, hindfoot, forefoot or abdomen.

As regards the time the schistosomulum remains in the skin following infection, it has been asserted that *S. mansoni* larvae start to leave the skin only on day 3 (Smith *et al.*, 1976). In the present study they have been found both in lymph nodes and in the lung on day 2. Those found in the lung probably entered a blood capillary rather than a lymphatic vessel on leaving the skin, and travelled by the blood vascular route. Investigating the behaviour of *S. haematobium* larvae in the skin, Smith *et al.* (1976) found that the parasites remained intradermal for a minimum of 3 days. In the present study, however, it was found that S. haematobium larvae behaved in the same way as S. mansoni in that they were found in lymph nodes on day 2.

Following infection by the tail-immersion method it was possible to monitor the presence of schistosomula in the various nodes along the relevant lymphatic routes. Schistosomula were present in the sciatic and inguinal nodes on day 2, the lumbar nodes on day 3 and in the renal and axillary nodes on day 4. This no doubt represents the chronological sequence of migration through the lymphatic nodes to the thoracic or right lymphatic duct and into the blood stream. On the basis of the time taken in passing through the lymph nodes, schistosomula found in the lung on day 2 and 3 after infection can be considered to be a pure blood-borne population. Parasites in the lung from day 4 onwards would almost certainly comprise a mixed population.

Based on their work with the hamster cheek pouch (HCP), Miller and Wilson (1978) conclude that in schistosomular migration from skin to lung the blood vascular route is of major importance. The present study shows, however, that the published experimental work of these authors warrants no such conclusion. The HCP is regarded as an immunologically priviliged site, based on its acceptance of grafts of normal or malignant tissues - either homologous or heterologous in origin - which become established and persist for lengthy periods. Various reasons to account for this property of the HCP have been suggested.

Billingham, Ferringan and Silvers (1960) and Billingham and Silvers (1962) have advanced the hypothesis that the pouch may owe its immunological privilege to its connective tissue layer acting as a barrier to prevent or impede the passage of antigens. Shepro, Kula and Halkett (1963) investigating the properties of the HCP, agree with Billingham _et al_. (1960) and Billingham and Silvers (1962) that its immunological privilege derives from its structure; in addition they point to the alymphatic nature of the pouch. In line with Shepro _et al_. (1963), to whom they do not refer, Miller and Wilson (1978) attribute the immunological privilege of the pouch to "the lack of an afferent lymphatic drainage". On this basis they selected the HCP as an experimental tool to measure the success of a schistosomular migration when the only exit from the site of infection is by way of the blood vascular route. Infecting via the HCP, Miller and Wilson (1978) found that there was no significant difference in the recovery of adult worms as compared with recovery when infecting via the abdominal skin of the hamster, where both blood vascular and lymphatic routes are available. As a result of this finding they conclude that the blood vascular route is the major route in the migration of the parasite from the abdominal skin.

Their conclusion should, however, be regarded with some reservations. Whether or not there exists a lymphatic drainage system in the HCP is a matter of controversy. Miller and Wilson (1978) do not refer to direct investigations of lymphatic drainage in the HCP but quote Billingham and Silvers (1962) and Tilney and Gowans(1971) to support their contention that "lymphatic drainage from the pouch is negligible or non-existent". However, no such statement can be found in the studies published by Billingham and Silvers (1962) - see page 259, or Tilney and Gowans (1971).

On the other hand Shepro, Eidelhoch and Patt (1960) implanted grafts of malignant tumors into the HCP. They found changes indicating an immunological response in the cervical lymph nodes. Miotti (1961) lists the submental node as draining the cheek pouch. In their histological studies Lindemann and Sträuli (1968) found lymphatics in the pouch but qualify their findings by describing two portions of the pouch - an oral and an aboral portion. They found lymphatics in the former but not in the latter. Using histochemical staining Goldenberg (1970) injected ferritin into the HCP and observed uptake of iron by regional lymph nodes, though at a lower rate than the uptake of ferritin from the lip of the hamster. In a carefully controlled experiment Goldenberg and Steinborn (1970), using India ink injections into the tissue of the pouch, observed the passage of dye to various regional lymph nodes. They interpret their findings as evidence for the existence of lymphatics in the pouch but state that drainage is both delayed and reduced.

Conversely, no evidence of lymphatics in the HCP was found by Shepro et al. (1963) on histological examination and injection of carbon particles, or by Witte, Goldenberg and Schricker (1965; 1968), who used fluorescent dyes.

In spite of the negative findings of workers investigating the existence of lymphatics in the HCP (Shepro et al., 1963; Witte et al., 1965; 1968; Barker and Billingham, 1971), it is difficult to ignore the evidence of lymphatic drainage from the pouch to the regional nodes (Shepro et al., 1960; Miotti, 1961; Goldenberg, 1970; Goldenberg and Steinborn, 1970) and positive histological findings by Lindemann and Sträuli (1968). The results of Lindemann and Sträuli (1968) no doubt carry more weight than the negative histological findings of Shepro et al. (1963). Failure to find lymphatics histologically could be due to limited search of an inadequate number of sections. It is also possible that no lymphatics were found in the HCP because Shepro et al. (1963); Witte et al. (1965; 1968) and Barker and Billingham (1971) failed to make allowance for drainage being both delayed and reduced (Goldenberg, 1970; Goldenberg and Steinborn, 1970) and did not wait long enough between injection of dyes and dissection.

When compared with normal abdominal skin, the different structure of pouch "skin" with its absence of hair, glands and vascular papillae except at the oral portion (Fulton, Jackson and Lutz, 1947; Priddy and Brodie, 1948) invalidates a comparison of recovery of parasites following infections at these two sites, quite apart from the view held by Miller and Wilson (1978) that the pouch is alymphatic.

If the HCP is to be used at all in comparing the blood vascular and lymphatic exit routes it should be done on the basis of histological differentiation of the pouch observed by Lindemann and Sträuli (1968) - the aboral alymphatic portion, and the oral portion containing lymphatics. Infections at these portions of the pouch with associated monitoring of regional lymph nodes would probably provide more reliable evidence as to the relative importance of the two routes. Even so the uniqueness of the pouch "skin" and the decreased and delayed lymphatic drainage from the pouch (Goldenberg, 1970; Goldenberg and Steinborn, 1970) would complicate the interpretation of results, whatever the findings might be.

For a valid comparison of the two routes it would be necessary to have a separate lymphatic site devoid of blood vessels and another, blood vascular but alymphatic, site, the tissue in each case being identical in all other respects. So far as is known this possibility does not exist in any mammalian species.

There is a wide divergence of opinion concerning the role of the lymphatic circulation in schistosomular migration between skin and lung. Standen (1953), who has observed the parasite only in the lymphatics of the skin, adheres to "the lymphatic theory". Phillips and Reid (1978) indicate that the lymphatics play no part in migration.

The number of blood capillaries in the skin is believed to vary from region to region and any particular region may vary substantially from individual to individual, the differences being influenced by age and minor pathology (Ryan, 1973). The same comments are equally applicable to lymphatic capillaries. The state of the vessels also depends on physiological requirements at any particular time. Wheater and Wilson (1979) found that schistosomula leave the skin mainly by the blood vessels. The authors suggest that the less important role of the lymphatics "may be because they are not so numerous as blood vessels in the dermis". The calibre of lymphatics exceeds that of the blood capillaries (Hudack and McMaster, 1932; Godart, 1968) and in addition the former are thin-walled and lack a well-defined basement membrane (Leak and Burke, 1966; 1968). Thus, in a random search for an intravascular migration route and in the absence of any special inhibitory factor, the lymphatics would seem to present no less an opportunity for migration than the blood capillaries. Peaking of S. mansoni parasites in the lung occurs 4 to 6 days after infection (Sher, Mackenzie and Smithers, 1974). It is worthy of note that in the present study parasites first appeared in lymph nodes closest to the thoracic and right lymphatic ducts 4 days after infection via the tail. On the other hand the blood vascular system would seem to present less of an obstacle to the passage of the parasites as evinced by their first appearance in the lung 2 days after infection.

Neither from the present study nor from reported work is it possible to conclude whether the lymphatic route is of major or minor importance in schistosomular migration. There can, however, no longer be any doubt as to the utilization of the lymphatic route by the schistosomulum. To obtain information on actual numbers using this route would require further investigation.

CHAPTER 8

SCHISTOSOMULAR MIGRATION: EXTRAVASCULAR ROUTES

8.1 INTRODUCTION

The blood circulatory system has not been universally accepted as the exclusive pathway used by the schistosomulum in its migration from the lungs to the liver. To account for the migration of the schistosomulum from the lungs to the liver by a pathway other than the blood circulatory system some workers have postulated an extravascular route. However, such a route remains the subject of controversy. It implies active penetration by the larva, after breaking out of the lung capillaries, through tissue structures intervening between lung and liver. The adherents to the concept of an extravascular route claim to have found parasites in the pleural cavity (Sueyasu, 1920; Goto, 1932; Sadun et al., 1958; Wilks, 1962; 1967) in the thoracic cavity (El-Gindy, 1950) and in the mediastinum (Narabayashi, 1917). Lampe (1927) traced the larval path and concluded that the parasite passes variously through the pleural cavity, the mediastinum or the "pericardial cavity". While some of these authors report the recovery of schistosomula from washings of pleural cavities it is doubtful from their accounts whether the schistosomula were in fact obtained solely from the pleural cavity. All these authors assert that the parasite pierces the diaphragm to reach the liver. This raises the question of the means by which the parasite is able to pass through various tissues. It is generally agreed that by the time the schistosomulum has reached the lung its enzyme content has been exhausted (Stirewalt and Kruidenier, 1961; Campbell, Frappaola, Stirewalt and Dresden, 1976). Furthermore, this type of migration would seem to imply some form of taxis particularly with regard to the transdiaphragmatic course. In the absence of such taxis, only a small proportion of schistosomula may be assumed to pass through the various tissue barriers, to reach the liver by chance and the remainder to pass randomly into other tissues ultimately to perish.

To test the claims advanced for an extravascular migration the following investigations were undertaken. At certain intervals after percutaneous infection via the tail, washings from pleural and peritoneal cavities of mice were examined for the presence of larvae. Lungs, diaphragms and livers were examined histologically to ascertain the presence of larvae lying outside blood vessels and for evidence of any cellular response. A comparative study was made of intravenously injected cercariae with a full enzyme-load on the one hand and 6 day old schistosomula, whose enzyme-load is likely to be exhausted, on the other. In each case lungs were removed and examined histologically 24 hours after infection to determine whether the parasite was extravascular or intravascular. In addition a comparison was made of the recovery rate of parasites at maturation in each batch of mice.

8.2 MATERIALS AND METHODS

Recovery of schistosomula from pleural* and peritoneal cavities

The pleural and peritoneal cavities of mice, infected with approximately 2000 *S. mansoni* cercariae by the tail-immersion method described earlier (see page 38), were examined. Mice were killed daily from days 2-16 with ether, the fur was moistened and the skin incised on the ventral surface. The skin was then removed from thorax and abdomen. To obtain a clear view of the lungs the xiphisternum was extended ventrally and a 26-gauge needle introduced into the thorax at the ventral border of the diaphragm immediately lateral to the xiphisternum. As the parietal pleural layer was pierced allowing air to enter, the lung was seen to collapse creating a true pleural cavity. A 1ml tuberculin syringe containing 0.6ml of Earle's balanced salt solution was fitted to the needle and the contents introduced into the pleural cavity. The washing was withdrawn with the same syringe and placed in a Universal container (plastic). A similar technique using a 5ml syringe fitted with a 26-gauge needle, was employed to inject 5ml of Earle's solution into the peritoneal cavity after exposing the peritoneum at a point in the centre of the midventral line. The peritoneal fluid and solution were circulated in the cavity by gently pressing and releasing the abdomen. Peritoneal washing was withdrawn from the cavity by the insertion of the needle into the flank where a pocket of fluid had formed behind the spleen. The fluid from each cavity was placed separately in Universal containers and centrifuged for 1 minute at 3000rpm. The excess fluid was removed leaving a residue of 1ml for further examination in a Sedgewick Rafter counting cell (Gallenkamp 1ml capacity) to determine presence (or absence) of schistosomula in each mouse. One drop of 1% neutral red was added to the contents of the counting cell in order to recognize phagocytic cells which might be adhering to parasites.

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* The difficulties of this technique with regard to washings from the pleural cavity are discussed in detail on page 293.

Histological examination

In order to compare locations reached in the lungs by enzyme-loaded cercariae and enzyme-exhausted schistosomula, the following investigation was carried out using two separate batches of mice. Approximately 100 S. mansoni cercariae were injected into a tail vein of each mouse in one batch. Approximately 100 6 day-old S. mansoni schistosomula recovered from the lungs (see page 93) of previously infected mice were introduced into a tail vein of each mouse in the other batch. A 1ml tuberculin syringe fitted with a 26-gauge needle was used. In the case of the cercariae it was possible to inject a volume up to 0.4ml without untoward effect despite the fact that the suspension was non-physiological, since it was administered very slowly. Twenty four hours after infection, the lungs were taken from each mouse and examined histologically.

Lungs diaphragms and livers taken from the mice percutaneously infected via the tail were examined histologically. This procedure was carried out daily for days 1-16 after infection (see page 121).

Adult worm recovery by perfusion

Two groups of 10 mice each were infected with *S. mansoni* cercariae or schistosomula respectively by injection into a tail vein. The schistosomula used for these infections were recovered from lungs (see page 93) of mice exposed 6 days previously (tail-immersion method). Two further groups with 10 mice in each group were infected with cercariae by the tail-immersion method and the ring method to serve as controls. Subsequent treatment of each group was identical. The mice were killed 6 weeks after infection by intraperitoneal injection of 0.2ml undiluted veterinary Nembutal (60mg/ml - Abbott Laboratories) containing 25 units/ml of heparin per animal. Besides killing the mouse, the Nembutal narcotizes the adult worms which are then easily flushed out of blood vessels by perfusion. The with a 26-gauge needle was used. In the case of the cercariae it was possible to inject a volume up to 0.4ml without untoward effect despite the fact that the suspension was non-physiological, since it was administered very slowly. Twenty four hours after infection, the lungs were taken from each mouse and examined histologically.

Lungs diaphragms and livers taken from the mice percutaneously infected via the tail were examined histologically. This procedure was carried out daily for days 1-16 after infection (see page 121).

Adult worm recovery by perfusion

Two groups of 10 mice each were infected with *S. mansoni* cercariae or schistosomula respectively by injection into a tail vein. The schistosomula used for these infections were recovered from lungs (see page 93) of mice exposed 6 days previously (tail-immersion method). Two further groups with 10 mice in each group were infected with cercariae by the tail-immersion method and the ring method to serve as controls. Subsequent treatment of each group was identical. The mice were killed 6 weeks after infection by intraperitoneal injection of 0.2ml undiluted veterinary Nembutal (60mg/ml - Abbott Laboratories) containing 25 units/ml of heparin per animal. Besides killing the mouse, the Nembutal narcotizes the adult worms which are then easily flushed out of blood vessels by perfusion. The

Table 27 Daily recovery of schistosomula from washings of pleural and peritoneal cavities of groups of mice (5/group) following percutaneous infection via the tail with approximately 2000 S. mansoni cercariae.

DAY	GROUP NO.	MOUSE NO.	PLEURAL CAVITY	PERITONEAL CAVITY
2	I	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
3	II	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
4	III	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
5	IV	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
6	V	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
7	VI	1	-	-
		2	-	-
		3	+	-
		4	-	-
		5	-	-
8	VII	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
9	VIII	1	-	-
		2	+	-
		3	-	-
		4	-	-
		5	-	-
10	IX	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
11	X	1	-	-
		2	-	-
		3	+	-
		4	-	-
		5	-	-
12	XI	1	-	-
		2	+	-
		3	-	-
		4	-	-
		5	-	-
13	XII	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
14	XIII	1	-	-
		2	-	-
		3	+	-
		4	-	-
		5	-	-
15	XIV	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	+	-
16	XV	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-

heparin was included in the mixture in order to avoid blood coagulation in the mouse. The worms were recovered from the portal circulation using the technique described by Smithers and Terry (1965). Mice were dissected to expose the thoracic and the abdominal cavities. A needle carrying the perfusate (0.85% sodium cloride and 1.5% sodium citrate) was placed in the left ventricle of the heart and the hepatic portal vein was severed to provide a drain. The citrated saline was then introduced under pressure to flush out the vascular system and remove the worms. The efficiency of the perfusion was judged by observing the colour of the kidneys, liver and spleen. Adult worms obtained were counted. The viscera with surrounding fat were examined and livers squashed between glass plates to reveal any remaining worms not dislodged by the perfusion.

8.3 RESULTS

Pleural cavity

Of the 75 mice used in the experiment, 6 yielded schistosomula recovered in washings of the pleural cavity from days 2-16 (Table 27). The 6 positive mice represent 8% of the total number of animals. The highest number of parasites recorded in any individual mouse was 2. Phagocytes in varying numbers were seen to be adhering to the surfaces of schistosomula recovered from washings (Plate

52). The mobility of schistosomula with cells adhering to them in large numbers, appeared to be decreased*.

Peritoneal cavity

Of the 75 mice used in the experiment none yielded schistosomula in washings of the peritoneal cavity from days 2-16 (Table 27).

Histological findings

Lungs of mice infected percutaneously via the tail with cercariae from days 1-16:-

Schistosomula were found in serial sections of whole lung from days 2-16. Haemorrhages were occasionally seen. Parasites were seen both intravascularly (see Chapter 6 - Schistosomular routes: the blood vascular system, page 123) and extravascularly. Those lying outside blood vessels were few in number and were located within alveoli (see Chapter 4 - Plates 3 and 4, pages 65 and 67); some of them were surrounded by cellular infiltrate. Erythrocytes and/or haem were seen in the lumen of the gut of

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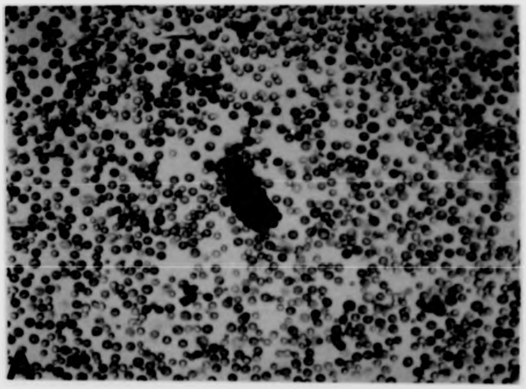
* A certain number of mice were discarded because of blood contaminant in the pleural washings. This was due to faulty technique, the blood probably coming from severed vessels when introducing the needle or as a result of puncturing a lung.

PLATE 52

Schistosomula (S. mansoni) recovered from pleural washings showing varying degrees of cellular adherence to the parasite. Stained neutral red



A) Moribund schistosomulum 12 days post-infection (X98)
B) Moribund schistosomulum 11 days post-infection (X158)
C) Moribund schistosomulum 11 days post-infection (X98)
D) Living schistosomulum 7 days post-infection (X98)
E) Moribund schistosomulum 7 days post-infection (X158)
F) Living schistosomulum 9 days post-infection (X98)
G) Living schistosomulum 9 days post-infection (X158)

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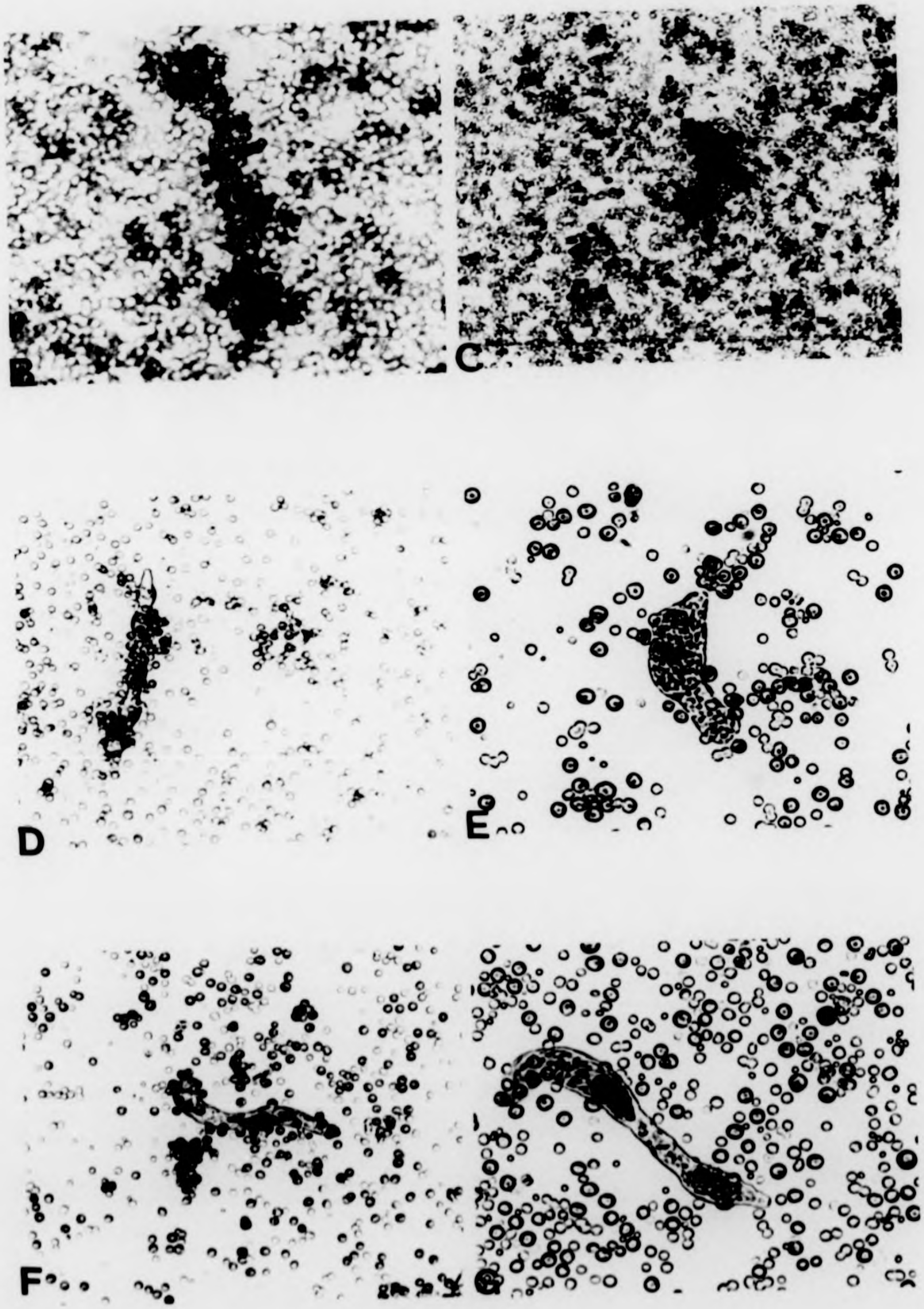

some schistosomula located within alveoli while the gut of parasites lying intravascularly appeared to be empty. Parasites were sometimes seen contiguous with the pleura and surrounded by cellular infiltrate. There was no evidence of larval penetration through the pleura. Exceptionally one schistosomulum was seen in a bronchiole, with accompanying tissue destruction, on day 8, from serially cut whole lungs - approximately 500 sections (Plate 53).

Diaphragms of mice infected percutaneously via the tail with cercariae from days 1-16:-

No schistosomula were seen within muscle fibres in serial sections of whole diaphragms from days 6-16. Where parasites were observed they were located as described in Chapter 6 - Schistosomular routes: the blood vascular system, page 137.

Livers of mice infected percutaneously via the tail with cercariae from days 1-16:-

Larvae were found in serial sections of the liver from days 6-16. There was no evidence of larval entry through the diaphragmatic surface of the liver. No parasites were seen in the hepatic parenchyma. No damage to the reticular network was observed. The larvae were only seen within blood vessels (see Chapter 6 - Schistosomular routes: the blood vascular system, page 166). As an illustration of migration by a tissue burrowing parasite see Plate 54 showing

PLATE 53

A and B) Serial sections of lung showing a schistosomulum (*S. mansoni*) in a bronchiole. Destruction of the endothelial lining of the bronchiole and cellular infiltrate are evident. 8 days post-infection 6µm Stained Verhoeff's and van Gieson (X390)

S - Schistosomulum

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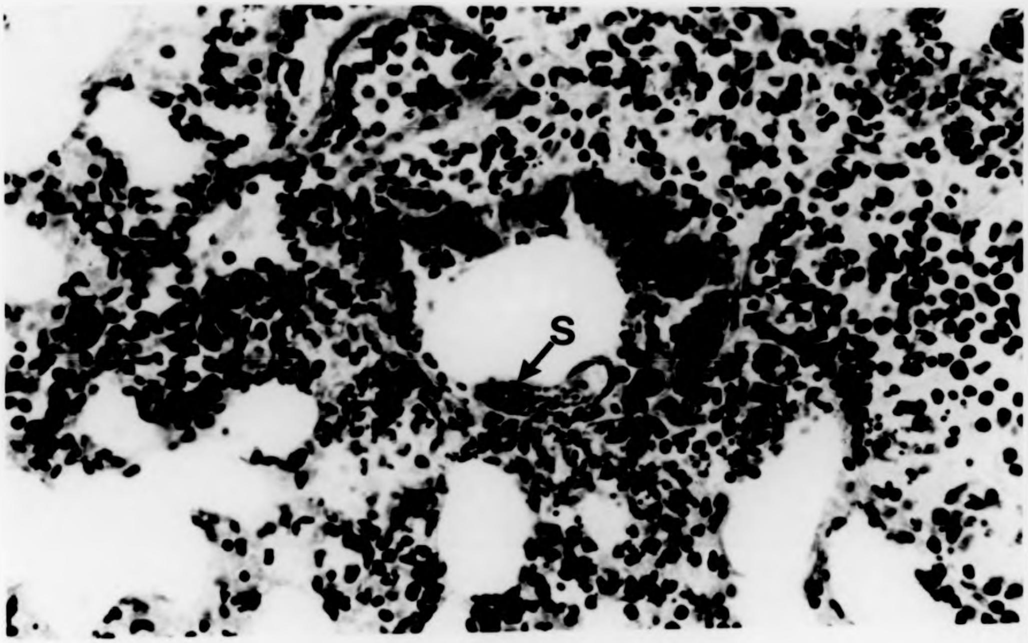
and cellular
infection
n (X390)



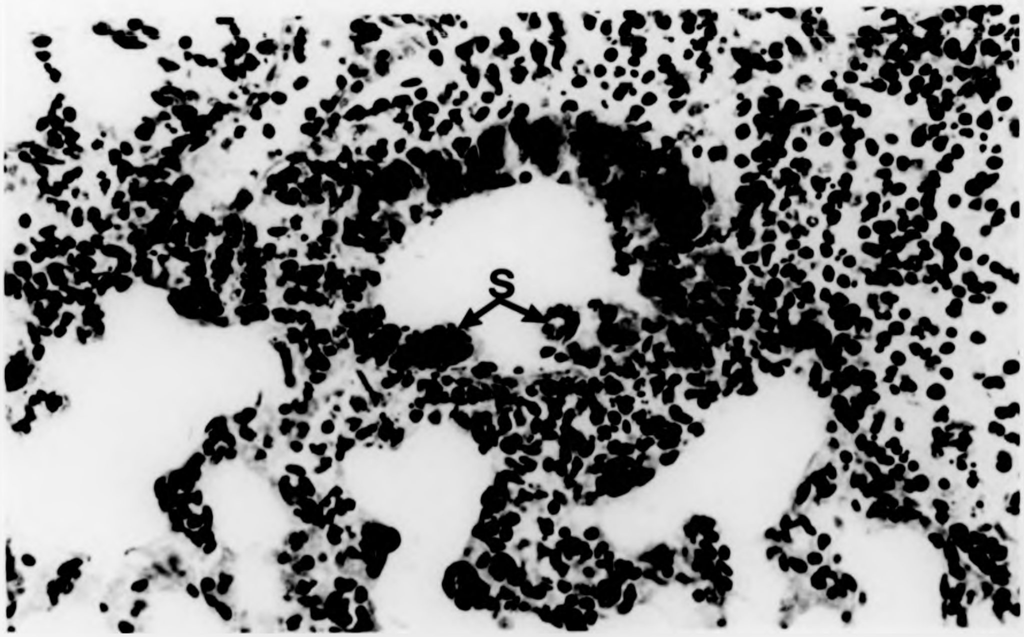


PLATE 54

A comparison between two types of migration:
active (A) and passive (B and C)

A) Larva of Fasciola hepatica in liver parenchyma showing damage to surrounding tissue. 11 days post-infection 5μm Stained H. and E. (X98)

B) Larva of S. haematobium in a vein in liver. 15 days post-infection 5μm Stained Lendrum (X625)

C) Larva of S. mansoni in a venule in liver. 9 days post-infection 5μm Stained H. and E. (X625)

L - Larva

on:

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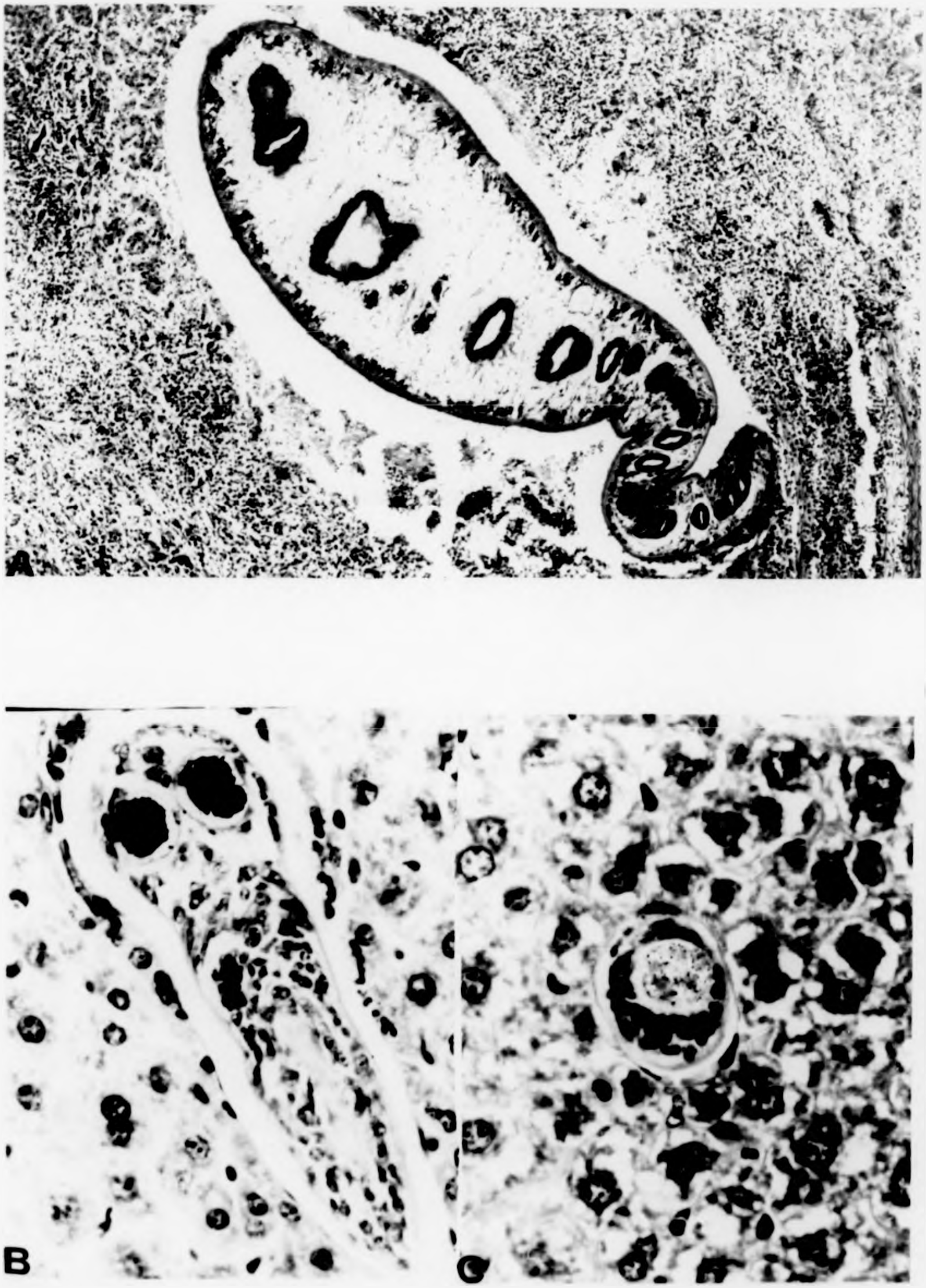
-infection

15 days

9 days post-



extensive damage caused by larval stage of Fasciola hepatica in the liver parenchyma (A); cf. S. mansoni and S. haematobium larvae lying within blood vessels (B and C).

Lungs of mouse 1 day after intravenous infection with 6 days old schistosomula:-

Schistosomula were seen only within blood vessels.

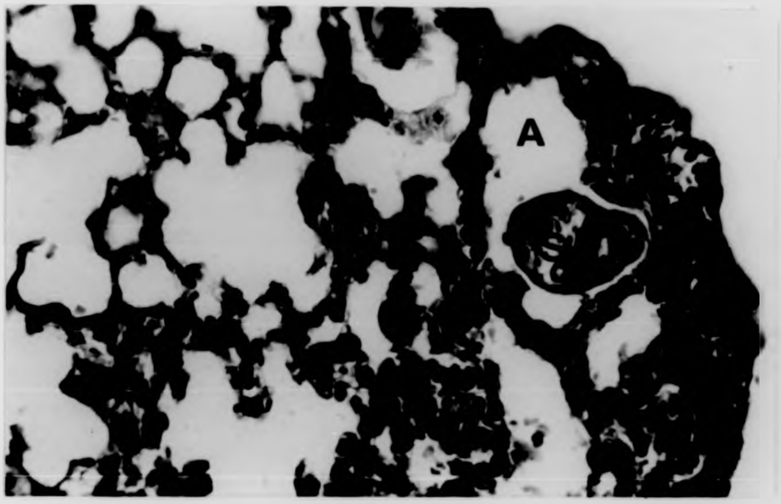
Lungs of mouse 1 day after intravenous infection with cercariae:-

Nearly all schistosomula were seen lying outside blood vessels in the alveoli and surrounded by inflammatory infiltration predominantly of the polymorphonuclear leucocytic type (Plate 55). A schistosomulum appeared to be emerging through the visceral pleura covering the lung surface, though artifact cannot be excluded (Plate 56).

Adult worm recovery

Tables 28-31 (Appendix, pp. 331 - 334) and Figures 20 and 21 relevant to 2 experimental groups and 2 control groups show number and percentage of adult worms recovered from liver perfusions 6 weeks after infection of mice with 100 cercariae or 50 schistosomula. The difference between the proportion of worms recovered is significant. This is shown clearly in Figure 20. There is no overlap between the percentage of worms recovered from the 2 experimental groups nor between the experimental groups and the control

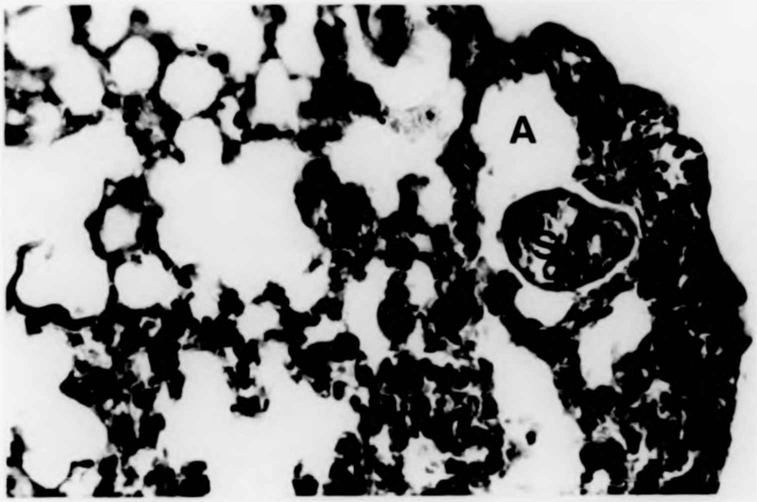
PLATE 55



Schistosomulum (S. mansoni) in a lung alveolus; adjacent cellular infiltrate is evident. 1 day post-infection by intravenous injection of cercariae into a tail vein 6μm Stained H. and E. (X390)

A - Alveolus
S - Schistosomulum

PLATE 55



Schistosomulum (S. mansoni) in a lung alveolus; adjacent cellular infiltrate is evident. 1 day post-infection by intravenous injection of cercariae into a tail vein 6μm Stained H. and E. (X390)

A - Alveolus
S - Schistosomulum

PLATE 56

A, B, C, D and E)	Serial sections of a schistosomulum (*S. mansoni*), in lung tissue adjacent to visceral pleura, following injection of cercariae. A cellular infiltrate surrounds the schistosomulum. 1 day post-infection 6µm Stained H. and E.
A and B)	Higher magnification of sections D and E (X390)
C, D and E)	(X98)

S - Schistosomulum

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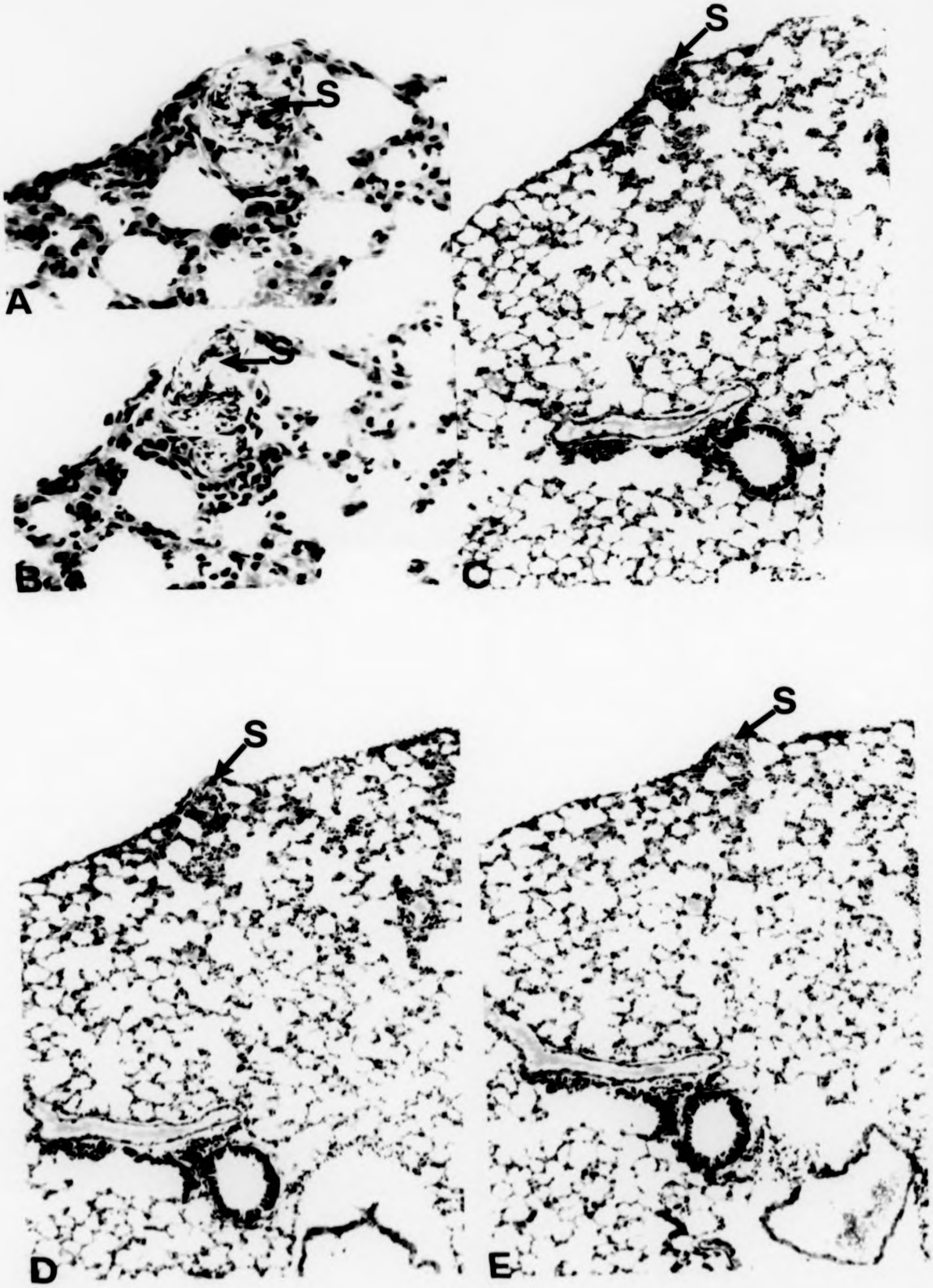

S
A
S
B
S
C
S
D
S
E

Fig. 20 The number of worms recovered by four different methods of infection, (for details, see text and tables).

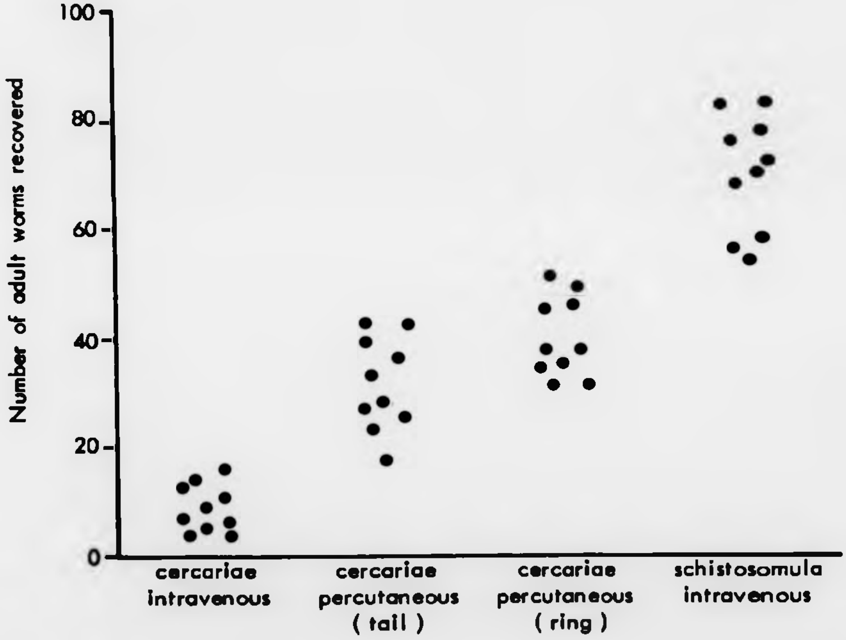
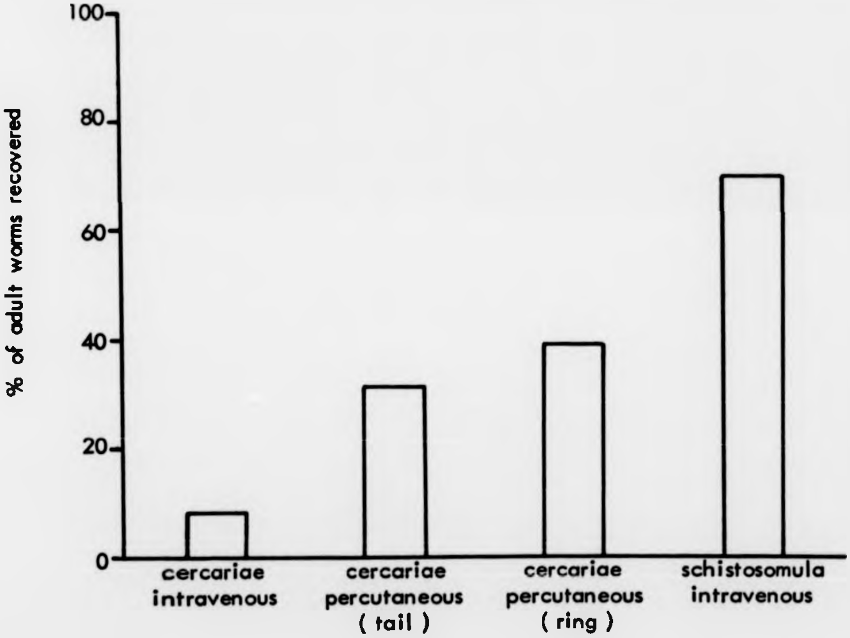


Fig. 21 Percentage of worms recovered by four different methods of infection, (for details, see text and tables).



group studied.

S. haematobium infections

Following percutaneous infections via the tail S. haematobium larvae were not seen extravascularly in histological sections of diaphragms, lungs and livers on days 8 and 16 after infection.

8.4 DISCUSSION

Ever since the second decade of this century, the hypothesis of an extravascular route has been advanced to account, in part or entirely, for schistosomular migration from lung to liver. The subject has remained contentious. Except for Lampe (1927) and El-Gindy (1950) none of the workers associated with investigations of the extravascular route has claimed it as the sole route of migration from lung to liver, but several have regarded it as the principal route of migration - Narabayashi (1917); Sueyasu (1920) and more recently Wilks (1962; 1967). Goto (1932) regards it as an alternative to the blood circulatory route without apportioning it a major or minor role. Faust and Meleney (1924) and Sadun et al. (1958) regard the extravascular route as being of minor importance.

The location of schistosomula in the lung after arrival by the blood circulatory system would be thought to give an initial indication of the likelihood or otherwise of extravascular migration following the pulmonary phase. Where schistosomula are found outside pulmonary blood vessels the host response to the parasite would provide additional information on the putative suitability of the extravascular route for larval migration from lung to liver.

From the present study it seems doubtful that schistosomula migrate out of the lung through the pleura other than to a negligible extent.

In the course of examination of serial sections of whole lungs of mice infected percutaneously via the tail, not a single schistosomulum was seen penetrating the pleural layer.

In the pulmonary phase of migration schistosomula were almost invariably seen intravascularly. A small number were seen extravascularly lying within alveoli and usually surrounded by an area of cellular infiltration. Occasionally parasites were located at the lung periphery contiguous with pleura thickened by cellular infiltration, sometimes appearing to protrude into the pleura, though it was not possible to say whether or not the pleura had been breached. In contrast to the findings in this study, Wilks (1962; 1967) found larvae mainly in alveoli. Bruce, Pezzlo, Yajima and McCarty (1974) in making an electron microscopy study of the pulmonary phase at 7 days post-infection found both intra- and extravascular parasites.

It is not clear why some of the schistosomula seen in lung sections are found in an extravascular situation nor why some containederythrocytes and/or haem. Possible explanations are here advanced to account for these observations. The rupture of the capillary wall may result from purely mechanical causes or as a result of enzymatic action. With regard to the former, the erythrocytes and/or haem seen in the lumen of the gut of some schistosomula are an indication that they have fed in the liver. It is conceivable, therefore, that these schistosomula have returned to the lungs from the liver. Possibly due to growth following feeding in the liver they have lost some of their potential for deformation and are unable to accommodate to the narrow confines of a pulmonary capillary. With regard to the possibility of breakdown of the capillary wall due to enzymatic action, a small number of schistosomula in the pulmonary phase may reach the lung with some residual enzyme(s), though why this should be used to break down the capillary wall is not clear. Altered carbon dioxide and oxygen tensions at this stage may possibly effect the behaviour of the parasite. In the event, judging from the cellular infiltration surrounding some of the extravascular schistosomula in the pulmonary phase, observed in serial sections, survival of such larvae seems improbable.

Out of several thousand serial sections of whole lungs a single schistosomulum was seen in a bronchiole on day 8 after infection. This cannot be regarded as supportive evidence for an extravascular route of migration via bronchi and trachea. An explanation for the presence of a parasite being in the bronchiole may lie in its having broken through a contiguous blood vessel wall after reaching the lungs from the right side of the heart. Alternatively on being carried by the blood stream from the pulmonary network to the left side of the heart it may have been pumped in the systemic circulation to vessels supplying the bronchiole and then broken out from a capillary. Faust and Meleney (1924) eliminated a possible extravascular route via bronchi/ trachea/ oesophagus/ stomach to mesenteric vessels, by subjecting schistosomula recovered from the lungs to hydrochloric acid equivalent in strength to that found in the stomach. The effect was lethal. It is of interest to note that Ransom and Cram (1921) investigation larvae of Ascaris lumbriocoides found that passage through the lungs, as well as growth and development were necessary to render them resistent to digestive juices. Schistosomula do not grow in the lung (see Chapter 4, page 79). Narabayashi (1917); Lampe (1927) and Wilks (1962; 1967), all adherents of the extravascular route hypothesis, claim that the extravascular exodus from the pulmonary phase for the most part takes place from the base of the lung. The claim is made on histological evidence of greater numbers of schistosomula at the bases than in other areas of the lung.

Glazier, Hughes, Maloney and West (1969) working with dogs found that alveolar capillaries are 4 times more numerous per unit volume of lung parenchyma at the base of the lungs as compared with the apex. On the assumption that this holds in mice, it would certainly be reasonable to find a greater concentration of parasites at the bases of the lungs. A concentration of schistosomula at the bases of the lungs would not, therefore, of itself mean that extravascular migration starts in this region even if the concept of extravascular migration is accepted.

As migration from the lung to diaphragm requires passage through the visceral and parietal pleural layers the recovery of parasites from the pleural and/or thoracic cavities would lend support to an extravascular transpleural migration from the lungs. In respect of pleural washings, the findings in this aspect of a possible extravascular migration in the present investigation generally accord with those of Faust and Meleney (1924); Miller (1976); and Miller and Wilson (1980); and differ on several points from those of Wilks (1962; 1967). The recovery of parasites in "significant numbers" from the pleural spaces as claimed by Wilks (1962; 1967) was not shared either by the present investigator, by Faust and Meleney (1924); Miller (1976) or by Miller and Wilson (1980).

In the present study parasites were recovered from pleural washings in only 8% of the experimental mice used. Although each mouse had been heavily infected with cercariae, the schistosomula recovered amounted to 1-2 in the 6 mice which had given positive results. The parasites recovered, though living, appeared to be in a state of degeneration, with impaired mobility and with varying numbers of leucocytes adhering to them. However, these findings must be regarded with some caution, since the parasites recovered from the pleural space or indeed their supposed recovery from the pleural space may perhaps be the result of faulty technique. While Wilks (1962; 1967) claims that the parasites recovered from the pleural cavity were alive and retained their mobility, the appearance of the larva found in the present investigation as well as the adherence of phagocytes to some of them suggested that they would not have been able to migrate beyond the pleural cavity. After infecting experimental mice with a small number (80) cercariae Wilks (1962; 1967) states that approximately 12% of these were recovered in the form of larvae from the pleura by the 14th day and in the declining percentages up to the 28th day after infection. The technique depends to a large extent on converting the potential space of the pleural cavity into a sac which can be irrigated and washed out for recovery of its contents. This is achieved by introducing air between the two pleural layers with consequent collapse of the related lung with it attached pleural covering. Detailed accounts of the technique used for washing out pleural cavities are not provided by Faust and Meleney (1924); Wilks (1967); Miller (1976); Miller and Wilson (1980). The reason for such divergent recovery rates of *S. mansoni* from pleural washings as cited by Wilks (1967) on the one hand and by Faust and Meleney (1924); Miller (1976); Miller and Wilson (1980) and the present author on the other, may lie in the different techniques employed. In working with small laboratory animals, the margin for error is small: it is difficult to exclude the possibility that contaminants in the form of blood cells or even parasites may have been introduced by inadvertently severing a blood vessel when opening the thorax. Conversely a low recovery rate may arise from failure actually to enter the pleural sac, washings being obtained merely from the mediastinal space and parietal pleural surfaces. Based on his claim of "significant numbers" of schistosomula recovered from the pleural sac, Wilks (1967) dismisses the blood vascular route as the exclusive or principal path of migration from lung to liver. It is not clear from his report whether by inference he considers the extravascular route from lung to liver as the principal or exclusive path. However, previously, Wilks (1962) stated that the results in his study indicated that the extravascular route is the principal one.

The possibility that schistosomula had left the pleura to penetrate the diaphragm was investigated by the present author. While schistosomula were found in the diaphragm they were almost invariably seen lying within blood vessels as was nearly always the case with larvae found in other tissues or organs supplied by the systemic blood circulation. The findings in the present investigation were derived from examination of serial sections of whole diaphragms taken each day from 5-16 days following infection. From the 6th to the 16th day parasites were seen, lying within blood vessels. Exceptionally parasites were seen lying adjacent to ruptured blood vessels and surrounded by cellular infiltrates. It is noteworthy that no evidence was seen of damage to muscle fibres, in contrast to the extensive damage caused by the newborn larvae of Trichinella spiralis (Despommier, 1977). Wilks (1962; 1967) found a few parasites in the diaphragm but does not state whether they were lying within or outside blood vessels. Faust and Meleney (1924) found them lying free in diaphragmatic tissue, surrounded by cellular infiltrate. They were unable to conclude whether the larvae had come from ruptured blood vessels or had directly penetrated the tissue. Miller (1976); Miller and Wilson (1980) obtained a small number of larvae from the diaphragm but as the recovery procedure consisted of mincing and incubation it was impossible to say whether they were intra- or extravascular. It seems possible that extravascular schistosomula found in the diaphragm had reached a dead end, without any prospect of proceeding further.

If schistosomula had traversed the diaphragm it was thought that some might be recovered from peritoneal washings though it is probable that schistosomula which had reached the peritoneal cavity by one means or another would be trapped and destroyed by the macrophages which abound there. In the present study an attempt was made to recover larvae from the peritoneal cavity. Faust and Meleney (1924) and Wilks (1967) conducted similar investigations. No parasites were recovered from the peritoneal washings by Faust and Meleney (1924) or by the present author. Wilks (1967) whose investigation included work with *S. mansoni*, *S. douthitti* and *Heterobilharzia americana* states that he recovered *S. douthitti* and *H. americana* but makes no mention of recovery of *S. mansoni*.

Cort (1921) cites the conclusions of Narabayashi (1916) and "Suyeyasa" (1919), working with large numbers of suckling mice infected with *S. japonicum*, that migration between lung and liver is extravascular. It is possibly of some significance that the experimental animals used in this investigation comprised suckling mice and that the method of infection was by "paddling". Their findings are based on serial sections of mice *in toto*.

Support for extravascular migration was sought by the present author who looked for evidence of active larval penetration of the liver capsule and parenchyma. Liver surfaces of infected mice were devoid of petechial haemorrhages and there was no damage to Glison's capsule or liver parenchyma as might be expected to follow active penetration by a parasite. *Fasciola hepatica* on entry to the liver through the capsule (Schumacher, 1956) and in its passage through liver parenchyma causes tissue destruction (Dawes, 1961). In serial sections of liver representing days 6-16 after infection, such schistosomula as were present, were invariably seen within blood vessels. Faust and Meleney (1924) investigating an extravascular route found parasites in the liver but "every one in a blood vessel". On the other hand, Wilks (1967) as well as frequently finding petechiae on the anterior surface of the liver, found a schistosomulum extravascularly in the liver parenchyma "immediately beneath the capsule". In the present study no evidence was found to support schistosomular penetration of the liver by an extravascular route.

The question as to whether schistosomula are physically equipped for extravascular migration raises comparisons with parasites known to migrate through tissue, such as *F. hepatica*, which is recognized as a true tissue burrower. Apart from leaving clear evidence of tissue destruction in its path, its caecal contents comprise disintegrated host tissue cells. *F. hepatica* is described as making "pincer-like" movements with its oral sucker as it gouges out pieces of tissue to make its way forward (Dawes, 1961). Wilks (1967) has suggested that the schistosomulum penetrates tissue by purely physical means - "probing" with anterior end while the body is alternately elongated and contracted to impart forward thrusts. This might be an explanation for a method of extravascular progression. However, though equipped with an oral sucker it is not recorded that the gut of the schistosomulum has been seen to contain the debris of disintegrated host cells. The absence of ingested host tissue would exclude it being a tissue feeder but not necessarily its using the oral sucker for burrowing in the manner of F. hepatica. However, the absence of any tissue damage in association with extravascular schistosomula, in the present investigation, does not support use of the oral sucker for tissue burrowing purposes.

With regard to possible biochemical means at its disposal the presence of enzyme(s) which some schistosomula may retain to varying degrees during migration, was investigated as a possible factor which might influence extravascular migration. This experiment compared the behaviour of enzyme-loaded cercariae on the one hand, and enzyme-depleted schistosomula on the other, injected intravenously into 2 groups of experimental animals. Nearly 8 times as many of the enzyme-depleted schistosomula were subsequently recovered from the liver in the form of adults as compared with recovery of adult successors of the enzyme-loaded cercariae. The fate of larvae derived from the injected cercariae appeared to be sealed in the pulmonary phase where large numbers of the cercarial successor larvae were seen in serial sections to be lying outside capillaries, and surrounded by cellular infiltrate. It is conceivable that on reaching the comparative stagnation in the pulmonary capillaries there was opportunity for enzymatic action to break down the capillary wall. These results do not support the hypothesis of an extravascular migration if this were wholly or in part dependent on the presence of lytic enzyme(s). The results of the present experiments accord closely with those obtained by Holanda, Pellegrino and Gazzinelli (1974); Holanda, Pellegrino, Gazzinelli and Ramalho-Pinto (1976) who recovered approximately 9% of intravenously injected cercariae in the form of adults. James and Taylor (1976) obtained an even lower return with approximately 6%. These workers neither comment on reasons for the low return of adults nor on the stage of migration at which the majority of the larvae perished. There is also a fair measure of agreement with the results of Holanda and Pellegrino (1974) who intravenously injected schistosomula which had been obtained on day 4 after infection, and recovered 64% of these as adults. The same workers intravenously injected larvae obtained from skin 30 minutes after infection and recovered 19.6% of these as adults. From these results it would seem reasonable to postulate that survival to adulthood is dependent on depletion of enzyme. In this connection it is of interest to note the conclusion of Miller and Wilson (1978) that the longer the time spent in the skin by the parasite (3-4 days) before migrating the greater its chance of survival to maturity though they do not associate their findings with depletion of enzyme.

The findings and observations of Wilks (1962; 1967) point unambiguously to an extravascular migration route from lungs to liver, and support earlier findings of Lampe (1927) and El-Gindy (1950) who were among workers to postulate the extravascular as being the sole route of migration. It has not been possible in the present study to corroborate the findings of Wilks (1962; 1967). Similarly Miller 1976; Miller and Wilson (1980) and Wheater and Wilson (1979)* have not found any evidence to support Wilks (1967) claim for the extravascular route.

It is conceivable that certain circumstances might favour an extravascular migration. Following a relatively unhindered penetration of the skin and a rapid exit by the blood vascular route from the skin, a proportion of larvae arriving at the lung might retain an enzyme-load sufficient to break out of lung capillaries and start an extravascular migration. In the absence of a fully developed host immune response, some of the parasites might succeed in reaching the liver. However, it is unlikely that such a pathway is in any way comparable in importance to the intravascular route.

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* Wheater and Wilson (1979) erroneously cite Kagan and Meranze (1955) as proposing an extravascular route from lungs to liver. The work of Kagan and Meranze (1955) deals with inflammatory response in mouse skin following cercarial infection.

SUMMARY AND CONCLUSION

As indicated in the introduction to this thesis, the main aim of the present study has been to elucidate the route(s) taken by the schistosomulum in migrating from skin to lung and from lung to liver. Considering the experimental and investigative work carried out during the past 60-70 years it is surprising to find that so much uncertainty still surrounds different aspects of schistosomular behaviour and the route(s) of migration of schistosomula in a mammalian host.

Deformation, growth and definition of the schistosomular stage

Before considering the route(s) of larval migration two questions were addressed:- (a) that of schistosomular deformation and its relationship if any, to growth; and (b) a definition of the end-point of the schistosomular stage.

It has been shown that the parasite has the ability to elongate and narrow itself; this potential for deformation is considered to facilitate larval accommodation within microvessels of the blood circulatory system and within lymph vessels. While parasites have been seen lying within microvessels, their actual mode of entry into vessels is unknown. During schistosomular migration from skin to lung and from lung to liver, growth, as defined in this study does not take place.

With regard to the end-point of the schistosomular stage, the commencement of growth (as defined, in Chapter 4) neatly signals the beginning of a new phase which will ultimately culminate in adulthood; it is, therefore, considered that the end of the schistosomular stage should be related to the beginning of growth. The term schistosomulum is applied to the parasite after it has entered the skin and until growth begins.

Route(s) of schistosomular migration

As indicated earlier it has been generally accepted that migration from skin to lung takes place through vessels of the blood circulatory system. There was some speculation concerning the lymphatics as an effective complementary route of migration from the skin. Various workers have assigned degrees of importance to either the blood vascular or the lymphatic routes of migration from the skin. While the results of the present study show that both the blood, and the lymphatic vessels without interruption at the lymph nodes, are used by schistosomula in migration from the skin, it is considered that apportionment of major importance to either route, on the basis of the present and all other studies so far conducted, has not been substantiated. The proportionate use of blood and lymph vessels by larvae migrating from the skin is unknown; and it is not possible by means of histological methods to make quantitative analyses. The migration of larvae from the skin is probably random and dependent on the relative availability of the respective vessels. Even if the ratio of blood to lymph vessels in the skin were known, however, it would still be impossible to assess the relative availability of these two routes, unless both were identical in all other respects.

With regard to migration from lung to liver the hypotheses propounded are: an intravascular pathway along vessels of the blood circulatory system; an extravascular pathway through tissues of the host; and on occasions a combination of these routes. It is concluded from the results of the present study that schistosomular migration of S. mansoni and S. haematobium in the mouse from lung to liver proceeds exclusively by vessels of the blood circulatory system and in the same direction as the blood flow.

Having entered the blood circulatory system the parasites are eventually dispersed through the host's body by the systemic circulation. In all likelihood the proportional distribution of parasites to various organs will be closely related to the percentages of cardiac output received in those tissues and organs. In this way a proportion of larvae reach the liver. There is evidence that parasites dispersed through the systemic circulation may be returned through the venous system to the lungs and then return again to the left side of the heart and be dispersed once more through the systemic circulation. The number of such cycles is assumed to be limited. It has been established in this study that schistosomula perish in various organs and tissues following dispersion in the systemic circulation. This is supported by observing schistosomula at the centre of surrounding cellular infiltrates and later by the appearance of granulomata.

Conclusion

As to the outcome of such basic studies, it would be unrealistic to expect an immediate application of the results to human schistosomiasis. In the author's opinion, the main importance of these investigations lies in elucidating a fundamental problem in the biology of the parasite which will contribute to the efforts of those in other disciplines engaged in the study of the disease. For example, workers dealing with the immunological, biochemical and pharmacological aspects of schistosomiasis can draw on knowledge educed from the present study, to pursue their endeavours. Moreover, information presented in this thesis will it is hoped be useful in comparative studies in which portals of entry and stages of migration have been manipulated. Various problems have arisen in the course of this study and it is suggested that these might form the bases of future investigations and provide a link between the present study and future work. These are listed below together with some methods which might be employed.

Suggestions for future research

1. Development of techniques to study *in vivo* infection, making microscopic observations of schistosomula in, for example, microvessels of the mesentery, using transillumination and implantation of transparent windows using epiillumination for long term microscopic observations.
2. Ultrastructural study by electron microscopy of localization of parasites in tissues as well as tissue damage caused by the parasite in, for example, the brain and kidney.
3. Histochemical studies using frozen sections from various organs to determine the content of lytic enzyme(s) in schistosomula.
4. A three dimensional study of the deformation and possible associated changes in volume of the organism in the schistosomular stage, for better appreciation of it's biophysical activity.

ACKNOWLEDGEMENTS

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APPENDIX

TABLE 1

(Methyl - 3H) THYMIDINE INCORPORATION BY S. HANSONI LARVAE

7 HOUR PULSE AT 8 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host lung tissue	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
1	3,1,0,0,3.	1.4	1.5	0.7	3,1,2,0,1.	1.4	1.1	0.5	0,1,1,1,0.	3,2,0,1,0.
2	0,0,2,0,1.	0.6	0.9	0.4	1,1,0,0,2.	0.8	0.8	0.4	2,1,3,0,1.	3,1,0,0,3.
3	2,2,0,1,0.	1.0	1.0	0.4	0,0,2,1,1.	0.8	0.8	0.4	1,0,0,1,0.	1,2,1,0,0.
4	3,0,0,2,0.	1.0	1.4	0.6	1,0,0,2,0.	0.6	0.9	0.4	0,1,0,2,0.	3,2,3,1,0.
5	0,0,2,1,1.	0.8	0.8	0.4	2,0,0,3,1.	1.2	1.3	0.6	3,1,1,4,0.	0,1,0,1,2.
									0,0,0,1,0.	4,1,1,3,0.
									2,2,1,0,0.	0,0,1,3,1.
									1,0,1,0,2.	0,2,1,0,0.
									1,2,0,1,0.	3,0,0,2,1.
									1,4,3,0,0.	1,0,0,1,0.

Counts were made over 5 unit areas for larvae and for each lung section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of lung tissue = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.0, the ± SD was 1.1 and the ± SE 0.1

TABLE 2

(Methyl - 3H) THYMIDINE INCORPORATION BY S. MANSONI LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
1	10. 2. 5. 7. 5.	5.8	2.9	1.3	3,1,0,2,1.	1.4	1.4	0.5	1,0,1,2,0.	2,1,0,3,3.
2	4. 0. 4.24. 4.	7.2	9.5	4.3	1,4,0,1,0.	1.2	1.6	0.7	2,1,2,0,0.	2,3,0,3,1.
3	36,16. 2,15. 8.	15.4	12.8	5.7	1,3,2,1,0.	1.4	1.1	0.5	3,0,0,0,0.	1,2,2,0,2.
4	5. 7. 4. 9. 3.	5.6	2.4	1.1	1,2,0,1,4.	1.6	1.5	0.7	2,2,1,1,0.	0,1,0,2,1.
									3,1,0,0,2.	1,2,2,1,0.
									1,2,1,0,1.	0,3,3,0 2.
									0,1,0,0,0.	4,1,1,2,3.
									1,0,0,0,0.	2,1,2,1,0.
									0,1,0,1,1.	1,1,0,2,1.
									2,2,1,0,1.	0,2,0,2,0.

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.2, the ± SD was 1.1 and the ± SE 0.1

TABLE 3

(Methyl - 3H) THYMIDINE INCORPORATION BY *S. MANSONI* LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
5	21,10,11, 5, 7,	10.8	6.2	2.8	2,2,3,4,1,	2.4	1.1	0.5	4,0,1,0,1,	0,1,0,1,1,
6	40,37,18, 5, 2,	20.4	17.6	7.8	4,1,3,4,7,	3.8	2.2	1.0	0,2,1,1,4,	0,1,2,2,1,
7	4, 0, 7, 1, 7,	3.8	3.3	1.5	1,4,8,0,2,	3.0	3.2	1.4	1,3,1,4,1,	1,1,1,2,1,
8	15, 7,12, 4, 7,	9.0	4.4	2.0	2,3,1,1,3,	2.0	1.0	0.4	1,3,2,0,0,	0,1,0,4,0,
9	16,11, 8, 8, 2,	9.0	5.1	2.3	3,2,2,0,1,	1.6	1.1	0.5	1,1,3,1,0,	0,0,1,0,0,
									1,2,0,1,1,	1,0,1,1,2,
									2,3,2,1,0,	0,0,0,1,3,
									0,0,1,0,0,	0,1,1,0,3,
									0,1,0,1,0,	3,3,1,2,1,
									2,2,3,0,4,	3,2,2,1,0,

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$)per unit area of background = $\frac{\text{total number of grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.2, the ± SD was 1.1 and the ± SE 1.1

TABLE 4

(Methyl - 3H) THYMIDINE INCORPORATION BY _S. MANSONI_ LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
10	10,13, 0, 0,12.	7.0	6.5	2.9	4,3,2,1,0.	2.0	1.6	0.7	2,1,0,2,0.	0,1,1,4,1.
11	12, 0. 2,27, 3.	8.8	11.2	5.0	4,5,0,1,2.	2.4	2.1	0.9	2,2,1,3,0.	0,0,1,2,0.
12	26, 0. 0. 0. 3.	5.8	11.4	5.1	3,0,2,4,0.	1.8	1.8	0.8	1,0,4,0,1.	0,1,0,0,3.
13	3, 0. 0. 0. 3.	1.2	1.6	0.7	1,2,0,1,4.	1.6	1.5	0.7	0,0,3,0,0.	2,0,3,3,2.
14	6, 0, 3, 0, 1,	2.0	2.5	1.4	0,3,2,1,0.	1.0	1.6	0.9	0,0,1,0,1,	1,0,1,1,2.
									0,0,4,2,0.	1,3,2,3,3.
									2,2,4,3,2.	0,1,3,0,1.
									0,0,2,4,0.	0,2,3,0,3.
									0,2,4,0,2.	0,0,3,1,0.
									1,1,2,1,0.	0,2,1,0,2.

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.3, the ± SD was 1.3, and the ± SE 0.1

TABLE 5

(Methyl - ^{3}H) THYMIDINE INCORPORATION BY *S. MANSONI* LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
15	13,17,16,11,16,	14.6	2.5	1.1	3,2,0,2,1,	1.6	1.1	0.5	3,1,3,3,0,	1,1,0,0,0,
									2,0,2,3,2,	0,2,2,1,2,
16	1, 3,13, 3, 1,	4.2	5.0	2.2	1,2,0,1,3,	1.4	1.1	0.5	3,1,0,2,2,	1,0,0,1,2,
									3,3,2,4,0,	1,3,2,3,2,
									3,1,3,2,3,	2,2,3,3,1,
									1,3,2,1,1,	1,2,2,4,2,
									2,4,1,2,4,	0,3,5,2,1,
									1,3,3,0,3,	0,3,2,2,3,
									1,3,4,4,1,	4,2,1,0,1,
									1,3,0,0,1,	2,1,0,1,2,

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.9, the ± SD was 1.3 and the ± SE 0.1

TABLE 6

(Methyl - 3H) THYMIDINE INCORPORATION BY S. MANSONI LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	$\pm$ SD	$\pm$ SE	No. grains/unit area over host liver cells	$\bar{x}$	$\pm$ SD	$\pm$ SE	No. grains/unit area over emulsion*	
17	18, 6, 2, 0, 4.	6.0	7.1	3.2	5,1,2,0,4.	2.4	2.1	0.9	1,2,2,0,0,	0,3,2,4,0,
18	5, 4, 4, 3, 2.	3.6	1.1	0.5	5,4,3,1,0.	2.6	2.1	0.9	0,0,1,2,0,	0,0,1,2,2,
									0,0,1,2,1,	1,1,2,1,1,
									3,1,2,0,1,	2,1,0,0,1,
									2,0,1,1,0,	0,2,1,1,0,
									1,3,0,2,1,	0,0,0,2,2,
									2,4,1,2,0,	0,3,2,3,2,
									2,5,0,2,3,	1,1,2,0,0,
									0,1,2,0,0,	0,0,2,0,0,
									3,2,1,1,0,	0,2,1,0,2.

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.1, the $\pm$ SD was 1.2 and the $\pm$ SE 0.1

TABLE 7

(Methyl - 3H) THYMIDINE INCORPORATION BY *S. MANSONI* LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
19	14, 20, 9, 9, 14,	13.2	4.5	2.0	4, 5, 5, 6, 3,	4.6	1.1	0.5	3, 4, 3, 1, 3,	4, 2, 4, 3, 4,
20	4, 1, 0, 2, 0,	1.4	1.7	0.7	4, 11, 5, 6, 5,	6.2	2.8	1.2	4, 3, 4, 3, 1,	1, 2, 2, 2, 1,
21	7, 8, 10, 8, 8,	8.2	1.1	0.5	3, 4, 5, 6, 2,	4.0	1.6	0.7	1, 1, 0, 0, 4,	1, 0, 0, 1, 0,
22	20, 11, 16, 5, 9,	12.2	5.9	2.6	4, 2, 9, 1, 0,	3.2	3.6	1.6	1, 1, 2, 3, 2,	2, 2, 1, 2, 1,
									0, 3, 2, 1, 3,	1, 1, 1, 0, 2,
									0, 4, 1, 3, 1,	2, 2, 0, 0, 1,
									1, 0, 3, 2, 4,	1, 0, 2, 2, 2,
									3, 1, 0, 3, 2,	2, 1, 2, 1, 2,
									3, 2, 1, 0, 3,	1, 1, 4, 1, 0,
									4, 1, 0, 2, 0,	2, 2, 3, 1, 0,

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 2.2, the ± SD was 1.8 and the ± SE 0.2

TABLE 8

(Methyl - 3H) THYMIDINE INCORPORATION BY *S. MANSONI* LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
23	30,42,26,31,28	31.4	6.2	2.8	1,1,1,1,2,	1.2	0.4	0.2	1,1,1,0,1,	2,0,2,1,0,
24	1, 2, 1, 0, 1,	1.0	0.5	0.3	2,2,1,1,0,	1.2	0.8	0.4	0,2,1,2,1,	3,1,1,1,2,
25	18,26,34,19,11,	21.6	8.7	3.9	9,4,0,0,1,	2.8	3.8	1.7	1,1,0,1,1,	0,1,0,2,0,
									0,0,1,2,0,	1,2,4,3,1,
									2,1,2,0,1,	1,1,4,2,1,
									4,0,2,2,3,	0,1,1,0,2,
									3,1,4,1,2,	0,3,2,1,3,
									1,2,1,2,2,	1,4,1,1,0,
									2,1,1,1,0,	2,1,2,1,2,
									4,0,2,1,2,	1,0,2,1,1,

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.4, the ± SD was 1.1 and the ± SE 0.1

TABLE 9

(Methyl - 3H) THYMIDINE INCORPORATION BY *S. HAEMATOBIUM* LARVAE

7 HOUR PULSE AT 8 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host lung tissue	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
1	3,0,2,1,2,	1.6	1.1	0.5	1,1,0,3,0,	1.0	1.2	0.5	0,0,1,2,1,	2,1,0,3,3,
2	2,1,1,3,0,	1.4	1.1	0.5	1,2,0,2,2,	1.4	0.9	0.4	1,2,2,0,0,	2,3,0,3,1,
3	2,1,0,0,0,	0.6	0.9	0.4	3,1,1,2,0,	1.4	1.1	0.5	1,0,1,0,1,	2,2,0,0,1,
4	1,1,2,2,1,	1.4	0.5	0.2	2,0,0,1,2,	1.0	1.0	0.4	2,2,1,1,0,	0,1,0,2,4,
5	0,0,2,0,1,	0.6	0.9	0.4	1,1,2,0,1,	1.0	0.7	0.3	0,1,0,3,2,	1,1,2,3,0,
									0,2,1,0,1,	0,3,2,0,2,
									0,1,0,0,0,	4,1,1,2,3,
									1,0,0,0,0,	2,1,2,1,0,
									0,1,0,2,1,	1,1,0,2,1,
									2,2,1,0,1,	0,3,0,1,0,

Counts were made over 5 unit areas for larvae and for each lung section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of lung tissue = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of backgroud = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 1.1 , the ± SD was 1.0 and the ± SE 0.1

TABLE 10

(Methyl - 3H) THYMIDINE INCORPORATION BY S. HAEMATOBIUM LARVAE

4 HOUR PULSE AT 16 DAYS - EXPOSED FOR 3 MONTHS

Parasite No.	No. grains/unit area over worm sections	$\bar{x}$	± SD	± SE	No. grains/unit area over host liver cells	$\bar{x}$	± SD	± SE	No. grains/unit area over emulsion*	
1	6, 4, 0,15, 3,	5.6	5.7	2.5	3,4,0,1,1,	1.8	1.6	0.7	1,0,0,0,1,	2,0,1,0,1,
2	25, 9,16, 0, 4,	8.2	9.1	2.6	4,1,0,2,1,	1.6	1.5	0.7	1,1,2,0,1,	1,1,3,0,2,
3	7,10, 3, 6, 2,	5.6	3.2	1.4	0,2,5,1,1,	2.3	2.5	1.5	0,3,2,0,2,	1,1,2,0,1,
									1,0,0,0,2,	3,2,0,1,0,
									2,0,2,0,1,	0,0,1,1,0,
									0,1,1,1,2,	0,2,2,1,1,
									0,0,3,0,1,	1,1,0,2,0,
									4,2,0,2,0,	0,2,1,0,0,
									1,1,0,1,1,	1,0,1,1,1,
									2,0,0,1,0,	1,1,0,2,0,

Counts were made over 5 unit areas for larvae and for each liver section, and 100 unit areas were counted over the emulsion

1 unit area = 225 μm^2

Mean grain density ($\bar{x}$) per unit area of worm = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of liver cells = $\frac{\text{total number grains counted}}{\text{5 unit areas}}$

Mean grain density ($\bar{x}$) per unit area of background = $\frac{\text{total number grains counted}}{\text{100 unit areas}}$

* With regard to the emulsion count, the mean ($\bar{x}$) was 0.9, the ± SD was 0.9 and the ± SE 0.1

TABLE 13

SCIATIC LYMPH NODE

Number of sciatic lymph nodes in which schistosomula were found by direct examination in 24 mice at successive daily intervals from 1 to 12 days after infection. Each animal was infected by the tail immersion method with approximately 2000 S. mansoni cercariae in a 10 ml suspension.

		Mouse number																								Total
Node	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	L + R as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	81.3
Right		+	+	-	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	
Left	3	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	89.6
Right		+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	
Left	4	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	98.0
Right		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Left	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Right		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Left	6	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	91.7
Right		+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	
Left	7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	93.8
Right		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	
Left	8	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	91.7
Right		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
Left	9	-	+	+	+	+	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	33.3
Right		+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	
Left	10	-	-	-	+	+	+	-	+	-	+	+	-	+	-	+	-	+	+	+	+	+	-	+	+	45.8
Right		+	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	+	
Left	11	-	-	-	+	-	-	-	-	+	+	+	+	-	+	-	+	-	+	+	+	+	-	+	+	35.4
Right			-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	
Left	12	-	+	-	-	-	+	-	-	+	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	41.7
Right		+	-	-	-	+	+	-	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	

+ = parasites present - = parasites absent 100% = a total count of 48 infected lymph nodes for each day

TABLE 14

LUMBAR LYMPH NODE

Number of lumbar lymph nodes in which schistosomula were found by direct examination in 24 mice at successive daily intervals from 1 to 12 days after infection. Each animal was infected by the tail immersion method with approximately 2000 *S. mansoni* cercariae in a 10 ml suspension.

Node	Day	Mouse number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total L + R as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	3	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	25.0
Right		-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	
Left	4	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	75.0
Right		-	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-	+	-	-	+	+	+	
Left	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	91.7
Right		+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	
Left	6	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	81.3
Right		+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	+	
Left	7	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	79.2
Right		-	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	
Left	8	+	+	-	+	-	-	+	-	+	-	-	+	+	+	-	-	+	-	+	-	-	-	+	-	45.8
Right		-	-	+	-	+	+	-	-	+	+	+	-	-	-	+	-	-	+	+	+	-	-	-	+	
Left	9	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	18.8
Right		-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	
Left	10	+	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	16.7
Right		-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
Left	11	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	14.6
Right		-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	
Left	12	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	14.6
Right		-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	

+ = parasites present   - = parasites absent   100% = a total count of 48 infected lymph nodes for each day

TABLE 15

RENAL LYMPH NODE

Number of renal lymph nodes in which schistosomula were found by direct examination in 24 mice at successive daily intervals from 1 to 12 days after infection. Each animal was infected by the tail immersion method with approximately 2000 S. mansoni cercariae in a 10 ml suspension

Side	Day	Mouse number																								Total L + R as %
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	4	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	+	-	-	14.6
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
Left	5	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	79.2
Right		-	+	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	+	+	+	-	+	
Left	6	+	+	-	+	+	-	+	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	-	75.0
Right		+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	
Left	7	+	+	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	-	-	62.5
Right		+	-	+	-	+	+	-	+	+	-	+	+	-	-	-	+	-	+	+	+	-	-	+	+	
Left	8	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-	33.3
Right		+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	+	-	+	+	
Left	9	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	29.2
Right		+	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	
Left	10	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+	+	-	18.8
Right		-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	
Left	11	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	25.0
Right		+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	+	-	-	-	+	-	
Left	12	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	8.3
Right		-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	

+ = parasites present — = parasites absent 100% = a total count of 48 infected lymph nodes for each day

TABLE 16

INGUINAL LYMPH NODE

Number of inguinal lymph nodes in which schistosomula were found by direct examination in 24 mice at successive daily intervals from 1 to 12 days after infection. Each animal was infected with the tail immersion method with approximately 2000 S. mansoni cercariae in a 10 ml suspension.

Node	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total L + R as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	12.5
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
Left	3		-	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	20.8
Right		+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	
Left	4	+	+	-	+	-	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	62.5
Right		-	+	+	+	+	-	+	+	-	+	+	+	-	-	+	-	-	-	-	-	+	-	-	+	
Left	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	77.1
Right		+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+	-	+	+	
Left	6	-	+	+	-	+	-	+	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	+	+	68.8
Right		-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	
Left	7	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	18.8
Right		+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
Left	8	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	18.8
Right		-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	
Left	9	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-		10.4
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	
Left	10	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	10.4
Right		+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	11	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	4.2
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

+ = parasites present   - = parasites absent   100% = a total count of 48 infected lymph nodes for each day

TABLE 17

AXILLARY LYMPH NODE

Number of axillary lymph nodes in which schistosomula were found by direct examination in 24 mice at successive daily intervals from 1 to 12 days after infection. Each animal was infected by the tail immersion method with approximately 2000 S. mansoni cercariae in a 10 ml suspension

Node	Day	Mouse number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total L + R as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Left	4	-	-	-	+	-	+	-	-	-	+	-	-	-	+	+	+	+	-	+	-	+	-	+	+	41.7
Right		-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	-	+	
Left	5	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	-	66.7
Right		+	-	+	+	+	+	+	+	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	+	
Left	6	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	66.7
Right		+	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	+	+	+	-	+	+	-	+	
Left	7	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	20.8
Right		-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	
Left	8	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	12.5
Right		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	
Left	9	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	6.3
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
Left	10	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.2
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
Left	11	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.2
Right		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
Left	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.1
Right		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

+ = parasites present   - = parasites absent   100% = a total count of 48 infected lymph nodes for each day

TABLE 18

POPLITEAL LYMPH NODE

Number of popliteal lymph nodes in which schistosomula were found in 24 mice at successive daily intervals from 1 to 12 days after infection.

Each animal was infected via the left hindfoot with approximately 500 S. mansoni cercariae.

Node	Day	Mouse number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total L as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Left	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Left	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	95.8
Left	5	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	91.7
Left	6	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	91.7
Left	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	95.8
Left	8	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	91.7
Left	9	+	-	-	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-	+	-	54.2
Left	10	+	-	+	-	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	29.2
Left	11	-	-	+	-	-	-	+	+	-	-	-	+	-	+	+	-	-	-	+	-	-	-	-	+	34.3
Left	12	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	20.8

+ = parasites present   - = parasites absent   100% = a total count of 24 infected lymph nodes for each day

TABLE 19

SCIATIC LYMPH NODE

Number of sciatic lymph nodes in which schistosomula were found in 24 mice at successive daily intervals from 1 to 12 days after infection.

Each animal was infected via the left hindfoot with approximately 500 *S. mansoni* cercariae.

		Mouse number																								Total I as %
Side	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	3	+	-	+	+	+	-	-	-	+	+	-	+	-	+	-	-	+	+	+	+	+	-	+	+	62.5
Left	4	+	+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	66.7
Left	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Left	6	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	87.5
Left	7	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	-	83.3
Left	8	+	-	-	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	-	66.7
Left	9	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+	37.5
Left	10	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	25.0
Left	11	+	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	29.2
Left	12	+	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	20.8

+ = parasites present - = parasites absent 100% = a total count of 24 infected lymph nodes for each day

TABLE 20

LUMBAR LYMPH NODE

Number of lumbar lymph nodes in which schistosomula were found in 24 mice at successive daily intervals from 1 to 12 days after infection.

Each animal was infected via the left hindfoot with approximately 500 *S. mansoni* cercariae.

		Mouse number																								Total L. as %
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Node	Day																									
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	3	+	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	25.0
Left	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Left	5	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	95.8
Left	6	+	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-	66.6
Left	7	+	+	-	+	+	-	+	+	+	+	-	-	-	+	+	+	+	-	-	+	+	-	+	+	66.6
Left	8	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	-	+	+	-	37.5
Left	9	-	+	+	-	-	+	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	+	-	41.7
Left	10	+	-	+	-	-	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	29.2
Left	11	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	20.8
Left	12	-	-	-	+	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	25.0

+ = parasites present   - = parasites absent   100% = a total count of 24 infected lymph nodes for each day

TABLE 21

RENAL LYMPH NODE

Number of renal lymph nodes in which schistosomula were found in 24 mice at successive daily intervals from 1 to 12 days after infection

Each animal was infected via the left hindfoot with approximately 500 *S. mansoni* cercariae.

Node	Day	Mouse 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total L. as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	4	-	-	-	-	•	-	•	•	•	-	•	•	-	-	•	•	•	•	•	•	•	•	-	-	58.3
Left	5	•	•	•	•	-	•	•	•	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•	-	87.7
Left	6	-	•	-	•	•	•	•	•	•	•	-	•	•	-	-	•	-	-	•	-	•	•	-	•	62.5
Left	7	•	•	•	•	•	•	-	•	-	-	•	-	•	-	•	-	•	-	•	•	•	-	•	•	66.7
Left	8	-	-	-	•	-	-	•	•	•	-	-	-	-	•	-	•	-	•	-	-	•	•	-	•	41.7
Left	9	-	•	-	•	-	-	•	•	-	-	•	-	-	•	•	•	-	-	-	-	•	•	-	-	41.7
Left	10	-	•	-	-	-	•	-	•	-	-	-	•	•	•	-	-	-	•	-	-	-	-	-	-	29.2
Left	11	-	-	-	•	•	-	-	-	-	-	•	•	-	•	-	-	-	•	-	-	•	-	•	-	33.3
Left	12	•	-	-	-	-	•	-	-	-	•	•	-	-	-	-	-	-	-	-	-	•	-	-	•	25.0

• = parasites present - = parasites absent 100% = a total count of 24 infected lymph nodes for each day

TABLE 22

INGUINAL LYMPH NODE

Number of inguinal lymph nodes in which schistosomula were found in 24 mice at successive daily intervals from 1 to 12 days after infection.

Each animal was infected via the left hindfoot with approximately 500 S. mansoni cercariae.

		Mouse number																							Total
NODE	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	L. as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	16.7
Left	3	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+	+	-	+	-	+	-	+	37.5
Left	4	+	-	+	+	+	-	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	-	+	-	62.5
Left	5	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	91.4
Left	6	-	+	-	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	45.8
Left	7	-	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	+	+	-	+	-	+	+	62.5
Left	8	+	-	+	-	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	50.0
Left	9	-	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	+	+	+	-	-	-	-	-	37.5
Left	10	-	-	-	-	+	+	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	+	+	37.5
Left	11	+	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	16.7
Left	12	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.3

+ = parasites present   - = parasites absent   100% = a total count of 24 infected lymph nodes for each day

TABLE 23

AXILLARY LYMPH NODE

Number of axillary lymph nodes in which schistosomula were found in 24 mice at successive daily intervals from 1 to 12 days after infection

Each animal was infected via the left hindfoot with approximately 500 _S. mansoni_ cercariae.

NODE	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total L as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Left	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	95.8
Left	5	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	91.7
Left	6	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	70.8
Left	7	-	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	75.0
Left	8	-	-	+	+	-	+	-	+	+	-	+	+	-	-	+	+	+	+	-	-	+	+	+	-	58.3
Left	9	-	-	+	+	+	-	-	+	-	+	-	-	+	+	-	+	-	-	+	+	+	-	+	-	50.0
Left	10	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	33.3
Left	11	-	+	-	-	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	29.2
Left	12	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	12.5

+ = parasites present - = parasites absent 100% = a total count of 24 infected lymph nodes for each day

TABLE 24 BRACHIAL LYMPH NODE

Number of brachial lymph nodes in which schistosomula were found in 12 mice at successive daily intervals from 1-12 days after infection. Each animal was infected via theleft and right forefoot with approximately 500 S. mansoni cercariae.

Node	Day	1	2	3	4	5	6	7	8	9	10	11	12	Total L + R as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	+	+	-	+	+	+	-	+	+	+	+	+	75.0
Right		-	+	+	+	+	+	+	+	+	-	-	-	
Left	3	+	+	+	+	+	+	+	+	+	+	+	+	95.8
Right		+	+	+	+	+	-	+	+	+	+	+	+	
Left	4	-	+	+	+	+	+	+	+	+	+	+	+	95.8
Right		+	+	+	+	+	+	+	+	+	+	+	+	
Left	5	+	+	+	+	+	+	+	+	+	+	+	+	100
Right		+	+	+	+	+	+	+	+	+	+	+	+	
Left	6	-	+	-	+	+	+	+	+	-	+	+	+	79.2
Right		+	-	+	+	+	-	+	+	+	+	+	+	
Left	7	+	+	+	+	-	+	+	-	+	+	+	-	66.7
Right		+	+	+	+	-	+	-	+	-	+	-	-	
Left	8	+	+	-	+	-	+	+	-	+	+	+	+	79.2
Right		+	+	-	+	+	+	-	+	+	+	+	+	
Left	9	+	-	-	-	-	-	+	-	+	-	+	+	45.8
Right		-	+	+	-	+	-	+	-	+	+	-	-	
Left	10	-	-	-	-	+	+	-	-	-	-	+	-	29.2
Right		-	+	+	-	+	-	-	+	-	-	-	-	
Left	11	-	+	-	-	-	-	-	+	-	-	-	-	12.5
Right		-	-	-	-	-	-	-	-	-	+	-	-	
Left	12	+	-	-	+	-	-	-	-	-	-	-	-	16.7
Right		-	-	+	-	+	-	-	-	-	-	-	-	

+ = parasites present - = parasites absent

100% = a total count of 24 infected lymph nodes for each day

TABLE 25 AXILLARY LYMPH NODE

Number of axillary lymph nodes in which schistosomula were found in 12 mice at successive daily intervals from 1-12 days after infection. Each animal was infected via the left and right forefoot with approximately 500 S. mansoni cercariae.

Node	Day	1	2	3	4	5	6	7	8	9	10	11	12	Total L + R as %
Left	1	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	
Left	2	-	-	-	-	-	-	-	-	-	-	-	-	0
Right		-	-	-	-	-	-	-	-	-	-	-	-	
Left	3	+	+	+	-	+	+	-	-	+	+	+	-	58.3
Right		+	-	+	+	+	+	-	-	-	-	+	-	
Left	4	+	+	-	+	+	+	+	+	+	+	+	-	91.7
Right		+	+	+	+	+	+	+	+	+	+	+	+	
Left	5	+	-	+	+	+	+	+	+	+	+	+	+	95.8
Right		+	+	+	+	+	+	+	+	+	+	+	+	
Left	6	-	-	+	+	-	+	+	-	+	+	+	+	72.9
Right		+	+	+	+	+	-	+	+	+	+	+	+	
Left	7	-	+	+	+	+	+	+	+	-	+	+	+	75.0
Right		+	-	+	+	+	-	+	+	-	+	-	+	
Left	8	+	+	-	+	+	+	+	+	-	+	-	+	72.9
Right		-	+	+	+	+	+	+	+	+	+	+	-	
Left	9	-	-	+	-	+	+	-	+	-	+	-	-	45.8
Right		+	-	+	+	+	-	-	+	+	-	-	-	
Left	10	-	-	-	+	+	+	+	-	-	+	+	-	58.3
Right		+	+	+	-	+	+	-	-	+	+	+	-	
Left	11	-	-	-	-	+	+	-	-	-	+	-	-	20.8
Right		-	-	+	-	+	-	-	-	-	-	-	-	
Left	12	-	-	-	+	-	-	+	-	-	+	-	-	20.8
Right		-	+	-	-	-	-	-	-	+	-	-	-	

+ = parasites present - = parasites absent

100% = a total count of 24 infected lymph nodes for each day

TABLE 28 Mice injected intravenously with 100 cercariae of S. mansoni per animal. Worm recovery from liver perfusions at 6 weeks after injection.

Mouse No.	No. of cercariae injected	Adult worms recovered		Total No. of adult worms recovered	% recovery of adult worms
		♂	♀		
1	100	5	4	9	9
2	100	9	5	14	14
3	100	2	2	4	4
4	100	9	7	16	16
5	100	6	5	11	11
6	100	5	2	7	7
7	100	7	6	13	13
8	100	3	2	5	5
9	100	2	2	4	4
10	100	4	2	6	6
Total	1000	52	37	89	8.9
Range		2-9	2-7	4-16	4-16

$\bar{X}$ (mean of total adult worms recovered) = 8.9

SD (standard deviation) = 4.4

SE (standard error of the mean) = 1.4

TABLE 29 Mice injected intravenously with 50 schistosomula of S. mansoni obtained from lungs of animals infected by tail-immersion 6 days previously. Worm recovery from liver perfusions 6 weeks after injection of schistosomula (for details see text).

Mouse No.	No. of schistosomula injected	Adult worms recovered		Total No. of adult worms recovered	% recovery of adult worms
		♂	♀		
1	50	18	18	36	72
2	50	15	12	27	54
3	50	19	15	34	68
4	50	21	17	38	76
5	50	14	14	28	56
6	50	22	17	39	78
7	50	21	20	41	82
8	50	19	16	35	70
9	50	16	13	29	58
10	50	23	20	43	86
Total	500	188	162	350	70
Range		15-23	12-20	27-43	54-86

$\bar{X}$ (mean of total adult worms recovered) = 35.0

SD (standard deviation) = 5.5

SE (standard error of the mean) = 1.8

TABLE 30 Mice exposed to 100 cercariae of S. mansoni by percutaneous (tail) infection method. Worm recovery from liver perfusions 6 weeks after infections.

Mouse No.	No. of cercariae	Adult worms recovered		Total No. of adult worms recovered	% recovery of adult worms
		♂	♀		
1	100	10	7	17	17
2	100	14	13	27	27
3	100	23	19	42	42
4	100	20	16	36	36
5	100	24	18	42	42
6	100	13	12	25	25
7	100	15	13	28	28
8	100	13	10	23	23
9	100	20	19	39	39
10	100	18	15	33	33
Total	1000	170	142	312	31.2
Range		10-24	7-19	17-42	17-42

$\bar{X}$ (mean of total adult worms recovered) = 31.2

SD (standard deviation) = 8.5

SE (standard error of the mean) = 2.7

TABLE 31 Mice exposed to 100 cercariae of *S. mansoni* by percutaneous (ring) infection method. Worm recovery from liver perfusions 6 weeks after injection.

Mouse No.	No. of cercariae	Adult worms recovered		Total No. of adult worms recovered	% recovery of adult worms
		♂	♀		
1	100	28	23	51	51
2	100	19	19	38	38
3	100	20	15	35	35
4	100	19	15	34	34
5	100	15	16	31	31
6	100	26	20	46	46
7	100	19	18	37	37
8	100	16	15	31	31
9	100	25	20	45	45
10	100	26	23	49	49
Total	1000	213	184	397	39.7
Range		15-28	15-23	31-51	31-51

$\bar{X}$ (mean of total adult worms recovered) = 39.7

SD (standard deviation) = 7.4

SE (standard error of the mean) = 2.4

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"It is not my intention to stick stubbornly to my opinions, but as soon as people urge against them any reasonable objections ... I'll give mine up and go over to the other side."

Anton van Leeuwenhoek, 1694.