

A STUDY OF THE INFECTIVITY OF THE CERCARIAE OF SCHISTOSOMA
MANSONI AND SCHISTOSOMA HAEMATOBIIUM

A thesis submitted for the degree of Doctor
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by

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A B S T R A C T

The effect of various factors on the infectivity of the cercariae of S.mansoni and S.haematobium were studied. It has clearly been shown that the infectivity of cercariae of both species is directly influenced by those factors.

Almost equal proportions of cercariae of both species died or were severely damaged during penetration of mammalian host skin: 24-48% died in mouse skin compared with only 11-19% in hamster skin. These differential losses in the skin of different hosts account for the fact that hamsters yield higher adult worm recoveries than mice. However, adult worm recoveries from animals infected with S.haematobium cercariae were much less, almost one third, than those from S.mansoni infections. Young mice were more susceptible to S.mansoni than old mice and this was mainly due to the low level of mortality of cercariae during penetration of young mouse skin. More cercariae of S.mansoni died, at least during the early stages of penetration, in the skin of infected animals than in the skin of normal previously non-infected animals. It is very difficult to explain this observation; it could be related to the immune state of the host or to local reaction, provoked by previous exposure to cercariae, at the site of penetration. No difference was found in the susceptibility of male or female mice to S.mansoni but male hamsters were more susceptible to S.haematobium than female hamsters.

The percentages of S.mansoni and S.haematobium cercariae which die, during penetration of host skin, steadily increased with increase in the post-emergence age of the cercariae and this

accounted for the observed decline in infectivity which accompanied ageing of the cercariae. It has been demonstrated that death of S.mansoni cercariae during penetration of host skin is probably due to the exhaustion of their stored energy reserves.

Ultra-violet irradiation affected the infectivity of cercariae of S.mansoni and S.haematobium by increasing the level of mortality of cercariae in the skin and delaying their migration in the lungs beyond days 3 - 4 postinfection. Gamma irradiation also inhibited the development of S.mansoni cercariae to the adult stage : cercariae were mainly destroyed in the liver although some form of damage occurred in the lungs as well. Maintenance of cercariae of S.mansoni and S.haematobium at low or high temperatures increased their mortality in the skin and consequently resulted in a marked reduction in the worm burdens of animals infected with these cercariae. Treatment of S.mansoni cercariae with sublethal concentrations of niclosamide (Bayluscide) had the same effect as temperature.

The in vivo development of S.haematobium was also studied. It followed the same general pattern as for S.mansoni and S.japonicum: six stages of development, characterized by morphological and histochemical criteria, were distinguished. However, the development of S.haematobium was slower (61-63 days) than S.mansoni (34-35 days) or S.japonicum (28-29 days).

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CHAPTER I

GENERAL INTRODUCTION

PART I: Objectives of the present work.

The main purpose of this work was to carry out detailed studies on the infectivity of the cercariae of S.mansoni and S.haematobium. However, as experienced by most workers, S.haematobium has proved to be a difficult parasite to maintain under laboratory conditions and consequently has not been studied as fully as S.mansoni.

Three groups of factors were investigated in terms of their influence on the infectivity of the cercariae. These were:

1. Host-related factors- such as the species, age, sex and immune state of the mammalian definitive host.
2. Parasite-related factors- which included the post-emergence age and the energy contents of the cercariae.
3. Physical and Chemical factors- such as ultra-violet and gamma irradiation, temperature and molluscicide (niclosamide).

Two criteria were first used to assess the infectivity of the cercariae: the levels of mortality of cercariae during penetration of host skin and the percentage recovery of adult worms from the liver and mesenteric veins. Later in the work a third criterion, the percentage recovery of schistosomula from lungs of infected animals, was employed.

A study of the lung migration of S.haematobium proved of great interest and led to an investigation of the development of S.haematobium in vivo using the hamster as a definitive host.

PART II: Review of the literature on shistosome cercariae and schistosomula

(a) General Comparative Description of Cercariae and Schistosomula:

(1) Cercariae - Schistosome cercariae have been described in detail by various authors (Cort, 1919; Archibald, 1931, 1932; Mohamed, 1932; Girges, 1934; Porter, 1938; Dawes, 1946; Lengy, 1962 and Smyth, 1962, 1966). Basically, the cercaria is a juvenile trematode with a bifurcated tail. The entire cercaria is covered by a thin tegument bearing minute spines and fine sensory bristles. The digestive system is rudimentary with a small mouth situated subterminally in the middle of a prominent well developed oral sucker, and an oesophagus leading to a pair of short intestinal caeca ending in the middle of the body. The nervous system consists of a diffuse mass of fibres behind the oral sucker forming a cerebral ganglion from which emerge three pairs of nerves. The excretory system consists of 4 - 6 pairs of flame cells and their ducts, a small excretory bladder and a long bifurcated duct. The reproductive system is represented by a small cluster of cells just posterior to the ventral sucker (acetabulum) which is much smaller than the oral sucker.

Recently the ultrastructure of the surface of the cercaria has been thoroughly studied. The cercarial tegument has been shown to be covered by a surface coat consisting of a layer of diffuse granular and fibrous material with short branched filaments (Kruidenier and Stirewalt, 1955; Lumsden and Foor, 1968; Inatomi et al., 1970 and Hockley, 1972). This surface coat may serve a variety of functions: sticking of cercariae together and to the host's skin during penetration, and protection of the cercaria

from its own enzymatic secretions during penetration (Kruidenier, 1953), permeability control in the cercariae (Morris, 1971) and protection of cercaria from large particles which might damage the surface by acting as gel filtration devices (Kent, 1967). The cercarial tegument is a single continuous cytoplasmic structure over the body and tail of the cercaria with a trilaminar outer membrane (Smith et al., 1969; Inatomi, et al., 1970; Hockley, 1970; Morris, 1971 and Hockley and McLaren, 1973). A variety of inclusions have been noted in the tegument. Mitochondria are sparsely distributed throughout the tegument and spines are common and similar to those of the adult worm. The cercarial tegument is, presumably, a protective layer with no absorptive function.

Schistosome cercariae possess two pairs of preacetabular glands and three pairs of postacetabular glands, both types of glands having massive ducts by which they empty their contents at the tip of the oral sucker. The morphology, staining reactions, histochemistry and chemical composition of the secretions of these glands have been fully studied (Miura and Kissichugaku, 1953; Stirewalt and Kruidenier, 1961; Ebrahimzadeh, 1970; Robson and Erasmus, 1970 and Dorsey and Stirewalt, 1971). The glands are morphologically similar except for their shape and in the nature of the secretory globules. The secretory globules of the preacetabular glands are of several types varying in size, shape and homogeneity while those of the postacetabular glands are of a single type, spheroidal to irregular in shape with many electron dense areas. Both glands have different staining reactions- alizarin used as an intra-vital stain stains the acidophilic preacetabular

a brilliant red colour when used as an intra-vital stain whereas the postacetabular glands do not stain. The basophilic postacetabular glands stain with PAS whereas the preacetabular glands remain colourless. Secretions of the preacetabular glands are primarily enzymatic in function helping the cercariae during active penetration of the host's skin while those of the postacetabular glands are adhesive, lubricative, protective and enzyme directive helping the cercariae during the early stages of penetration while still looping on the skin surface.

Being free living organisms cercariae are not well adapted to saline and serum. They become shrunken and non-motile after a few minutes of immersion in these media. Upon immersion in immune serum a characteristic reaction known as the Cercarien-
hullenreaktion (CHR) occurs. This was first observed by Vogel and Minning (1949) and later by various workers (Standen, 1952; Kagan, 1955; Stirewalt, 1955; Evans and Stirewalt, 1959; Smit, 1961; Kemp, 1970 & 1972; Hockley, 1970 and Ebrahimzadeh, 1972). This reaction assumes the form of an envelope - the pericercarial envelope - which is formed as follows: within five minutes of immersion in antiserum, cercariae secrete orally large amounts of sticky material by which they become permanently attached to the substrate. During the first twenty minutes, the interspace between the outer and inner bounds of the tegument continues to swell until the cercaria is enclosed in tight transparent sheaths. The cercarial movement is restricted to the sheath but the cercariae remain viable and quite active up to 24 hours. Any cercaria which

breaks the sheath could not form a new sheath. The CHR reaction may be formed as a result of an interaction between factors in the antiserum and the cercarial tegument coat (Liu and Bang, 1950; Standen, 1952; Kagan, 1955; Kagan and Levine, 1956 and Kemp, 1970). The serum factor has been shown to be an antibody of the class IgG (Ebrahimzaden, 1972 and Kemp, 1972). Another serological reaction of schistosome cercariae, which may be essentially another manifestation of the same phenomenon responsible for the CHR, is known as "Agglutination". Under certain undefined conditions cercariae become sticky in antiserum and will agglutinate if present in large numbers (Liu and Bang, 1950; Stirewalt and Evans, 1955; Evans and Stirewalt, 1957 and Kusel, 1970).

(2) Schistosomula: Post-penetration schistosome larvae, i.e. schistosomula, have been described by Miyagawa (1912); Narabayshi and Kyoto (1914); Cort (1921); Faust and Meleny (1924); Gordon and Griffith (1951) and Stirewalt (1963). Schistosomula have no tail, so that locomotion is restricted to the inchworm movement accomplished by progressive alternate attachment of the oral and ventral suckers. They are spinose, wrinkled, flaccid and worm like. In contrast to cercariae, the contents of the penetration glands of schistosomula are completely evacuated owing to their exhaustion during penetration of the host's skin. Another radical difference from cercariae is the loss or alteration of the surface coat of the cercarial tegument. Schistosomula are intolerant to water but very tolerant to normal saline or serum. No CHR occurs when schistosomula are placed in antiserum.

Recent EM studies have confirmed the fact that the surface of the

schistosomulum is different from that of the cercaria (Bruce et al., 1970; Rifkin, 1971 and Hockley and MacLaren, 1973). A surface coat is still present on the newly penetrated schistosomulum but it is thinner and more dense and granular than the cercarial coat. Unlike the cercaria, the surface coat on the schistosomulum (30 min. post-penetration) has numerous sub-tegumental cells connected to the tegument, but the tegument adjacent to the sub-tegumental cells is filled with laminated bodies. In the 3 hour schistosomulum the number of layers in the outer membrane increased from three to seven (Hockley and McLaren, 1973).

(b) The Production of Cercariae from the Snail-Intermediate Host:

(1) Factors affecting the emergence of cercariae: At temperatures between 10°C and 30°C and possibly higher, light is the principal stimulus causing the release of cercariae of both S.mansoni and S.haematobium. However, cercariae are shed in smaller numbers in darkness and periodic peaks of output occur even in the absence of light because of the presence of an innate rhythm (McClelland, 1965). These characteristics were established by Schreiber and Schubert (1949) and by Luttermoser (1955) in respect of S.mansoni cercariae and are shared by a variety of species of cercariae including S.bovis (Lengy, 1962) and also by S.haematobium. (McClelland, 1967). Elevated temperature has the supplementary effect of causing greater output than illumination alone but, within the range specified, temperature is of secondary importance as far as S.mansoni and S.haematobium are concerned. Mass emergence of cercariae appears to be limited to a pH range of 6.5 to 9.5 (Smyth, 1966). In the laboratory the patterns of output of S.haematobium and S.mansoni cercariae are constant but different from one another. When Bulinus nasutus and Biomphalaria sudanice are exposed to light and heat continuously from 08.30 to 17.30 or 18.30 hours, the output pattern shows only one peak during the day - two hours after application of the stimulus in the case of S.mansoni and after four hours in S.haematobium (McClelland, 1967). Barbosa et al., (1954) carried out a series of experiments in Brazil in which naturally and experimentally infected B.glabrate were kept in aquaria in the open. The pattern of cercarial output corresponded to the curves for temperature and intensity of illumination. Large numbers of cercariae were produced from 9 a.m. onwards with an increase to a peak at 3 p.m. which then declined and few cercariae emerged between 7 p.m. and 9 a.m. These findings agree

with those of Faust (1934), Giovannola (1936), and McClelland (1967) and with the patterns of recovery of cercariae from natural waters in Puerto Rico recorded by Rowan (1958) and Maldonado (1959). In Tanzania, the peak of S. mansoni cercarial shedding period in a natural water course occurred between 10.00 and 14.00 hours (Webbe and Jordan, 1966). Pitchford and Visser (1966) established the pattern of cercarial shedding in South Africa as follows: For S. haematobium and S. mansoni between 11.00 and 15.00 hours with a peak at 13.00 hour; for S. mattheei an equal night and day shedding of cercariae with a peak at 09.00 and 23.00 hours; for S. bovis a diurnal pattern from 07.00 - 11.00 hours with a peak at 09.00 hour and for S. rodhaini a nocturnal pattern from 21.00 - 03.00 hours with a peak at 21.00 hour. Cercariae of S. japonicum are most abundant in the field during the early part of the night with a peak at 11.00 p.m. and a minimum concentration at 3.00 p.m. Prolonged exposure to light is necessary so that the cercariae are most abundant during the early parts of the night (Pesigan et al., 1958). Ferrisia tenuis sheds cercariae intermittently within narrow temperature limits - at 27°C. but not if it rises to 29°C. or falls below 26°C. (Gadgil and Shah, 1955, 1956).

(2) Numbers of Cercariae Produced: Numbers vary greatly, both from day to day and from snail to snail (Schreiber and Schubert, 1949 and McClelland, 1967). In general, few cercariae are produced daily when a snail first becomes infected, then the number increases over a period of days or weeks until it reaches a constant level, which is maintained until a few days before death of the snail or its self-cure (McClelland, 1965). Among equally susceptible snails the size governs the number of cercariae shed

large snails shedding more than small ones (Barretto and Barbosa, 1959). The output from B. glabrata is high, 1000 - 3000 cercariae/ day. African Biomphalaria spp. shed fewer cercariae - usually 500/ day or less though up to 1000/ day have been obtained from B. pfeifferi (Gordon et al., 1934) and B. sudanica (McClelland, 1967). Bulinus (Physopsis) globosus in West Africa produced up to 400 S. haematobium cercariae daily (Gordon et al., 1934) and output of naturally infected Bulinus (Ph.) nasutus in East Africa rarely exceeded 2000/ day but high counts were more common at the end of the transmission season than at the beginning (Jordan and Webbe, 1969). McClelland (1967) recorded that the mean % daily output of individual naturally infected Bulinus nasutus varied between 14 and 4,119, 34% of 205 daily counts being less than 501 cercariae. Webbe and James (1972) have found that the numbers of S. haematobium shed varied considerably but the level of output of individual Bulinus globosus seldom exceeded 2000 and of Bulinus truncatus 600. Large numbers of cercariae of S. haematobium were obtained from Bulinus (Ph.) ugandae and Bulinus (Bulinus) truncatus but few from Bulinus forskali (Cowper 1953 and Malek 1959). Ferrisia tenuis in India shed only four or five cercariae at a time (Gadgil and Shah, 1956). Pesigan et al., (1958) showed that when infected Oncomelania spp. snails were allowed to shed naturally, the mean produced daily was 15 but as many as 160 could be obtained.

(c) Penetration of Host Skin by Cercariae:

(1) Mechanism of penetration: The first observations on the penetration of host skin by cercariae were those by Hitchcock (1949). He noted that at the beginning of penetration, S. mansoni

cercariae were perpendicular to the skin and that the tail waved violently but usually did not break off from the body until the body had completely penetrated. This work was followed by more extensive studies, mainly on S.mansoni (Gordon and Griffiths, 1951, 1953; Standen, 1953 and Stirewalt and Hackey, 1956). Before penetrating, the cercariae explore the surface of the skin searching for a suitable point of entry—often skin irregularities. Usually, mucous secretions from the postacetabular glands are deposited on the skin surface thus serving as adhesive attachment points. Then, the cercaria orientates itself vertically to the skin surface and undergoes muscular contraction of the body. These changes in body shape provide a means of enlarging the entry pore and eventual entry of cercaria into the skin leaving the tail behind. Once within the stratum corneum the cercaria, now called a schistosomulum, abandons its boring movements and enters a resting phase varying from 10 min. to 24 hours. Forward movements of the schistosomulum are now in a horizontal direction by quivering contractions of the musculature around the preacetabular glands from which secretions are ejected thus helping the schistosomulum in migrating to the deeper layers of the skin.

The pattern and rate of migration of S.mansoni schistosomula in mouse skin was studied by Stirewalt (1959 b) who noted that schistosomula followed the route of least resistance in the dermis. Migration of several schistosomula in a common tunnel was observed rather frequently. The rate of migration varied in different types of skin and was slowest in that of the tail.

The dermis of the abdominal and ear skin was invaded almost immediately. Effective invasion of veins or venules occurred within two days in abdominal skin, three days in ear skin and five days in tail skin. No schistosomula were found in abdominal skin after 4 days or in ear skin after 5 days but they remained in the tail for at least 11 days.

Rai and Clegg (1968) were the first to show that some cercariae of the bird schistosome, Austrobilharzia terrigalensis, normally died during penetration of host skin and that the level of death was related to the type of host skin traversed: a higher level of deaths (85%) was found in duckling skin than in seagull or budgerigar skin (40% and 29% respectively). The same phenomenon was also observed in S.mansoni by Clegg and Smithers (1968) who showed that more cercariae died during penetration of rat skin (59%) than during penetration of mouse (27 - 46%) or hamster skin (11 - 16%). It seemed that the majority of deaths, in both schistosomes occurred during the first 10 min. of penetration when the cercariae were trying to traverse the Malpighian layer of cells and the basement membrane into the dermis.

(2) Factors triggering penetration: Faust and Meleny (1924) first commented that there was no chemical attraction between host's skin and cercariae of S.japonicum. Bolwig (1955), studying the behaviour of the cercariae of S.haematobium and S. bovis, concluded that although the cercariae did not actively seek the host, two main factors caused them to become attached to the host's skin: some component of the sebaceous gland secretion and the

temperature differential between the skin and water. Wagner (1959) suggested that free fatty acids stimulated cercariae of Schistosomium douthitti to penetrate mouse skin. Clegg (1969) found that cholesterol greatly stimulated penetration of skin by cercariae of the bird schistosome, A. terrigalensis, but that it did not act as an attractant, while MacIniss (1969) showed that short chain fatty acids and amino acids stimulated penetration of S.mansoni cercariae. This has been confirmed by Schiff et al. (1972) who have shown that unsaturated fatty acids in dermal lipids are the penetration stimuli for S.mansoni and S.haematobium cercariae. Stirewalt (1971) and Austin et al. (1972) have shown that skin surface lipids of man and rat, particularly fatty acid polyol ester and free fatty acids, strongly stimulated the penetration mechanism of S.mansoni cercariae. Gilbert et al. (1972) believe that phospholipids are involved in the penetration of S.mansoni cercariae.

(3) Cercarial enzymes involved in penetration: Evidence of the involvement of enzyme activity on entry and migration in skin by schistosome cercariae has been accumulating over the years (Levine et al., 1948; Stirewalt and Evans, 1952; Lewert and Lee, 1954; Lee and Lewert, 1957; Doelin et al., 1960; Milleman and Mergenhogen, 1960; Gazzinelli and Pellegrino, 1964 and Stirewalt and Walters, 1973). The enzyme hyaluronidase is produced by S.mansoni cercariae during penetration of host's skin. Histochemical studies showed that other enzymes such as mucopolysaccharidase, polysaccharidase and collagenase were involved in penetration of host skin. It seems that the bulk of these

enzymes originate from the preacetabular glands, but some may come from the postacetabular glands.

(4) Experimental methods for converting cercariae to

schistosomula : Recently, there has been a tremendous interest in the mechanism of conversion of schistosome cercariae to schistosomula and in the biology of the schistosomulum as a whole. This has arisen due to the suggestion by Smithers and Terry (1967) that host immunity may be directed against the schistosomula, and the demonstration by Hsu et al., (1969) of the active role of these larval stages in inducing immunity. Thus, many methods have been designed for the production of schistosomula in bulk and with relative ease:-

(a) Jensen's dialysis membrane: Jensen et al., (1965) successfully converted cercariae of S. mansoni to schistosomula by culturing the cercariae in the presence of chick embryo or mouse tissue, under dialysis membrane in Rose multipurpose chambers in Medium 190 and 20% serum. Growth and development of such schistosomula was normal but much slower than in vivo.

(b) Stirewalt's penetration membrane: Stirewalt et al., (1966) devised a simple apparatus for the collection of large numbers of schistosomula. It consists of a modified Rose chamber, containing a membrane prepared from dried rat skin, and a water jacket at 37°C. A suspension of cercariae is added to the water jacket; these pass through the skin becoming schistosomula which are collected in Hanks's salt solution. 35% of the cercariae may be converted by this method in about 4 hours and up to 45,000 schistosomula could be collected.

(c) Clegg and Smithers's membrane: Clegg and Smithers (1972) modified Stirewalt's membrane by using the abdominal skin of freshly killed mice from which the gel-like dermal tissue has been removed by vigorous rubbing. This membrane is stretched across a glass penetration apparatus having a cercarial suspension on the upper side and warm Earle's balanced saline, containing 0.5% of lactalbumin hydrolysate, 100 units/ml. penicillin and 100 ug/ml. streptomycin, in which the schistosomula are collected. 20 - 30% of the applied cercariae can be successfully converted to schistosomula.

(d) Eveland's technique: Eveland (1972) found that when cercariae of S.mansoni were injected directly into the peritoneal cavities of mice, approximately 15% of them were recovered as schistosomula. Also, within 2 hours of implantation of millipore diffusion chambers, containing cercariae, in the peritoneal cavities of mice almost 80 - 90% of the cercariae were converted to schistosomula.

(e) Gilbert's technique: Gilbert et al., (1972) have found that S.mansoni cercariae could be transformed to schistosomula by adding them to certain chemicals. This chemical stimulus for transformation resulted from contact with human or rat skin lipid and fractionation of the latter led to localization of the active component in a fraction containing phospholipids.

(f) Colley and Wikel's Syringe technique: (1974) These workers have developed a very simple method of conversion of cercariae to schistosomula by agitating the cercariae vigorously with a 22 G-needle

and syringe to remove their tails and then transferring them to a culture medium composed of 97% medium RPMI, 3% normal serum and 2% penicillin-streptomycin. Subsequent incubation at 37°C in a 5% CO₂ atmosphere produced schistosomula in a few hours.

(d) Development of Schistosomula in the Definitive Host:

(1) Migratory stages: Miyagawa (1912) and Miyagawa and Takemoto (1921) were the first to show that schistosomula migrated from the skin to the lungs of mice via the blood vessels. However they supported Narabayshi's and Kyoto (1914) suggestion that the lymphatic vessels might be another route of migration. Faust et al., (1934) confirmed Miyagawa's hypothesis that migration from the skin to the lungs was within the blood stream. However, there is no general agreement about the route of migration of schistosomula from the lungs to the liver. Miyagawa (1912) and Miyagawa and Takemoto (1921) concluded that the principal route from the lungs to the liver was through the pulmonary veins to the heart and arterial circulation and then to the portal vein. Faust et al., (1934) investigated this problem by examining washings from the peritoneal and pleural cavities of infected animals and also the blood vessels of the lungs, liver, spleen and gastrointestinal tracts. The presence of schistosomula in the left portion of the heart the appearance of haemorrhages in the gastrointestinal capillaries and the scarcity of schistosomula in the pleural cavity led Faust et al., (1934) to confirm Miyagawa's conclusions. Similar observations were also reported by Koppisch (1937), Yolles et al., (1949 and Olivier (1952).

Other workers have suggested a completely different route of migration of schistosomula from the lungs to the liver: that schistosomula migrate from the lungs to the pleural cavity and then

through the diaphragm into the peritoneal cavity from which they penetrate directly into the liver (Narabayshi and Kyoto 1914, 1915; Sueyasu, 1920; Cort, 1921 and La Rue, 1951). The frequent occurrence of schistosomula in the pleural cavity, mediastinum and diaphragm supports this view. Similar observations suggesting a direct lung to liver migration have been reported in schistosome infections in birds (McMullen and Beaver, 1945). This view was later supported by Wilks (1967) who was working with S.mansoni, Heterobilharzia americana and Schistosomatium douthitti. The presence of large numbers of schistosomula in the pleural cavity, during the period when their numbers were decreasing in the lungs and increasing in the liver led him to suggest the direct route of migration. Effective use of both routes for the migration of schistosomula from the lungs to the liver was suggested by Gotto (1932) who found only a few schistosomula of S.japonicum in the mediastinum and diaphragm of dogs and rats but many in the pleural cavities of mice.

(2) Development Stages: Miyagawa (1912) and Narabayshi and Kyoto (1916), studying some of the post-cercarial stages of S.japonicum, noticed forms such as the closed-gut forms in which haematin pigments were seen in the well developed guts and longer worms, over 1.0 mm. long, in which the sex glands were well developed. Faust et al., (1934) classified 24 stages of development of S.japonicum in mice and rabbits and they used the Greek alphabet to designate these stages. They reckoned that the minimum time for sexual maturity of the worms was approximately 28 days. Similar observations were provided by Cort (1921). Clegg (1961, 1965) studied the optimum rate of development of S.mansoni in mice. He divided this into six stages each recognized by morphological,

cytological or histochemical criteria: (1) Lung form, (2) Closed-gut form, (3) Organogeny, (4) Gametogeny, (5) Egg shell formation, (6) Oviposition. It took 34 - 35 days for sexual, maturation of the worms. However, the in vitro development proceeded to the gametogeny stage about 1 week later than in vivo but mating did not always occur.

Studies on the mating behaviour of schistosomes have shown that females can not reach sexual maturity without the presence of males (Moore et al., 1954 and Armstrong, 1965). It has been suggested that mating is accompanied by thigmotaxis and trial and error rather than by specific chemotactic responses. Male worms seem to produce a substance (pheromone) which promotes the development of females of the same species and which inhibits the development of another clasped male (Armstrong, 1965). Michaels (1969) found that neither mating nor stimulation of the rate of oviposition depended on intact testes, sperms or substances secreted with sperm. The mechanism by which the male stimulates the maturation of the female was not known; it was either, a nutritional, hormonal or chemical stimulus. There was some evidence to point out that linear receptors in both sexes of worms determined mating and mating behaviour. Taylor et al. (1969) reported parthenogenesis in S.mattheei - females reached sexual maturity in single sex infections of mice and some non-viable eggs were produced. This was not observed in other species of schistosomes (Taylor, 1971a). Erasmus (1973) has found that the presence of sperm in the female oviduct was not the major stimulus inducing maturation of the worm.

CHAPTER II

GENERAL MATERIALS AND METHODS

PART I: SNAILS

(a) Species of Snails used: During the course of the present work two species of snails were used:

(1) Biomphalaria glabrata (Say) (Puerto Rico), used for cycling of S.mansoni (Sambon, 1907) and was derived from a laboratory colony originally obtained from the U.S.A. Army Tropical Research Medical Laboratory in San Juan, Puerto Rico.

(2) Bulinus (Bulinus) truncatus (Audouin) (Sudan), used for cycling of S.haematobium (Bilharz, 1852) was maintained from a batch of wild snails collected from irrigation canals in Khartoum North, Sudan, and kindly supplied by Dr. M. A. Amin.

(b) Maintenance of snails: Snails were kept, in water, in glass tanks (7 litres) with a thin layer of autoclaved gravel on the bottom and some aquatic weeds such as Valisneria sp. The tanks were continuously aerated by Hy-Flo pumps and attached air-stones. Certain points were always observed for the proper maintenance of snail colonies:

(1) Type and volume of water used: Tap water was allowed to stand for two weeks before use in order to allow the chlorine to evaporate and excess salts such as calcium and magnesium to settle out. This "conditioned water" could then be safely used for snail colonies. The maintenance of high densities of snails results in reduced growth rates, lowered fecundity and resistance of the snails to infection (Malek, 1950; Chernin et al., 1956; Chernin and Michelson, 1957a,b; Lagrange, 1957; Wright, 1960 and Coles, 1973). Consequently care was taken not to overcrowd the snails in the tanks: a maximum of only 5 adult snails per

litre was maintained. This was rather similar to the levels used by Claugher (1960) and Webbe and James (1971a) for the successful maintenance and breeding of snails.

(2) Food of snails: Dried lettuce (prepared by removing the midrib of each leaf, boiling in water for 2 - 3 min. followed by rapid cooling in cold water and drying in an oven) was fed to adult snails three times a week.

Newly hatched snails would not eat dried lettuce and were fed on dried fish meals such as Tetramin.

(3) Temperature of water: The aquaria were maintained at a constant temperature of 25 - 27° C. This has been shown to be a crucial factor influencing the growth and fecundity of the snails and the intra-molluscan development of the parasite (Schiff, 1964; Sturrock, 1966a).

(4) Type of lighting: The aquaria were illuminated, by using warm-white fluorescent tubes, for 12 hours per day, usually from 9 a.m. to 9 p.m. This was controlled by time switches.

In general, snails were always kept in a healthy condition by avoiding adding excess food and immediate removal of dead snails, both factors being favourable to the development of bacterial decay and decomposition. Under these conditions water had to be changed only once every month.

However, sometimes some harmful organisms, introduced through the aquarium weeds or on the shells of field snails, were seen invading the tanks. Some of these (described by Deschiens et al, 1953; Coelho, 1957; Claugher, 1960; Khalil, 1961; Wajdi, 1964 and Webbe and James 1971a) included annelid worms such as Chaetogaster, ostracods such Cypridopsis, turbellaria such as Macrostomum and some brown algae. Claugher's method of narcotization snails in a

2% solution of ethyl carbamate whenever harmful organisms were seen invading the tanks was not followed. This was because heavy infestations of tanks by these organisms was not observed. Cleaning gravel, aquarium plants, tanks and snail shells was a sufficient remedy.

(c) Breeding of snails: Many methods have been designed for breeding the aquatic snail hosts of schistosomes (Brumpt, 1928, 1936 & 1941; Cowper, 1946; Lee and Lewert, 1956; Standen, 1949a,b & 1951; Hopf and Muller, 1962 and McClelland, 1964). Breeding tanks were kept under the same condition as for maintenance tanks except that fewer snails (5-10) were kept in larger tanks. An abundance of aquatic plants and polyethene sheets were provided for the deposition of egg masses.

PART 2: SCHISTOSOMES

(a) Source of eggs used for infection of snails: Male albino mice (4-5 weeks old) were used for routine cycling of S.mansoni and male hamsters (6-8 weeks old) were used for routine maintenance of S.haematobium.

Schistosome eggs were extracted from mice and hamster livers by macerating the tissue, straining it through a wire mesh filter (mesh 36/in.) and washing in physiological saline, followed by sedimentation in urine glass for 20 min. This was repeated 2-3 times until the supernatant was clear. A final washing and sedimentation in distilled water at 4-5°C then followed. The sediment then contained a ready good concentrate of eggs.

(b) Hatching of eggs: Warm water at 25-27°C was added to the egg concentrate in petri dishes. These were then left in an

illuminated incubator at 29 - 30°C for 1 hour at the end of which the majority of eggs hatched.

(c) Infection of snails: Snails were individually infected with 6 - 8 miracidia in haemagglutination plates containing 1 ml. of distilled water. The plates were covered with a sheet of glass and left under aquarium conditions for 3 - 5 hours.

(d) Screening of snails: Infected snails were kept undisturbed, during the prepatent period, under aquarium conditions. Three weeks after infection the snails were placed in 3" X 1" tubes containing 3 - 5 ml. of distilled water, and placed under a bright light for 2 - 4 hours to stimulate the shedding of the cercariae. The cercariae could easily be detected and all the shedding snails were then kept separately. The remaining negative snails were similarly screened every 3 days till the 60th day after infection.

(e) Infection of animals:

(1) Method of infection: All adult animals were infected by the ring method described by Smithers and Terry (1965a). The animals were anaesthetized by an intra-peritoneal injection of a barbiturate. Their fur was shaved from the lower abdomen and they were then laid on their backs between wooden strips permanently fixed to a base board. The abdomen was then moistened with water and nickel-plated rings, holding the cercarial suspension, were placed on the animal's lower abdomen. The rings were heavy so that they formed a seal with the wet skin thus preventing the escape of water underneath.

The rings used for mice measure 1.3 cm. inside diameter and are 2 cm. high, weigh 12 gm. and hold 1.2 ml. of water. Those

used for hamsters measure 3 cm. inside diameter, 1.0 cm. high, weigh 25 gm. and hold 5.0 ml. of water.

Very young mice, up to 7 days old, were infected by taping each one over a circular well (2.5 cm. high, measuring 1.5 cm. inside diameter and holding 1.3 ml. of water) attached to a petri dish so that the abdominal surface was in contact with a suspension of cercariae in the well.

At the end of the infection the water remaining in the rings and the wells was thoroughly searched for any remaining cercariae.

(2) Use of anaesthesia: The correct level of anaesthesia was induced by an intra-peritoneal injection of 1:10 dilution of Nembutal (Pentobarbitone sodium) in saline. The dosage level was estimated from the body weight: mice were given 7 mg. of Nembutal/100 gm. of body weight and hamsters 6 mg. /100 gm.

(3) Collection and estimation of the number of cercariae: Cercariae were collected by placing infected snails in a small volume of water and exposing them to strong illumination at 25-27°C for at least 2 hours in the case of S.mansoni and for 4 hours for S.haematobium.

The cercarial suspension was carefully agitated with side to side movements of the beaker so as to ensure a uniform suspension of cercariae. An automatic syringe, attached to a pipette and preadjusted to deliver a constant volume, was used to withdraw five 0.5 ml. aliquot samples from the suspension. These samples of cercariae were separately placed on a squared glass plate, killed and stained with Lugol's iodine and counted under a dissecting microscope. If the samples differed from one another by more than 10% fresh samples were counted. The mean of five counts

was then used to estimate the number of cercariae to be used for infections. For estimation of worm recovery, a minimum of 20 animals (for each separate experiment) were each exposed to 60 cercariae in the case of S.mansoni and 100 cercariae for S.haematobium.

(f) Perfusion of animals for recovery of adult worms: The adult worms were recovered from the infected animals by perfusion with citrated saline (0.85% sodium chloride and 1.5% sodium citrate) according to Smithers and Terry (1965a). The pressure for perfusion was provided by a rotary peristaltic pump with a foot operated switch.

The animals were killed by an intra-peritoneal injection of undiluted heparinized Nembutal (2500 units of heparin/100 ml. of Nembutal)- this caused all the worms to relax their hold on the walls of the blood vessels so that they were easily washed out. The abdominal and thoracic cavities were opened and the animal was attached by spring clips to a vertical board of Perspex having a horizontal tray of wire mesh gauze (below the suspended animal) for collection of the worms.

The hepatic portal vein was cut open, the perfusion needle was inserted into the left ventricle of the heart and the pump started. At the end of perfusion, which took $\frac{1}{2}$ min. for mice and 2 min. for hamsters, the needle was removed and the operator's fingers were gently washed to remove any worms attached to them. The perfused worms were then collected from the tray by thoroughly washing them off with saline into a petri dish.

(g) Recovery of schistosomula from the skin of infected

animals: This was done as described by Clegg and Smithers (1968). The animals were infected, as described previously, with 1000-2000 cercariae within a 10 min. period. After an additional 5 min. the animals were killed by cervical dislocation and the infected area of the skin was washed with aquarium water and rapidly excised. The skin was then chopped into small pieces and incubated in 10 ml. of Hanks's saline at a pH of 7.4 for 1-2 hours in a water bath at 37°C. The schistosomula were isolated by sieving the suspension through small wire mesh filters placed in 15 ml. centrifuge tubes. They were concentrated in 0.5 ml. of Hanks's saline by centrifuging for 1 min. at 3000 r.p.m. and the number of dead or severely damaged schistosomula was assessed by a dye-exclusion technique. A drop of 0.03% methylene blue in Hanks's saline was added to the schistosomula. Dead schistosomula or severely damaged ones (moribund) were contracted and showed eversion of the ventral sucker, with very feeble or no movement at all. The stain deeply penetrated all these while any living ones were completely unstained. However, if the schistosomula were left in contact with the stain for a long time some living ones became slightly stained due to attachment of the stain on to particles on the outer surface of the schistosomula. Care was taken not to count such schistosomula as dead ones.

200-300 schistosomula were counted in each experiment and the number of dead or non-viable schistosomula was expressed as a percentage of the total.

A minimum of 20 animals were used (in each experiment) for estimation of the percentages of the cercariae dying during penetration of the skin.

(h) Recovery of schistosomula from the lungs of infected animals: was carried out as described by Clegg (1965). The animals were killed with chloroform and the body cavity was opened to expose the heart and lungs. 10 ml. of Hanks's saline, containing 10 units of Heparin/ 1 ml. of Hanks's saline, was injected into the right ventricle to perfuse the lungs which expanded and became white due to the removal of blood. The left ventricle was punctured to allow the perfused blood to flow away from the lungs.

The lungs were dissected out, finely chopped into small pieces and incubated in 10 ml. of heparinized Hanks's saline at a pH of 7.4 for 3 hours in a water bath at 37°C. The schistosomula were then collected by a similar technique to the skin recovery. If there were lots of red blood cells in the sediment they were lysed by the addition of 2 ml. of distilled water followed immediately by 2 ml. of 2X Hanks's saline. A final centrifugation for 1 min. at 3000 r.p.m. concentrated the schistosomula.

For the estimation of the percentages of schistosomula recovered from the lungs, a minimum of 20 animals for each experiment were used.

PART 3: HISTOLOGICAL STUDIES: All tissue specimens collected for histology were fixed in 10% formol saline, dehydrated and embedded in paraffin wax. Sections were cut at about 7 microns and stained routinely with haematoxylin and eosin.

PART 4: STATISTICAL ANALYSIS: This was carried out with an Olivetti computer programme for standard forms of analysis.

CHAPTER III

EFFECT OF HOST-RELATED FACTORS ON THE INFECTIVITY OF THE CERCARIAE OF S.MANSONI AND S.HAEMATOBIMUM

PART 1: The effect of the species of mammalian host on the infectivity of S.mansoni and S.haematobium cercariae

Introduction

Mammalian hosts of different species differ markedly in their susceptibility to schistosome infection. Hamsters are more susceptible to S.mansoni than mice, cotton rats or rabbits (Standen, 1949b; Stirewalt et al., 1951; Smithers and Terry, 1965a and Taylor and Andrews, 1973) while white rats are poor hosts to S.mansoni rejecting their infection 4 weeks after exposure to cercariae (Lincicome, et al., 1963; Ritchie et al., 1963; Sadun and Bruce, 1964 and Smithers and Terry, 1965c).

Clegg and Smithers (1968) have shown that large numbers of S.mansoni cercariae die, or are severely damaged, within a few minutes of penetrating the skin of mice, rats and hamsters. This initial loss varies considerably in different hosts and is the main factor influencing the higher adult worm recovery from hamsters than from mice or rats. Rai and Clegg (1968) have demonstrated a similar effect with the cercariae of the bird schistosome, Austrobilharzia terrigalensis : 29% die in budgerigar skin, which is a natural host for this schistosome compared to 85% in duckling skin which is completely resistant to infection.

Standen (1949b), Moore and Meleny (1954) and Taylor and Andrews (1973) have shown that hamsters are better hosts for S.haematobium than mice. However, no studies have been done on the penetration of S.haematobium into mammalian host skin.

The main objectives of this experiment were to find out

if S. haematobium cercariae die during penetration of mammalian host skin, and whether losses in the skin may account for the higher recovery of adult worms from the liver and mesenteric veins.

Materials and Methods

Two sets of experiments were carried out:

(a) The first experiment was designed to compare the mortality of cercariae of S. mansoni and S. haematobium during penetration of mouse and hamster skin.

(b) The second experiment was conducted to find out if there was any correlation between the level of death of cercariae of both species of schistosomes in the skin, with the recovery of adult worms from the liver and mesenteric veins.

The abdominal skins of mice and hamsters which were exposed to cercariae of S. mansoni and S. haematobium were studied using histological methods.

Results

Death of cercariae during penetration of host skin: Analysis of the results of this experiment (Tables 1 and 2 and Figs. 1a and 2a) showed that the mean percentages of the level of death of S. haematobium and S. mansoni cercariae during penetration of mouse skin were almost the same, being 29.8% and 33.3% respectively with no significant difference ($p > 0.05$). There was also no significant difference ($p > 0.05$) between the levels of death of S. haematobium and S. mansoni cercariae during penetration of hamster skin: 15.9% and 14.7% respectively. However, it is evident that a higher proportion of the cercariae of S. haematobium and S. mansoni died in mouse rather than in hamster skin and this difference is

TABLE 1

Percentages of dead S. haematobium schistosomula recovered from the skin of adult mice and hamsters.

Species of host	No. of animals	Mean % dead schistosomula	Range	S.D. \pm	S.E. \pm
Mouse	10	29.8	26-34	2.85	0.9
Hamster	10	15.9	9-22	3.8	1.2

Mice v Hamsters

Degrees of Freedom 18

t 9.30

p < 0.01

TABLE 2

Percentages of dead S.mansoni schistosomula recovered from the skin of adult mice and hamsters.

Species of host	No. of animals	Mean % dead schistosomula	Range	S.D. \pm	S.E. \pm
Mouse	10	33.3	25-47	7.0	2.1
Hamster	10	14.7	10-18	3.7	1.0

Mice v Hamsters

Degrees of freedom 18

t 8.14

p < 0.01

T A B L E 3

Comparison of the percentages of S. haematobium cercariae recovered as dead schistosomula from the skin of mice and hamsters with those recovered as adult worms.

Species of host	% Dead Schistosomula				% Adult Worms				Mean % Cercariae not penetrating
	No. of animals	Mean	Range	S.D. ± S.E.	No. of animals	Mean	Range	S.D. ± S.E.	
Mouse	10	37.8	28-46	2.8 ± 1.5	20	8.0	3-15	2.6 ± 0.6	2
Hamster	10	15.2	8-22	5.0 ± 1.6	20	18.6	12-29	5.0 ± 1.2	3

Mice v Hamsters

Degrees of freedom 18

t 8.68

p < 0.01

Mice v Hamsters

Degrees of freedom 38

t 8.65

p < 0.01

TABLE 4

Comparison of the percentages of S. mansoni cercariae recovered as dead schistosomula from the skin of mice and hamsters with those recovered as adult worms from the liver and mesenteric veins.

Species of host	% Dead Schistosomula				% Adult Worms				Mean % Cercariae not penetrating
	No. of animals	Mean	Range	S.D. ± S.E. ±	No. of animals	Mean	Range	S.D. ± S.E. ±	
Mouse	10	36.7	25-43	6.4 2.1	20	34.2	20-51	8.5 1.9	3
Hamster	10	13.8	10-17	2.9 0.9	20	64.4	48-85	8.3 1.8	4

Mice v Hamsters

Degrees of freedom 18

t 9.61

p < 0.01

Mice v Hamsters

Degrees of freedom 38

t 9.82

p < 0.01

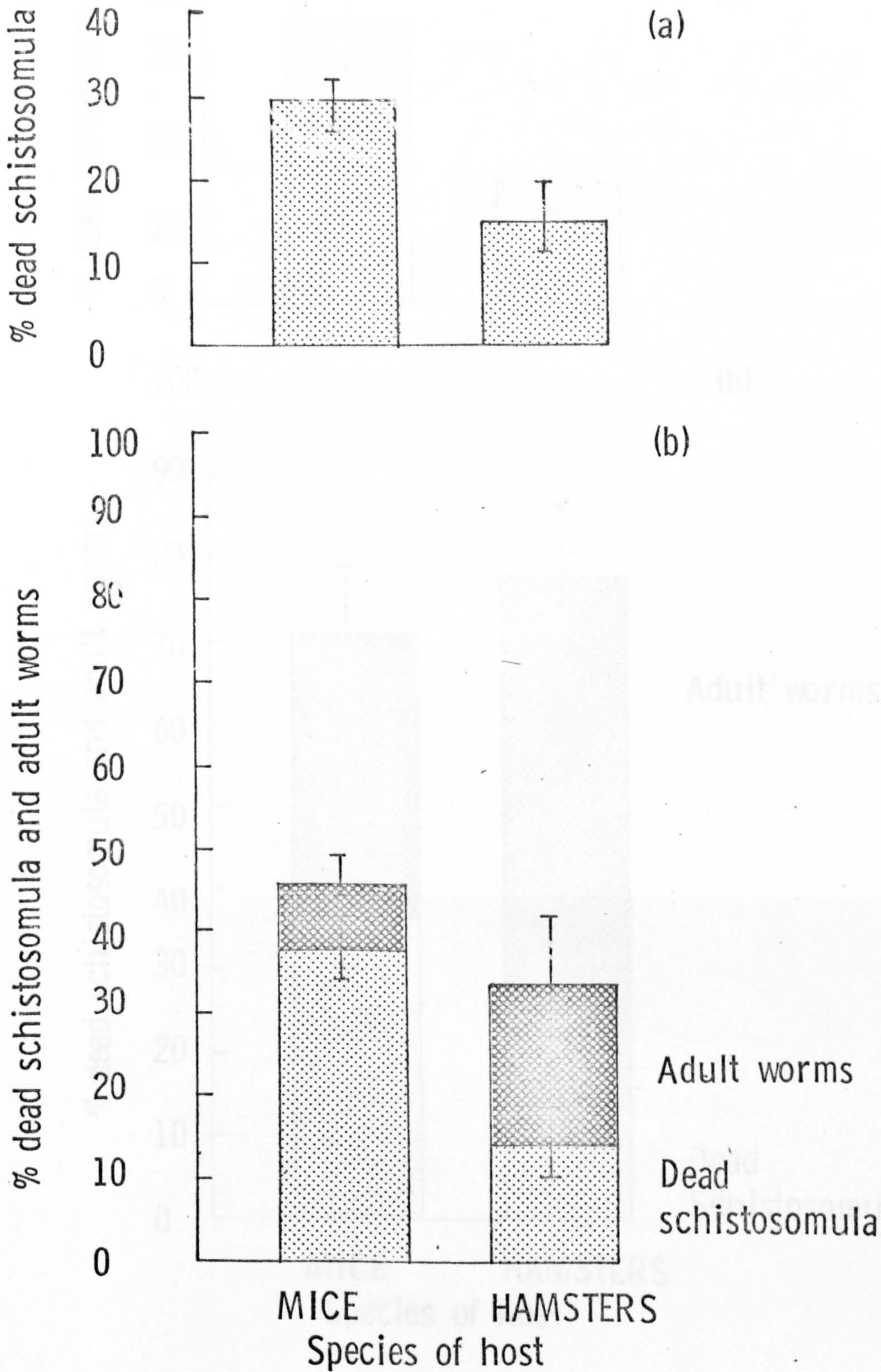


Fig. 1: a Percentages of dead *S. haematobium* schistosomula recovered from the skin of adult mice and hamsters. Each column represents the mean of 10 determinations.

b Comparison of the percentages of *S. haematobium* cercariae recovered as dead schistosomula from the skin of adult mice and hamsters with those recovered as adult worms. Each column represents the combined results of two simultaneous experiments.

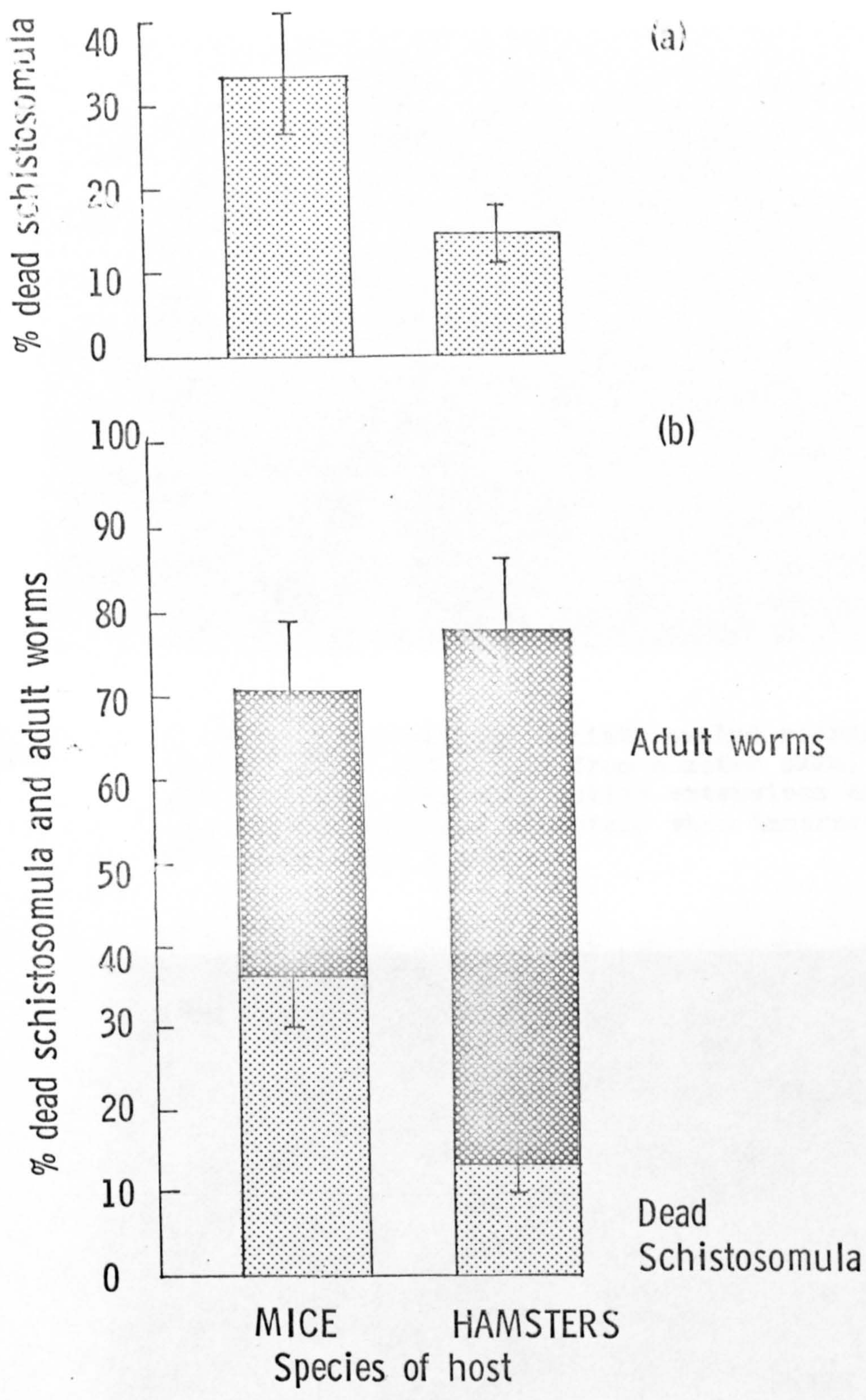


Fig. 2: a Percentages of dead *S.mansoni* schistosomula recovered from the skin of adult mice and hamsters.
b Comparison of the percentages of *S.mansoni* cercariae recovered as dead schistosomula from the skin of mice and hamsters with those recovered as adult worms.



PLATE 1: A living S. haematobium schistosomulum recovered, 15 min. after infection, from hamster skin. Living schistosomula show very active extensions and contractions and are unstained when immersed in Methylene blue. X 400.



PLATE 2: A dead S. haematobium schistosomulum recovered, 15 min. after infection, from hamster skin. Dead schistosomula are immobile, contracted and granular and readily pick up methylene blue stain X 200.



PLATE 3: A living S.mansoni schistosomulum recovered, 15 min. after infection, from mouse skin. X 400.

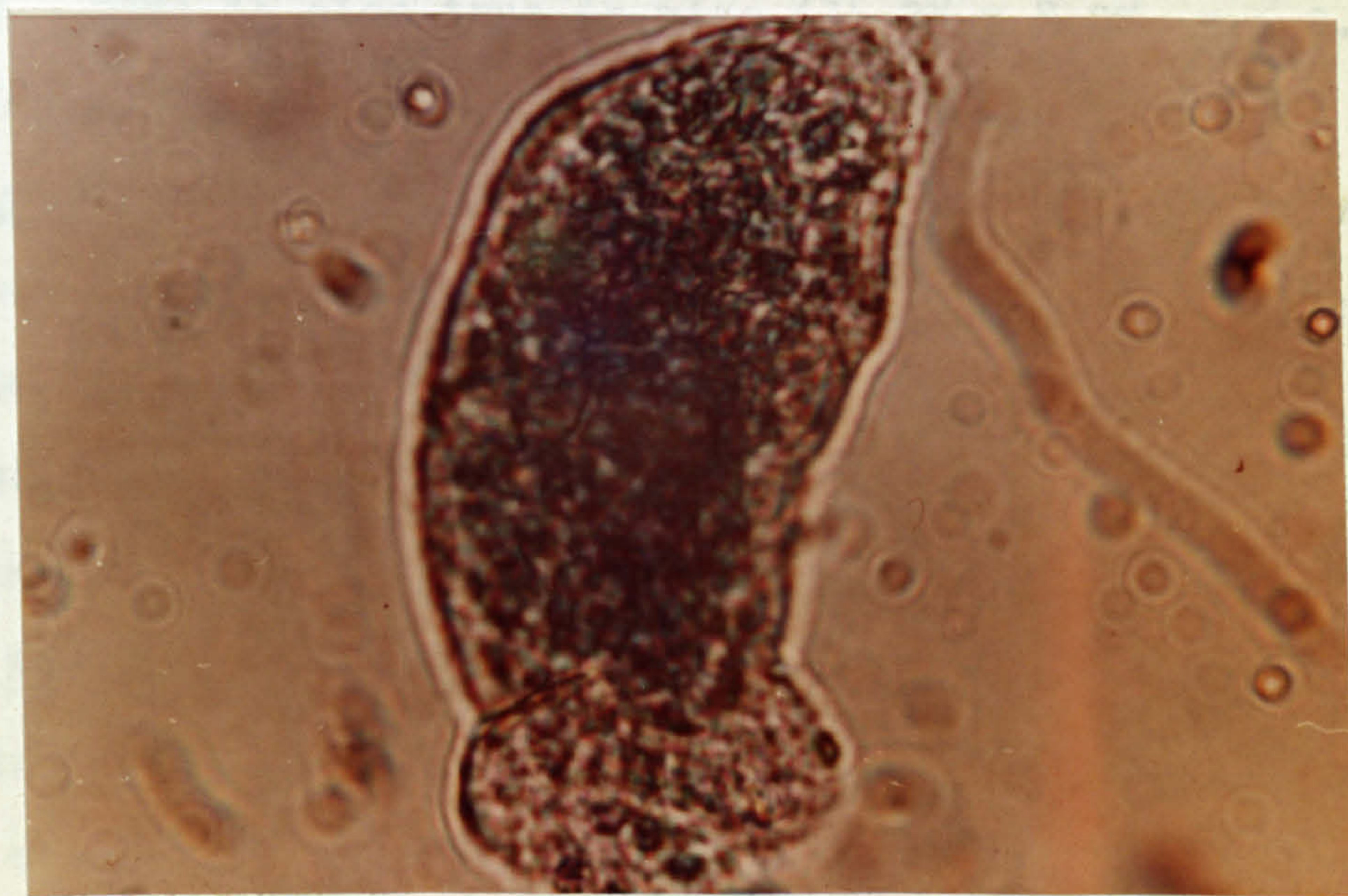


PLATE 4: A dead S.mansoni schistosomulum recovered, 15 min. after infection, from mouse skin. X 400.

statistically significant ($p < 0.01$ and $p < 0.01$ respectively).

Comparison of death of cercariae in the skin with the recovery of adult worms: As indicated in Table 3 and Fig. 1b the mean recovery of adult worms from hamsters infected with S.haematobium is greater than that from mice (18.6% and 8.0% respectively) and the difference is highly significant ($p < 0.01$). This differential mortality is partly due to the fact that fewer cercariae (15.2%) die in hamster than in mouse skin (37.8%).

In S.mansoni infections hamsters yielded higher adult worm recovery (64.4%) than mice (34.2%) and the difference between the recoveries was highly significant ($p < 0.01$). This is mainly due to the observation that only a small proportion of cercariae die in hamster skin (13.8%) while up to (36.7%) died in mouse skin.

In general, hamsters yielded higher adult worm recovery when infected with S.mansoni than S.haematobium (64.4% v 18.6%, $p < 0.001$) and the same is also true in mice (34.2% v 8.0%, $p < 0.001$).

No difference was noticed in the shape or size of schistosomula of S.mansoni and S.haematobium (Plates 1 - 4). There was no noticeable difference in the number of S.haematobium cercariae failing to penetrate mouse and hamster skin (2% and 3% respectively). The same was also true in S.mansoni infections of mice and hamsters (3% and 4% respectively).

Histological studies: These are shown for S.haematobium in (Plates 5 - 8). At 1 min. after exposure, the cercariae have lost their tails and were attached to the outer skin surface while the contents of the post-acetabular glands were evacuated. After 5 and 10 min. the cercariae have entered into the epidermis and are then called schistosomula. They turned parallel to the skin surface and were located between the stratum corneum and the Malpighian layer of cells some of which were squeezed flat. After

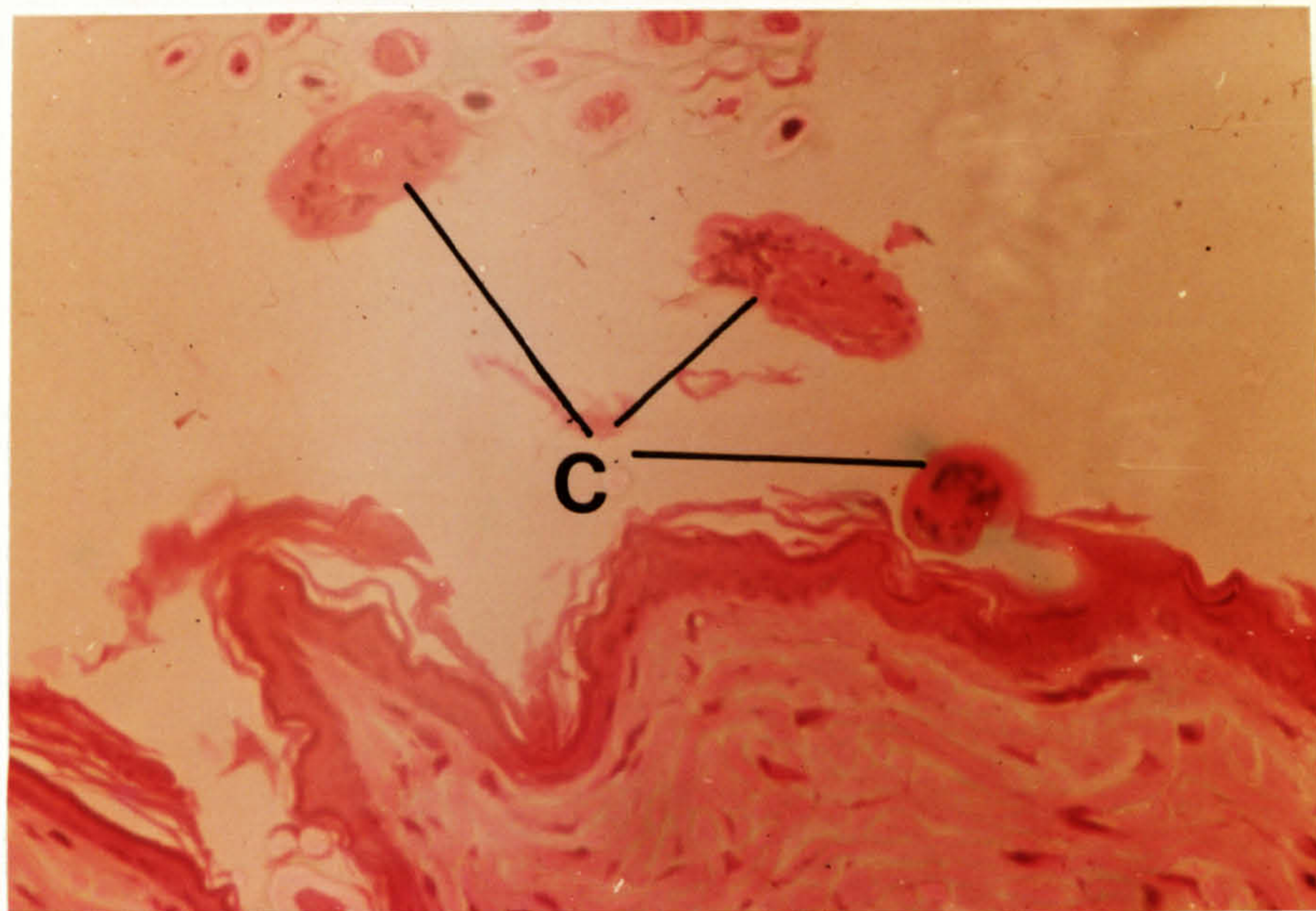


PLATE 5: Section of hamster abdominal skin at 1 min. after exposure to S. haematobium cercariae. The cercariae (C) have lost their tails and one of them can be seen attempting to penetrate into the skin. (H. & E. stain) X 400.

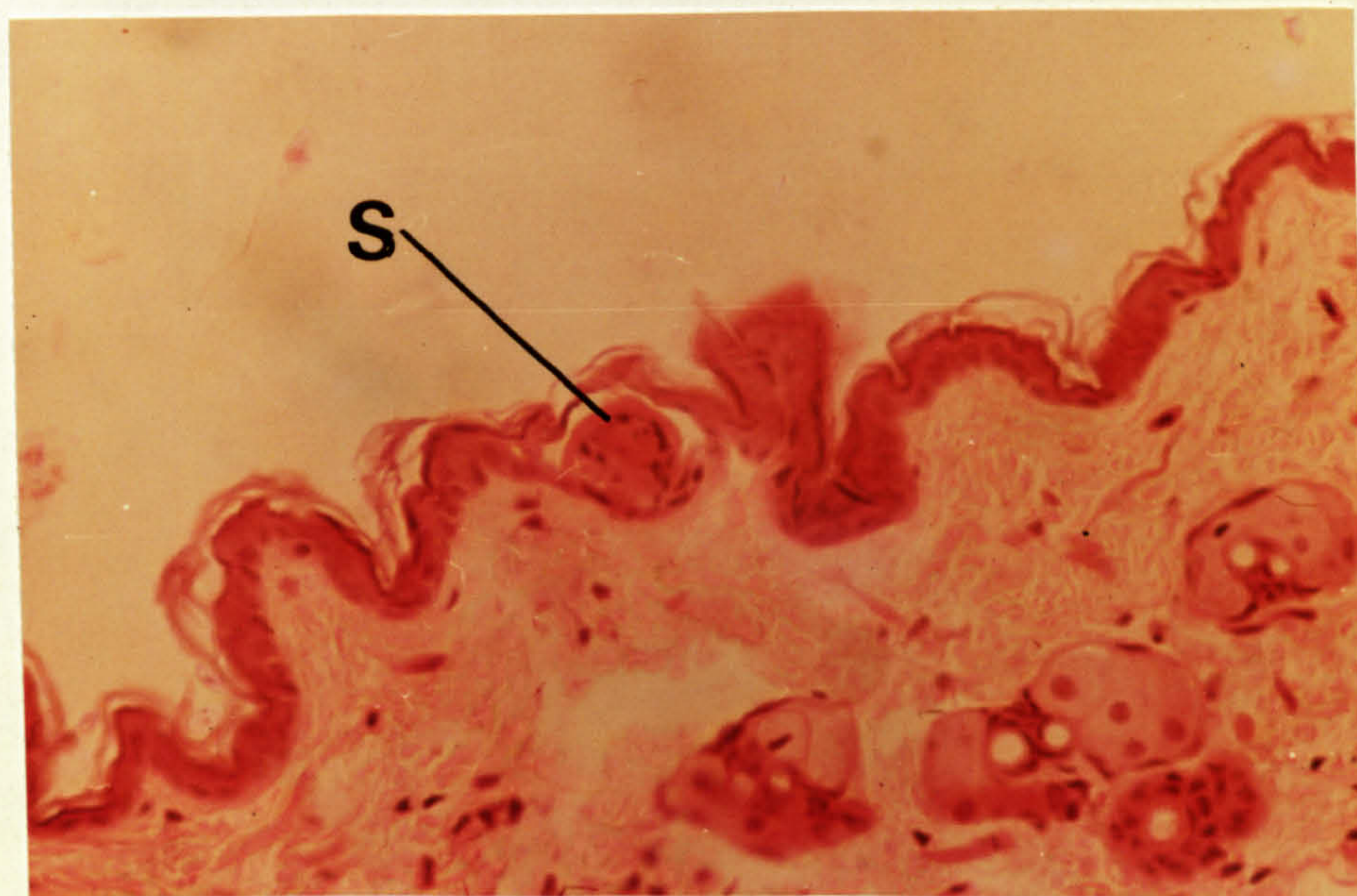


PLATE 6: Section of hamster abdominal skin at 5 min. after exposure to S. haematobium cercariae. A schistosomulum (S) is seen located in the epidermis, parallel to the skin surface, between the stratum corneum and the Malpighian layer of cells. (H. & E. stain) X 400.

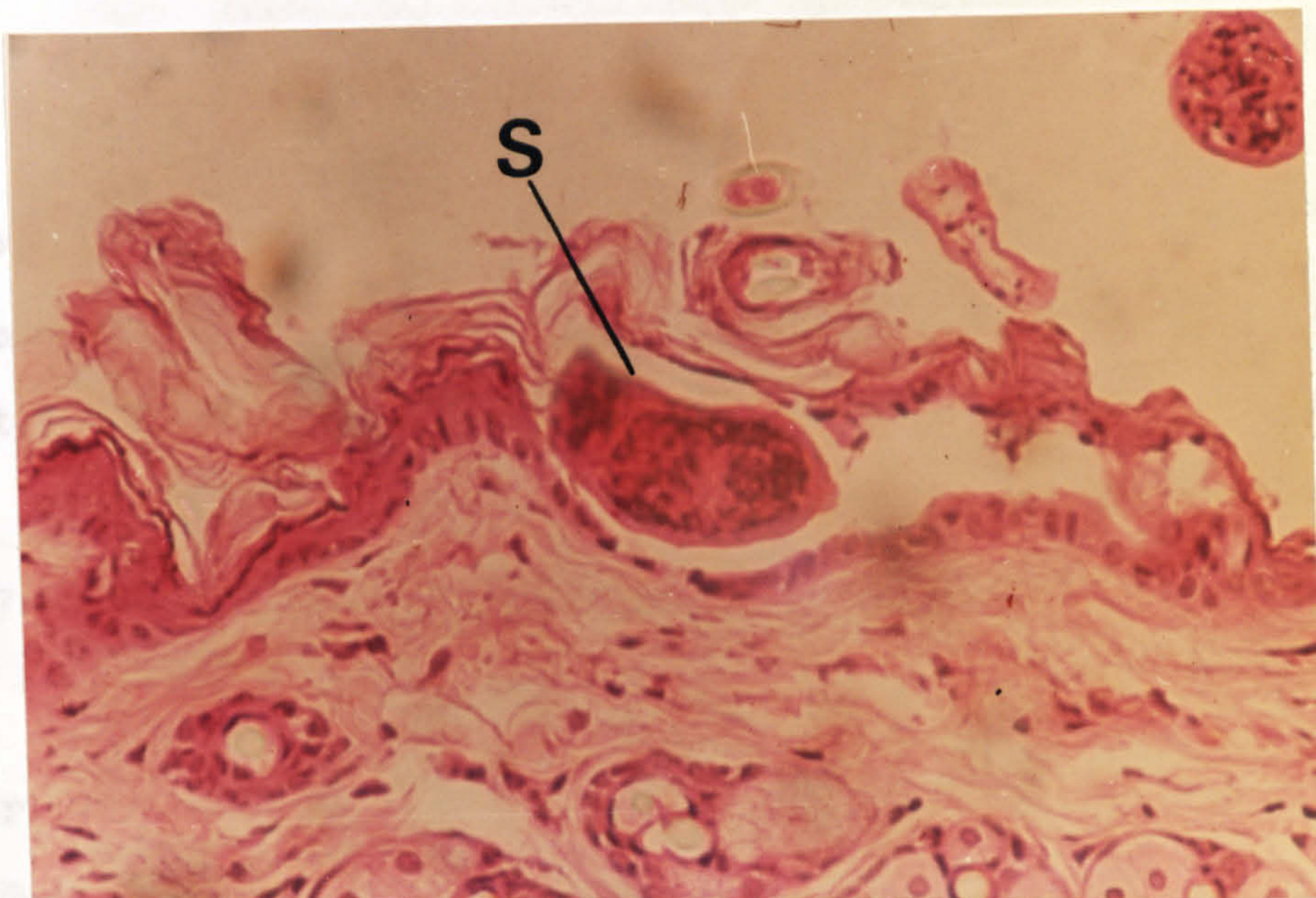


PLATE 7: Section of hamster abdominal skin at 10 min. after exposure to S. haematobium cercariae. A schistosomulum (S) is still seen in the epidermis with a penetration tunnel around it. (H. & E. stain) X 400.

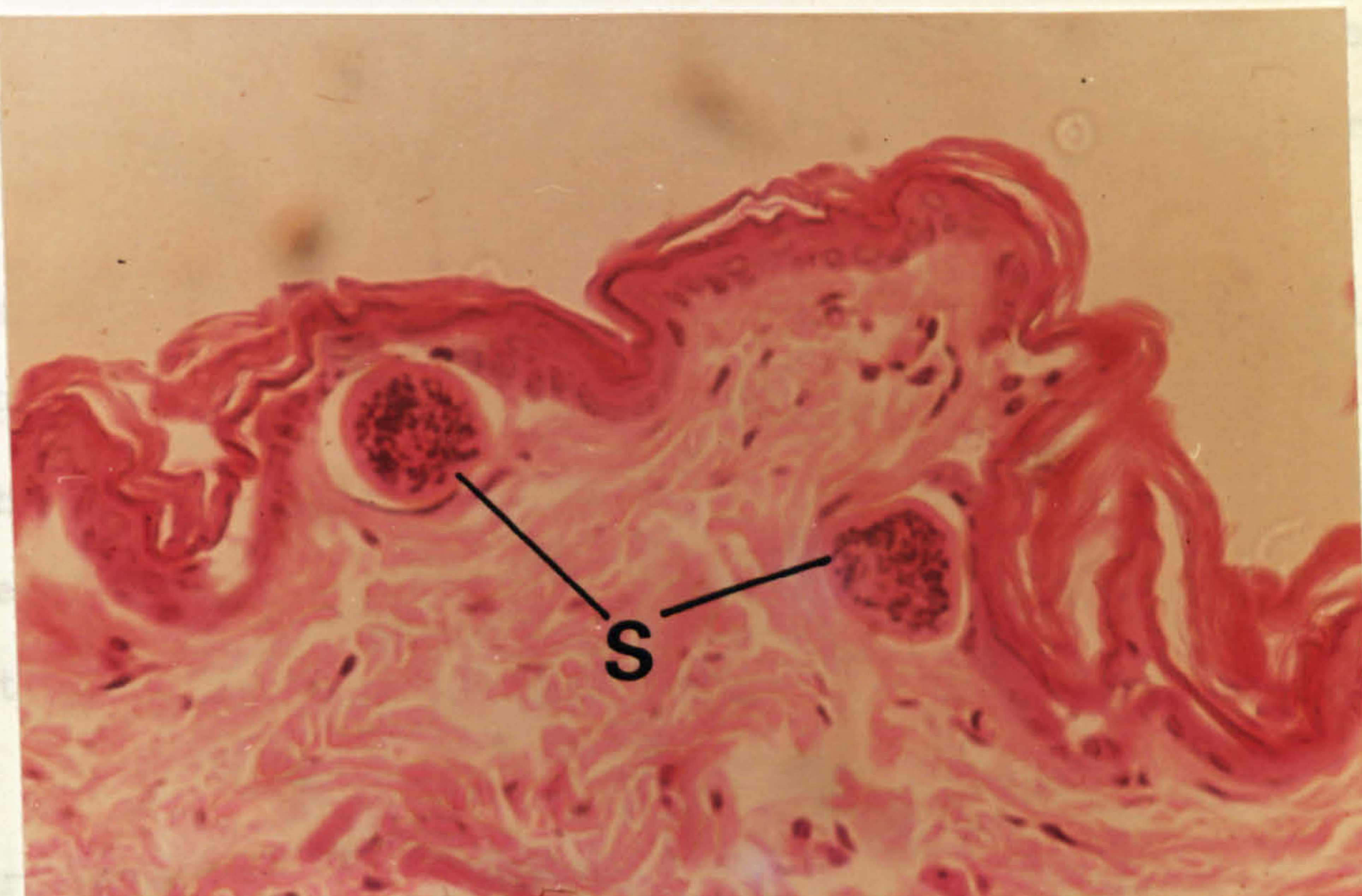


PLATE 8: Section of hamster abdominal skin at 15 min. after exposure to S. haematobium cercariae. Two schistosomula (S) can be seen in the dermis. (H. & E. stain) X 400.

15 min. the schistosomula have succeeded in passing through the epidermis and are now located, beneath the Malpighian layer of cells, in the dermis. A penetration tunnel is always recognizable around the migrating schistosomula. Similar findings to these were observed in the case of S.mansoni.

Discussion

The present results demonstrate very clearly for the first time that S.haematobium cercariae die or are severely damaged during penetration of mammalian host skin. They behave similarly to S.mansoni cercariae in that 30 - 38% die in mouse skin and only 15 - 16% die in hamster skin. This variation in the level of death might be related to the ease with which the basement membrane or the Malpighian layer of cells in the epidermis can be lysed by cercarial enzymes (Lewert and Lee, 1954 and Clegg and Smithers, 1968).

The present results for S.mansoni agree with those of Clegg and Smithers (1968) who have shown that the higher recoveries of adult worms from hamsters compared with those from mice infected with S.mansoni were mainly due to the lower mortality levels of cercariae during penetration of hamster skin. These differential losses in the skin of different hosts seem to account also for the fact that adult worm recovery from hamsters infected with S.haematobium is more than twice that in mice.

It is evident from this study that hamsters and mice are less susceptible to S.haematobium than to S.mansoni. Similar observations were reported by Kuntz and Malakatis (1955b) who obtained 15% adult

worm recovery from Cercopithecus sp. infected with an Egyptian strain of S.haematobium while higher recoveries were recorded for S.mansoni (Kuntz et al., 1953). Jordan and Goatly (1966) found that a Mwanzan strain of S.haematobium was not very infective to Cercopithecus aethiops centralis: only 2% adult worm recovery and scanty egg production for only nine weeks after the prepatent period. In contrast to their resistance to S.haematobium, the monkeys were very susceptible to S.mansoni infections: 30% adult worm recovery and high level of egg production for 67 weeks. Taylor and Andrews (1973) have recently demonstrated that the infectivity and pathogenicity of different species of schistosomes differed from each other and were related to the species of mammalian host used. Hamsters yielded higher adult worm recoveries than mice when exposed to the Nigerian strain of S.haematobium and their total tissue egg loads were higher than in mice. The same observation was also noted in animals infected with a Puerto Rican strain of S.mansoni. However, in terms of adult worm recovery and egg load, S.mansoni was far more infective to mammalian hosts than S.haematobium.

The histological studies on the rate of migration of schistosomula of S.mansoni and S.haematobium in the skin of mammalian hosts show that within 15 minutes of penetration both parasites traversed the epidermis and were located in the dermis. Clegg and Smithers (1968) and Rai and Clegg (1968) reported similar findings for S.mansoni and A.terrigalensis. The mortality of most of the cercariae of S.mansoni and A.terrigalensis during penetration of the skin occurs within 10 minutes after penetration when the cercariae are still in the epidermis trying to traverse the Malpighian layer of cells. It could be that cercariae of S.haematobium suffer damage or death while they are in this location since they migrate in the skin as do S.mansoni and A.terrigalensis cercariae.

The lower susceptibility of animals to S.haematobium than to S.mansoni is not principally due to a higher proportion of S.haematobium cercariae dying in the skin. It seems reasonable to assume that S.haematobium schistosomula suffer damage at some other stage of migration beyond the skin or that they lack some vital nutritional or physiological requirements within their definitive host.

The present observations on the susceptibility of mice and hamsters to S.mansoni and S.haematobium show that, in contrast to S.mansoni, S.haematobium shows only limited potentialities for establishing optimal host-parasite relationships in laboratory animals. Hamsters and baboons are good experimental hosts to S.haematobium (Jordan et al., 1967; Wright and Bennett, 1967a,b; Wright and Knowles, 1972; Webbe and James, 1971b; Taylor and Andrews, 1973 and Webbe et al., 1974). Further evidence for this host-specificity of S.haematobium comes from field surveys which showed that wild animals are not very susceptible to this parasite (Brumpt, 1928; Azim and Cowper, 1950; Kuntz and Malakatis, 1955a,c; Nelson, 1960; Capron et al., 1965 and Gear et al., 1966).

PART 2: The effect of the age of the host on the infectivity of S.mansoni cercariae

Introduction

The age of the definitive host has been shown to affect the number of S.mansoni cercariae recovered as adult worms from the hepatic portal system. Young mice exposed to the same batch of S.mansoni cercariae as old mice yielded a higher worm recovery (Stirewalt, 1952). This observation was confirmed by Lewert & Mandlowitz (1963) who found that young mice (19 - 28 days) were more susceptible to S.mansoni than old mice (about 1 year). Purnell (1966a) showed that 5-day-mice were particularly susceptible to S.mansoni, but this high susceptibility decreased sharply and reached a steady adult level after about 1 month of age. Da Motta et al., (1965) and Pellegrino and Katz (1969) recommended the use of baby suckling mice in experimental schistosomiasis because of their high susceptibility to infection. Purnell (1966a) could not demonstrate, in S.haematobium, a similar relationship between the percentage worm recovery and the age of the hamster host. This might be due to the fact that he could not infect very young hamsters because they had not been weaned and were eaten by their mothers when returned to them. The youngest hamsters he used were around 14 days old.

This study was carried out to determine whether losses of S.mansoni cercariae, during penetration of mammalian host skin, were influenced by the age of the host and whether these losses are related to the adult worm recovery.

Materials & Methods

Mice varying in age from 2 days to 28 - 35 days were infected with S.mansoni cercariae within 2 hrs. of emergence from the snail intermediate host and the number of dead schistosomula recovered from the skin was determined as described previously. Due to the limited numbers of skin recoveries which can be made at one time, all the animals of different ages could not be infected from the same batch of cercariae. A group of mice (28 - 35 days old) was used as the control, and compared with two other groups of mice of different ages. Three replicates of each experiment were carried out.

On the basis of the findings of the above investigation an experiment was designed to compare the mortality of schistosomula in the skin with the recovery of adult worms using 2 and 28 - 35 days old mice. Two groups of mice of these ages were used : the first was infected with 1000 - 2000 cercariae/ animal for skin recovery and the second with 60 - 80 cercariae/ animal for adult worm recovery.

Results

Death of cercariae during penetration of the skin of mice of different ages: Table 5 and Fig. 3a summarize the results of this experiment which showed that the mean percentages of dead schistosomula recovered from the skin increased with increase in the age of the mice. Very few cercariae died in the skin of 2 day old-mice (9.1%) compared to more than one third in the skin of adult mice of 28 - 35 days of age (33.7%) and the difference between these two groups is highly significant ($p < 0.01$). No difference was noted ($p > 0.05$) in the level of death in 22 day old mice (27.2%) and 28 - 35 day old mice (33.7%).

T A B L E 5

Percentages of dead S.mansoni schistosomula recovered from the skin of mice of different ages.

Age of mice (days)	No. of animals	Mean % dead schistosomula	Range	S.D. \pm	S.E. \pm
2	15	9.1	8-11	1.9	.6
7	15	14.2	12-18	1.8	.6
12	15	16.3	15-21	2.2	.7
17	15	19.4	15-23	2.6	.9
22	15	27.2	20-34	4.5	1.3
28-35, (CONTROL)	45	33.7	27-45	3.2	1.3

2 days v 28-35 days
 Degrees of freedom 28
 t 11.97
 p < 0.01 S.D.

7 days v 28-35 days
 Degrees of freedom 28
 t 10.17
 p < 0.01

12 days v 28-35 days
 Degrees of freedom 28
 t 9.59
 p < 0.01

17 days v 28-35 days
 Degrees of freedom 28
 t 7.43
 p < 0.01

22 days v 28-35 days
 Degrees of freedom 28
 t 2.47
 p > 0.05

TABLE 6

Comparison of the percentages of S. mansoni cercariae recovered as dead schistosomula from the skin of young and old mice (2 and 28-35 days respectively) with those recovered as adult worms.

Age of Mice (days)	% Dead Schistosomula				% Adult Worms				Mean % cercariae not penetrating		
	No. of animals	Mean	Range	S.D. ± S.E. ±	No. of animals	Mean	Range	S.D. ± S.E. ±			
2	10	8.5	4-13	3.1	1.0	20	64.8	55-86	7.2	1.6	5
28-35	10	36.4	28-42	4.9	1.6	20	40.8	25-52	7.5	1.7	6

2 days v 28-35 days

Degrees of freedom 18

t 15.1

p < 0.01

2 days v 28-35 days

Degrees of freedom 38

t 10.28

p < 0.01

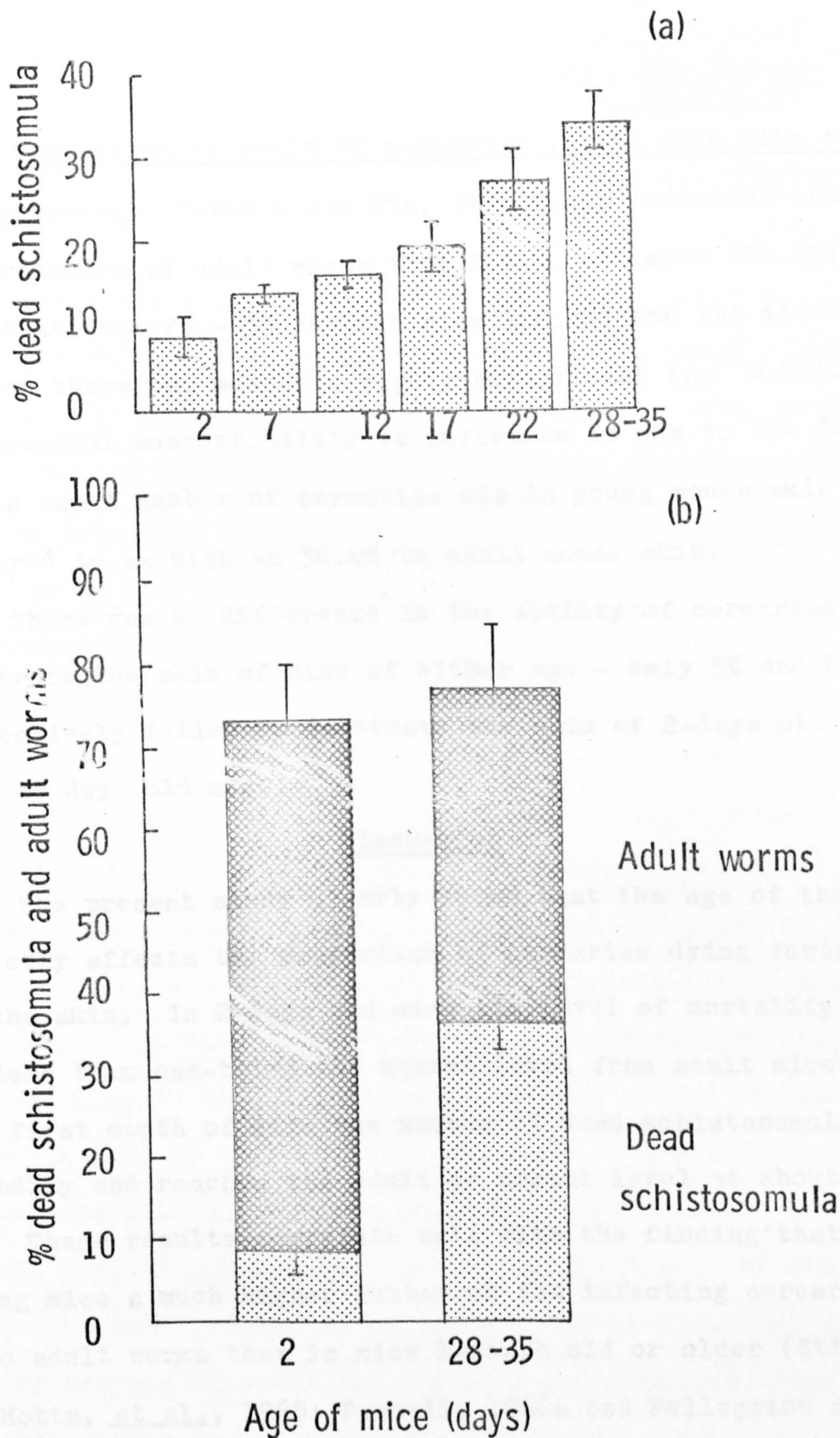


Fig. 3: a Percentages of dead *S.mansoni* schistosomula recovered from the skin of mice of different ages.

b Comparison of the percentages of *S.mansoni* cercariae recovered as dead schistosomula from the skin of young and old mice (2 and 28 - 35 days respectively) with those recovered as adult worms.

Comparison of death of cercariae in the skin with the adult worm recovery: Table 6 and Fig. 3b clearly indicate that the mean recovery of adult worms from 2 day old mice (64.8%) is higher than that from 28 - 35 day old mice (40.8%) and the difference between these two means is highly significant ($p < 0.01$). This differential susceptibility to infection is due to the fact that only a small number of cercariae die in young mouse skin (8.5%) compared to as high as 36.4% in adult mouse skin.

There was no difference in the ability of cercariae to penetrate the skin of mice of either age - only 5% and 6% respectively failed to penetrate the skin of 2-days old mice and 28 - 35 day old mice.

Discussion

The present study clearly shows that the age of the mouse host directly affects the proportion of cercariae dying during penetration of the skin. In 2-days old mice the level of mortality in the skin is less than one-third the normal level from adult mice. During the first month of life the number of dead schistosomula rises steadily and reaches the adult or normal level at about 28 - 35 days.

These results correlate well with the finding that in very young mice a much higher number of the infecting cercariae develop into adult worms than in mice 1 month old or older (Stirewalt, 1952; Da Motta, et al., 1965; Purnell, 1966a and Pellegrino and Katz, 1969). The most obvious conclusion is that the higher susceptibility of young mice to infection is due to young mouse skin being a less formidable barrier to penetration than old mouse skin. Experiments which demonstrate this connection show clearly that a low level of mortality of cercariae in the skin of 2-day old mice results

in high adult worm recovery, whereas the greatly increased losses (more than three times) in the skin of 28 - 35 day old mice almost exactly account for the drop in the adult worm recovery.

Lewert and Lee (1954) have demonstrated that the movement of helminth infective larvae through the skin of their definitive hosts causes extensive alterations in the basement membrane and ground substance of the host's connective tissues. These changes are due to enzymatic activity of those penetrating helminths and take the form of softening and changing to a soluble state of the acellular barriers. These authors have also shown that the acellular ground substance of the dermis of mice become more dense and highly polymerized with age - this results in inhibition of larval penetration in old mice i.e. there is greater resistance of the host basement membrane and ground substance to alteration by cercarial enzymes. Lewert and Mandlowitz (1963) considered that these changes in density were responsible for the resistance of old mice to infection with S.mansoni. This explanation was strengthened by the fact that old mice of hybrid LAF strain, which are slow ageing, are susceptible as CF mice, less than one month old, to S.mansoni. The connective tissue of old LAF mice have the same properties as young animals, i.e. they contain large amounts of water and water soluble compounds and are less highly polymerized. Further evidence for this idea comes from studies on scroubitic mice. The acellular elements of the connective tissue in the skin of these mice are altered in a greater accumulation of water and water soluble compounds (Lewert and Mandlowitz, 1963).

Scrobutic mice had twice as many schistosomes as normal mice of similar age and sex when infected with S.mansoni. Animals treated with cortisone are more easily penetrated with cercariae due to an increase in the density of the basement membrane of the skin. Large doses of cortisone (0.7 mg. / day for 6 days) enhanced their resistance to S.mansoni infection as compared to untreated animals. X-irradiation seems to alter the acellular elements of the skin of old mice in the direction of closer resemblance to the plasticity and density of the acellular components of the skin of young mice - these animals have infection rates similar to those of young mice. In young hypophysectomized rats there is also a reduction in the degree of glycoprotein alteration of the skin during penetration of S.mansoni cercariae - this was correlated with inhibition of penetration. These animals resemble normal aged rats in that they have thick basement membrane and more highly polymerized basement membrane and ground substance of the dermis.

PART 3: The effect of sex of the host on the infectivity of
S.mansoni and S.haematobium cercariae

Introduction

The effect of sex of the host or of sex hormones on the infection of animals with Schistosoma spp. has been studied in some detail (Berg, 1957a, b; Coker, 1958; Robinson, 1960; Weinmann and Hunter, 1960; Purnell, 1966a; Taylor et al., 1971b and Wright and Knowles, 1972). Berg (1957a) found that castration of mice and injection of testosterone reduced the worm burdens of these animals when they were infected with S.mansoni. Robinson (1960) confirmed Berg's findings and reported that treatment of mice with massive doses of stilboestrol had adverse effects on the S.mansoni worms they harboured. These effects included a delay in development of both sexes to maturity, the development of many males with some accessory gonadial tissue, probably ovarian, and a reduction in the length of male worms compared to worms from untreated hosts. Treatment of mice with cortisone increased their natural resistance to S.mansoni infection since fewer worms were recovered from treated animals than from untreated ones (Coker, 1958 and Weinmann and Hunter, 1960). Purnell (1966a) has shown that male animals were more susceptible to S.mansoni than female animals. Similar findings have been reported in other host-parasite models: in chicks infected with Ascaridia galli (Todd and Hollingsworth, 1952); in dogs infected with Toxocara canis (Ehrenford, 1957); in hamsters infected with Nippostrongylus muris (Haley, 1958) and in mice infected with Aspiculuris tetraptera (Mathies, 1959).

However in the case of S.haematobium there has been some controversy as to which sex of animal is more susceptible. Purnell (1966a) has shown that male hamsters are more susceptible to S.haematobium than females and that male animals had a predominance of male worms. Wright and Knowles (1972) seem to contradict this opinion: they produced some evidence that female hamsters were slightly more susceptible to S.haematobium than males and that there was a tendency for the sex ratios of the parasite to be biased in favour of male worms in female hosts.

The present work was carried out to compare the susceptibility of male and female animals to S.mansoni and S.haematobium and to find out whether death of cercariae during penetration of host skin could be related to the adult worm recovery.

Materials and Methods

Two experiments were carried out in duplicate:

(a) Male and female animals of the same age (4 - 5 weeks for mice and 5 - 6 weeks for hamsters) and strain, were infected with freshly shed cercariae of S.mansoni and S.haematobium. Schistosomula were recovered from the skin, 15 min. after penetration, and a record was made of the percentages of dead ones.

(b) The second experiment was designed to compare the mortality of S.mansoni and S.haematobium cercariae during penetration of the skin with the adult worm recoveries from the liver and mesenteric veins of animals infected with the same batch of cercariae.

Results

Death of cercariae during penetration of the skin of male and female animals: The results of this experiment are shown in Tables 7 & 8 and Figs. 4a & 5a. It is quite evident that, for both S.mansoni and S.haematobium, there is no significant difference ($t= 0.3$ $p > 0.5$) ($t= 0.4$ $p > 0.5$) in the percentages of cercariae which died during penetration of the skin of male or female animals. (24.3% v 25.0% for S.mansoni and 19.3% v 18.4% in S.haematobium).

Comparison of death of cercariae in the skin with the adult worm recovery: Tables 9 & 10 and Figs. 4b & 5b summarize the results of this experiment. Male animals were slightly more susceptible, in terms of adult worm recovery, to S.mansoni than females (37.2% v 34.1%) but the difference was not statistically significant ($t= 1.0$ $p > 0.05$). Similarly there was no difference the levels of death of cercaria in the two sexes (37. % v 39.2%, $p > 0.5$). However, the adult worm recovery from male hamsters infected with S.haematobium was statistically greater than that from female hamsters (17.1% v 11.8%; $t= 5.39$ and $p < 0.01$). This difference in the adult worm recovery could not be related to differences in the level of mortality of cercariae during penetration of the skin of either sex of host (16.8% v 16.9%; $t= 0.46$ and $p > 0.5$).

Discussion

It is evident from this study that the sex of the mammalian definitive host has a direct influence on its subsequent adult worm burden. This is particularly true in S.haematobium infections where male hamsters yielded higher worm

TABLE 7

Percentages of dead S.mansoni schistosomula recovered from the skin of adult male and female mice.

Sex of Mouse	No. of animals	Mean % dead schistosomula	Range	S.D. \pm	S.E. \pm
Male	10	24.3	20-29	3.6	1.1
Female	10	25.0	20-32	4.4	1.4

Male v Female

Degrees of freedom 18

t 0.30

p > 0.5

T A B L E 8

Percentages of dead S. haematobium schistosomula recovered from the skin of male and female hamsters.

Sex of hamster	No. of animals	Mean % dead schistosomula	Range	S.D. \pm	S.E. \pm
Male	10	19.3	10-23	4.2	1.3
Female	10	18.4	10-25	5.5	1.7

Male v Female

Degrees of freedom 18

t 0.42
 p > 0.5

TABLE 9

Comparison of the percentages of *S. mansoni* cercariae recovered as dead schistosomula from the skin of male and female mice with those recovered as adult worms.

Sex of mice	% Dead Schistosomula				% Adult Worms			
	No. of animals	Mean	Range	S.D. ± S.E. ±	No. of animals	Mean	Range	S.D. ± S.E. ±
Male	10	37.1	31-42	3.7 1.1	20	37.2	19-63	10.4 2.4
Female	10	39.2	31-45	5.4 1.7	20	34.1	19-48	8.7 1.9

Male v Female

Degrees of freedom 18

t 0.78

p > 0.5

Male v Female

Degrees of freedom 38

t 1.03

p > 0.05

T A B L E 10

Comparison of the percentages of *S. haematobium* cercariae recovered as dead schistosomula from the skin of male and female hamsters with those recovered as adult worms.

Sex of Hamsters	No. of animals	% Dead Schistosomula			% Adult Worms			Mean % cercariae not penetrating	
		Mean	Range	S.D. ± S.E. ±	Mean	Range	S.D. ± S.E. ±		
Male	10	16.8	10-25	6.1 1.9	20	17.1	12-23	3.4 0.8	4
Female	10	16.9	11-27	5.1 1.6	20	11.8	8-17	2.6 0.5	5

Male v Female

Degrees of freedom 18

t 0.467

p > 0.5

Male v Female

Degrees of freedom 38

t 5.39

p < 0.01

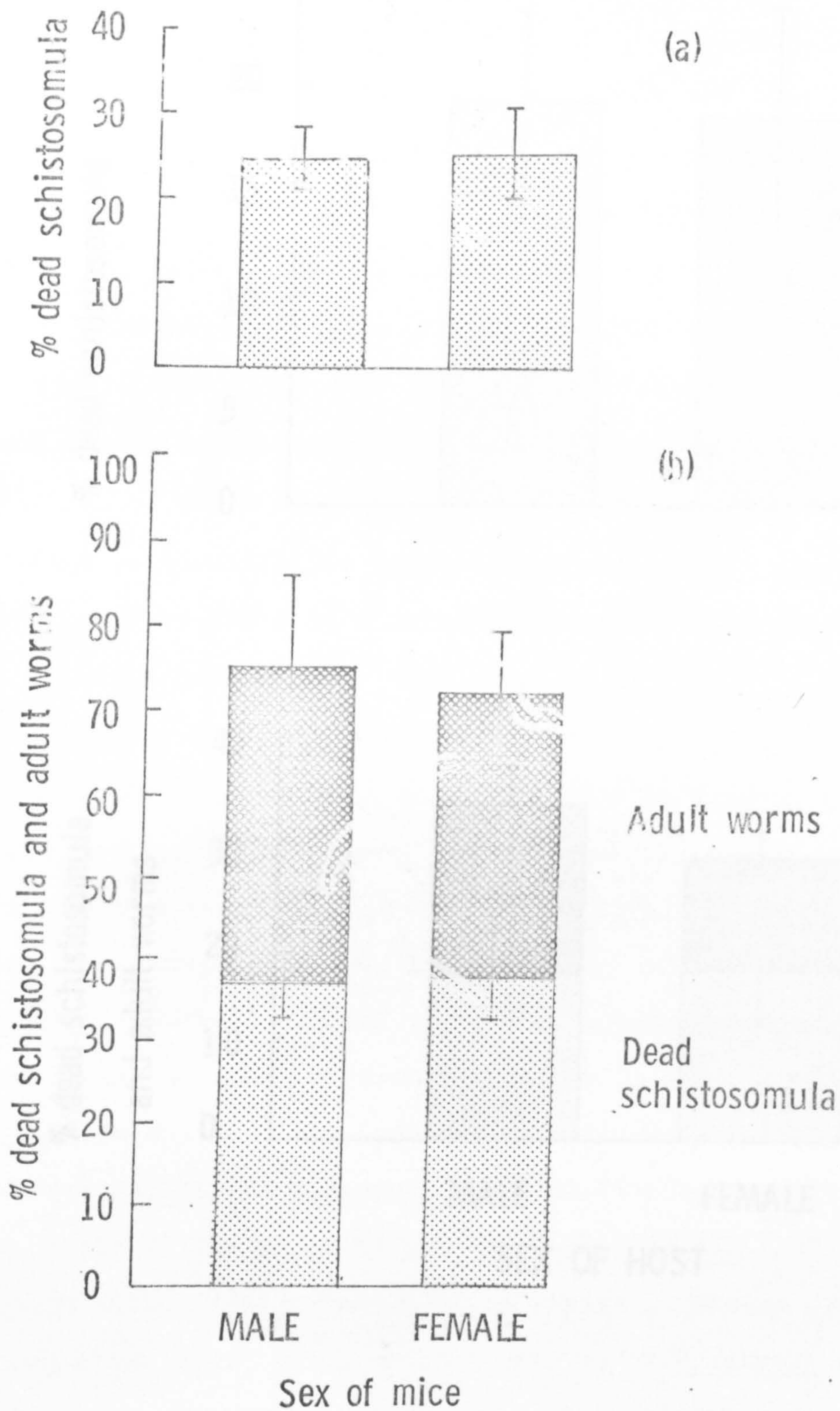


Fig. 4: a Percentages of dead *S. mansoni* schistosomula recovered from the skin of male and female mice.

b Comparison of the percentages of *S. mansoni* cercariae recovered as dead schistosomula from the skin of male and female mice with those recovered as adult worms.

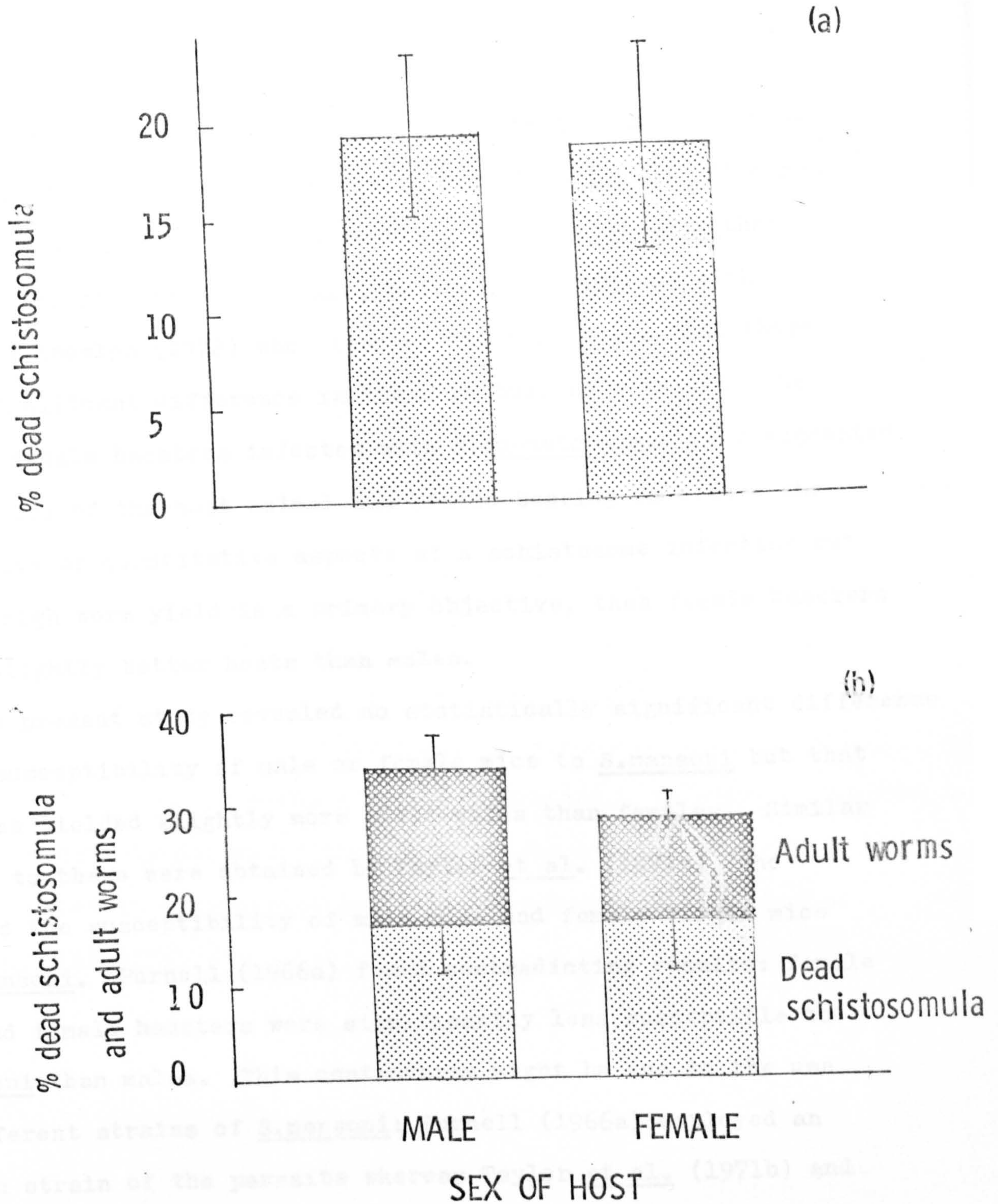


Fig. 5: a Percentages of dead *S. haematobium* schistosomula recovered from the skin of male and female hamsters.

b Comparison of the percentages of *S. haematobium* cercariae recovered as dead schistosomula from the skin of male and female hamsters with those recovered as adult worms.

recovery rates than female hamsters exposed to the same batch of cercariae. This is in accordance with Purnell's (1966a) findings that male hamsters were more susceptible to S.haematobium than females. The present results are, however, in variance with Wright and Knowles (1972) who reached the conclusion that there was no significant difference in the worm burdens obtained from male and female hamsters infected with S.haematobium. They suggested that the sex of the host animal has little bearing on either the qualitative or quantitative aspects of a schistosome infection but that if high worm yield is a primary objective, then female hamsters may be slightly better hosts than males.

The present study revealed no statistically significant difference in the susceptibility of male or female mice to S.mansoni but that male mice yielded slightly more adult worms than females. Similar results to these were obtained by Taylor et al. (1971b) who compared the susceptibility of male mice and female virgin mice to S.mansoni. Purnell (1966a) found contradicting results: female mice and female hamsters were significantly less susceptible to S.mansoni than males. This controversy might be due to the use of different strains of S.mansoni: Purnell (1966a) employed an African strain of the parasite whereas Taylor et al. (1971b) and the present author used a Puerto Rican strain. Recent studies have shown clear differences between strains of S.mansoni and S.haematobium in their infectivity and pathogenicity to mammalian hosts (Saoud, 1965; 1966a; Wright and Bennett, 1967a, b; Wright and Knowles, 1972 and Webbe and James, 1971b).

Differences in the susceptibility of male and female hamsters to S.haematobium is not due to variations in the mortality of cercariae during penetration of host skin. Almost equal numbers of cercariae died in the skin of male and female animals. This may indicate that migrating schistosomula might be subjected to different physiological conditions within different sexes of the host. In fact there is evidence that the hormonal balance of the host is responsible for differential susceptibility of different sexes of host to schistosome infection. Taylor et al. (1971b) found that lactating mice were resistant to infection with S.mansoni and they suggested that hormonal influences associated with lactation may affect susceptibility of mice to schistosome infection. Berg (1957a,b) found that, in castrated animals, the migration of S.mansoni worms might be inhibited. In other helminths, Mathies (1959) demonstrated that gonadectomy of either sex of mice resulted in a reduction in their worm burden when infected with A.tetraptera.

PART 4: The effect of prior infection of the host on the proportion of S.mansoni cercariae which die during penetration of the skin

Introduction

Various workers have shown that animals with an established schistosome infection can be very resistant to reinfection (see review by Smithers and Terry, 1969). This resistance seems to be partly located in the skin since the inflammatory responses of the skin of previously infected animals are more intense, localized and developed earlier than in the skin of normal animals (Sueyasu, 1920; Watari, 1936; Kagan and Meranze, 1955; Magalhaes and Coelho, 1957; Lin and Sadun, 1959; Lichtenberg and Ritchie, 1961; Davis et al., 1963; Radke and Sadun, 1963 and Hsu et al., 1971). Clegg and Smithers (1968) have shown that in rats immunized with S.mansoni there was a noticeable increase in the level of death of challenging S.mansoni cercariae during penetration of the skin. However they related this increase in the level of death to local action of the skin, due to previous exposure to large numbers of immunizing cercariae, rather than to specific cell-mediated or humoral-mediated immune responses. Conversely, Colley et al. (1972) have recently shown a very clear immune dermal reaction in mice, previously infected with S.mansoni, to a challenging infection of cercariae or cercarial extract.

The present study was designed to find out if the presence of an already established infection with S.mansoni affects the proportion of cercariae which die during penetration of the skin of mice.

Materials and Methods.

Two groups of male adult mice (4-5 weeks old) were used in this experiment. The first was infected with 50-60 cercariae per mouse and the second was kept as uninfected control group. Ten weeks later, when the infection was mature, both groups of mice were simultaneously challenged with 1000 cercariae/mouse. Schistosomula were recovered from the skins of these animals at 15 min., 24 and 48 hours after challenge and the proportion of them dying in the skin during penetration was compared in both groups.

Results.

Table 11 and Fig. 6 summarize the results of this experiment. It is evident that the mean percentages of dead schistosomula recovered from the skin of previously infected animals, 15 min. after challenge, was significantly higher ($t=10.9$; $p < 0.01$) than in the normal non-infected control animals being 50.3% and 29.8% respectively. This difference persisted during the first two days after challenge, mortality levels of 55.7% and 40.0% being noted at 24 hours after challenge ($t=7.0$; $p < 0.01$) and 53.7% and 42.2% at 48 hours ($t=6.6$; $p < 0.01$) for previously infected and non-infected groups respectively. It is clear that no marked increase was noted in the level of death of cercariae during penetration of the skin of previously infected animals, at 24 and 48 hours (55.7% and 53.7%) over the initial level of death at 15 min. which was 50.3%. Student's t-test of these figures shows no significant difference at all (15 min. v 24 hrs., $t=2.7$; $p > 0.05$) and (15 min. v 48 hrs., $t=1.0$; $p > 0.05$).

Discussion.

The results of this experiment show that there is a large increase in the level of death of schistosomula in the skin of

T A B L E 11

Percentages of dead of *S. mansoni* schistosomula recovered from the skin of immune and normal non-infected mice challenged with 1000 cercariae each.

Time after challenge	Non-Infected Mice				Previously Infected Mice			
	No. of animals schistosomula	Mean % dead	Range	S.D. ± S.E. ±	No. of animals schistosomula	Mean %	Range	S.D. ± S.E. ±
15 minutes	20	29.8	25-47	6.1 1.4	20	50.3	40-59	5.8 1.3
24 hours	20	40.0	29-50	7.2 1.6	20	55.7	44-65	6.6 1.5
48 hours	20	42.2	33-50	4.9 1.1	20	53.7	42-66	5.8 1.3

15 min. 'Infected' v 15 min. 'Non-Infected'
 Degrees of freedom 38
 t 10.9
 p < 0.01

24 hrs. 'Infected' v 24 hrs. Non-Infected'
 Degrees of freedom 38
 t 7.03
 p < 0.01

48 hrs 'Infected' v 48 hrs. 'Non-Infected'
 Degrees of freedom 38

t 6.61
 p < 0.01

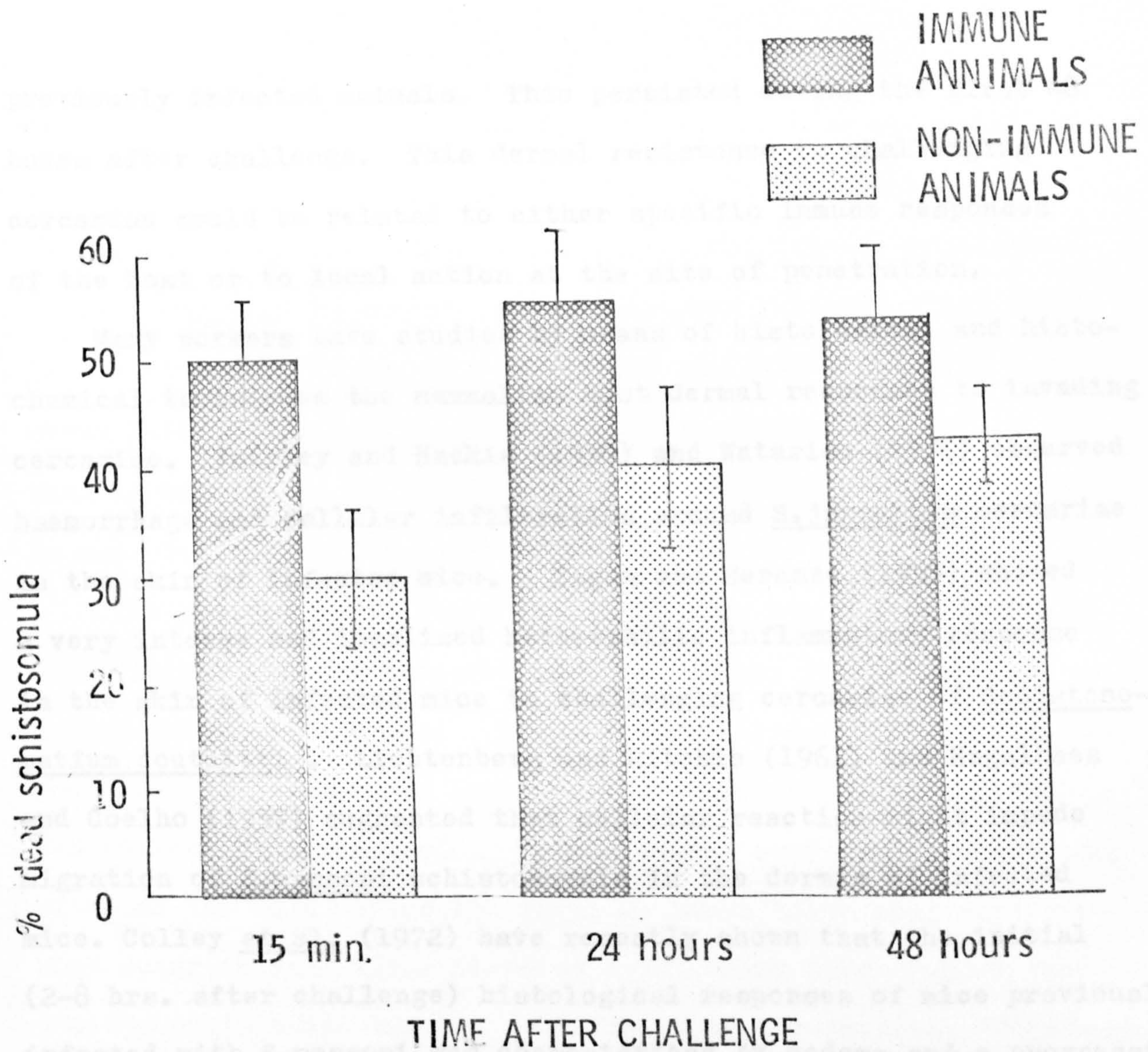


Fig. 6: Percentages of dead S.mansoni schistosomula recovered, at 15 min. 48 hours after infection, from the skin of immune and normal non-immune mice.

previously infected animals. This persisted during the first 48 hours after challenge. This dermal resistance to challenging cercariae could be related to either specific immune responses of the host or to local action at the site of penetration.

Many workers have studied by means of histological and histochemical techniques the mammalian host dermal responses to invading cercariae. Fairley and Mackie (1930) and Watariae (1936) observed haemorrhage and cellular infiltration around S.japonicum cercariae in the skin of infected mice. Kagan and Meranze (1955) showed a very intense and localized heterophilic inflammatory response in the skin of infected mice to challenging cercariae of Schistosomatum douthitti. Lichtenberg and Ritchie (1961) and Magalhaes and Coelho (1957) suggested that cellular reaction might impede migration of S.mansoni schistosomula in the dermis of infected mice. Colley et al. (1972) have recently shown that the initial (2-8 hrs. after challenge) histological responses of mice previously infected with S.mansoni was characterized by oedema and a progressive polymorphonuclear infiltration. By 16 and 24 hours the schistosomula were the foci of intense mixed cell infiltrates and by 36 and 48 hours dense mononuclear infiltrates were evident. Normal mice showed only oedema and slight polymorphonuclear infiltrates and by 16 hours most of the schistosomula had migrated through the dermis. Passive transfer of serum from infected mice immunized normal mice, which then responded with an early (5 hours) polymorphonuclear infiltrates against schistosomula. Lymphoid cells from infected mice passively immunized normal mice which developed a late (30 hours)

mononuclear infiltrate against either cercariae or cercarial extract. It is not, however, clear if cellular response to schistosomula is stimulated by living schistosomula in the dermis, or whether it is a response to schistosomular antigens released after death of schistosomula in the dermis. Clegg and Smithers (1968) have shown that 30% of S.mansoni cercariae die during penetration of skin of uninfected mice and that no histological distinction could be made between living and dead schistosomula.

The present study shows that following the initial level of death in the skin of infected mice no further deaths occurred during the next 48 hours. Clegg and Smithers (1968) have already found similar results in immune rats. They concluded that destruction of schistosomula by the immune reaction does not take place in the skin during the first 24 hours after challenge but must occur at some later stage of development since few of the schistosomula would survive to maturity in immune rats. They suggested that these initial increases in the level of death might be due to a local action at the skin. Stirewalt (1958) has shown that penetration of S.mansoni cercariae into mouse tail skin resulted in transient thickening of the epidermis which may be responsible for the fact that fewer cercariae enter that tail when re-exposed to infection within 2 days. This local action may also take the form of non-specific inflammatory responses and not specific cell-mediated or antibody - mediated responses.

The relationship of the innate defense mechanisms of infected animals to acquired immunity in schistosomiasis is far from being understood and needs further investigation.

CHAPTER IV

EFFECT OF PARASITE-RELATED FACTORS ON THE INFECTIVITY OF THE CERCARIAE OF S. MANSONI AND S. HAEMATOBIIUM

PART 1: The effect of ageing on the infectivity of S. mansonii and S. haematobium cercariae

Introduction

Miller and Edney (1957) first showed that the number of schistosomes (Schistosomatium douthitti) recovered as adult worms from the rat was drastically reduced with increases in the age of the cercariae following emergence from the snail intermediate host. A fuller study was conducted by Olivier (1966) who demonstrated a similar effect with the cercariae of S. mansonii in mice: the infectivity of the cercariae, in terms of the number surviving to mature adult worms, was reduced to half after 8 hours and almost completely by 24 hours. Similar results were obtained by Purnell (1966b) and Stirewalt and Fregeau (1968). However, there is no information about the relationship between the age of S. haematobium cercariae and their infectivity. The objective of this work was to determine whether deaths of S. mansonii and S. haematobium cercariae, during penetration of mammalian host skin, are influenced by the age of the cercariae and whether increases in mortality account for the lower recovery of adult worms from animals infected with old cercariae.

Materials and Methods

Cercariae of S. mansonii and S. haematobium were collected from their snail-intermediate hosts at hourly intervals, and allowed to age under standard aquarium conditions at a temperature of 25- 27°C for 2-24 hours. Two sets of experiments were conducted:

- a) It was first decided to find out if ageing of cercariae of S. mansonii and S. haematobium affected their death during penetration

of mammalian host skin. Cercariae aged 2, 4, 6, 8, 10, 18 and 24 hours were used to infect animals from which schistosomes were recovered from the skin at 15 min. after infection.

b) The second set of experiments was designed to find out if there was any correlation between ageing of S.mansoni and S.haematobium cercariae and their ability to mature into adult worms. A comparison was made between the mortality of cercariae (of both species of parasite) aged 2 and 10 hours and the percentage recoveries of adult worms from animals infected with cercariae of these ages.

Results

Death of cercariae during penetration of host skin: The results of this experiment are shown in Tables 12 and 13 and Figs. 7a and 8a. The level of death of S.mansoni schistosomes increased steadily with increase in the ages of the cercariae. With 2 hour old cercariae the mean percentage of dead schistosomes was only 29.0% but with 18 and 24 hour old cercariae mortality increased greatly to 75.1% and 83.5% respectively- the increases being highly significant ($t=16.2$; $p < 0.01$ and $t=23.8$; $p < 0.01$).

The same situation occurs in S.haematobium: the mean percentages of dead schistosomes when 2 hour old cercariae were used was 16.6% and these increased to 36.5%, 46.0% and 51.9% with 10, 18 and 24 hour old cercariae- the difference between the means and that at 2 hour being highly significant ($t=11.7$; $p < 0.01$; $t=15.5$; $p < 0.01$ and $t=18.6$; $p < 0.01$).

Comparison of death of cercariae in the skin and the adult worm recovery: Table 14 and Fig. 7b show that, in the experiment with S.mansoni, the mean percentage recovery of adult worms from

T A B L E 12

Percentages of dead S.mansoni schistosomula recovered from the skin of adult mice infected with cercariae of increasing age.

Age of cercariae (hours)	No. of animals used	Mean % dead schistosomula	Range	S.D.±	S.E.±
2	10	29.0	23-34	3.9	1.2
4	10	37.5	36-42	3.0	1.0
6	10	45.0	35-51	4.1	1.3
8	10	50.0	40-58	7.1	2.6
10	10	57.6	50-66	7.3	2.3
18	10	75.1	64-84	7.9	2.5
24	10	83.5	70-90	6.1	1.9

4 hrs. v 2 hrs.
Degrees of freedom 18
t 5.2
p < 0.01

6 hrs. v 2 hrs
Degrees of freedom 18
t 8.54
p < 0.01

8 hrs. v 2 hrs.
Degrees of freedom 18
t 8.25
p < 0.01

10 hrs. v 2 hrs.
Degrees of freedom 18
t 10.9
p < 0.01

18 hrs. v 2 hrs.
Degrees of freedom 18
t 16.27
p < 0.001

24 hrs. v 2 hrs.
Degrees of freedom 18
t 23.8
p < 0.001

T A B L E 13

Percentages of dead *S. haematobium* schistosomula recovered from the skin of hamsters infected with cercariae of increasing ages.

Age of cercariae (hours)	No. of animals	Mean % dead schistosomula	Range	S.D.±	S.E.±
2	10	16.6	10-19	2.4	0.7
10	10	36.5	30-46	4.7	1.5
18	10	46.0	37-51	5.2	1.6
24	10	51.9	41-58	5.8	1.7

2 hrs. v 10 hrs

Degrees of freedom 18

t 11.75

p < 0.01

2 hrs. v 18 hrs.

Degrees of freedom 18

t 15.5

p < 0.001

2 hrs. v 24 hrs.

Degrees of freedom 18

t 18.67

p < 0.001

T A B L E 14

Comparison of the percentages of *S. mansoni* cercariae recovered as dead schistosomula from the skin of mice (Infected with 2 and 10 hrs. old cercariae) with those recovered as adult worms.

Age of Cercariae (hrs)	% Dead Schistosomula				% Adult Worms				
	No. of animals	Mean	Range	S.D. ± S.E. ±	No. of animals	Mean	Range	S.D. ± S.E. ±	Mean % cercariae not penetrating
2	10	33.3	23-40	5.6 1.8	20	44.3	25-72	14.2	
10	10	59.1	50-66	6.0 1.9	20	23.0	15-30	4.15	

2 hrs. v 10 hrs.

Degrees of freedom 18

t 9.0

p < 0.01

2 hrs. v 10 hrs.

Degrees of freedom 38

t 6.14

p < 0.01

T A B L E 15

Comparison of the percentages of young and old (2 and 10 hours respectively) S. haematobium cercariae recovered as dead schistosomula from the skin of adult hamsters with those recovered as adult worms.

Age of cercariae (hours)	% Dead Schistosomula			% Adult Worms							
	No. of animals	Mean	Range	S.D. ± S.E. ±	No. of animals	Mean	Range				
2	10	16.6	10-21	2.5	0.8	20	25.2	12-40	7.6	1.7	5
10	10	36.0	28-41	4.7	1.5	20	14.3	8-21	7.1	2.5	12

Mean % cercariae not penetrating

2 hrs. v 10 hrs.

2 hrs. v 10 hrs

Degrees of freedom 18

Degrees of freedom 38

t 11.85

t 3.12

p < 0.01

p < 0.01

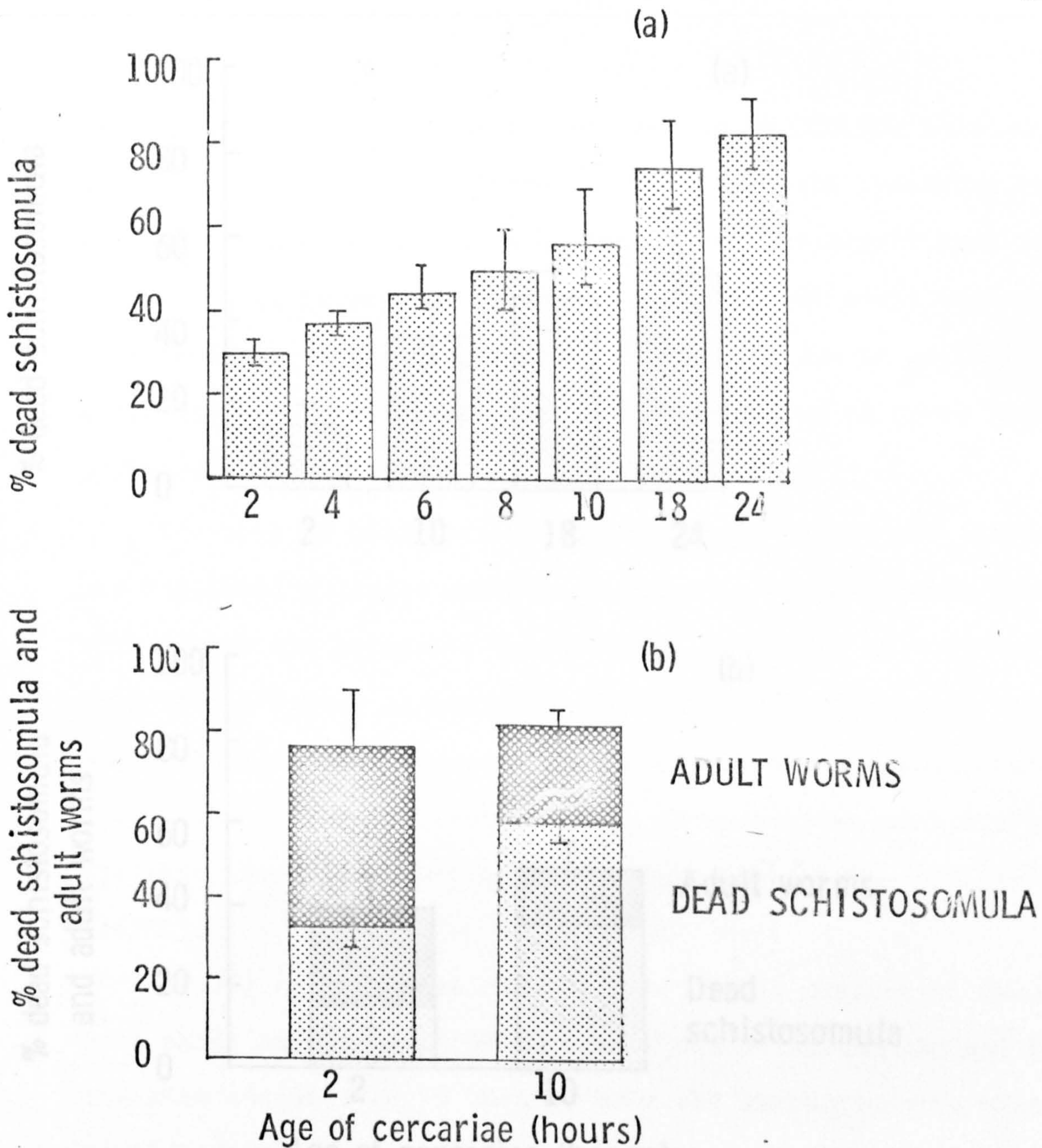


Fig. 7: a Percentages of dead *S.mansoni* schistosomula recovered from the skin of mice infected with cercariae of increasing ages.

b Comparison of the percentages of fresh and old cercariae (2 and 10 hours respectively) of *S.mansoni* recovered as dead schistosomula from the skin of mice with those recovered as adult worms.

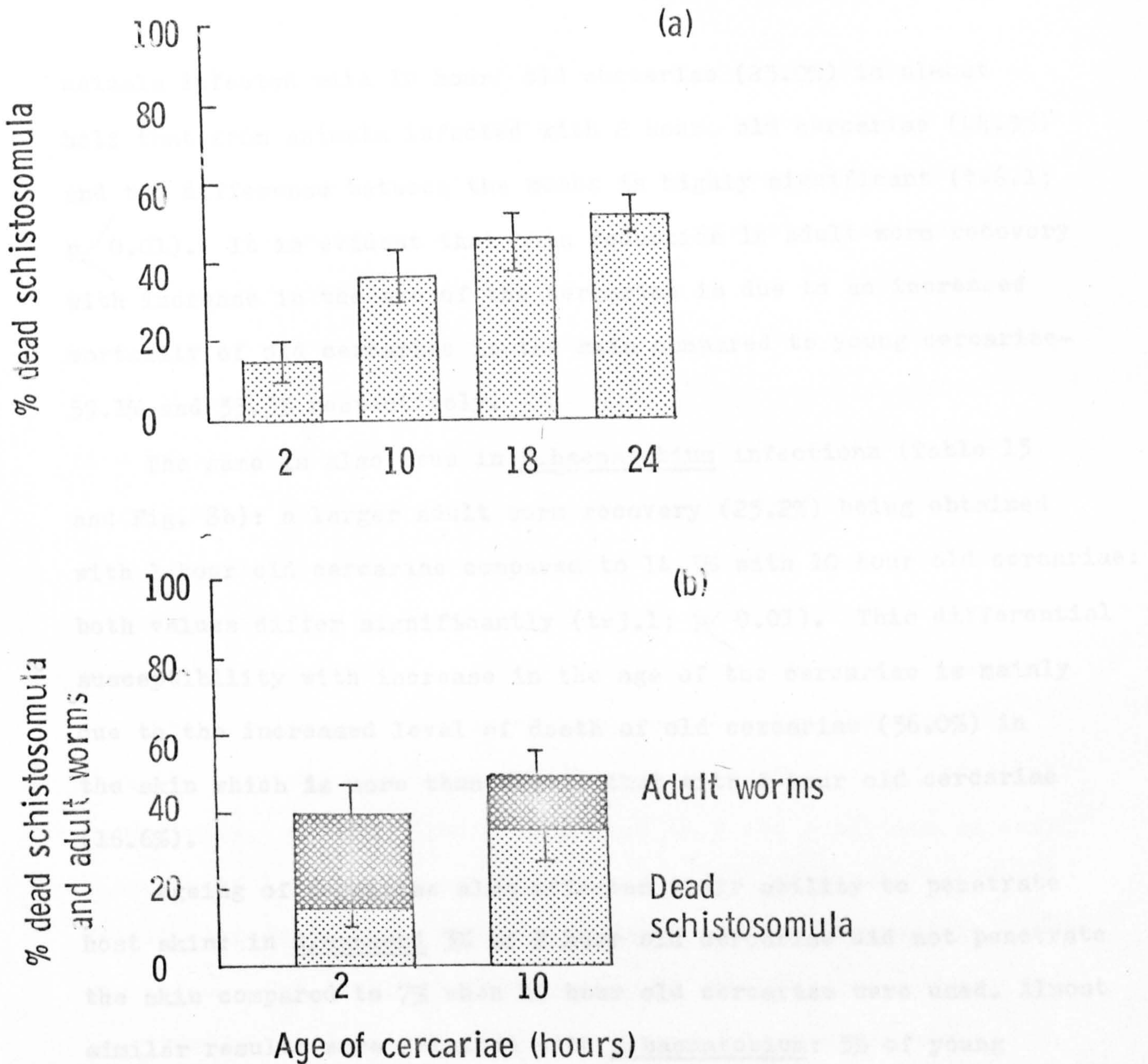


Fig 8: a Percentages of dead *S. haematobium* schistosomula recovered from the skin of hamsters infected with cercariae of increasing ages.

b Comparison of the percentages of fresh and old cercariae (2 and 10 hours respectively) of *S. haematobium* recovered as dead schistosomula from the skin of hamsters with those recovered as adult worms.

animals infected with 10 hour old cercariae (23.0%) is almost half that from animals infected with 2 hour old cercariae (44.3%) and the difference between the means is highly significant ($t=6.1$; $p < 0.01$). It is evident that this reduction in adult worm recovery with increase in the age of the cercariae is due to an increased mortality of old cercariae in the skin compared to young cercariae—59.1% and 33.3% respectively.

The same is also true in S. haematobium infections (Table 15 and Fig. 8b): a larger adult worm recovery (25.2%) being obtained with 2 hour old cercariae compared to 14.3% with 10 hour old cercariae: both values differ significantly ($t=3.1$; $p < 0.01$). This differential susceptibility with increase in the age of the cercariae is mainly due to the increased level of death of old cercariae (36.0%) in the skin which is more than double that with 2 hour old cercariae (16.6%).

Ageing of cercariae also affected their ability to penetrate host skin: in S. mansoni 3% of 2 hour old cercariae did not penetrate the skin compared to 7% when 10 hour old cercariae were used. Almost similar results were obtained with S. haematobium: 5% of young cercariae did not penetrate the skin while 12% of old cercariae failed to penetrate.

Discussion

This study shows that ageing of S. mansoni and S. haematobium cercariae after emergence from the snail-intermediate host is directly related to losses in the skin of the definitive host soon after penetration. As the cercariae age the percentages dying in the skin rises steadily and after 10 and 24 hours, respectively, mortality is almost doubled and trebled. These results correlate

well with Olivier's findings (1966) that S.mansoni cercariae lose half and almost all their infectivity 8 and 24 hours after emergence from the snail, judged by the number which survive to become adult worms in the hepatic portal system. The inference that the loss of infectivity observed by Olivier (1966) is due to increased mortality of older cercariae in the skin was confirmed experimentally (Fig. 7band 8b). The results on S.haematobium cercariae show that they behave similarly to S.mansoni : after 10 hours their infectivity is reduced to half and this is due to the increased mortality of such cercariae in the skin. Ageing of cercariae also interferes with their ability to penetrate into the host's skin and these results are in accordance with Stirewalt and Fregeau (1968).

The results of the present study confirm the observation that the post-emergence age of schistosome cercariae affects their infectivity. Olivier (1966) suggested that the depletion of energy reserves of S.mansoni cercariae during their short non-feeding free-living state could account for decreased infectivity in older cercariae. The level of glycogen is, in fact, almost reduced to one-fourth of the initial value in S.mansoni cercariae which have been swimming in water for 18 hours after emergence from the snail (Bruce et al., 1969). These findings suggest that a low level of energy reserves may be, in part, responsible for decreased infectivity of cercariae with increase in age, but the evidence is circumstantial and the next experiment was thus designed to investigate this question.

PART 2: Relationship between the energy contents of *S.mansoni* cercariae and their death during penetration of mouse skin.

Introduction

Schistosome cercariae are free-living organisms with glycogen reserves stored in cells surrounding the penetration glands and in the tail (Stirewalt and Kruidenier, 1961 and Rai and Clegg, 1968). They accumulate these reserves while still embedded in sporocysts within the snail-intermediate host (Cheng and Synder, 1962). As soon as the cercariae emerge from the snail they start using these reserves- the size of which determines their longevity (Ginetsinskaja, 1960).

Work done by Rai and Clegg (1968) suggested that there might be a relationship between the glycogen reserves of schistosome cercariae and the proportion of them which die during penetration of host skin. They found that dead bird schistosomula of *A. terri-galensis*, appeared to have used 80% of the glycogen reserves originally present in the cercariae while living ones used only 30%: it is this depletion of energy reserves which might be responsible for death of cercariae during penetration of host skin.

The purpose of the present experiment was therefore to determine whether the level of death of *S.mansoni* cercariae, during penetration of mouse skin could be reduced by increasing the energy reserves of the cercariae while they are still within the snail.

Materials and Methods

A batch of 60 infected *B.glabrata* was divided into two equal groups. The first was kept in 2% solution of glucose under semi-sterile

conditions by wiping the snail' shells with 70% ethanol, rinsing the snails in five changes of distilled water and placing them in the dark in a clean-air hood at 25-27°C. The second group was similarly treated but maintained in "conditioned" aquarium water and fed on lettuce. After two days, both groups of snails were washed in distilled water and allowed to shed for 2 hours in aquarium water. The cercariae collected from both groups of snails were immediately used for the recovery of schistosomula from mice (4-5 weeks old) as described previously.

The two groups of snails were then each separately maintained in conditioned aquarium water and fed on lettuce. Freshly shed cercariae collected from these snails at two daily intervals, until day 8 after the start of the experiment, were used for the recovery of schistosomula from mouse skin.

Results

Table 16 and Fig. 9 summarize the results of this experiment. The mean percentages of dead schistosomula recovered from the skin of mice infected with "glucose-fed cercariae" on day 2 is much lower (18.9%) than that from mice infected with normal untreated cercariae (32.6%) and the difference between the two means is evidently highly significant ($t=8.0$; $p < 0.01$). This reduction in mortality is also observed on day 4, 20.2% and 28.6% of the infecting cercariae died when treated and untreated cercariae were used respectively. However, on days 6 and 8 the mortality of treated cercariae returned to normal being 28.3% and 31.9% respectively and for control untreated cercariae being of the values 30.3% and 33.6%. No

T A B L E 16

Comparison of the percentages of dead *S. mansoni* schistosomula recovered from the skin of mice infected with normal and with glucose-fed cercariae.

Days after Glucose- Feeding	Control		Cercariae		Glucose-Fed		Cercariae			
	No. of animals	% Dead schistosomula	S.D.±	Range	S.E.±	No. of animals	% Dead schistosomula	S.D.±	Range	S.E.±
2	20	32.6	5.9	25-40	1.5	20	18.9	3.2	11-24	0.8
4	20	28.6	4.2	23-34	1.0	20	20.2	4.4	13-22	1.1
6	20	30.3	4.8	23-36	1.0	20	28.3	5.4	14-32	1.3
8	20	33.6	5.5	25-41	1.4	20	31.9	5.9	24-44	1.5

2 days Control v 2 days Glucose-Fed
 Degrees of freedom 38
 t 8.08
 p < 0.01

4 days Control v 4 days Glucose-Fed
 Degrees of freedom 38
 t 5.69
 p < 0.01

6 days Control v 6 days Glucose-Fed
 Degrees of freedom 38
 t 0.64
 p > 0.05

8 days Control v 8 days Glucose-Fed
 Degrees of freedom 38
 t 0.926
 p > 0.05

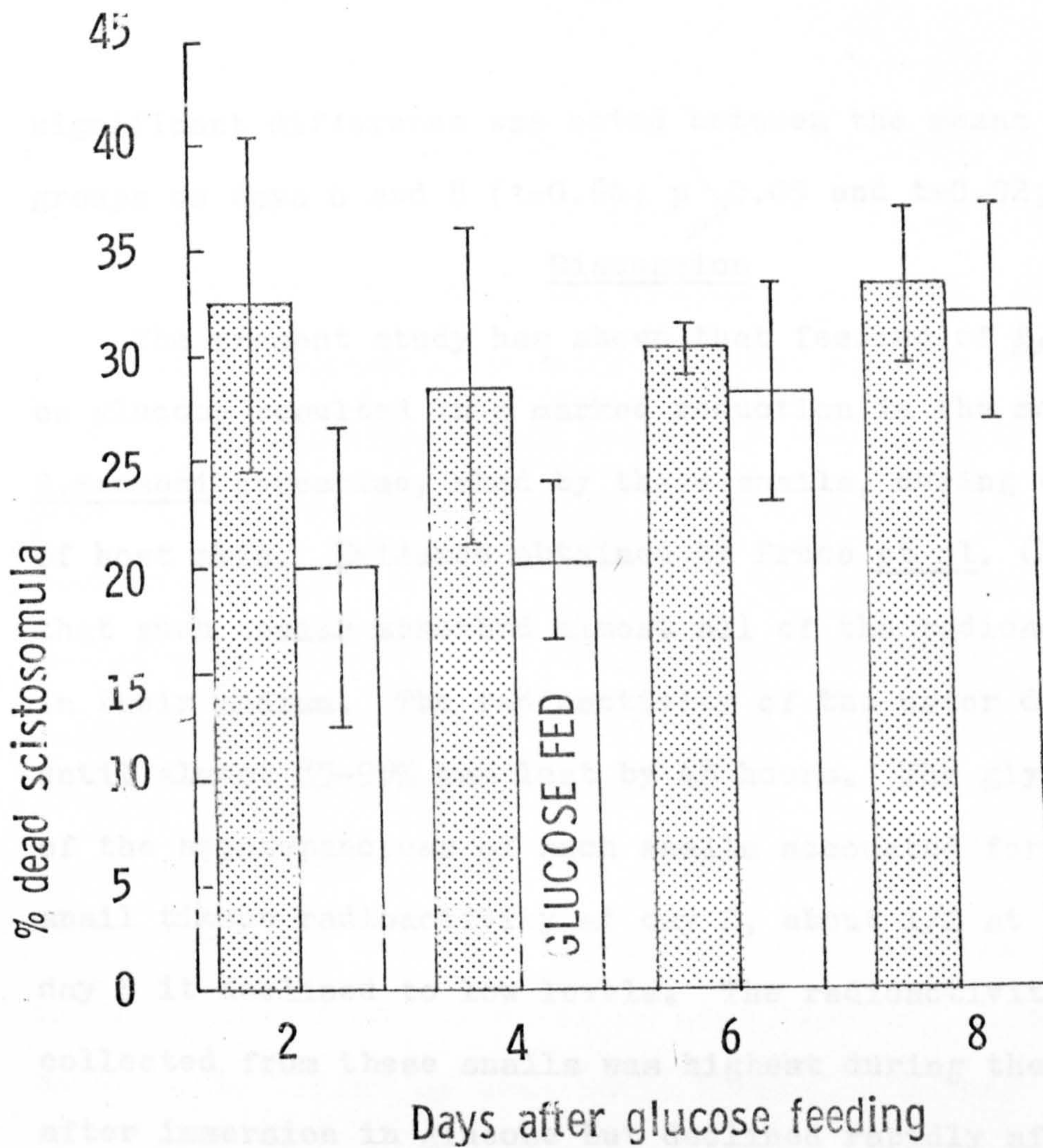


Fig. 9 : Percentages of dead S.mansoni schistosomula recovered from the skin of mice infected with "glucose-fed cercariae" and with normal untreated cercariae.

significant difference was noted between the means of these two groups on days 6 and 8 ($t=0.64$; $p > 0.05$ and $t=0.92$; $p > 0.05$).

Discussion

The present study has shown that feeding of B.glabrata snails on glucose resulted in a marked reduction in the mortality of S.mansoni cercariae, shed by these snails, during their penetration of host skin. Evidence obtained by Bruce et al. (1969) has shown that such snails absorbed almost all of the radioactive glucose in their medium. The radioactivity of the water decreased gradually until almost 85-95% was lost by 48 hours. The glycogen contents of the hepatopancreas of such snails accounted for 1/6 of the total snail tissue radioactivity at day 2, about 1/2 at day 5 but by day 8 it declined to low levels. The radioactivity of cercariae collected from these snails was highest during the first few days after immersion in glucose but declined rapidly afterwards. Christie et al. (1974) have also shown that B.glabrata absorbed glucose containing radioactive ^{14}C from the surrounding medium and that this radioactivity was passed on to the shed cercariae.

Clegg and Smithers (1968) could not conclusively determine the cause of death of S.mansoni cercariae during penetration of host skin. The possibility that cercariae might develop sensitivity to water during the first 10 min. of penetration (due to transition to water sensitive schistosomula) and die because they were still in contact with water in the keratin layer of the epidermis was eliminated. This was shown by the fact that a high proportion of the cercariae died when they were allowed to penetrate skin from a medium of balanced saline instead of water. These authors suggested

two possible hypotheses for explaining the death of cercariae in the skin. This might be due to the presence of a toxic substance in the skin in which case the epidermis of mice and rats (more resistant to cercarial penetration than hamster skin) might contain more of this substance than hamster's skin. Alternatively, cercariae might die due to exhaustion of their energy reserves during the very active phase of penetration of the skin. Rai and Clegg (1968) conducted some studies on the mechanism of death of cercariae of A. terrigalensis during penetration of host's skin. They demonstrated that the mechanism of death is not due to some physiological deficiency of the penetrating cercariae, such as inability to adjust to sharp changes of temperature or osmotic pressure involved in transition from sea water to host tissues. Schistosomula might, however, be killed by some toxic humoral substance or due to exhaustion of energy reserves of the cercariae during penetration. They have shown that the percentages of cercariae which died during penetration of isolated epidermis was related to the thickness of the barrier: 5-6% only died during penetration of one or two layers but penetration of three layers increased the level of death during penetration to 16% and of four layers to 37% i.e. almost equal to the level of death during penetration of intact host skin. These authors attempted to reduce the proportion of death of cercariae of A. terrigalensis during penetration of host skin, by increasing the glycogen reserves of the cercariae by incubation of freshly shed cercariae in 2% glucose solution. However, this treatment did not decrease the proportion of deaths of cercariae during penetration. They suggested that cercariae were unable to absorb glucose from

the surrounding medium or to synthesize glycogen from absorbed glucose. In fact, Bruce et al. (1969) have shown that young S.mansoni cercariae (3 hours old) do not absorb exogenously supplied glucose due to the availability of the huge masses of stored glycogen. However, old cercariae (18 hours old) swimming freely in water have lost almost 70% of the original reserves and can then absorb glucose from the surrounding medium.

It is most likely therefore that S.mansoni cercariae collected from "glucose-fed" snails had a very high level of energy which was exhibited by a marked reduction in their mortality during penetration of host skin.

CHAPTER V

DEVELOPMENT OF S. HAEMATOBIIUM IN VIVO

Introduction

Few detailed studies have been conducted on the intramammalian phase of development of schistosomes. Miyagawa(1912) was one of the first pioneers in this field of study: he recognized two different stages of development of S. japonicum in mice- the "closed gut form" in which haematin pigments were seen in the well developed gut and longer worms, over 1.0 mm. long, in which the sex glands were well developed. Cort (1921) studied the in vivo development of S. japonicum in mice and rabbits and noted that there was a range of individuals recovered on any one day thus suggesting that the worms take different times in maturing. He estimated that it took 28 days for the development of sexual maturity. Faust and Meleny (1924) and Faust et al. (1934) classified 24 stages of development of S. japonicum in mice and rabbits and they used the Greek alphabet to designate these stages. The earliest stage described by them was found in the peripheral lymph nodes, the second stage was in the inguinal lymph nodes and lungs while all other stages were recovered from the liver.

Clegg (1965) was the first to study in a systematic and detailed nature the development of S. mansoni in mice. He divided the development into six stages each readily recognizable by morphological, cytological or histochemical criteria. The first stage was the "lung form" (7 days) and this feeds on red blood cells and gives rise to pigment formation but mitosis was not noticed. Mitosis takes place and gut formation occurs in the second stage, "Closed gut form" (day 15). In stage 3 or "Organogeny" (21 days)

the sexes can be differentiated - males develop 2 testes and lateral extensions of the body and females possess a narrow uterus. Stage 4 (28 days) is characterized by gametogeny in which mating occurs and the males have developed 8 testes containing spermatozoa and the females possess a small ovary. In stage 5 (30 days) eggshell protein is synthesized by the vitelline cells which give a bright orange reaction with diazo salts. In stage 6 eggs are produced on the 34 - 35 th days and migration of paired schistosomes to mesenteric veins of the host occurs.

Similar studies have not been reported for S.haematobium. The only observation on the intra-mamalian phase of development of S.haematobium is that of Leiper (1915a) who made drawings of a series of developmental stages but he did not describe or compare the stages nor did he distinguish the sexes. He found that it took two months for the parasite to develop into mature egg-producing adults.

Studies of the in vivo or in vitro development of schistosomes are valuable in drug screening tests and in immunological investigations. The paucity of information on host-parasite relationships in S.haematobium led to the present study which describes the development of S.haematobium in vivo using the hamster as a definitive host.

Materials and Methods

Groups of hamsters were infected with 300 - 400 cercariae each. As many schistosomula as possible were recovered at daily intervals from the skin, lung and liver of these animals:

(a) Skin recovery: was carried out, 15 min. after penetration according to Clegg and Smithers (1968) technique.

(b) Lung recovery: was performed at daily intervals from day 3 to 11 postinfection from a group of 20 animals.

(c) Liver recovery: Schistosomula were collected from the liver, from one animal per day, from days 12 to 63 postinfection. The early stages of development in the liver were very minute and were collected as follows:

The animals were killed by an intra-peritoneal injection of heparinized Nembutal (10 units heparin / 1 ml. of Nembutal) and perfused with heparinized Hanks's saline according to Smithers and Terry (1965a). The whole perfused blood was collected in a beaker and centrifuged in 30 ml. 'Sterilin tubes' for 1 min. at 3000 r.p.m. The supernatant was pipetted off leaving a 2 ml. sediment of blood. This was lysed by the addition of 10 ml. of distilled water followed immediately by 10 ml. of x2 Hanks's saline. This was done twice until the suspension was clear. The schistosomula were then concentrated in 2 ml. of suspension by pipetting off the supernatant.

From day 22 - 63 the schistosomula were large and were recovered from the liver by ordinary perfusion collecting them on a wire mesh sieve. Whenever any stage of development was recognized this was confirmed in at least four more animals.

Different stages of development were recovered on any one day but only the most fully developed worms were examined - thus this study represents the "optimum" rate of development of S. haematobium in the hamster. Although there was a wide variation in the rate of development yet the "optimum" rate was always constant and occurred on the same day.

Camera lucida drawings and measurements were made from at least 25 specimens of each stage recognized. Schistosomula recovered from the skin and lung were relaxed by keeping them overnight between wet blotting paper in a refrigerator at 4°C. All other stages were fixed under slight pressure in 70% ethanol. Stages 1 and 2 were examined unstained but stages 3 and 4 were stained in aceto-orcein or acetic acid alum-carmin and stages 5 and 6 in Fast Red.

Aceto-orcein: Schistosomula were stained in a filtered saturated solution of the stain for 20 min. at 37°C. They were then differentiated in acid alcohol, dehydrated in increasing grades of alcohol, cleared in xylene and mounted in D.P.X.

Acetic acid alum-carmin: The specimens were stained in a 1 : 2 dilution of the stain for 20 - 60 min. depending on the size of the specimens. They were then processed in the usual way and mounted in D.P.X.

Fast Red: Specimens were stained in a 1% freshly prepared filtered solution of the stain and processed as for the above stains.

Results

Six stages of development were recognized:

Stage 0: "Skin form or freshly penetrated schistosomulum." The cercariae of S. haematobium have preacetabular and postacetabular penetration glands (Plate 9) whose contents are evacuated during penetration of host skin and transformation to schistosomula. These organisms measure (162X40 u) and possess physiological and morphological characteristics similar to those of other schistosomes (Stirewalt, 1963; and Clegg and Smithers, 1968).

Stage 3 (7 days) "Gang form": was found in the lungs from the 3rd day post-infection onwards. Their numbers steadily increased until peak concentrations were reached on the 9th day post-infection, and then declined to low levels on the 11th day (Fig. 10). These forms showed some signs of feeding on red blood cells since

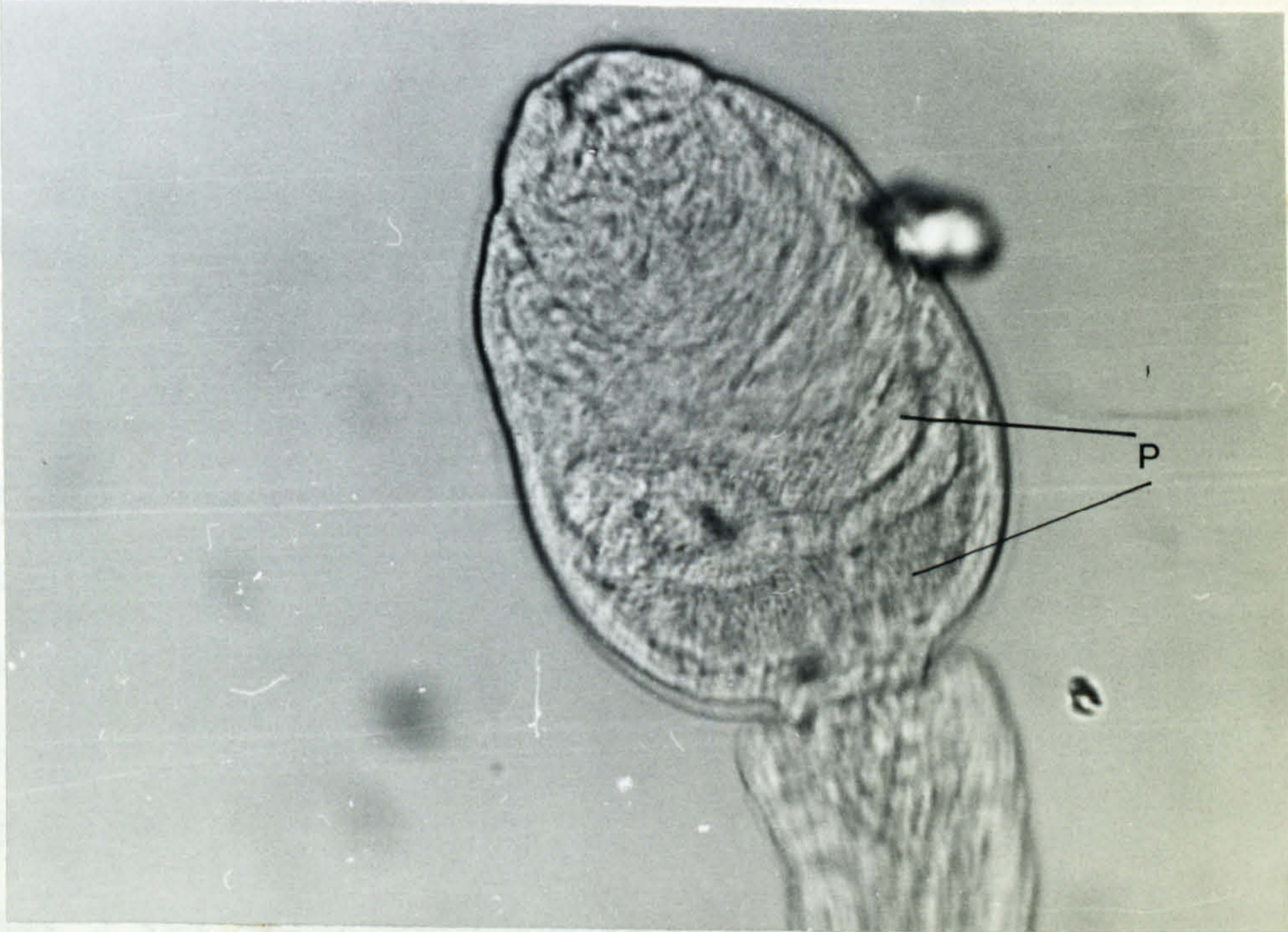


PLATE 9: Anterior end of S. haematobium cercaria. Penetration glands (P) can be seen. X 400.

differentiated by distinctive morphology. Males are larger than females and have developed the lateral extensions of the body extending from the ventral sucker (Plate 15).

Stage 4 (28 days) "Gonotogony": This is the first day when mating had occurred and females were enclosed in the gynaeophoric sheath of the male (Plate, 16). The females have a small ovary, an oviduct and a well developed uterus (Plate 17). Males are still larger than the females and have developed four testes containing spermatocytes recognizable in cross-section as elongated structures

Stage 1 (9 days) "Lung form": was found in the lungs from the 3rd day post-infection onwards. Their numbers steadily increased until peak concentrations were reached on the 9th day post-infection, and then declined to low levels on the 11th day (Fig. 10). These forms showed some signs of feeding on red blood cells since black pigment (probably haematin) was seen in their gut (Plate 10). They are longer than the skin form measuring 260X 30 u.

Stage 2 (18 days) "Closed-gut form" . Schistosomula were found in the liver from the 9th day post-infection onwards. The gut started developing as two short caeca on either side of the ventral sucker with masses of haematin pigment in them showing that feeding on red blood cells had started. Increase in size gradually occurred during the next few days and on day 18 the gut caeca had completely joined behind the ventral sucker. Males (Plate 11) are larger in size than females (Plate 12).

Stage 3 (24 days) "Organogeny": At this time the worms were easily differentiated by functional morphology. Males are larger than females and have developed the lateral extensions of the body and one small testis is just visible behind the ventral sucker (Plates 13 and 14). Females have developed a narrow uterus extending from the ventral sucker (Plate 15).

Stage 4 (28 days) "Gametogeny": This is the first day when mating had occurred and females were enclosed in the gynaecophoric canal of the male (Plate, 16). The females have a small ovary, an ootype and a well developed uterus (Plate 17). Males are still larger than the females and have developed four testes containing spermatozoa recognizable in aceto-orcein squashes of the worm (Plate 18)



PLATE 10: A living S. haematobium schistosomulum recovered from the lungs of a hamster at 9 days post-infection. X 200.

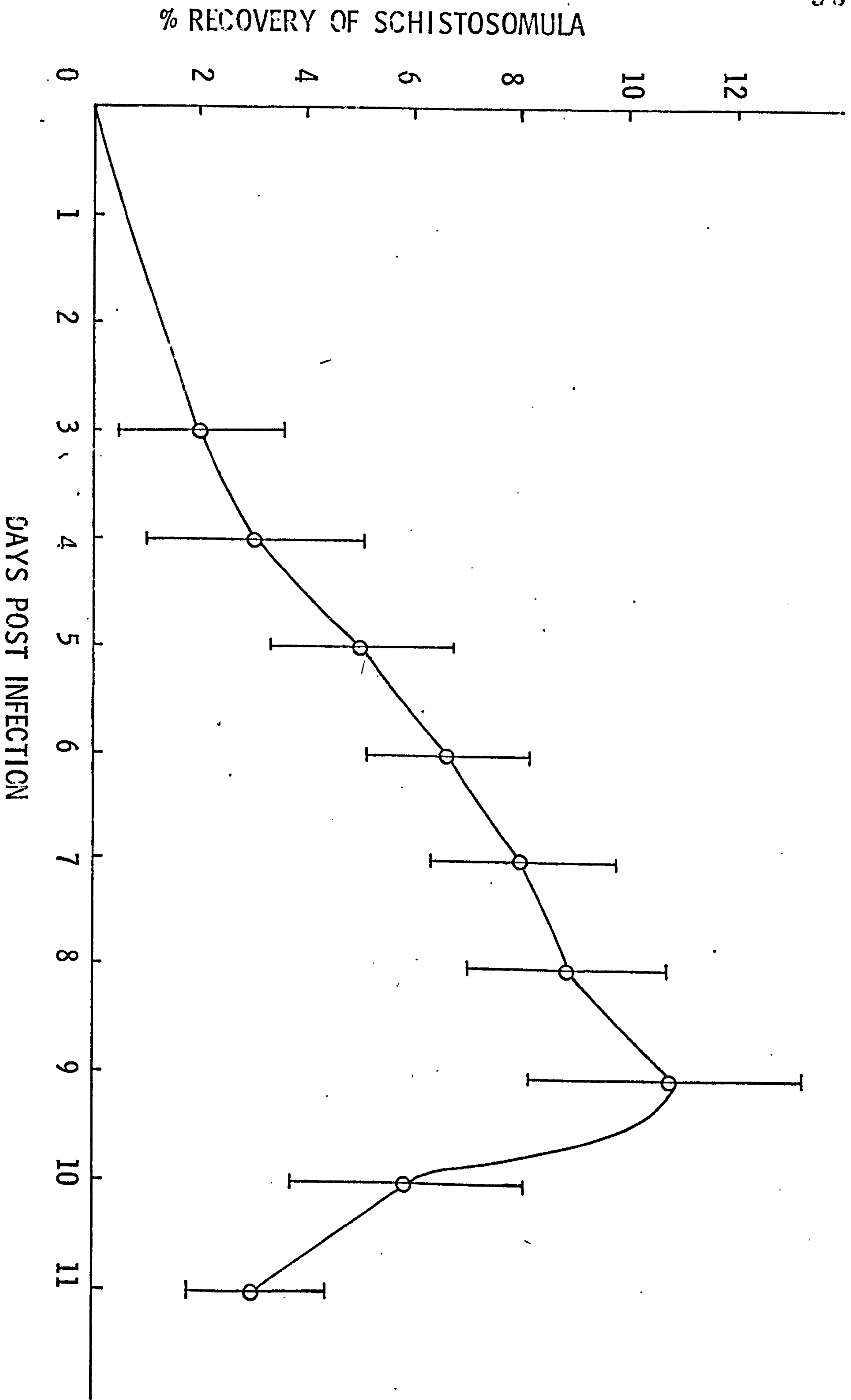


Fig. 10: Percentages of *S. haematobium* schistosomula recovered, at daily intervals, from the lungs of hamsters infected with freshly shed cercariae.



PLATE 11: Male S.haematobium schistosomulum ("Closed-gut" form) recovered from the liver of a hamster at 18 days post-infection. X 200.

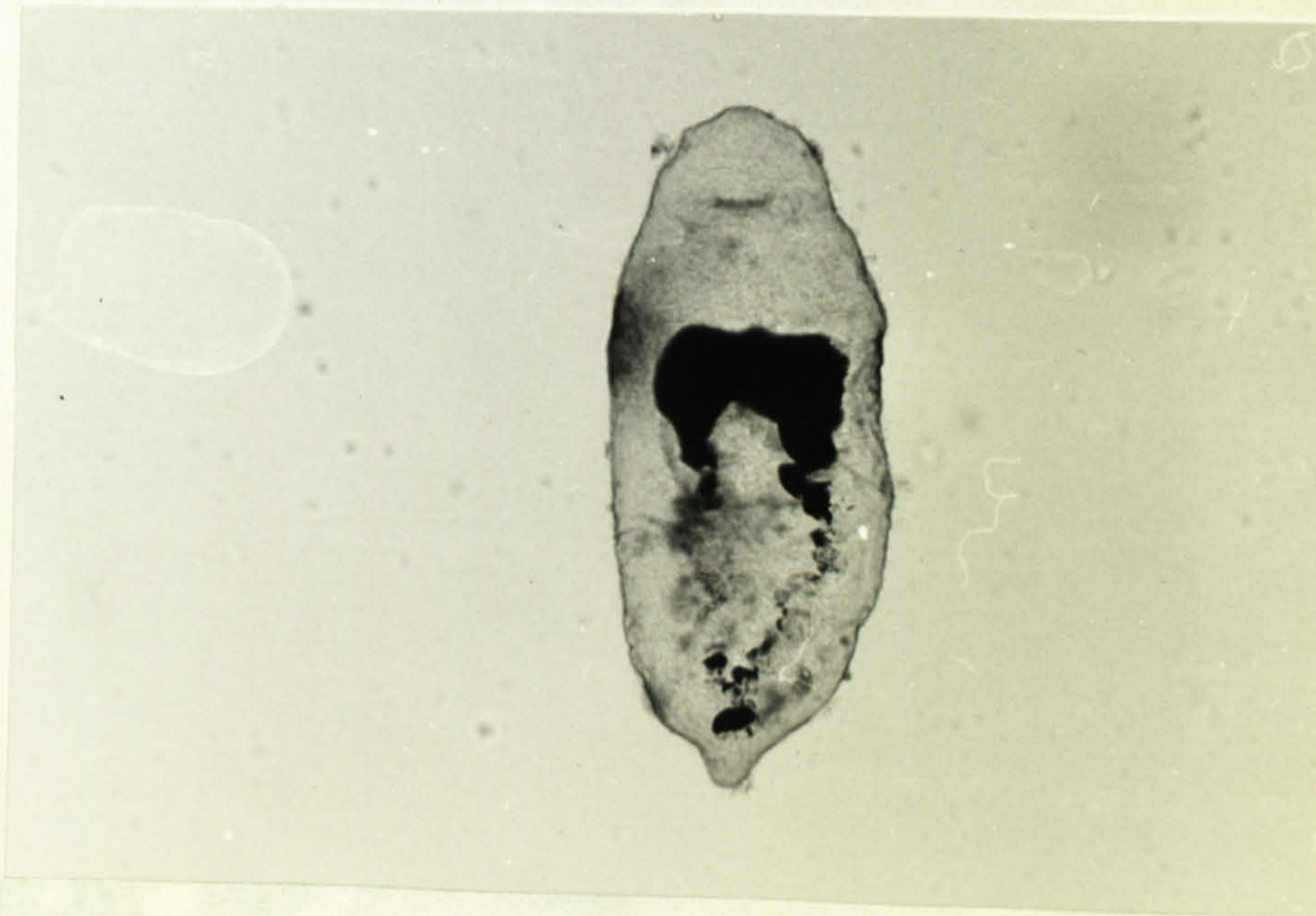


PLATE 12: Female S.haematobium schistosomulum ("Closed-gut" form) recovered from the liver of a hamster at 18 days post-infection. X 200.



PLATE 13: Stage 3 ("Organogeny"- day 24). Male S. haematobium worm showing one small testis (T). (Acetic acid alum-carminc stain) X 100.

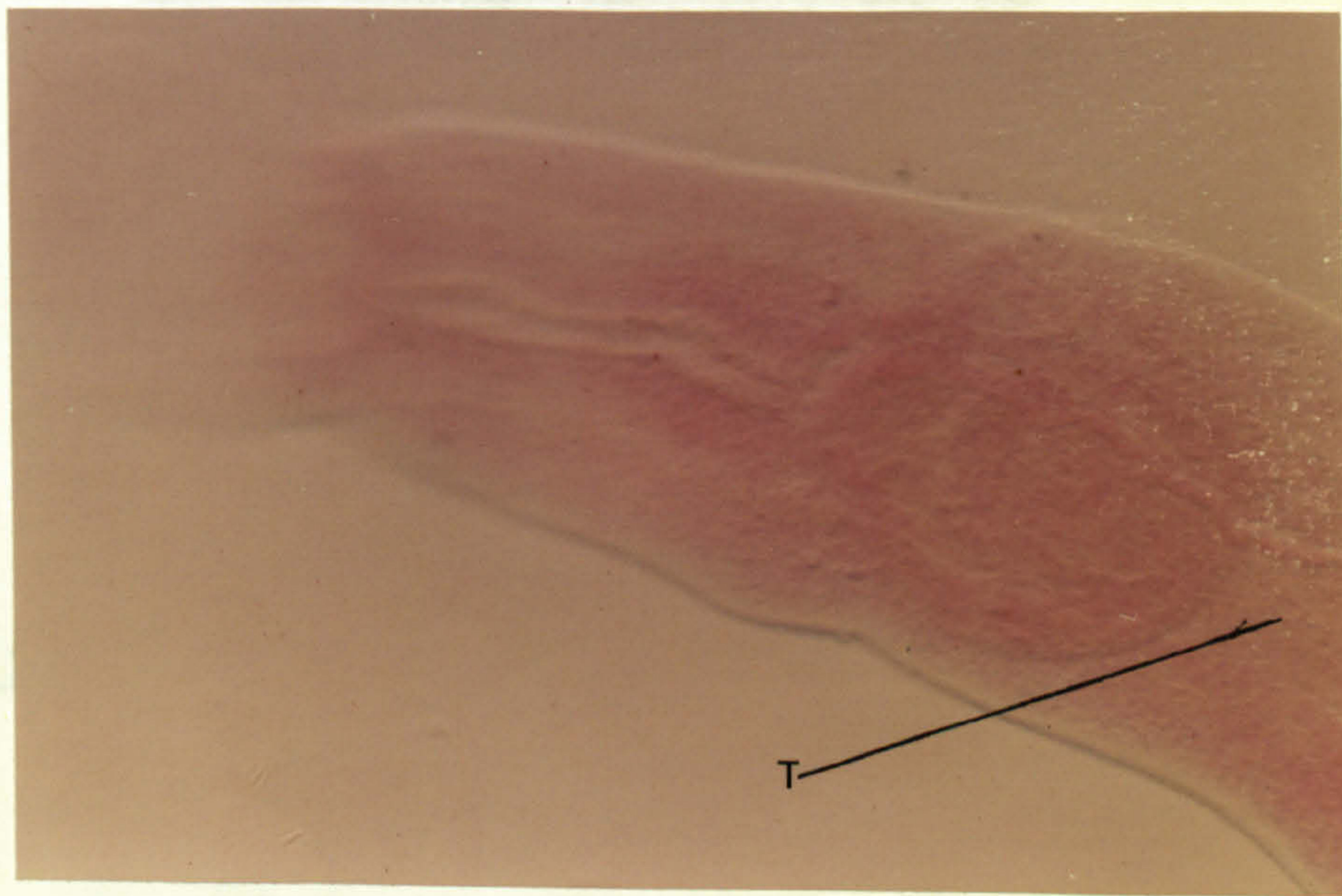


PLATE 14: Stage 3 ("Organogeny"- day 24). Anterior end of male S. haematobium worm showing testis (T). (Acetic acid alum- carmine stain) X 300.

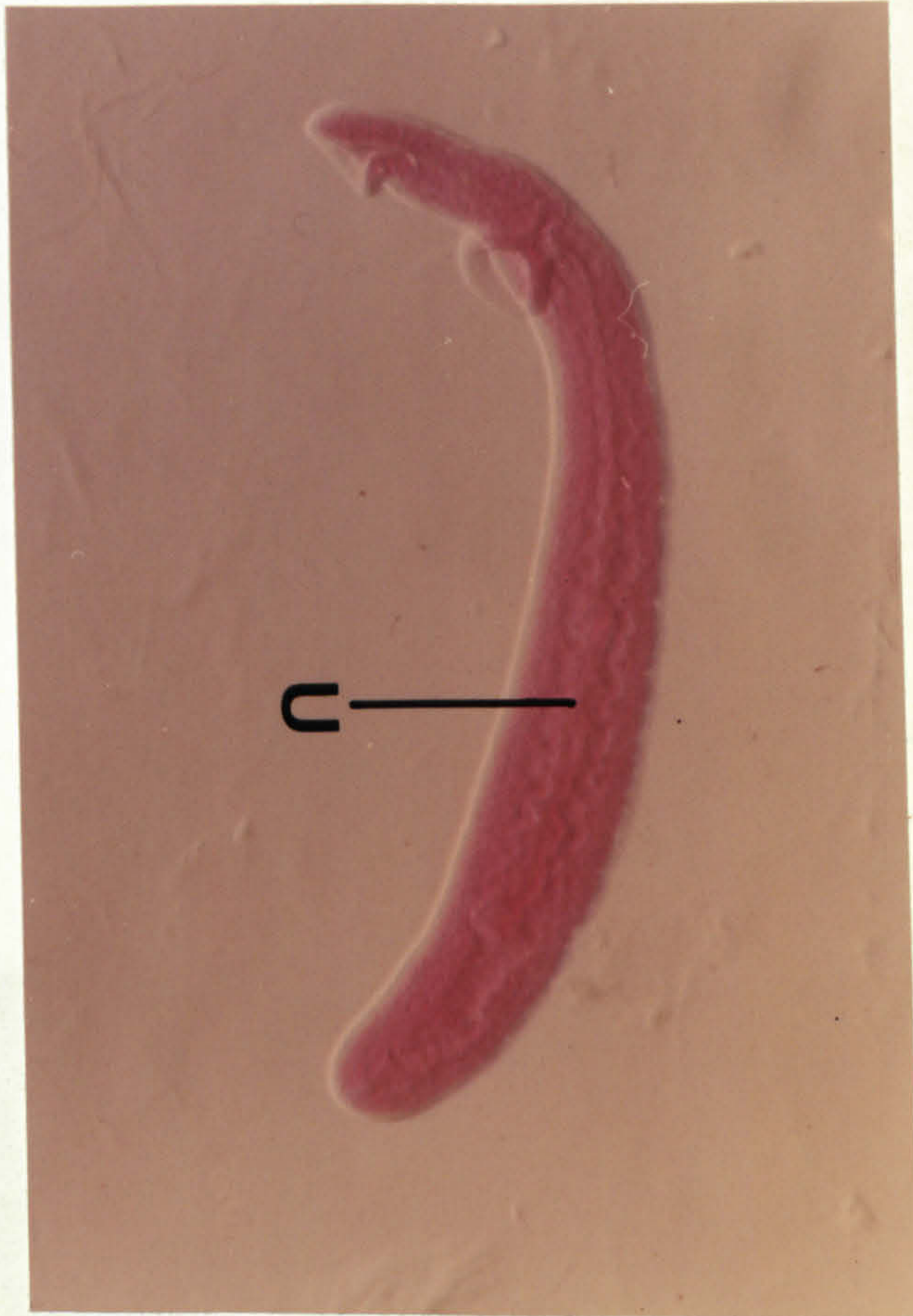


PLATE 15: Stage 3 ("Organogeny"- day 24). Female *S. haematobium* worm showing a narrow uterus (U). (Acetic acid alum-carmin stain) X 100.

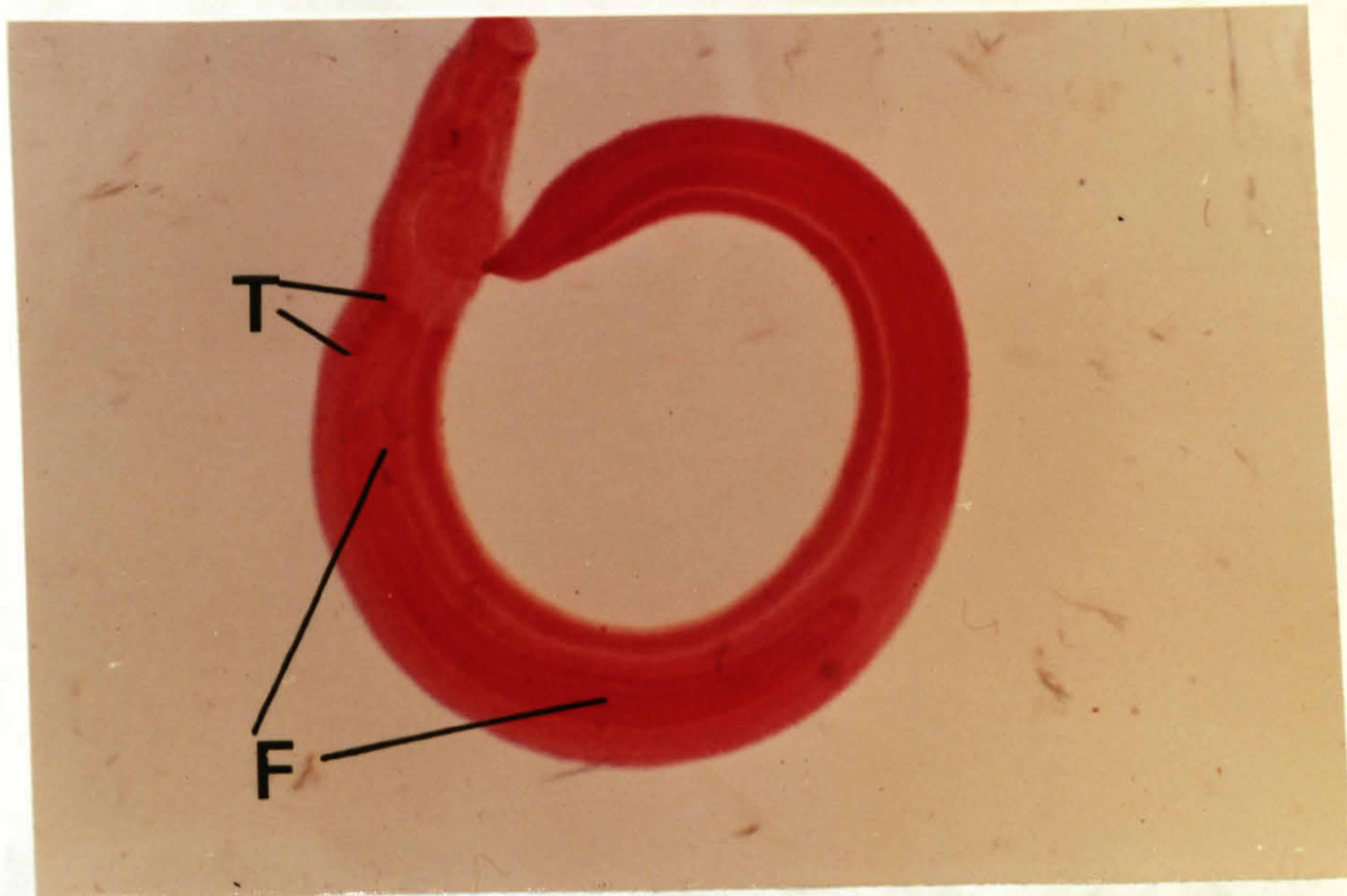


PLATE 16: Stage 4 ("Gametogeny"- day 28). S. haematobium worm pair. The female (F) is enclosed in the gynaecophoric canal of the male. Notice the four large testes (T) of the male. (Aceto-orcein stain) X 40.

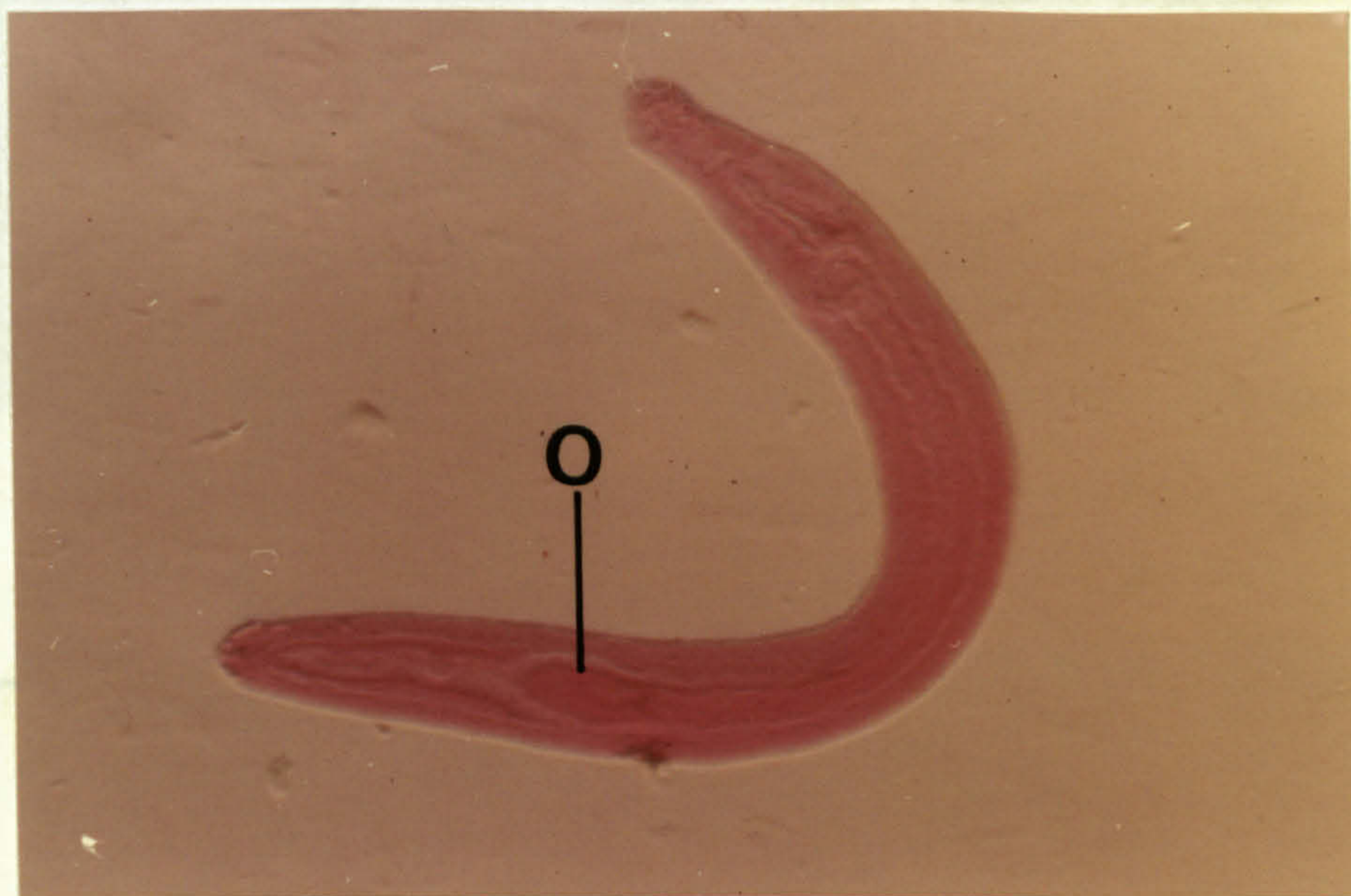


PLATE 17: Stage 4 ("Gametogeny"- day 28). Female S. haematobium worm showing a well-developed ovary (O). (Acetic acid alum-carmin stain) X 100.

Stage 3 (33 days) "Egg-shell formation": This is the first day when females become longer than males (Plate 19). The females have developed few vitelline follicle cells posterior to the well developed ovary. The egg-shell protein is synthesized by these follicles and gives a characteristic orange-red reaction with Fast Red (Plate 20). This reaction is given by the remains and histology of the egg-shell.



PLATE 18: Squashed testis of S. haematobium male worm (day 28 post-infection) showing spermatozoa (sp). (Aceto-orcein stain). Oil-immersion, X 1000.

(Saito, 1957; Saito and Clegg, 1961). The stages of development characterized by growth of the male and female worms (Saito, 1957; Saito and Clegg, 1961; Clegg and Saito, 1963) and form the initial stage of development. Clegg (1963) noted that S. haematobium cercariae could not be grown in culture media and suggested that this might be due to the fact that cercariae require some stimulus associated with penetration of host skin before they can survive and develop in vitro cultures.

Stage 5 (53 days) "Egg-shell formation": This is the first day when females become longer than males (Plate 19). The females have developed few vitelline follicle cells posterior to the well developed ovary. The egg-shell protein is synthesized by these follicles and gives a characteristic orange red reaction with Fast Red (Plate 20). This reaction is given by the tyrosine and histidine residues of the protein.

Stage 6 (61-63 days) "Oviposition": The females have developed the complete set of vitelline glands which are densely packed, posterior to the ovary, along both sides of the body (Plate 22). Eggs are seen in the uterus showing that the females have reached sexual maturity on one of those days (Plates 23 and 24). No further change occurs in the male except the formation of seminal vesicles (Plate 21).

Discussion

The in vivo development of S. haematobium follows the same general pattern as for S. mansoni (Clegg, 1965) and S. japonicum (Suliman, personal communication) (Table 17). Six stages of development characterized by morphological and histochemical criteria were distinguished. The transition of schistosome cercariae to schistosomula is accompanied by many changes (Stirewalt and Kruidenier, 1961; Stirewalt, 1963 and Clegg and Smithers, 1968) and forms the initial stage of development. Clegg (1965) showed that S. mansoni cercariae could not be grown in culture media and suggested that this might be due to the fact that cercariae require some stimulus associated with penetration of host skin before they can survive and develop in vitro culture.

TABLE 17

Comparison of the rates of development of S. mansoni, S. haematobium and S. japonicum, in vivo

Stage of development	DAYS POST-INFECTION		
	<u>S. mansoni</u> (Clegg, 1965)	<u>S. haematobium</u> (Present Study)	<u>S. japonicum</u> (Suliman, 1974 personal communication)
Lung form	7-8	9	4-6
Closed-gut	15	18	10
Organogeny	21	24	16
Gametogeny	28	28	22
Egg-shell formation	30	53	27
Oviposition	34-35	61-63	28-29



PLATE 19: Stage 5 ("Egg-shell formation"-day 53). *S. haematobium* worm pair. The female is longer than the male and vitelline follicles start developing. (Fast red stain) X20.



PLATE 20: Stage 5 ("Egg-shell formation"-day 53). Posterior end of *S. haematobium* worm pair showing vitelline follicles in the female (V). (Fast red stain) X 100.



PLATE 21: *S. haematobium* male worm recovered on day 63 post-infection from the liver of a hamster. (Fast red stain) X 20.

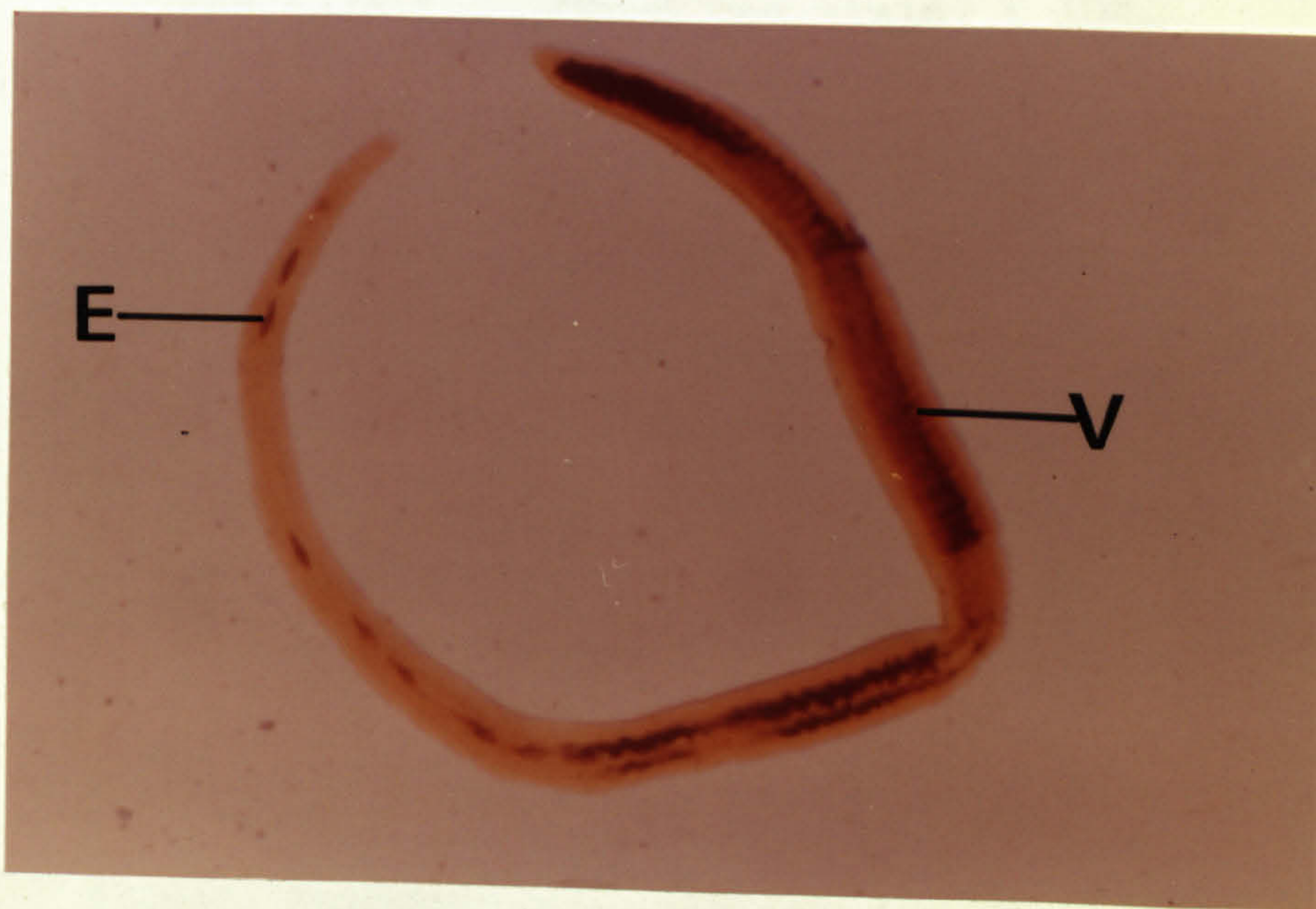


PLATE 22: *S. haematobium* female worm recovered on day 63 post-infection from the liver of a hamster. Note the well developed vitellaria (V) and eggs (E). (Fast red stain) X 20.



PLATE 23: Anterior end of *S. haematobium* female worm ("Oviposition"-day 61-63) showing vitellaria (V), vitelline duct (VD) and eggs (E). (Fast red stain) X 100.

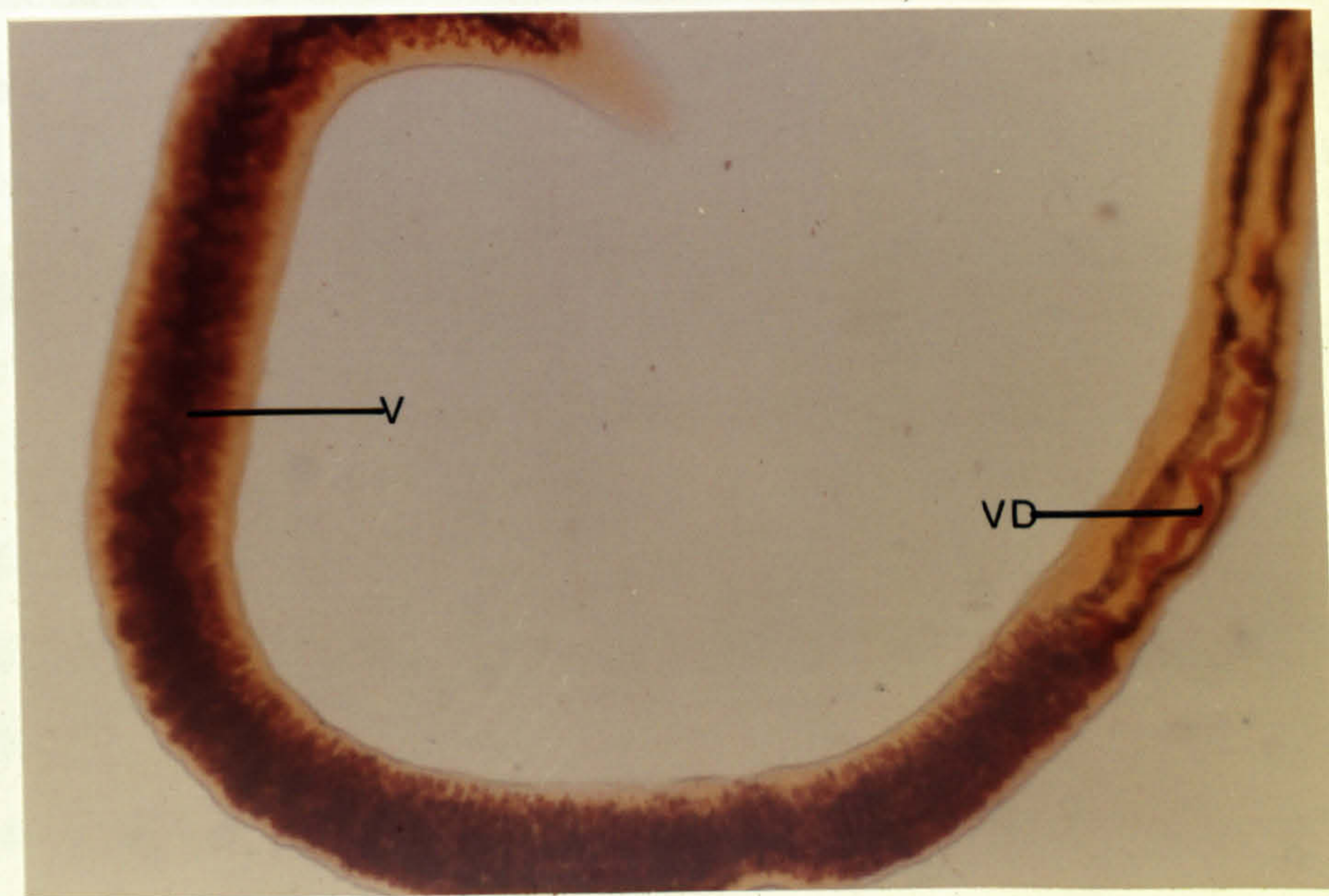


PLATE 24: Posterior end of *S. haematobium* female worm ("Oviposition"-day 61-63) showing densely packed vitellaria (V) and vitelline duct (VD). (Fast red stain) X 100.

The migration of S. haematobium in the lungs of infected animals took longer (9 days) than that of other schistosomes. Olivier (1952), Lichtenberg and Sadun (1963) and Clegg (1965) recorded peak concentrations of S. mansoni in the lungs of mice on days 6-8 post-infection. S. japonicum and Schistosomatium douthitti migrate very quickly through the lungs of mice and peak concentrations were recorded on day 4 post-infection (Olivier, 1952). In fact the percentage recoveries of schistosomula of these two species is so small that it is probably due to their quick passage through the lung capillaries. The accumulation of S. haematobium schistosomula in the lungs and their slow passage to the portal system may be due to an abnormal host reaction and may explain the host-specificity of this parasite. Other schistosomes, S. mansoni and S. japonicum, are highly successful parasites of a variety of laboratory and wild animals and their schistosomula do not accumulate in the lungs but reach the portal system without any delay. Although cytological tests were not performed on lung forms of S. haematobium, due to the scarcity of specimens recovered, it might be possible that growth had not started. Clegg (1965) could not find a single mitosis in 2000 lung schistosomula of S. mansoni thus suggesting that they do not grow in the lung capillaries. This was confirmed by in vitro studies which showed that inhibition of growth of S. mansoni in the lungs is due to the inability of schistosomula to grow for the first 5 days after penetrating the skin. Schistosomula recovered from the skin 30 min. after penetration and cultured in vitro did not commence growth,

as shown by cell division, before the 6th day in culture. Clegg (1965) suggested that inhibition of growth of S.mansoni in the lungs might be an adaptation which prevents excessive damage to the lung capillaries of the definitive host. In contrast, S.japonicum and Schistosomatium douthitti cause severe damage and haemorrhages by their growth in the lungs of their definitive hosts. S.mansoni, however, produced a relatively small number of haemorrhage and minimal injury to the lungs of the same host (Olivier, 1952). The closed-gut forms of S.haematobium observed in this study resemble those of S.mansoni (Clegg, 1965) and S.japonicum (Suliman, personal communication) except that they occur later than in the two other species. Males are slightly larger than the females indicating that, from this early stage of development, males grow faster than females. Clegg (1965) found that this is the first stage in the development of S.mansoni when cell division occurs: males showed 100-200 inhibited mitoses and females 60-80 after incubation in colchicine and employing the aceto-orcein technique.

The next two stages of development of S.haematobium, organogeny and gametogeny, almost immediately followed the closed-gut form. This is again similar to the situation in S.mansoni (Clegg, 1965) and S.japonicum (Suliman, personal communication). In contrast to the in vivo development of other schistosomes this study indicates a considerable delay in the development of S.haematobium female worms to sexual maturity. The reproductive system of the female worm is a delicately co-ordinated system easily disturbed by a variety of factors. Little is understood about the structure and

functioning of the reproductive system of either male or female schistosomes. In fact, one of the most important features affecting the functioning of the female of S.mansoni is the presence or absence of the male (Erasmus, 1973). Severinghaus (1928) and Sagawa et al. (1928) were among the first to show that S.japonicum females remain sexually immature in unisexual infections and reach only one fifth of the body length of mated females. Similarly the females of S.mansoni, S.haematobium and Schistosomatium douthitti fail to develop to maturity in the absence of males (Brumpt, 1936 and Short, 1952). In fact, females which have developed in the absence of males have two morphological differences from females found in a bisexual infection. The first is that the ovary is smaller than normal and is capable of producing ova but these do not possess the characteristic cortical glands of normal ova (Erasmus, 1973). This may be due to the absence of certain metabolites such as amino acids. Senft (1969) suggested that contact of the female with a male may help in transfer of amino acids absorbed by the male into the female across the tegument. The second major difference is the complete inability of the vitelline cells of the female to mature. These two differences contrast with observations on females from a bisexual infection (Erasmus, 1973).

In the present study, failure of females of S.haematobium to develop to sexual maturity is not due to the absence of males since paired worms were always seen and, as noted by various workers, (Wright and Bennett, 1967a,b and Wright and Knowles, 1972) there is always an abundance of males in infections with S.haematobium.

However, dependence on male worms for maturation of females is not always necessary since females of Schistosomatium douthitti (Short, 1952), Heterobilharzia americana (Armstrong, 1965) and S.mattheei (Taylor et al., 1969) will all produce eggs in the absence of males although these eggs may not always be viable.

Various experiments have conducted to explain the way in which the male schistosome stimulates the maturation of females. Severinghaus (1928) proposed that maturation of S.japonicum females depend on a hormone produced by the males. Vogel (1947) suggested that the same mechanism which brings mated females to maturity may act upon homosexually clasped males to inhibit their normal development. Armstrong (1965) suggested that development to sexual maturity required tactile stimulation. Male worms seem to produce a hormone which may promote the development of females of the same species but prevents the development of another clasped male. Michaels (1969) could not determine if a chemical factor was involved in successful pairing of S.mansoni worms. Shaw (1974) conducted some in vitro studies on the nature of the stimulus provided by the male which influences the sexual development of S.mansoni females. He concluded that residence in the gynaecophoric canal was essential for development of the female but insemination was not a major stimulus. The intimate contact offered by the male to the female and the many sensory structures on the tegument of both male and female worms indicate that the development stimulus might be one of physical contact. However, the possibility of chemical stimulation within the gynaecophoric canal cannot be excluded.

Although in the present study males were present and paired

with females, it is possible that the males were not mature enough to stimulate the maturation of the females. Evidence to dismiss this possibility was produced by Armstrong (1965) who showed that X-irradiation of S.mansoni worms did not prevent them to mate and the females eventually laid eggs. Michaels (1969) found that anorchid males of S.mansoni stimulated females to lay eggs just as fertile males did.

In this study, a considerable variation in growth has been observed and not all the developing schistosomes reached sexual maturity in 61-63 days. Similar observations were noted in the development of S.mansoni (Clegg, 1965) and S.japonicum (Cort, 1921 and Suliman, personal communication). In the case of S.mansoni this might be because many schistosomula remained in the lungs for quite some time: up to day 28 post-infection (Clegg, 1965). The same situation might be occurring in S.haematobium thus explaining this wide variation in growth .

CHAPTER VI

EFFECT OF PHYSICAL AND CHEMICAL FACTORS ON THE INFECTIVITY
OF THE CERCARIAE OF S. MANSONI AND S. HAEMATOBIIUM

PART 1: The effect of ultra-violet irradiation on S. mansoni
and S. haematobium cercariae

Introduction

Radiation of different wavelengths causes severe damage to a wide range of living organisms. This damage may be associated with changes in normal physiological processes and, at high levels of irradiation, with cytological interference and eventual death. Ultra-violet irradiation is similar in its action on living organisms to X-rays or Gamma irradiation. In fact, different stages of helminths are adversely influenced by U. V. irradiation. The effects of U. V. irradiation on eggs of helminths have been studied by several workers (Schleip, 1923; Ruppert, 1924; Seide, 1925; Nolf, 1932; Jones et al., 1940 and Jones and Hollaender, 1942, 1944). Stowens (1942) observed that exposure to U. V. irradiation would prevent the normal development of Trichinella spiralis larvae, and that female worms which developed after small doses of irradiation were sterile. Keeling (1960) has shown that larvae of Nippostrongylus muris were completely immobilized when exposed to U. V. irradiation at a distance of 0.5 cm. for four min. Larvae which received smaller doses of irradiation and which appeared normal were incapable of reaching maturity in rats. Schistosome cercariae were found to be very sensitive to U. V. irradiation (Krakower, 1940 and Tomberg and Lagrange, 1952). Standen and Fuller (1959) have demonstrated that exposure of cercariae of S. mansoni to U. V. irradiation resulted in inhibition, in mice exposed to them, of

their development to the adult stage. No similar studies were conducted for S. haematobium.

The present work was designed to find the possible sites and mechanism of failure of irradiated cercariae of S. mansoni and S. haematobium during their migration pathways in the definitive host.

Materials and Methods

A Hanovia U. V. lamp (with a mercury-vapour U-shaped bulb) emitting wavelengths in the region of 2537°A was used to irradiate freshly shed cercariae of S. mansoni and S. haematobium. Before use, the lamp was switched on for 10-15 min. so as to ensure its maximum output (Stiff, 1971). The distance of the lamp was controlled by an adjustable head and kept constant at 16.5 cm. from the cercarial suspension. The cercariae were irradiated by placing them directly under the lamp and exposures were timed by a stop watch. The cercarial suspension never exceeded 10 ml. in volume since U. V. irradiation does not penetrate well in liquid media beyond that distance.

Four experiments were conducted and each was repeated:

- a) It was first decided to find out how irradiation affected the viability and behaviour of the cercariae. Embryo dishes, containing 80-100 cercariae in 5 ml. of suspension were exposed to irradiation for 5-60 seconds and subsequently observed for some time. At all exposures, an embryo dish containing normal non-irradiated cercariae was set up as a control.
- b) The second experiment was designed to compare the death of irradiated (5-20 sec.) and normal non-irradiated cercariae during penetration of host skin.

- c) The migration of irradiated (10 sec.) and non-irradiated cercariae to the lungs of infected animals was followed by making lung recoveries on days 3, 5, 7 and 10 for S.mansoni and on days 4, 6, 9 and 11 for S.haematobium: these intervals cover the migration of both schistosomes in the lungs.
- d) A combined experiment was set up to compare the mortality of irradiated (10 sec.) and non-irradiated cercariae, during penetration of host skin, with the recovery of adult worms from the liver and portal system.

Results

Effect of irradiation on the viability and behaviour of the cercariae: Cercariae of S.mansoni and S.haematobium were severely damaged at 30-60 sec. exposures to irradiation. They immediately became sluggish and gathered in the centre of the dishes moving very slowly. Eversion of the ventral sucker, loss of tail and complete cessation of nephridial movements soon (1-3 min.) occurred indicating death of the cercariae. However, exposures at 5-20 sec. seemed to have no visible effect on the cercariae.

Death of cercariae during penetration of host skin: Tables 18 & 19 and Figs. 11a & 12 a show the results of this experiment. The level of death of S.mansoni cercariae during penetration of mouse skin corresponds to previous results (Species of host) when normal non-irradiated cercariae were used (30.4%). However, these increased to 42.4%, 50.1%, 58.6% and 68.4% when the cercariae were exposed to ultra-violet irradiation for 5, 10, 15 and 20 sec. respectively. There is a significant difference between the levels

T A B L E 18

Percentages of dead *S. mansoni* schistosomula recovered from the skin of adult mice infected with cercariae exposed to different levels of U.V. irradiation.

Time of irradiation (sec.)	No. of animals used	Mean % dead schistosomula	Range	S.D.±	S.E.±
Control	10	30.4	26-36	3.5	1.1
5	10	42.4	35-47	3.6	1.1
10	10	50.1	43-55	4.6	1.4
15	10	58.6	50-66	6.6	2.1
20	10	68.4	61-78	5.7	1.8

Control v 5 sec.

Degrees of freedom 18

t 7.52

p < 0.01

Control v 10 sec.

Degrees freedom 18

t 10.67

p < 0.01

Control v 15 sec.

Degrees of freedom 18

t 11.76

p < 0.01

Control v 20 sec.

Degrees of freedom 18

t 17.52

T A B L E 19

Percentages of dead S. haematobium schistosomula recovered from the skin of hamsters infected with U.V. irradiated and with non-irradiated cercariae.

Level of irradiation (sec.)	No. of animals	Mean % dead schistosomula	Range	S.D.±	S.F.±
Control	10	19.3	9-23	4.2	1.3
10	10	33.4	21-45	7.1	2.2
20	10	52.8	42-60	7.3	2.3

Control v 10 sec.

Degrees of freedom 18

t 5.56

p < 0.01

Control v 20 sec.

Degrees of freedom 18

t 12.48

p < 0.01

of death of normal non-irradiated cercariae and that observed with irradiated cercariae at all exposures ($p < 0.01$).

S. haematobium cercariae are also similarly affected by irradiation. Only 19.3% of normal non-irradiated cercariae died during penetration of hamster skin but this increased to 33.4% and 52.8% at 10 and 20 sec. exposures. These levels are significantly different from that of normal non-irradiated cercariae ($t=5.5$; $p < 0.01$ and $t=12.4$; $p < 0.01$).

Lung migration: The results of this experiment (Tables 20 and 21 and Figs. 11b and 12b) showed that very few dead immature S. mansoni schistosomula (0.5%) (Plate 25) were recovered from the lungs, on day 5 post-infection and none at all thereafter, of mice infected with cercariae irradiated at 10 sec. exposure. These results contrast sharply with those observed in non-irradiated group since living mature schistosomula (Plate 26) were recovered from the lungs on day 3 post-infection (3.5%) and the percentage recoveries increased to 11.7% and 17.0% on days 5 and 7 post-infection and then declined to 3.4% on day 10.

Similar results were obtained with S. haematobium: only 0.3% dead immature schistosomula were recovered from the lungs of irradiated group on day 4 post-infection compared to 3.1% of living mature schistosomula from the normal non-irradiated group. The percentage recoveries on days 6, 9 and 11 postinfection were 6.6%, 10.7% and 3.1% while none were recovered from irradiated group.

Comparison of death of cercariae in the skin with the recovery of adult worms: Analysis of the results of this experiment (Tables 22 and 23 and Figs. 11c and 12c) show that no adult worms were



PLATE 25: A dead S.mansoni schistosomulum recovered from the lungs of a mouse infected with irradiated cercariae. X 200.



PLATE 26: A living S.mansoni schistosomulum recovered from the lungs of a mouse infected with normal non-irradiated cercariae. X 200.

T A B L E 20

Percentage recoveries of *S. mansoni* schistosomula from the lungs of mice infected with irradiated and with non-irradiated cercariae.

Days post-infection	Non-Irradiated Cercariae					Irradiated Cercariae						
	No. of animals recovery	Mean %	Range	S. D. ±	S. E. ±	Mean % Cercariae not penetrating	No. of animals recovery	Mean %	Range	S. D. ±	S. E. ±	Mean % Cercariae not penetrating
3	20	3.5	2-6	1.5	0.3	2	20	0.5	-	0.3	0.3	10
5	20	11.75	9-17	2.4	0.6	1	20	0.0	-	-	-	9
7	20	17.0	12-21	2.0	0.4	3	20	0.0	-	-	7	11
10	20	3.4	2-5	1.0	0.2	2	20	0.0	-	-	-	8

T A B L E 21

Percentage recoveries of S. haematobium schistosomula from the lungs of mice infected with U.V. irradiated and with non-irradiated cercariae.

Days post-infection	Non-Irradiated Cercariae				Irradiated Cercariae			
	No. of animals recovery	Mean %	Range	S.D. ±	No. of animals recovery	Mean %	Range	S.D. ±
4	20	3.1	2 - 7	2.1	20	0.3	- -	-
6	20	6.6	5 - 9	1.5	20	0.0	- -	-
9	20	10.7	8 - 13	2.4	20	0.0	- -	-
11	20	3.1	2 - 5	1.3	20	0.0	- -	-

Mean % cercariae not penetrating
 Mean % cercariae not penetrating

recovered from animals infected with irradiated cercariae of S.mansoni or S.haematobium. This was partly due to an increased level of death of irradiated cercariae of both species (52.4% and 28.9% respectively) in host skin during penetration. The mean percentage recovery of adult worms from animals infected with non-irradiated cercariae of S.mansoni and S.haematobium were quite normal: up to 33.9% and 16.9% respectively were noted. These recoveries correlate well with the levels of mortality of cercariae in the skin (30.5% and 16.5% respectively) and are in accordance with results recorded in previous experiments.

Ultra-violet irradiation affected the ability of S.mansoni cercariae to penetrate host skin: only 2% of normal non-irradiated cercariae did not penetrate the skin while up to 10% of irradiated cercariae failed to penetrate host skin. The same is also true in S.haematobium: 3% and 11% of non-irradiated and irradiated cercarias respectively failed to penetrate host skin.

Histological observations: These are shown for S.mansoni in Plates 27-32. In mouse skin, fixed 15 min. after cercarial penetration, schistosomula were seen located in the dermis just beneath the Malpighian layer of cells in the epidermis, in both irradiated and non-irradiated groups. Although more of the irradiated schistosomula were apparently dead no cellular reaction or any abnormalities were observed in this group. Sections of the lungs of mice infected with non-irradiated cercariae showed intact feather-like schistosomula situated in the alveoli. No such organisms were seen in the irradiated group. Sections of the liver of mice, infected with non-irradiated cercariae, revealed

T A B L E 22

Comparison of the percentages of *S. mansoni* cercariae (irradiated at 10 sec. exposure and non-irradiated control) recovered as dead schistosomula from the skin of mice with those recovered as adult worms.

Level of Irradiation (sec.)	No. of animals	Mean Range S.D.± S.E.±			No. of animals	Mean Range S.D.± S.E.±			Mean % cercariae not penetrating		
		% Dead Schistosomula	% Adult Worms			% Dead Schistosomula	% Adult Worms				
Control	10	30.5	21-40	6.4	2.0	20	33.9	22-41	4.0	0.9	2
10	10	52.4	48-66	7.8	2.4	20	0.0	-	-	-	10

Control v 10 sec.

Degrees of freedom 18

t = 7.25

p = < 0.01

T A B L E 23

Comparison of the percentages of *S. haematobium* cercariae (irradiated and non-irradiated) recovered as dead schistosomula from the skin of hamsters with those recovered as adult worms.

Level of irradiation (sec.)	% Dead Schistosomula				% Adult Worms				Mean % Cercaria not penetrating		
	No. of animals	Mean	Range	S.D.±	S.E.±	No. of animals	Mean	Range		S.D.±	S.E.±
Control	10	16.5	10-21	3.6	1.1	20	16.9	10-30	4.8	1.0	3
10 sec.	10	28.9	23-42	6.7	2.1	20	0.0	-	-	-	11

Control v 10 sec.
 Degrees of Freedom 18
 t = 5.10
 p < 0.01

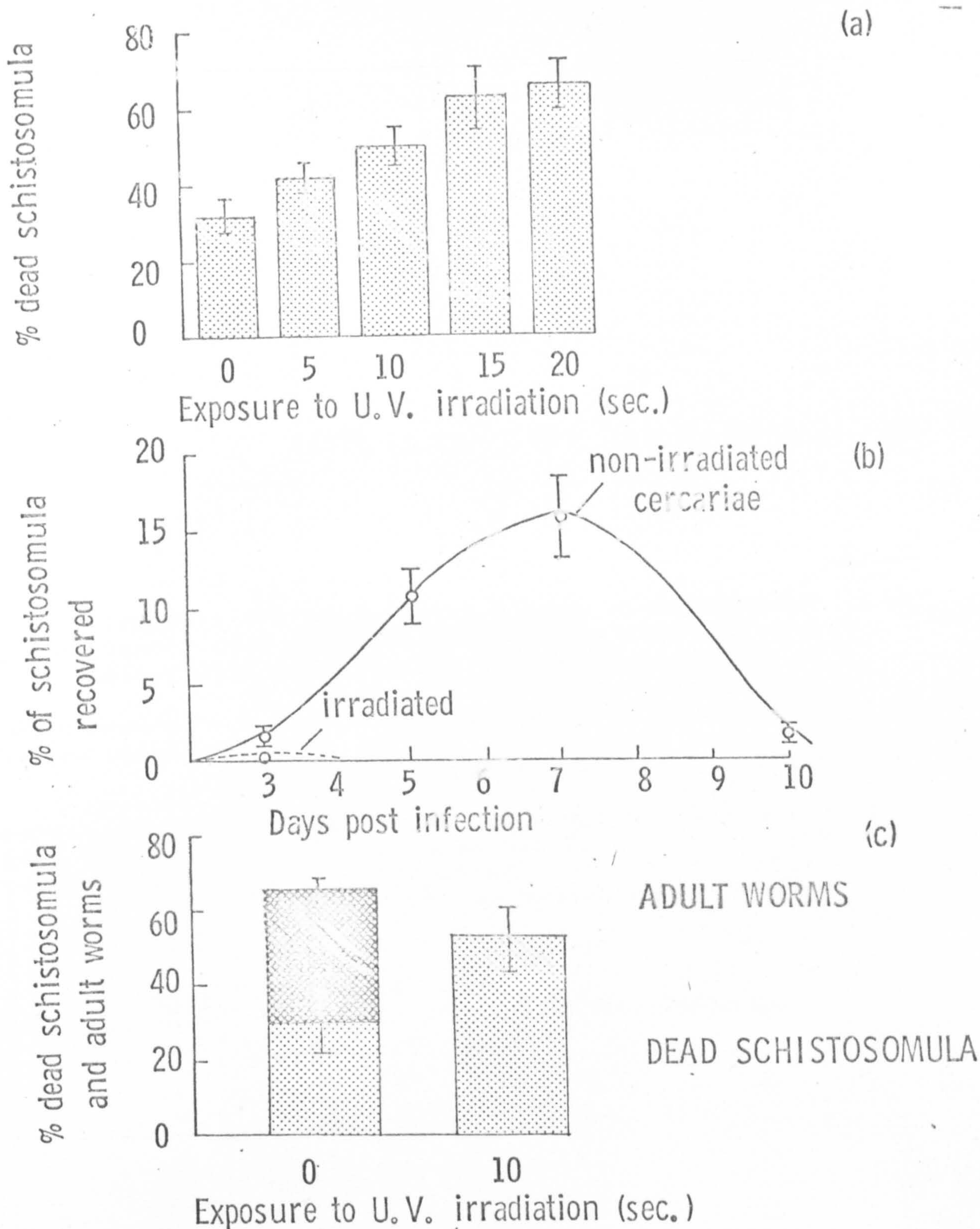
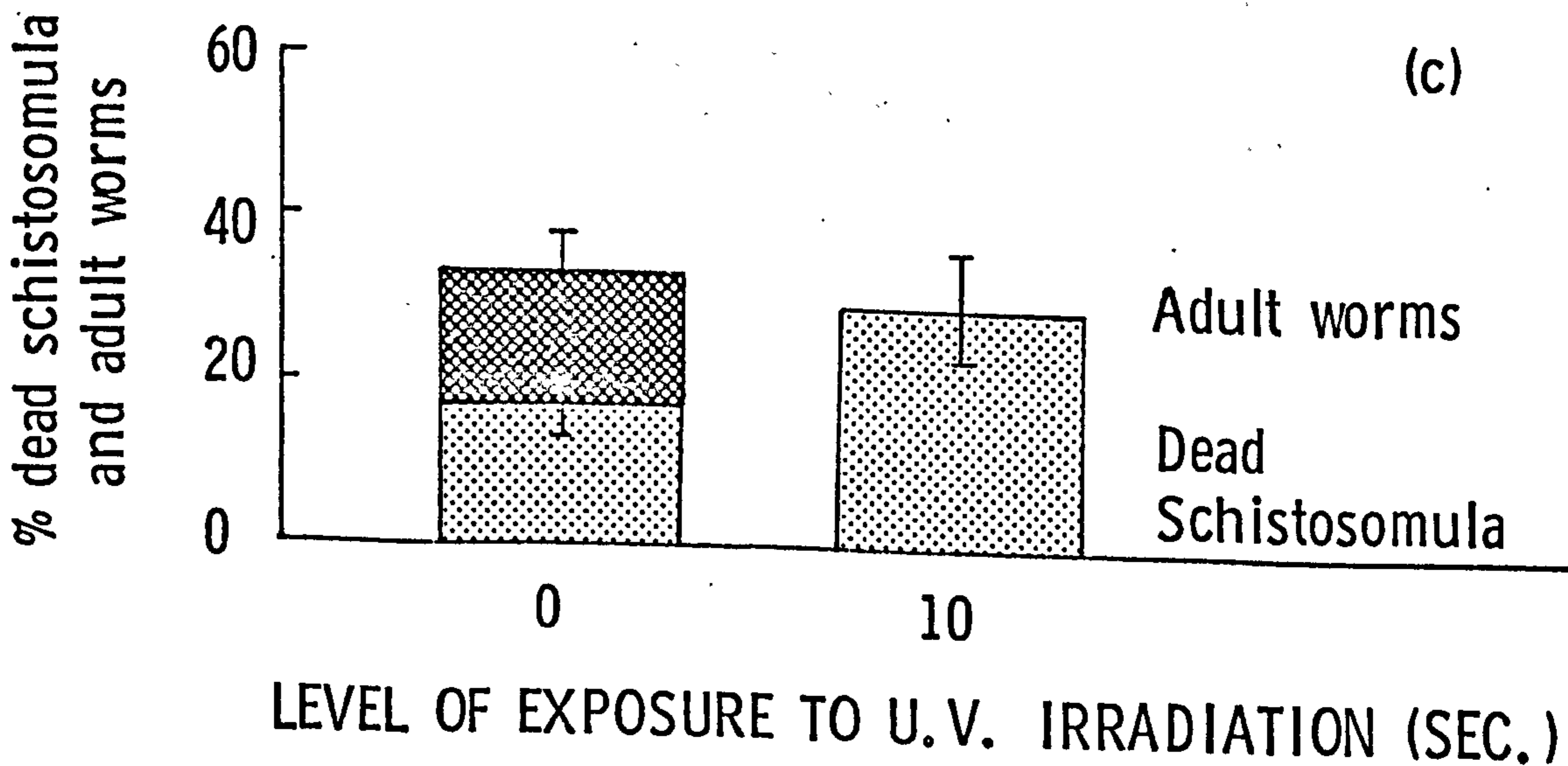
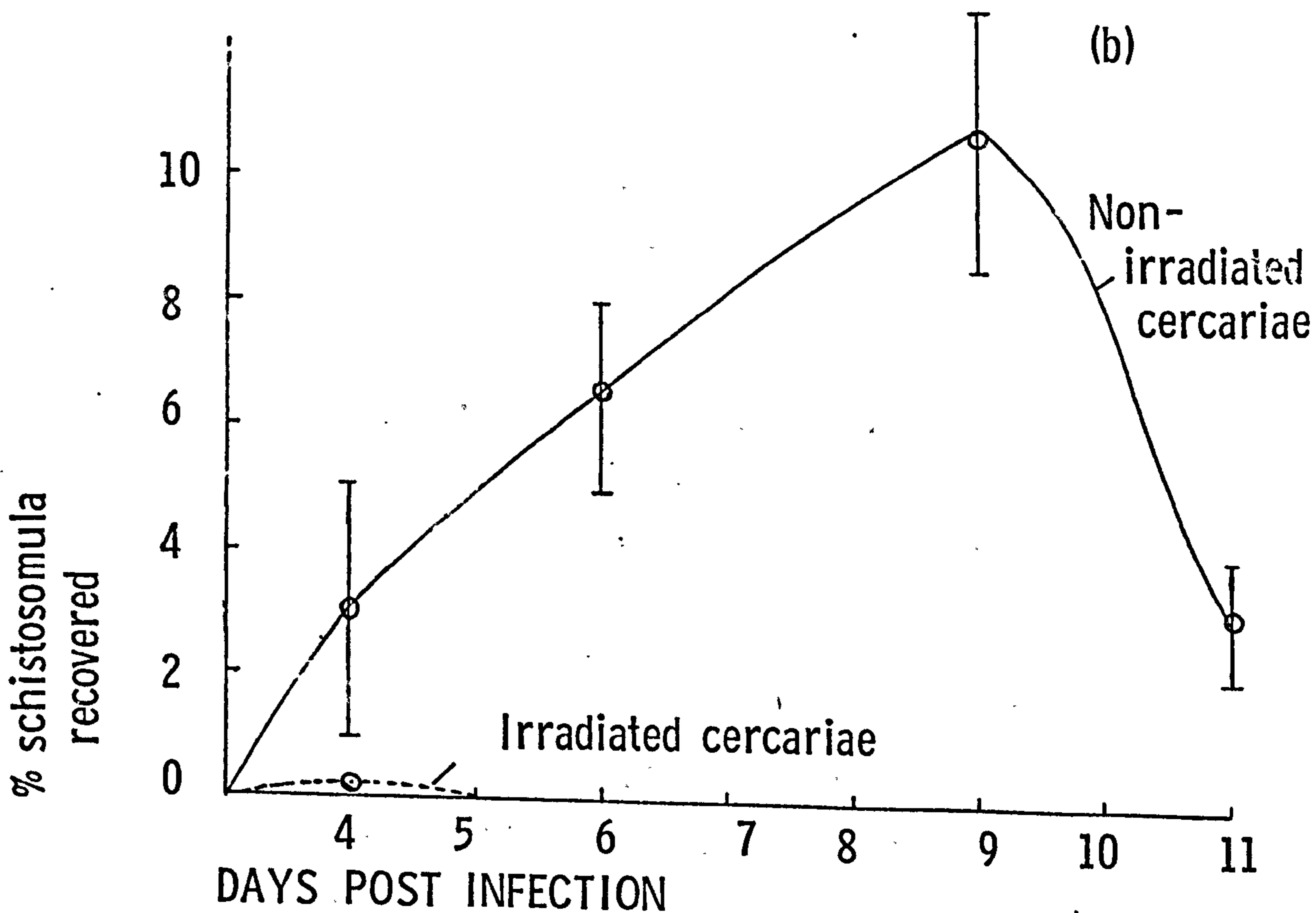
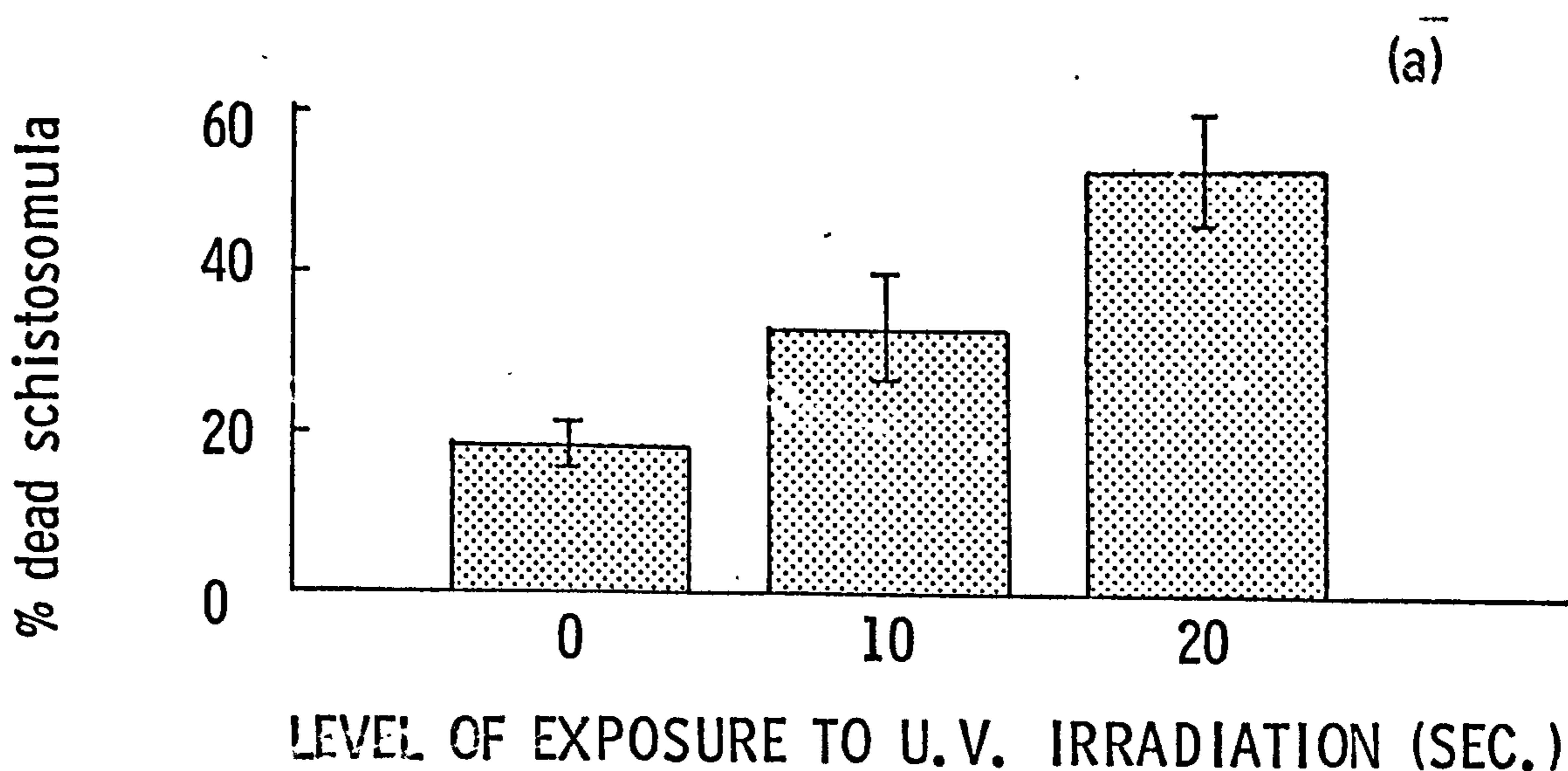


Fig. 11: a Percentages of dead *S.mansoni* schistosomula recovered from the skin of mice infected with cercariae exposed to different levels of ultra-violet irradiation.

b Percentages of *S.mansoni* schistosomula recovered from the lungs of mice infected with irradiated and with non-irradiated cercariae.

c Comparison of the percentages of *S.mansoni* cercariae recovered as dead schistosomula from the skin of mice (infected with irradiated and with non-irradiated cercariae) with those recovered as adult worms.

- Fig. 12: a Percentages of dead S.haematobium schistosomula recovered from the skin of hamsters infected with cercariae exposed to different levels of ultra-violet irradiation.
- b Percentages of S.haematobium schistosomula recovered from the lungs of hamsters infected with irradiated and with non-irradiated cercariae.
- c Comparison of the percentages of S.haematobium cercariae recovered as dead schistosomula from the skin of hamsters (infected with irradiated and with non-irradiated cercariae) with those recovered as adult worms.



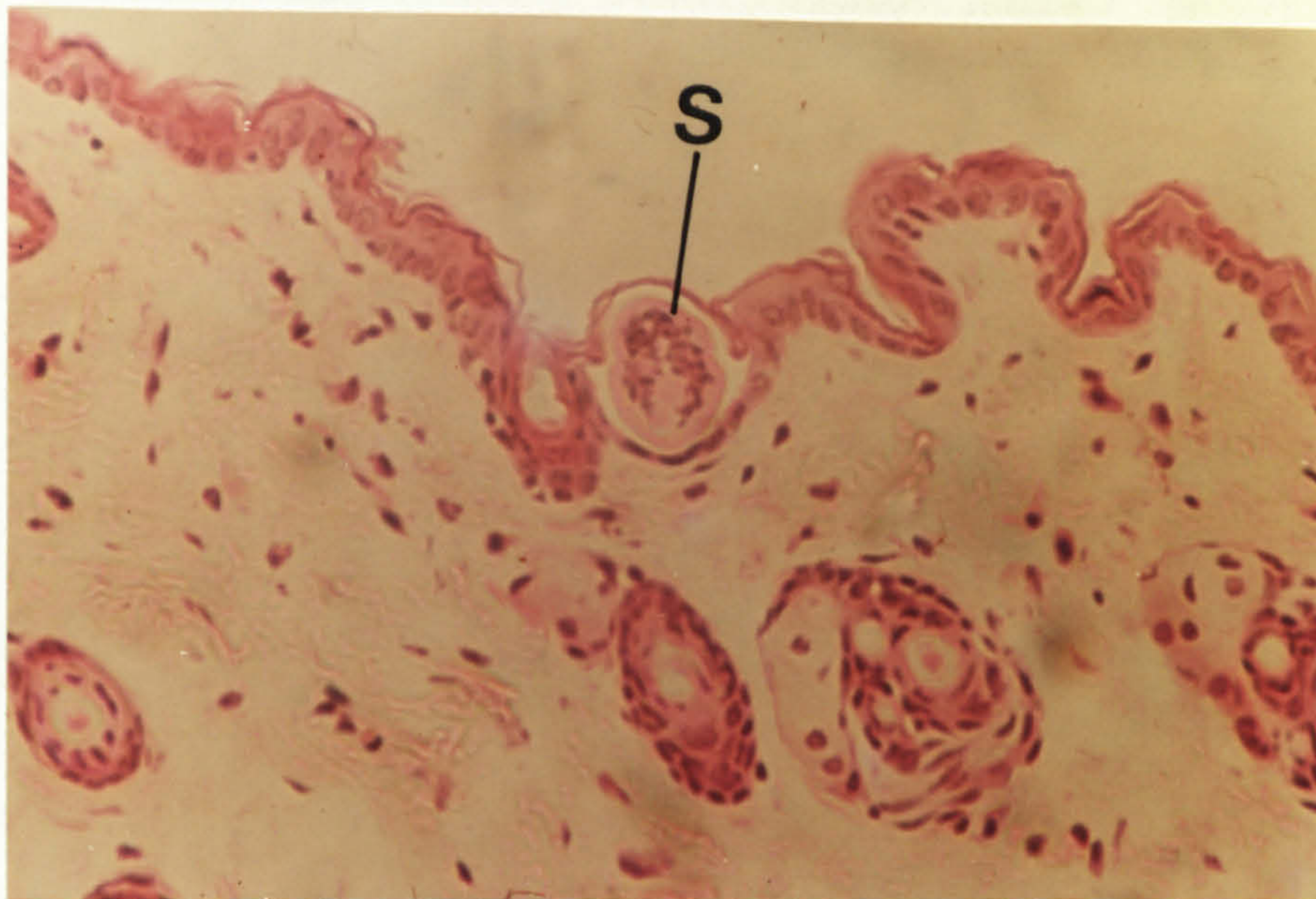


PLATE 27: Section of mouse abdominal skin at 10 min. after infection with irradiated S.mansoni cercariae. Penetration occurs normally since a schistosomulum (S) can be seen in the epidermis with no cellular infiltration or tissue reaction around it. (H. & E. stain) X 400.

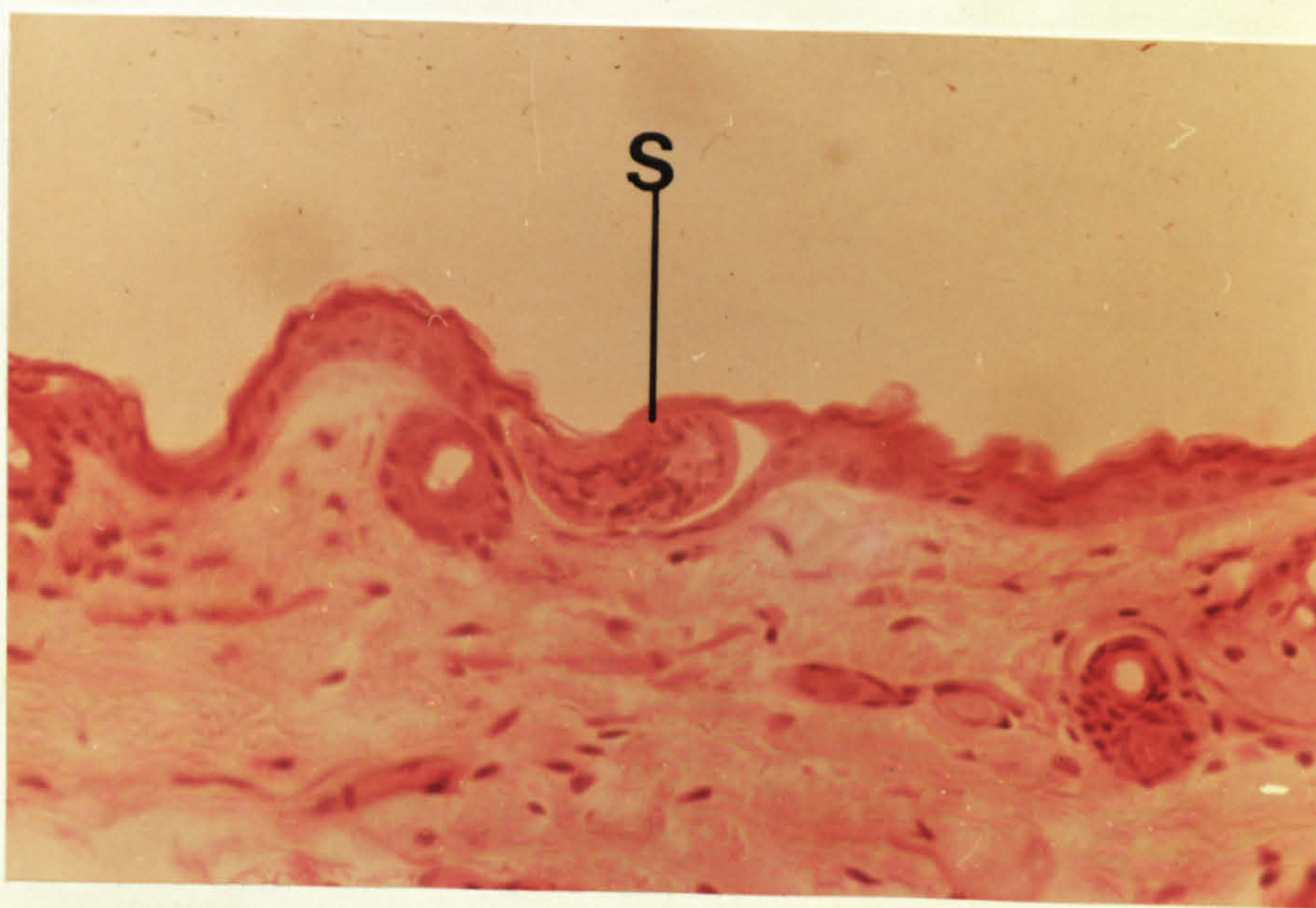


PLATE 28: Section of mouse abdominal skin at 10 min. after infection with normal non-irradiated S.mansoni cercariae. A schistosomulum (S) can be seen in the epidermis. (H. & E. stain) X 400.

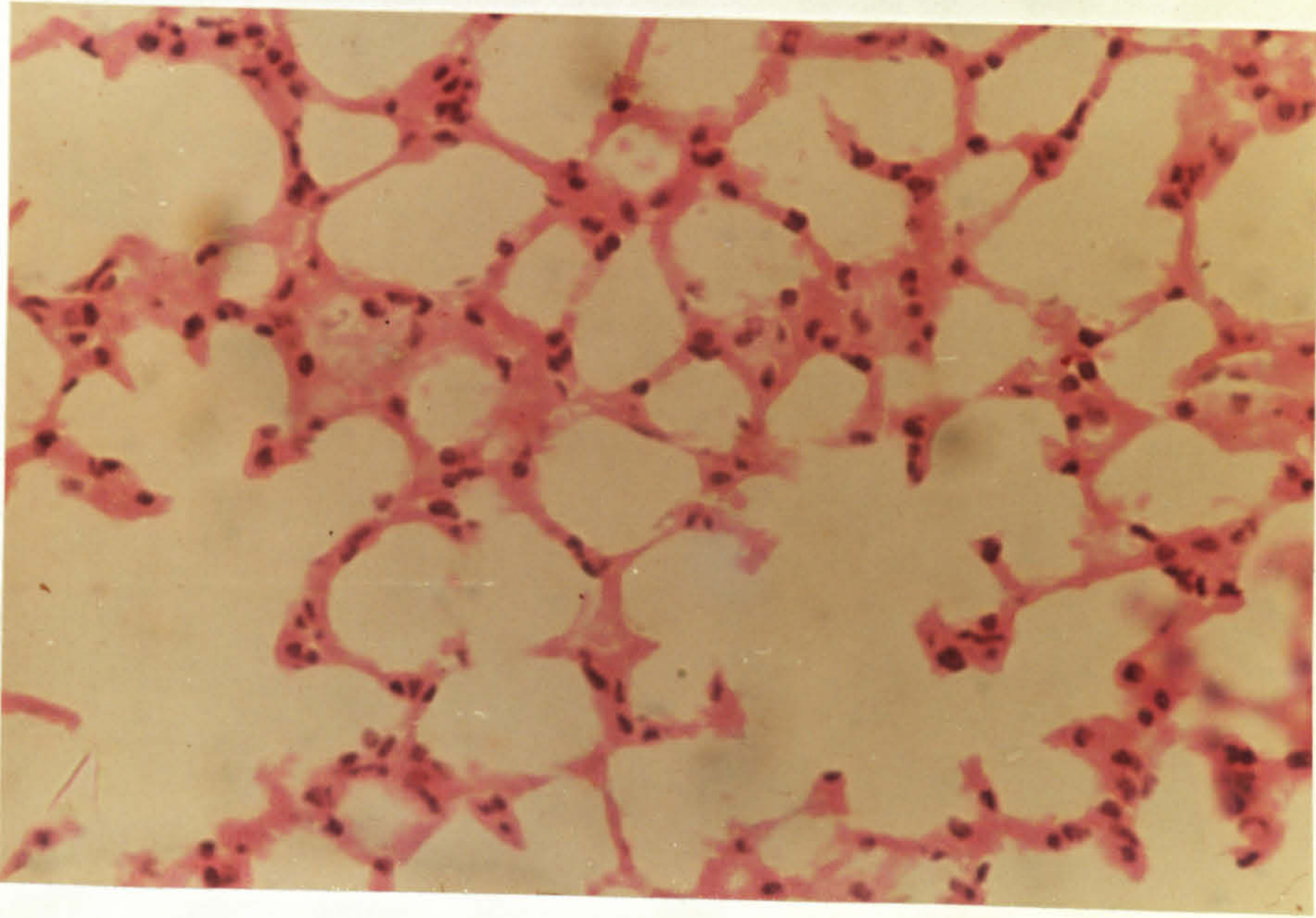


PLATE 29: Section of lungs of a mouse at 7 days after infection with irradiated S. mansoni cercariae. No schistosomula can be seen. (H. & E. stain) X 400.

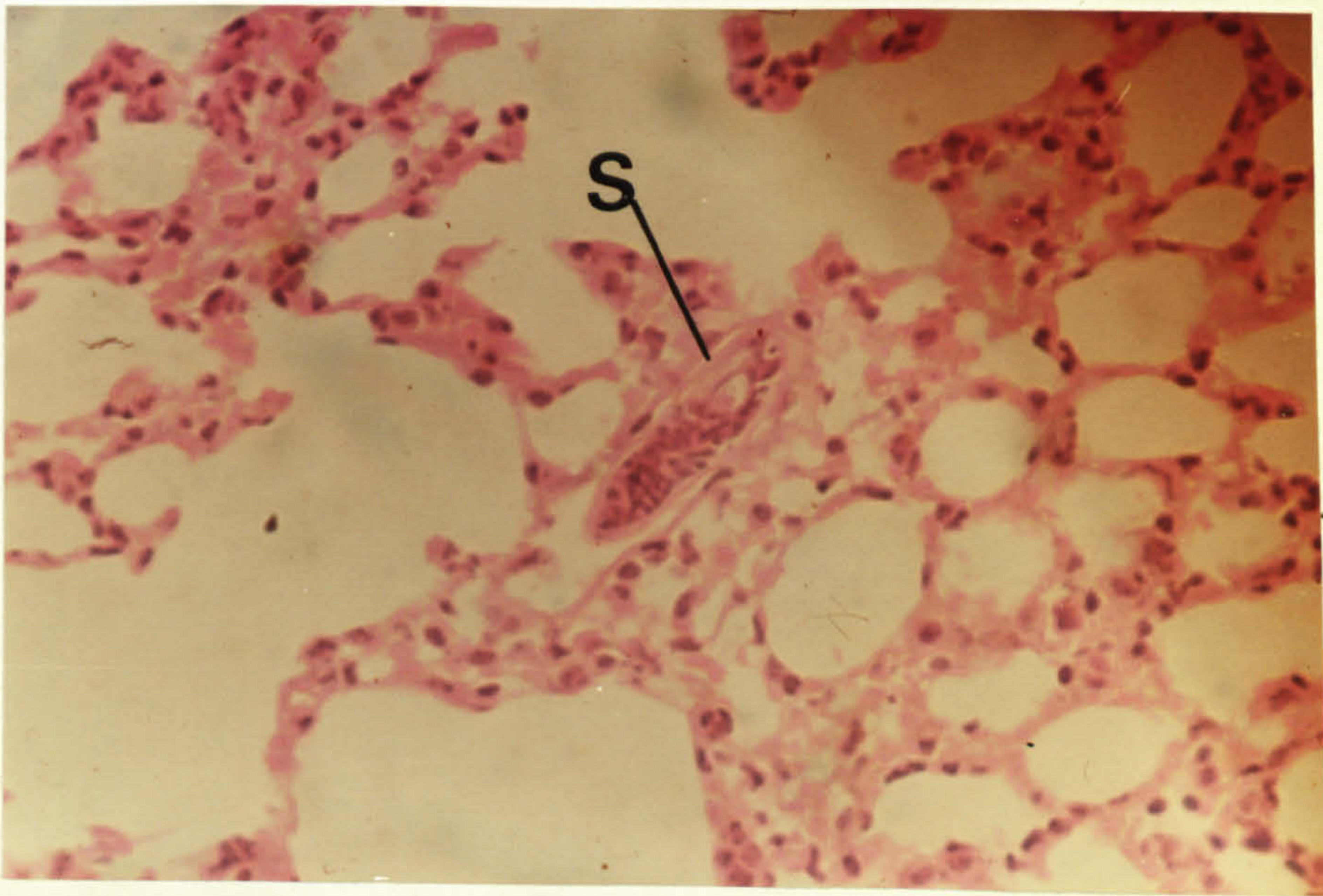


PLATE 30: Section of lungs of a mouse at 7 days after infection with normal non-irradiated S. mansoni cercariae. A schistosomulum (S) can be seen in an alveolus. (H. & E. stain) X 400.

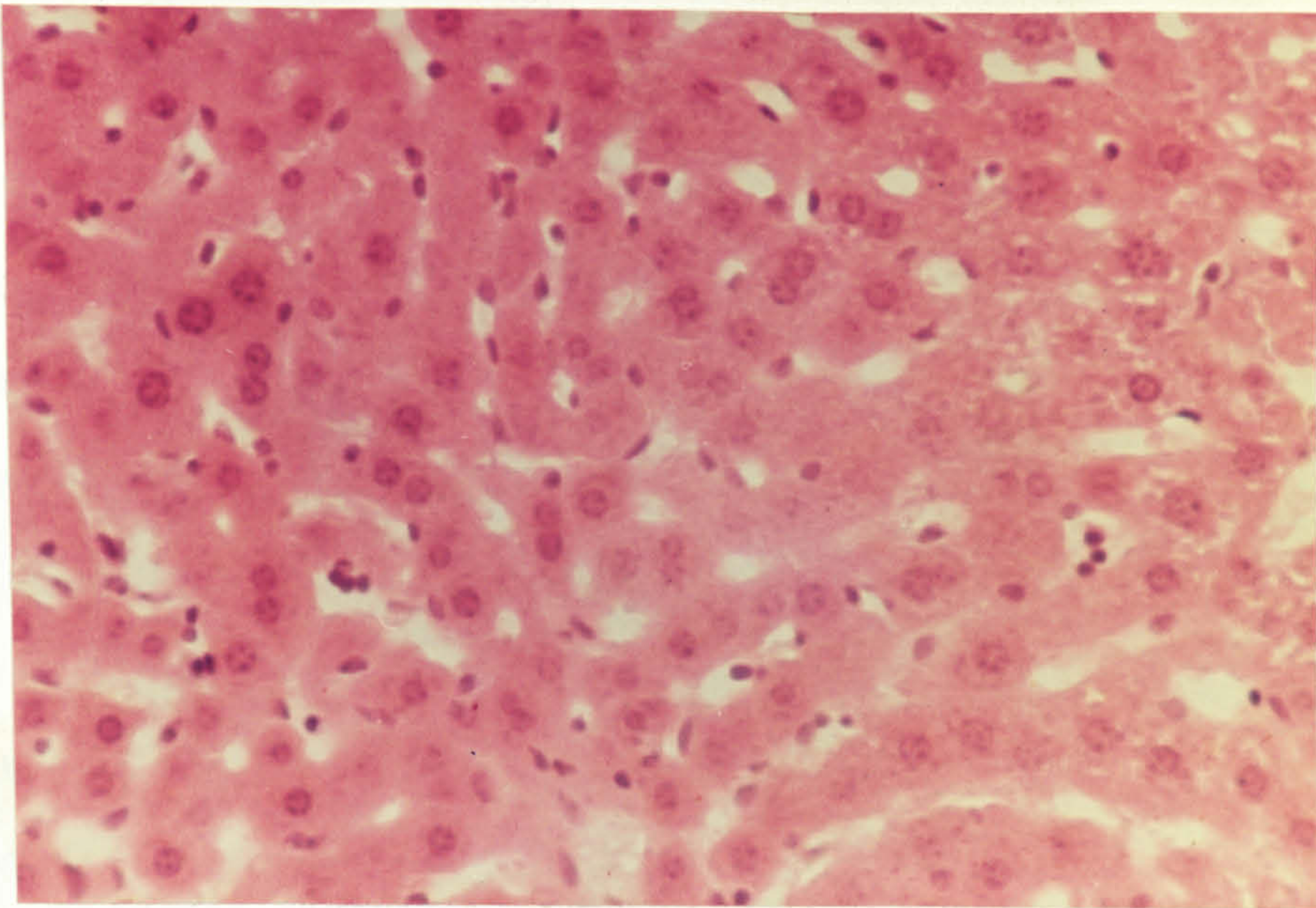


PLATE 31: Section of mouse liver at 42 days after infection with irradiated S.mansoni cercariae. Normal liver histology is seen indicating failure of the cercariae to develop into adult worms. (H. & E. stain) X 400.

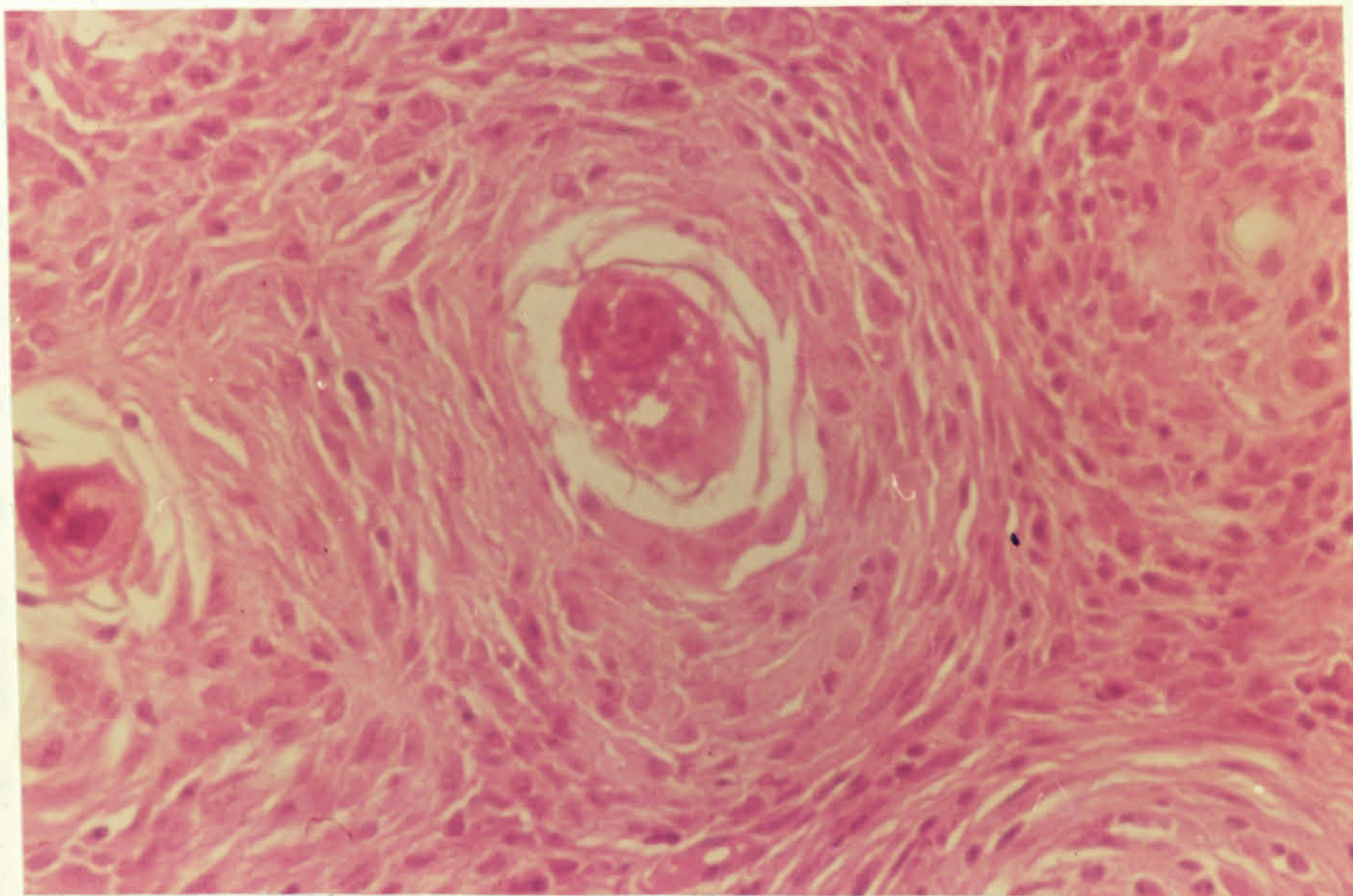


PLATE 32: Section of mouse liver at 42 days after infection with normal non-irradiated S.mansoni cercariae. Note the presence of a well developed granuloma containing an egg. (H. & E. stain) X 400.

the development of mature infection as indicated by the presence of well formed granuloma. However, in the irradiated group the liver showed normal histology with no signs of schistosome infection. Similar results to these were observed for S. haematobium.

Discussion

This study clearly establishes the inimical effect of ultraviolet irradiation on cercariae of S. mansoni and S. haematobium. The majority of damage to cercariae occurred during penetration of host skin. The levels of mortality of non-irradiated cercariae of S. mansoni (30.4%) and S. haematobium (19.3%) were quite normal and similar to results obtained in previous experiments. However, with irradiated cercariae their mortality steadily increased until high levels of 68.4% (S. mansoni) and 52.8% (S. haematobium) at 20 sec. exposure were reached. The lung migration of irradiated cercariae did not proceed beyond days 3-4 post-infection. Almost all the schistosomula that managed to pass undamaged through the skin seemed to have died on route to the lungs. This was revealed by the fact that, in animals infected with irradiated cercariae no living schistosomula were detected by either the direct recovery technique or by histologic means. In contrast, the lung migration of non-irradiated cercariae of S. mansoni was normal in all respects and confirmed results obtained by Lichtenberg and Sadun (1963) and Clegg (1965) who found that migration of S. mansoni in the lungs of mice followed a general pattern whereby peak concentrations were attained on day 7 post-infection with smaller recoveries on either sides of the peak. Sher et al. (1973) obtained similar results with S. mansoni except that the peak concentration was on days 4-6

post-infection depending on the strain of mice used. The lung migration of normal non-irradiated cercariae of S. haematobium was investigated for the first time in this study. It followed the same pattern as for S. mansoni but it took longer attaining a peak two days later.

The results of this study show that irradiation of cercariae of S. mansoni and S. haematobium for intervals as short as 10 sec. inhibited their development to the adult stage. These results confirm observations of Standen and Fuller (1959) on S. mansoni cercariae. In general, the intensity of U. V. irradiation used in the present study seems to have damaging effects on cercariae of S. mansoni and S. haematobium similar to those produced by high doses of other sorts of irradiation. Hsu et al. (1963) and Lichtenberg and Sadun (1963) have shown that exposure of cercariae of S. mansoni to 48,000 r from X-ray and Gamma ray sources, respectively, prevented them from migrating from the skin where they eventually died.

The activity of U. V. irradiation against viruses, bacteria and fungi reaches a maximum at about 2650° A (Hollaender, 1955). This wavelength is also at a peak of the absorption spectrum for nucleic acids. Keeling (1960) suggested that wavelengths of 2650° A and higher are effective through their action on the nuclear components which are vital in the duplication of chromosomal structures during growth. Irradiated larvae of N. muris can penetrate the skin and successfully pursue the first stages of migration through the host tissues but are unable to develop to

maturity. He suggested that failure of irradiated larvae might be a result of chromosome damage. This may operate by affecting some vital organ or tissue thus inhibiting the development of the larvae, e. g. death of cells in the neuro-secretory system responsible for exsheathment of the larvae at the third stage might cause destruction of the larvae in the lungs. Another possible mechanism of action of U. V. irradiation could be due to a general reduction of efficiency of irradiated larvae during migration thus enabling the host tissue reactions to destroy the parasite. Ellis and Wells (1941) found that U. V. irradiation inhibits the action of many enzymes including those concerned with oxidation and digestion. It is quite possible that schistosome cercariae might be similarly affected by U. V. irradiation thus explaining their failure to develop to maturity in mice.

One implication of this cercaricidal property of U. V. irradiation lies in the possibility that water bodies in bilharziasis endemic areas might be protected against cercarial contamination due to the action of natural U. V. irradiation, but this might have little effect since irradiation penetrates only the top layers of water and is quickly absorbed by turbid water (Prah, 1973). Another application of U. V. irradiation can also be in the attenuation of helminth larvae for their use in eliciting immune responses in experimental models. The dose of irradiation should be calibrated so that schistosome cercariae would migrate normally and with equal distribution in the different organs of the host but without developing to maturity. This is the ideal situation

for stimulating immune responses in animals infected with cercariae attenuated with X-rays or Gamma rays (Villella, et al., 1961; Lichtenberg and Sadun, 1963; Radke and Sadun, 1964 and Erickson and Caldwell, 1965).

PART 2: The effect of Gamma irradiation on S.mansoni cercariae.

Introduction

Ionizing irradiation results in inhibition of development of schistosome cercariae to varying extents depending on the dosage of irradiation. Vellella et al. (1961) and Vellella and Weinbren (1965) reported that gamma irradiation of S.mansoni cercariae at 2,000-2,500 r produced massive deformation and eventual sterility of the resulting adult worms. Hsu et al. (1963) found that when S.japonicum cercariae were irradiated at 1700 r, from an X-ray source, the resulting schistosomula were destroyed mainly in the liver. At higher doses of up to 48,000 r the sites of destruction were in the skin and the lungs. In general, doses of irradiation greater than 2,000-2500 r resulted in failure of the cercariae to develop into mature adult worms. Similar results to these were obtained by Lichtenberg and Sadun (1963), Radke and Sadun (1963), Erickson and Caldwell (1965) and Erickson (1965). The majority of these studies were based on histopathological and histological observations.

The main objectives of the present study was to find out, by direct recovery of intact schistosomula from the skin, lungs and liver, the fate of irradiated cercariae of S.mansoni during their migration in the definitive host.

Materials and Methods

Irradiation of the cercariae was performed with a "Gammacell" cobalt-60 irradiator at the National Institute for Medical Research (Mill Hill). Sterilin plastic tubes, containing 25 ml. of cercarial suspension were exposed to one level of irradiation : 6,000r. Three

sets of experiments were performed:

- a) The death of irradiated and non-irradiated cercariae during penetration of host skin was investigated.
- b) The migration of irradiated and non-irradiated cercariae to the lungs of mice was followed by making lung recoveries from groups of mice on days 5 and 7 post-infection.
- c) The death of irradiated and non-irradiated cercariae during penetration of host skin was compared with the recovery of adult worms from the liver and mesenteric veins.

Results

Death of cercariae during penetration of host skin: Analysis of the results of this experiment (Table 24 and Fig. 13a) showed that the mean percentage of dead schistosomula from the skin of mice infected with irradiated cercariae (32.5%) almost equalled that from mice infected with normal non-irradiated cercariae (28.5%) with no significant difference between them ($t=1.6$; $p > 0.05$).

Lung migration: The results of this experiment (Table 25 and Fig. 13b) showed that, on day 5 post-infection, there was no significant difference ($t=0.5$; $p > 0.05$) between the mean percentage recovery of schistosomula from the lungs of mice infected with irradiated cercariae (10.7%) and non-irradiated cercariae (12.9%). However, on day 7 post-infection, the mean percentage recovery of schistosomula was significantly lower in the irradiated group (10.9%) than in the non-irradiated group (17.1%). The difference between the means was significant ($t=2.2$; $p < 0.01$).

Comparison of death of cercariae in the skin with the adult worm recovery: The results of this combined experiment (Table 26 and Fig. 13c) indicated that while only 40% of the irradiated

T A B L E 24

Percentages of dead *S.mansoni* schistosomula recovered from the skin of mice infected with gamma irradiated and with non-irradiated cercariae.

Level of irradiation (r)	No. of animals	Mean % dead schistosomula	Range	S.D.±	S.E.±
Control	10	28.5	22-40	6.1	1.9
6,000	10	32.5	24-48	7.8	2.4

Control v 6,000 r

Degrees of Freedom 18

t= 1.66

P > 0.05 N.S.

T A B L E 25

Percentage recoveries of S.mansoni schistosomula from the lungs of mice infected with gamma-irradiated and with non-irradiated cercariae.

Days post-infection	Irradiated cercariae					Non-irradiated cercariae					
	No. of animals	Mean % recov.	Range	S.D. \pm	S.E. \pm	No. of animals	Mean % recov.	Range	S.D. \pm	S.E. \pm	Mean % cercariae not penetrating
5	20	10.7	12-25	6.1	2.2	20	12.9	11-23	7.9	1.7	6
7	20	10.9	5-20	5.3	1.2	20	17.1	11-32	5.5	2.2	8

5 days Irradiated v Non-irradiated

Degrees of Freedom 38

t = 0.49

p > 0.05

7 days Irradiated v Non-irradiated

Degrees of Freedom 38

t = 2.18

p < 0.01

T A B L E 26

Comparison of the percentages of *S. mansoni* cercariae (exposed to 6,000 r and non-irradiated) recovered as dead schistosomula with those recovered as adult worms.

Level of irradiation (r)	% Dead Schistosomula				% Adult Worms				
	No. of animals	Mean	Range	S.D. ± S.E. ±	No. of animals	Mean	Range	S.D. ± S.E. ±	Mean % cercariae not penetrating
Control	10	47.5	40-52	4.5 1.6	20	28.2	20-35	8.2 1.8	6
6,000	10	48.1	39-54	5.2 1.3	20	0.0	-	-	7

Control v 6,000 r

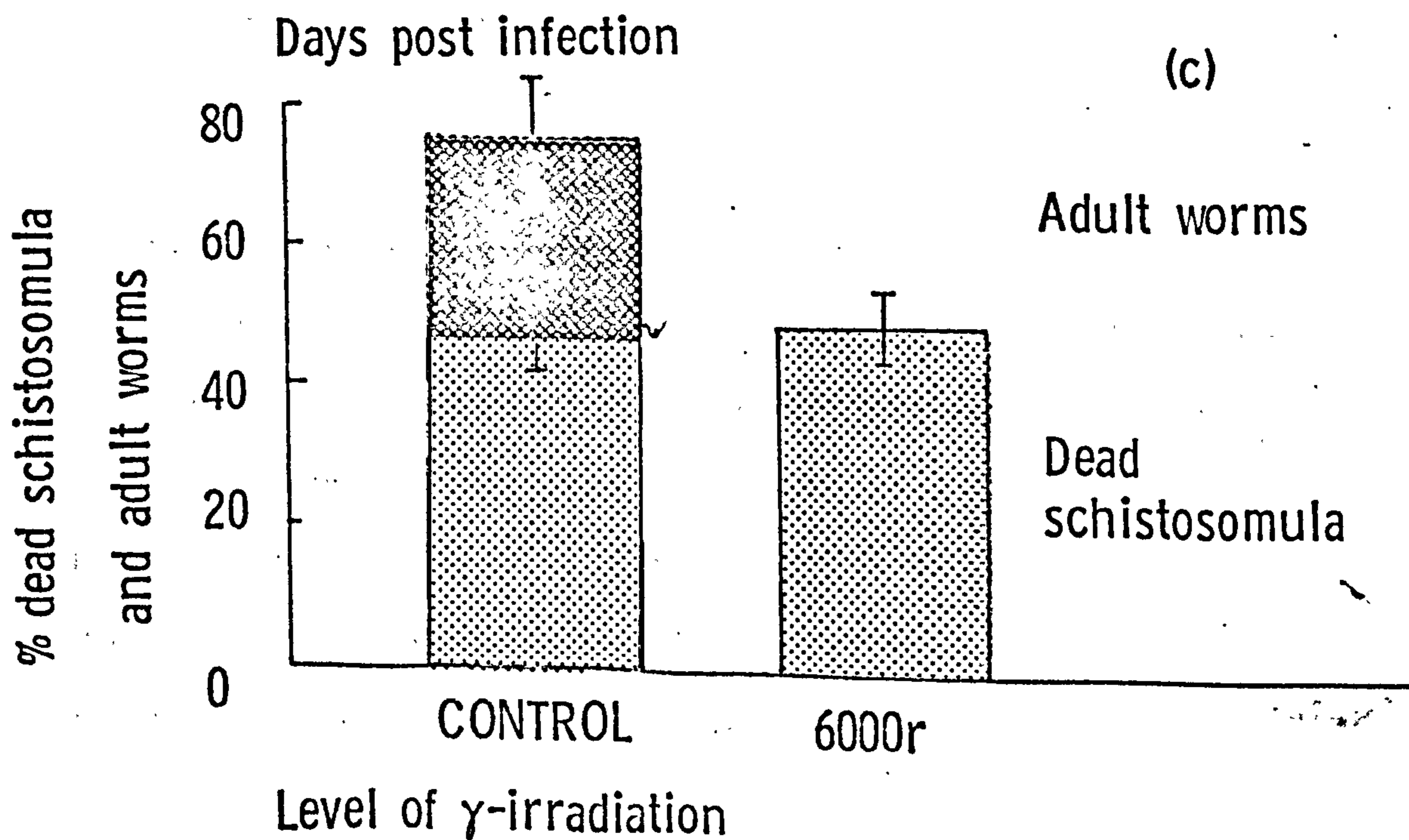
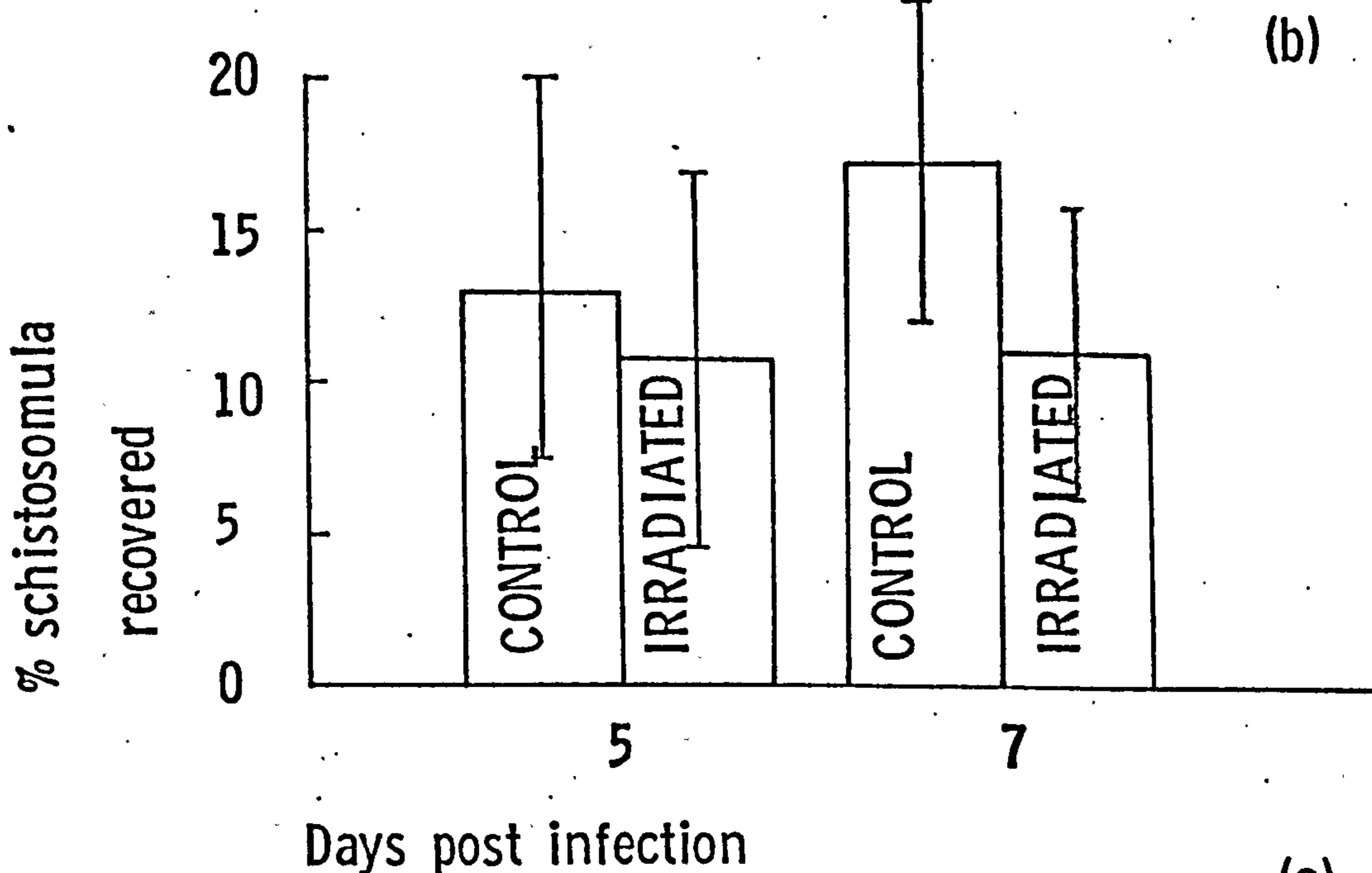
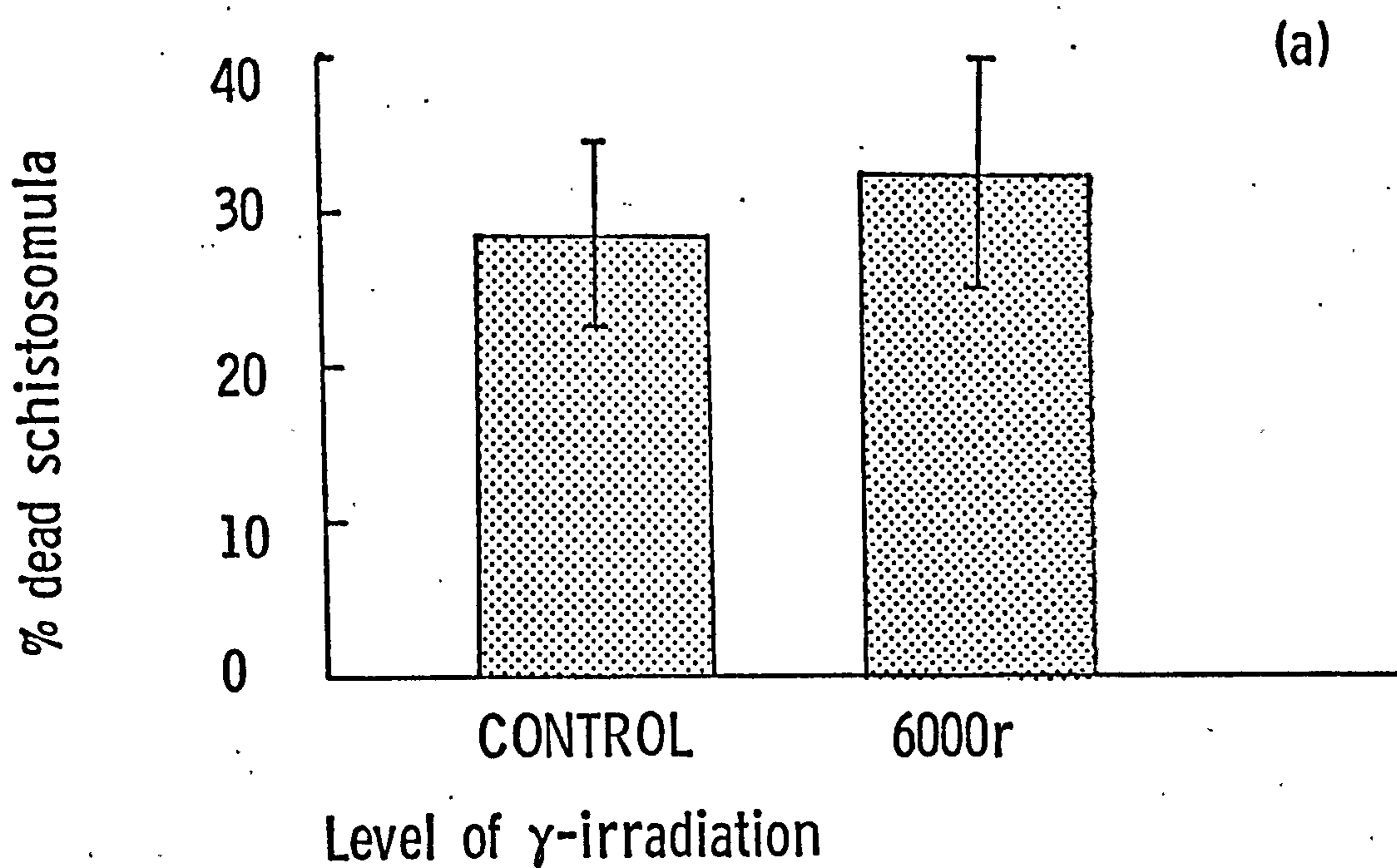
Degrees of Freedom 18

t= 0.24

p < 0.05 N.S.

Fig. 13

- a Percentages of dead S.mansoni schistosomula recovered from the skin of mice infected with gamma-irradiated and with non-irradiated cercariae.
- b Percentages of S.mansoni schistosomula recovered from the lungs of mice infected with gamma-irradiated and with non-irradiated cercariae.
- c Comparison of the percentages of S.mansoni cercariae recovered as dead schistosomula from the skin of mice (infected with irradiated and with non-irradiated cercariae) with those recovered as adult worms.



cercariae died in the skin no adult worms were recovered from mice infected with these cercariae. The results for non-irradiated cercariae were in general agreement with and extended those observed previously. There was a strong correlation between death of cercariae in the skin (47.6%) and the adult worm recovery (30.0%). These adult worms were normal in all respects and had fully mature eggs in their uteri. Gamma irradiation had no effect on the ability of cercariae to penetrate host skin since only 6% and 7% of non-irradiated and irradiated cercariae failed to penetrate mouse skin.

Discussion

The data of the foregoing experiments clearly indicate that migration and maturation of S.mansoni cercariae within the host were adversely affected by gamma irradiation. However, penetration of irradiated cercariae into the definitive host skin seemed to proceed normally. Erickson and Caldwell (1965) found that no effect on penetration or maturation was evident when S.mansoni cercariae were irradiated with 1,000 r of gamma irradiation. However, when cercariae were irradiated at 2,500-3,000 r they developed into worms in which egg production was greatly reduced as compared with normal worms. When irradiated at 4,000 r the worms did not survive 8 weeks in mice. Erickson (1965) conducted some studies on the time and sites of death of irradiated S.mansoni cercariae in mice. Schistosomes irradiated at 8,000 r from a Co-60 source penetrated normally into mice and migrated to the lungs at about the same rate as normal non-irradiated ones but fewer in numbers. Except for a lower worm count in the irradiated group

little difference was found in worm distribution in the two groups prior to day 11 post-infection. From the 11th day to the 35th day post-infection more worms were recovered from the lungs of mice infected with irradiated cercariae than from non-irradiated groups. This suggested a decreased ability of irradiated worms to migrate from the lungs to the liver: some of these worms eventually died in the lungs and were eliminated by host-tissue reaction while still there. The first histological evidence of death of irradiated worms was seen in the liver at day 22 post-infection. This was the site where the majority of death of irradiated cercariae occurred. In fact, only a very small number of worms could be recovered from mice infected with irradiated cercariae and the majority of these worms were stunted and appeared to be moribund (Erickson, 1965). However, migration of schistosomula to the lungs of infected animals, in the present study, was impeded by irradiation. This was the first site where a decrease in number of schistosomula started to occur. Further damage and death of schistosomula might have occurred in the liver since all irradiated cercariae failed to mature into adult worms.

Hsu et al. (1963) reported that the fate of S.japonicum schistosomula in mice varied with the amount of X-irradiation to which the cercariae had been exposed. With X-ray exposures of 1700, 3,000 and 6,000 r the schistosomula were destroyed in the liver; with doses of 12,000 r in the skin, lungs and liver; with 24,000 r the schistosomula were destroyed in the skin, seldom in the lungs but never in the liver and with 48,000 r all the schistosomula perished in the skin. The fate of schistosomula and their

histopathology in monkeys and mice were found to be generally similar. In fact, Hsu et al. (1963) saw an analogy with the destruction of normal non-irradiated cercariae in an immune host. Lichtenberg and Sadun (1963) investigated the parasite migration and host tissue reaction in mice infected with irradiated cercariae of S.mansoni. They found that the more intense the irradiation, the less the schistosomula migrated into the host tissues and the more intense the inflammation in the tissue where the schistosomula died. At a dosage of 50,000 r most of the schistosomula died in the skin and the main pathological change was inflammation of the skin with ulceration and vasculitis; at 5,000 r most of the schistosomula perished in the lung and there were many granulomatous foci in these organs. At 2500 r few worms developed into stunted adults in the portal system and there were granulomata and foci of necrosis in the liver. The tissue reactions around irradiated larvae were similar to those produced by normal cercariae in an abnormal host.

Comparison of the effects of Gamma irradiation on cercariae of S.mansoni with that of ultra-violet irradiation tends to suggest that U. V. irradiation is more damaging than gamma irradiation. U. V. irradiated cercariae of S.mansoni and S.haematobium do not reach the lungs of infected animals while gamma irradiated cercariae of S.mansoni do get to the lungs but in smaller proportions than non-irradiated cercariae. In fact, similar observations to these were reported for Nippostrongylus braziliensis by Keeling (1960), Jennings et al. (1963) and Kassai et al. (1966).

PART 3: The effect of temperature on the infectivity of S.mansoni and S.haematobium cercariae

Introduction

Several workers have studied the effect of temperature on the behaviour and viability of schistosome cercariae (Gordon et al., 1934; Porter, 1938; Krakower, 1940 and Jones and Brady, 1947). The relationship between temperature and the infectivity of cercariae was first studied by Foster (1964) who reported that, at temperatures ranging from 18°C to 30°C, penetration and maturation of S.mansoni cercariae in mice was not affected. However, others have found that the ability of S.mansoni cercariae to penetrate mouse skin was strongly influenced by cercarial maintenance temperatures from 10°C to 40°C (DeWitt, 1965; Stirewalt and Fregeau, 1965 and Purnell, 1966b). Cercariae maintained at any of these temperatures were infective to mice but their infectivity, in terms of adult worm recovery, was markedly reduced both at low and high temperatures (DeWitt, 1965 and Purnell, 1966b). Chappell and Coles (1973) have recently demonstrated that the infectivity of S.mansoni cercariae was reduced as a result of cooling to 0°C.

The objective of this work was to determine whether deaths of S.mansoni and S.haematobium cercariae, during penetration of mammalian host skin, were influenced by the cercarial maintenance temperatures and whether losses at this stage were related to the adult worm recovery.

Materials and Methods

Cercariae of S.mansoni and S.haematobium were collected within 2 hours of emergence from their snail-intermediate hosts and kept in aquarium water at temperatures of 10°C and 40°C, in thermostatically

controlled incubators, for 2 hours. Another batch of cercariae, similarly collected, was maintained for 2 hours under standard aquarium conditions at a temperature of 25-27°C and used as a control. Two sets of experiments were carried out:

- a) The first experiment was designed to compare the levels of death of cercariae of S.mansoni and S.haematobium, maintained at the above temperatures, during penetration of host skin.
- b) A combined experiment was designed to compare the mortality of cercariae of S.mansoni and S.haematobium in the skin with the recovery of adult worms from animals infected with cercariae maintained at the above temperatures.

Results

Death of cercariae during penetration of host skin: As shown in Table 27 and Fig. 14a the mean percentages of dead schistosomula of S.mansoni recovered from the skin of mice infected with cercariae maintained at 10°C and 40°C (55.3% and 58.6%) were significantly greater than that recovered from mice infected with cercariae maintained at 25-27°C (30.9%). The difference between the means at 10°C and 25-27°C ($t=6.0$; $p < 0.01$) and 40°C and 25-27°C ($t=8.6$; $p < 0.01$) are highly significant.

Table 28 and Fig. 15a show a similar situation in the case of S.haematobium: high levels of death (30.0% and 35.0%) at 10°C and 40°C compared to only 15.0% at 25-27°C and the difference between the means at 10°C v 25-27°C and 40°C v 25-27°C are highly significant ($t=7.6$; $p < 0.01$ and $t=8.0$; $p < 0.01$).

Comparison of death of cercariae in the skin with the adult worm recovery: Table 29 and Fig. 14b show that the mean percentage

T A B L E 27

Percentages of dead S.mansoni schistosomula recovered from the skin of mice infected with cercariae maintained at three different temperatures.

Temperature °C	No. of animals	Mean % dead schistosomula	Range	S.D.±	S.E.±
10	10	53.3	43-66	7.0	2.2
25-27	10	30.9	24-41	7.8	2.5
40	10	58.6	50-70	7.3	2.3

10°C v 25-27°C

Degrees of Freedom 18

t = 6.0

p < 0.01

40°C v 25-27°C

Degrees of Freedom 18

t = 8.59

p < 0.01

T A B L E 28

Percentages of dead *S. haematobium* schistosomula recovered from the skin of hamsters infected with cercariae maintained at three different temperatures.

Temperature °C	No. of animals	Mean % dead schistosomula	Range	S.D.±	S.E.±
10	10	30.0	21-35	6.1	2.1
25-27	10	15.0	11-18	4.5	1.0
40	10	35.0	28-40	6.3	2.5

10 C v 25-27 C

Degrees of freedom 18

t 7.65

p < 0.01

40 C v 25-27 C

Degrees of freedom 18

t 8.0

p < 0.01

T A B L E 29

Comparison of the percentages of *S.mansoni* cercariae (maintained at three different temperatures) recovered as dead schistosomula from the skin of mice with those recovered as adult worms.

Temperature °C	% Dead Schistosomula				% Adult Worms				Mean % cercariae not penetrating		
	No. of animals	Mean	Range	S.D.±	S.E.±	No. of animals	Mean	Range		S.D.±	S.E.±
10	10	60.4	51-73	7.2	2.3	20	29.5	17-45	9.4	2.1	6
25-27	10	39.1	33-41	2.6	0.8	20	44.9	22-59	17.1	3.8	1
40	10	64.3	55-73	7.3	2.3	20	19.7	10-34	7.5	1.7	9

10 C v 25-27 C
Degrees of Freedom 18

t = 8.68

p < 0.01

10 C v 25-27 C
Degrees of Freedom 38

t = 3.46

p < 0.01

40 C v 25-27 C
Degrees of Freedom 18

t = 10.28

p < 0.01

40 C v 25-27 C
Degrees of Freedom 38

t = 5.87

p < 0.01

T A B L E 30

Comparison of the percentages of *S. haematobium* cercariae (maintained at three different temperatures) recovered as dead schistosomula from the skin of hamsters with those recovered as adult worms.

Temperature °C	% Dead Schistosomula				% Adult Worms						
	No. of animals	Mean	Range	S.D.±	S.E.±	No. of animals	Mean	Range	S.D.±	S.E.±	Mean % cercariae not penetrating
10	10	29.0	23-31	6.5	1.9	20	9.4	6-11	7.0	3.1	7
25-27	10	17.0	12-20	5.1	2.0	20	16.3	12-20	10.0	2.3	2
40	10	40.0	29-42	2.3	1.7	20	7.5	3-10	3.9	1.9	10

10 C v 25-27 C

Degrees of Freedom 18

t = 8.2

p < 0.01

40 C v 25-27 C

Degrees of Freedom 18

t = 9.6

p < 0.01

10 C v 25-27 C

Degrees of Freedom 38

t = 5.6

p < 0.01

40 C v 25-27 C

Degrees of Freedom

t = 6.5

p < 0.01

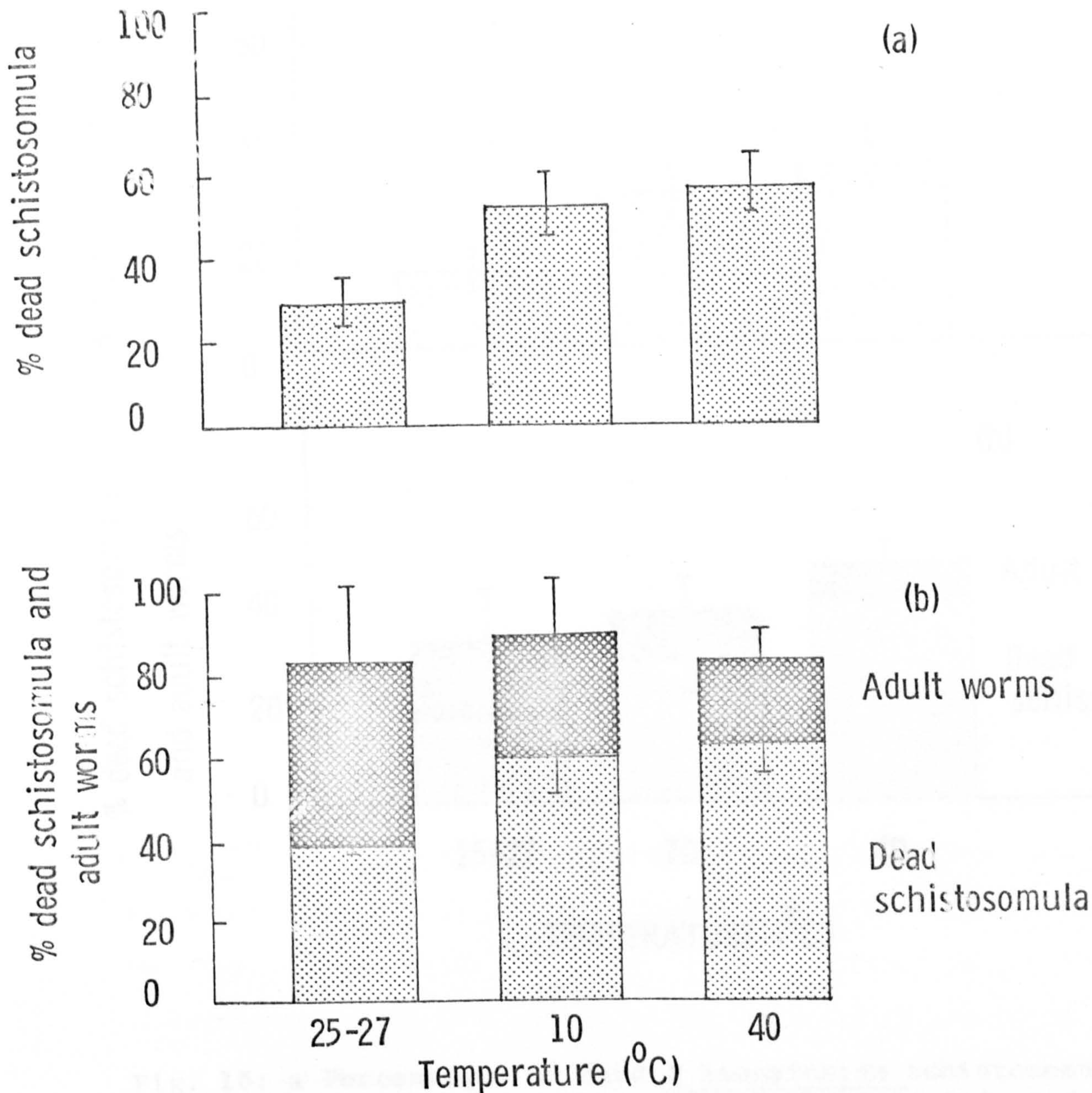


Fig. 14: a Percentages of dead *S. mansoni* schistosomula recovered from the skin of mice infected with cercariae maintained at three different temperatures.

b Comparison of the percentages of *S. mansoni* cercariae recovered as dead schistosomula from the skin of mice (infected with cercariae maintained at three different temperatures) with those recovered as adult worms.

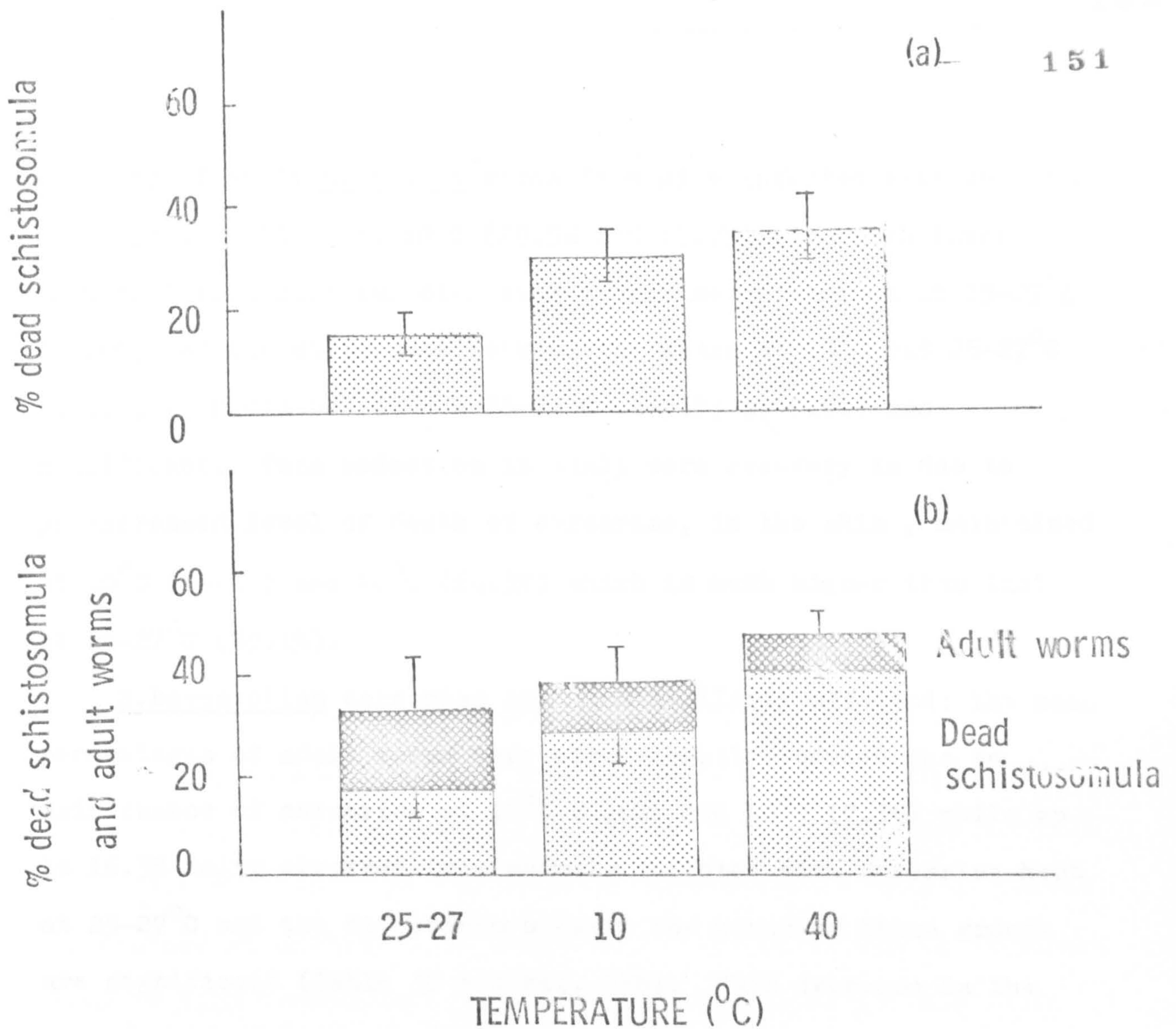


Fig. 15: a Percentages of dead *S. haematobium* schistosomula recovered from the skin of hamsters infected with cercariae maintained at three different temperatures.

b Comparison of the percentages of *S. haematobium* recovered as dead schistosomula from the skin of hamsters (infected with cercariae maintained at three different temperatures) with those recovered as adult worms.

recovery of adult S.mansoni worms from mice infected with cercariae maintained at 10°C and 40°C (29.5% and 19.7%) were much lower than that from mice infected with cercariae maintained at 25-27°C (44.9%) and the difference between the means at 10°C and 25-27°C ($t=3.4$; $p < 0.01$) and 40°C v 25-27°C ($t=5.8$; $p < 0.01$) was significant. This reduction in adult worm recovery is due to an increased level of death of cercariae, in the skin, maintained at 10°C (60.4%) and 40°C (64.3%) which is much higher than that at 25-27°C (39.1%).

S.haematobium cercariae are also similarly affected: The mean percentages of adult worms were significantly reduced due to maintenance of cercariae at 10°C (9.4%) and 40°C (7.5%) while up to 16.3% being observed from animals infected with cercariae kept at 25-27°C and the differences between the means of these groups are significant (Table 30 and Fig. 15b). This decrease in the recovery of adult worms is due to a marked increase in the mortality of cercariae during penetration of the skin: 29.0% and 40.0% being observed after maintenance of cercariae at 10°C and 40°C as compared with 17.0% at 25-27°C.

Both high and low temperatures affected the ability of S.mansoni cercariae to penetrate host skin: 6% and 9% of cercariae maintained at 10°C and 40°C did not penetrate host skin compared to only 1% of cercariae maintained at 25-27°C. Similar figures were observed in S.haematobium: 7%, 10% and 2%.

Discussion

It is evident from this study that the infectivity of cercariae

of S.mansoni and S.haematobium was markedly affected by the cercarial maintenance temperatures. The levels of mortality of cercariae of both species, during penetration of host skin, were increased due to maintenance of cercariae for only 2 hours at 10°C and 40°C. These results correlate well with the finding that the recovery of adult worms from animals infected with cercariae maintained at these temperatures was drastically reduced.

DeWitt (1965) and Purnell (1966b) reported that temperature played a major role in determining the infective potentiality of S.mansoni cercariae. The worm burdens of animals infected with cercariae maintained at high or low temperatures for some time were far less than that from animals infected with cercariae maintained at ambient room temperature. Purnell (1966b) suggested that at high temperature S.mansoni cercariae move rapidly thus losing most of their endogenous energy reserves and subsequently resulting in a reduction of infectivity of cercariae. This connection has been conclusively demonstrated in the present study (Expt. b). Chappell and Coles (1973) found that cooling of S.mansoni cercariae resulted in a pronounced reduction in their infectivity. They considered that this reduction was not due to exhaustion of endogenous energy reserves of the cercariae but that some other casual relationships might exist. It might be probable that maintenance of cercariae at low temperatures results in suppression of some vital activity or physiological process thus causing increased mortality of such cercariae during penetration of host skin.

PART 4: The effect of sublethal concentrations of the molluscicide niclosamide (Bayluscide) on the infectivity of S.mansoni cercariae

Introduction

The use of molluscicides is one of the most effective measures available at present for the control of schistosomiasis. Yet, due to the instability of some molluscicides under field conditions and the abundance and diversity of water courses to be treated a proportion of snail-habitats may be exposed to only sublethal doses of the molluscicides. It has strongly been stressed that studies on the effects of such sublethal concentrations on the larval stages of the parasite and on the intra-molluscan development were urgently required (WHO, 1965). Sturrock (1966b) was the first to conduct such studies: he investigated the effect of sublethal concentrations of the molluscicide niclosamide on the development of S.mansoni within their snail-intermediate hosts. Similar work was also carried out by Massoud and Webbe (1969) and Hira and Webbe (1972) on N-tritylmorpholine and Triphenyl lead acetate.

However, no studies were carried out on the effect of sublethal concentrations of any molluscicide on the infectivity of schistosome cercariae. The only work reported on cercariae was that of the effect of some molluscicides on their viability. Many workers (Azevedo and Pequito, 1961; Gönner, 1961; Buttner, 1961 and Webbe, 1961) have shown that cercariae are very sensitive to niclosamide and that 100% mortality occurred within 5-25 min. after exposure to concentrations of 0.2-0.7 mg. /l. Bruaux and Gillet (1961) reported that niclosamide was 750 times more effective against S.mansoni cercariae than copper sulphate.

The present work was carried out to study the effect of concentrations of niclosamide (5, 2-dichloro-4-nitro-salicylic-anilide) sublethal for cercariae on the infectivity of S.mansoni cercariae in terms of their mortality during penetration of host skin and their subsequent maturation to adult worms.

Materials and Methods

Niclosamide is a fast-acting molluscicide highly effective against Biomphalaria spp. and Bulinus spp. at concentrations ranging from 0.2-0.5 mg./l. under laboratory conditions and at 1 mg./l. applied over a period of 8 hours (Gonnert, 1961) under field conditions.

The formulation of niclosamide used in this study was a 70% wettable powder which was dissolved in dechlorinated tap water to make a 10 mg./l. stock solution. The different concentrations used in this study were then prepared by serial dilutions using an Agla microsyringe (accuracy of delivery \pm 0.0005 ml.). Three experiments were carried out in duplicate:

- a) It was first decided to test the effect of different concentrations on niclosamide on the behaviour and viability of cercariae so as to choose two sublethal concentrations for use in subsequent experiments. For this purpose 80-100 freshly shed cercariae, in 0.1 ml. of suspension, were added to 5 ml. of freshly prepared test solutions in embryo dishes which were observed up to 4 hours after treatment. A dish containing untreated water and cercariae was set up as a control.
- b) The second experiment was carried out to compare the mortality of molluscicide treated and normal untreated cercariae during penetration of adult mouse skin. Freshly shed cercariae were

maintained at 25-27°C in 0.05 and 0.02 mg./l. of niclosamide for two hours before infection of mice.

c) The third experiment was designed to compare the mortality of treated and untreated cercariae, during penetration of host skin, with the recovery of adult worms from the liver and mesenteric veins.

Results

Behaviour and viability of cercariae: The results of this experiment showed that concentrations of 0.2-1.0 mg./l. were lethal to cercariae. Cessation of movement and death occurred within 5-20 min. after exposure to these concentrations. Exposure to 0.1 mg./l. produced immobility of cercariae after 15 min. but 100% mortality was not achieved before 2 hours. No cercariae were visibly affected by exposure to concentrations of 0.02 or 0.05 mg./l. It was thus decided to use these two sublethal concentrations in subsequent experiments.

Death of cercariae during penetration of host skin: The results of this experiment (Table 31 and Fig. 16a) show that exposure of S.mansoni cercariae for 2 hours in 0.05 and 0.02 mg./l. of niclosamide has markedly increased the level of mortality of cercariae in the skin to 58.2% and 48.9% respectively compared to 28.5% with normal untreated cercariae. Student's t-test shows that the differences between the mean mortality at 0.05 mg./l. versus control ($t=13.6$; $p<0.01$) and 0.02 mg./l. versus control ($t=7.6$; $p<0.01$) are significant.

Comparison of death of cercariae in the skin with the recovery of adult worms: The results of this combined experiment are shown in Table 32 and Fig. 16b. The mean percentage recovery of adult worms from mice infected with cercariae exposed to 0.02 mg./l. (21.7%) is

T A B L E 31

Percentages of dead S.mansoni schistosomula recovered from the skin of mice infected with cercariae exposed to different concentrations of niclosamide.

Concentration of niclosamide (mg. /l.)	No. of animals	Mean % dead schistosomula	Range	S.D.±	S.E.±
Control	10	28.5	23-34	3.9	1.2
0.02	10	48.9	40-61	7.6	2.4
0.05	10	58.2	52-68	5.6	1.8

Control v 0.02 mg. /l.

Degrees of Freedom 18

t= 7.65

p < 0.01

Control v 0.05 mg. /l.

Degrees of Freedom 18

t= 13.6

p < 0.001

T A B L E 32

Comparison of the percentages of *S.mansoni* cercariae (exposed to different concentrations of niclosamide) recovered as dead *Schistosomula* from the skin of mice with those recovered as adult worms.

Concentration of niclosamide (mg./l.)	% Dead <i>Schistosomula</i>				% Adult Worms				Mean % cercariae not penetrating		
	No. of animals	Mean	Range	S.D.±	S.E.±	No. of animals	Mean	Range		S.D.±	S.E.±
Control	10	30.5	24-44	7.7	3.4	20	51.4	31-72	13.0	2.9	3
0.02	10	50.9	38-60	12.4	3.9	20	21.7	7-39	8.9	1.9	7
0.05	10	71.5	63-82	6.3	2.0	20	2.8	1-8	3.3	0.7	11

Control v 0.02 mg. /l.

Control v 0.02 mg. /l.

Degrees of Freedom 18

Degrees of Freedom 38

t= 6.029

t= 8.04

p < 0.01

p < 0.01

Control v 0.05 mg. /l.

Control v 0.05 mg. /l.

Degrees of Freedom 18

Degrees of Freedom 38

t= 12.25

t= 15.5

p < 0.01

p < 0.01

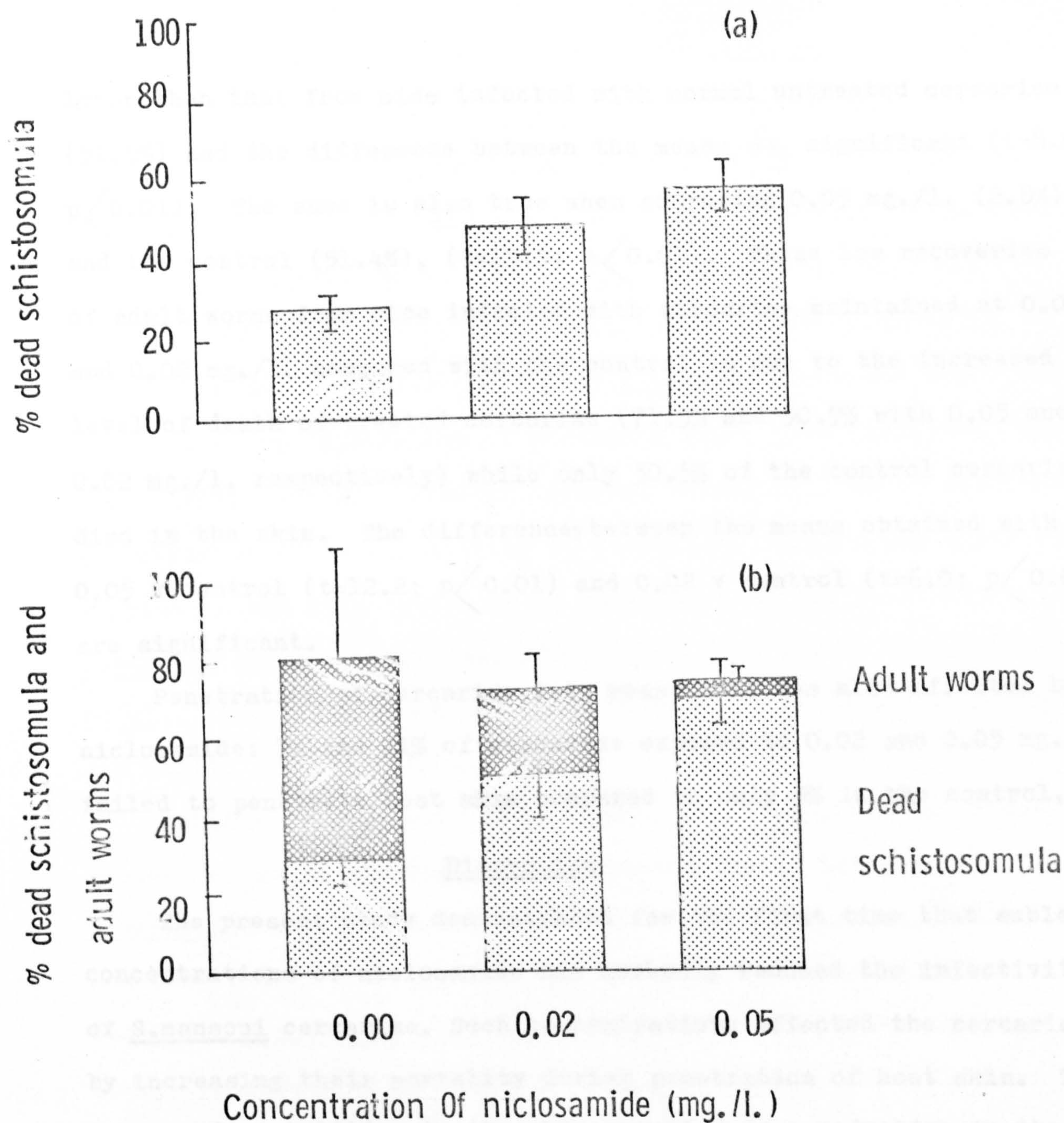


Fig. 16: a Percentages of dead *S. mansoni* schistosomula recovered from the skin of mice infected with cercariae treated with different concentrations of niclosamide.

b Comparison of the percentages of *S. mansoni* cercariae recovered as dead schistosomula from the skin of mice (infected with cercariae treated with different concentrations of niclosamide with those recovered as adult worms).

lower than that from mice infected with normal untreated cercariae (51.4%) and the difference between the means is significant ($t=8.0$; $p < 0.01$). The same is also true when comparing 0.05 mg./l. (2.8%) and the control (51.4%), ($t=15.5$; $p < 0.01$). These low recoveries of adult worms from mice infected with cercariae maintained at 0.05 and 0.02 mg./l. compared with the control is due to the increased level of death of treated cercariae (71.5% and 50.9% with 0.05 and 0.02 mg./l. respectively) while only 30.5% of the control cercariae died in the skin. The differences between the means obtained with 0.05 v control ($t=12.2$; $p < 0.01$) and 0.02 v control ($t=6.0$; $p < 0.01$) are significant.

Penetration of cercariae into mouse skin was also affected by niclosamide: 7% and 11% of cercariae exposed to 0.02 and 0.05 mg./l. failed to penetrate host skin compared to only 3% in the control.

Discussion

The present study demonstrated for the first time that sublethal concentrations of niclosamide has markedly reduced the infectivity of S.mansoni cercariae. Such concentrations affected the cercariae by increasing their mortality during penetration of host skin. These increased mortalities in the skin resulted in a reduction in the recovery of adult worms from the liver and mesenteric veins.

A similar effect was observed by Kuntz and Stirewalt (1946) of the effect of DDT on the infectivity of S.mansoni cercariae. They found that treatment of the cercariae with minute amounts of DDT caused a marked reduction in their infectivity.

Schistosome cercariae are also sensitive to the majority of other chemicals and/or physio-chemical changes in their media. Salinity is toxic to cercariae (Krakower, 1940 and Ingalls, 1946)

and so is chlorine (Olivier et al., 1945; Frick and Hillyer, 1966 and Fripp et al., 1972, 1973). The concentration of calcium and magnesium ions in water has also been shown to affect the infectivity of S.mansoni cercariae (Lewert et al., 1966). Stirewalt and Fregeau (1965) have shown that distillation of water makes it unsuitable as an exposure medium to S.mansoni cercariae since the penetration and maturation of cercariae under this condition were drastically reduced. However, mineral reconstitution of distilled water restored the infectivity of cercariae. Creams and ointments applied on the skin proved to be inhibiting to the penetration of S.mansoni cercariae (Atkins, 1947; Levine and Kuntz, 1948; Hunter et al., 1952, 1956; Moon and Hunter, 1955; Campbell and Cuckler, 1961 and Austin and Frappaolo, 1973).

Various workers have studied the effect of sublethal concentrations of molluscicides on the intra-molluscan development of S.mansoni and on the snail-intermediate host (Sturrock, 1966b; Massoud and Webbe, 1969 and Hira and Webbe, 1972). Sturrock (1966b) found that when B.sudanica tanganyicensis were treated with sublethal concentrations of niclosamide, both before and after infection with S.mansoni, there was no significant difference in the infection rates between the groups. He found that sublethal concentrations of niclosamide delayed the development of S.mansoni in the snails while Massoud and Webbe (1969) found that sublethal concentrations of N-tritylmorpholine stimulated the growth of sporocysts in B. glabrata treated in the early and late prepatent period of infection. Hira and Webbe (1972) have shown that sublethal concentrations of triphenyl lead acetate did not affect the

infection rate in treated snails but the development of S.mansoni sporocysts in the snails was delayed. As to cercarial production, Sturrock (1966b) and Hira and Webbe (1972) have shown that this was higher in the molluscicide-treated snails than in the normal untreated snails. However, Massoud and Webbe (1969) did not find any difference between the cercarial production rates in treated and untreated snails. In general, treatment of infected Biomphalaria spp., with sublethal concentrations of any of the three molluscicides mentioned, did affect their survival to varying extents depending on the maturity of the infection.

The cercaricidal properties of niclosamide, as exhibited in this study, adds an additional merit to the efficacy of this molluscicide as compared to other molluscicides.

CHAPTER VII.

GENERAL DISCUSSION.

The infectivity of schistosome cercariae is determined by a combination of factors including the species, age, sex and immune state of the mammalian definitive host, the condition of the cercariae within the snail-intermediate host and as free living organisms and the physical and chemical factors to which the cercariae are exposed in the water. Previous workers have investigated the effects of these various factors by simply determining the numbers of cercariae penetrating into the definitive host and the ability of these cercariae to mature into adult worms. The present study reveals the significance of the skin as a barrier in determining the infectivity of schistosome cercariae. It has previously been reported that cercariae are non-specific as to their penetration behaviour. However, there is a very strong innate immune response by the host skin to invading cercariae. This varies from host to host and plays a major role in determining the subsequent ability of the cercariae to mature into adult worms. Coutinho-Abath and Jampolsky (1957) found that S.mansoni cercariae could penetrate into the skin of pigeons, which are completely resistant hosts, but failed to migrate beyond the epidermis where they eventually died. Similar findings were reported by McFarlane (1952) who demonstrated that Cercariae parocellata, the cercariae of a bird schistosome, failed to penetrate further than the epidermis in the skin of man. This phenomenon also occurs in the skin of susceptible hosts since Batten (1956) found that many cercariae of Schistosomatium douthitti remained in the dermis and subcutaneous tissues of mice where they

were subjected to severe tissue reaction, in the form of local histiocytes resulting in eventual death of the cercariae. It is now known that differences in the proportion of cercariae dying in the skin can partly account for differential susceptibility of some types of mammalian hosts to schistosome infections. Clegg and Smithers (1968) have shown that up to one half of the cercariae of S.mansoni which penetrate the skin of rats die within 10 min. of penetration, whereas about 30% die in mouse skin and only 10% in hamster skin. This differential level of mortality of cercariae in the skin accounts for the fact that hamsters yield higher adult worm recoveries than mice. Rai and Clegg (1968) found a similar relationship in experimental infections of budgerigar and ducklings: 29% of cercariae die in the skin of the budgerigar, which is a natural host for this schistosome i. e. A. terrigalensis, compared to 85% in the skin of the duckling which is completely resistant to infection with this parasite. The present study has, likewise, shown that the skins of mice and hamsters also play a major role in determining their susceptibility to infection.

More S.haematobium and S.mansoni cercariae die during penetration of mouse than hamster skin and this partly accounts for the differential susceptibility of these animals to infection. Both mice and hamsters are far less susceptible to S.haematobium than to S.mansoni but the present study shows that this is not attributable to differential skin deaths since almost equal proportions of cercariae of both species die in the skin. Similar observations were recorded by Clegg and Smithers (1968) in infections of white

rats with S.mansoni. The lower recovery of adult worms from white rats was not entirely due to a higher proportion of cercariae dying in the skin. It was suggested that rats, being very poor hosts of S.mansoni, developed a very rapid and effective acquired resistance to infection which is superior to that of mice, hamsters or monkeys exposed to the same stimulus (Smithers and Terry, 1969). Mice and hamsters are, however, adequate hosts for schistosomes and it is unlikely that they respond to infection as rapidly as rats. It is quite possible that the lower susceptibility of these animals to S.haematobium, as compared with S.mansoni, might be because developing worms of S.haematobium need different physiological, nutritional or biochemical requirements to those of S.mansoni. Although it is very difficult and unsafe to extrapolate from in vitro studies to in vivo situations, in vitro culturing of both S.mansoni and S.haematobium in the same media and under the same conditions might reveal if the above hypothesis is correct. In fact, it has recently been shown that S.haematobium can be grown in the same culture media as S.mansoni (Smith and Webbe, in preparation).

In general, S.haematobium has been shown to be a very host-specific parasite. Wild animals are not usually very susceptible to this parasite (Brumpt, 1928; Azim and Cowper, 1950; Kuntz and Malakatis, 1955 a,c; Capron et al., 1965 and Gear et al., 1966). Natural infections have been reported in a baboon, guenon and a chimpanzee (Nelson, 1960; De Paoli, 1965 and Taylor et al., 1972). There are no records of infection of domestic animals with S.haematobium except the finding that domestic pigs in Nigeria could

be infected with this parasite (Hill and Onabamiro, 1960). However, Saoud (1966b) could not infect pigs with an Egyptian strain of S. haematobium. Attempts to infect sheep with S. haematobium showed that they were very resistant to infection (Leiper, 1915b; McHattie and Chadwick, 1932; McHattie et al., 1933 and Saeed, 1970). S. mansoni is not as host-specific as S. haematobium- natural or experimental infections occur in many types of animals such as primates, rodents, insectivores, marsupials and cattle (Amorim, 1953; Barbosa et al., 1953; Kuntz and Malakatis, 1955 a&c; Martins et al., 1955; Nelson, 1960; Pitchford and Visser, 1962; Loos, 1964; Andrade, 1964; Barretto et al., 1964; and Saeed and Nelson, 1974). Fenwick (1969) reported finding a tribe of wild baboons in Tanzania maintaining the life cycle of S. mansoni without any human intervention. S. japonicum is the least host-specific of the human schistosomes: a majority of domestic and wild animals are highly susceptible to it thus increasing the animal reservoir sources of the parasite and aggravating its control (Mao, 1948; Pesigan et al., 1958; Hsu and Hsu, 1962 and Ho Yi-hsun, 1963). Other aspects of this differential infectivity of S. mansoni and S. haematobium are also revealed in this study. In S. mansoni only 20-30% of the cercariae which penetrate the host skin could not be accounted for by adding the percentage loss in the skin and the percentage recovery of adult worms. Some schistosomula may remain in the skin for up to 11 days post-infection as shown by Stirewalt (1959b). Some may exhibit "surfacewards migration": these may reappear on the skin surface but having evacuated their penetration glands cannot re-enter the skin and

eventually die there. In S. haematobium infections a larger proportion of the penetrating cercariae (55-60%) cannot be accounted for in the skin or in the portal system. Studies on the lung migration of S. haematobium have shown that the percentage recovery of schistosomula from this site is much smaller than that of S. mansoni. Since the same recovery technique was used for both parasites it seems that this difference in recovery rates is possibly due to more rapid susceptibility of schistosomula of S. haematobium to the host reaction than S. mansoni.

Similar host specificity has been noted in other parasites. Betterton (1974) has recently studied the host specificity of the eye-fluke, Diplostomum spathaceum, in brown and rainbow trouts. Rainbow trout is more susceptible to infection, under laboratory and also under field conditions than brown trout thus indicating that ecological factors are not responsible for this host specificity. Recovery of cercariae from host tissues and histological studies of the skin indicated that cercariae were able to survive penetration since a differential mortality of cercariae was not noted in the different hosts' skin or tissues. Two hypotheses were put forward to explain this host specificity: muscles of brown trout might present a more effective barrier to migration of cercariae than those of rainbow trout. Another possibility is that cercariae may be disorientated by the lack of specific stimuli or presence of conflicting stimuli. The penetrated larvae may reach the head region of brown trout, just as in susceptible rainbow trout, but are not strongly stimulated to leave the circulatory system and hence remain

there where they eventually die: only one or two cercariae come under the influence of a weak stimulus and may migrate to the eyes where they mature into adult worms.

In the case of S.mansoni mice and hamsters become more resistant to infection with increase in age (Stirewalt, 1952; Purnell, 1966a; Pellegrino and Katz, 1969 and Ghandour and Webbe, 1973). It is tempting to compare this "age resistance" to situations under field conditions. Epidemiological evidence indicates that, in many endemic areas, schistosomiasis is a disease of the young and that with advancing age there is a decreased passage of eggs and a reduction in the clinical symptoms (Fisher, 1934; Bassenes and Pantoja, 1947; Gerber, 1952; Pesigan et al., 1958 and Gothe, 1963). Clarke (1966) has shown that, in Rhodesia, the prevalence of infection with S.mansoni and S.haematobium increased to a peak between 7-15 years of age and then declined to very low levels in adults. Attempts to relate these differences in prevalence rates to age resistance were dismissed. Kloetzel and Rodrigues da Silva (1967) found that resistance to infection with S.mansoni, as shown by a decrease in egg production, in a population of adults first exposed to the disease depended upon the duration of infection and not directly upon age. It thus seems that age resistance has no role in the epidemiology of schistosomiasis: man acquires resistance to reinfection due to repeated exposures to cercariae under field conditions.

The skin of previously infected hosts presents a stronger barrier to penetration of S.mansoni cercariae than that of normal: previously

uninfected hosts. A question of great interest arising from the demonstration of this dermal resistance in laboratory hosts is whether human skin presents a substantial barrier to cercarial penetration. Under natural conditions man is repeatedly exposed to schistosome cercariae due to contact with cercarial-infested water. The observation of acquired immunity noted under field conditions might be partly explained on the basis of this dermal resistance to cercarial penetration but the degree and nature of this protection are hard to explain.

Cercariae of S.mansoni and S.haematobium both show rapid decline in their infectivity with increase in their post-emergence ages. This is due to exhaustion of their energy reserves. Under field conditions the life span and infectivity of schistosome cercariae are much less than that under laboratory conditions. This is due to the existence of various injurious factors. Mechanical agitation causes a marked reduction in the infectivity of S.mansoni cercariae. Upathum (1973a) reported that this is the cause of loss of infectivity in S.mansoni cercariae after passing over a waterfall. Other extrinsic factors affecting the infectivity of cercariae under field conditions include water flow (Rowan and Gram, 1959; Radke et al., 1961 and Webbe, 1966). It was suggested that cercariae become fatigued in fast flowing water (Radke et al., 1961). Ultra-violet irradiation, turbulence, pollution and other physical and chemical factors all cause a marked reduction in the infective potentiality of schistosome cercariae (Madonado, 1959).

Ultra-violet irradiation has a damaging effect on cercariae of S.mansoni and S.haematobium which are similarly influenced. Prah (1973) found that sublethal exposures (40-60 sec. at a distance of 30 cm.) of miracidia of S.mansoni and S.haematobium to artificial ultra-violet irradiation resulted in their inability to penetrate into susceptible snail hosts. He found that sunlight contains natural ultra-violet irradiation which shortens the life span of miracidia. In fact, sunlight contains ultra-violet irradiation of long wavelengths from about 3,000 \AA to 3,900 \AA and also the most energetic and injurious short wavelength ranging from 2,000-3,000 \AA and including the most energetic peak of 2537 \AA (Stiff, 1971). Ultra-violet irradiation is known to have biological effects and direct sunlight falling on surface layers of lakes, streams and water falls produces varying degrees of disinfection by reducing bacterial contamination (Stiff, 1971). This property of ultra-violet irradiation might reduce the life span and infectivity of schistosome cercariae under natural conditions but the significance of this natural control measure is limited by the fact that turbid water absorbs ultra-violet irradiation (Prah, 1973). Another practical application of the ionizing effect of ultra-violet irradiation could be in immunization procedures for schistosomiasis. Cercariae could be attenuated and used as immunizing agents. This approach could be promising especially in endemic areas where trials could be carried out to test the value of live vaccines. At present, use is made of gamma rays and X-rays in attenuating schistosome cercariae (Villegla et al., 1961; Smithers, 1962; Radke and Sadun, 1963; Hsu et al., 1963

and Taylor, personal communication). However, field trials might be conducted in endemic areas far from centres where sophisticated means of irradiation, such as those mentioned, are available. A simple "Ultra-violet lamp" could then be used thus solving the problem. This might be a very optimistic outlook, but the increasing incidence of schistosomiasis demands the use of all feasible and safe control measures.

Low and high temperatures cause a decline in the infectivity of S.mansoni and S.haematobium cercariae which are similarly affected. Schistosome miracidia are also similarly influenced (Purnell, 1966b and Prah, 1973). However, it does not seem that temperature is a very important factor affecting the infectivity of schistosome cercariae under field conditions since the water temperature in endemic areas seldom falls below 16°C or rises above 34°C. Ghandour (1971) found that the water temperatures in irrigation canals in the Gezira area of the Sudan ranged between 16°C and 33°C and Upatham (1973b) recorded annual temperature range in water courses in St. Lucia of 22-30°C.

Sublethal concentrations of the molluscicide (niclosamide) produced a marked reduction in the infectivity of S.mansoni cercariae. This observation has profound epidemiological implications. In some schistosomiasis endemic areas, such as Lake Volta in Ghana and possibly Lake Nasser in Egypt, control of the disease is creating various problems. This is due to the enormous size of these water bodies thus rendering mollusciciding as a means of control very expensive and rather impracticable. Another disadvantage of using

molluscicides is the fact that these two lakes are of great economic importance in providing fishing opportunities and therefore a source of protein: both lakes have large quantities of edible fish. Sub-lethal concentrations of niclosamide might be cheap to apply and would not kill the fish population but would effectively reduce the danger of cercarial contamination. The only limitation to the use of this molluscicide is the difficulty that might be encountered in its application in these large water masses.

The study of the in vivo development of S. haematobium provides valuable information on some aspects of the biology of this parasite. Although no difference was found in the levels of mortality of cercariae of S. mansoni and S. haematobium during penetration of mammalian host skin, a considerable difference was noted in the recovery of schistosomula from the lungs of infected animals. At all intervals after infection fewer schistosomula of S. haematobium than S. mansoni were recovered from the lungs. This indicates that even from this early stage in development S. haematobium seems to be less adapted to its mammalian host. The fact that the migration of S. haematobium in the lungs takes longer than S. mansoni also suggests that the longer contact period with this highly immunogenic organ may elicit more effective and rapid immune responses than those induced by S. mansoni and may explain why S. haematobium is such a host-specific parasite. Although the in vivo development of S. mansoni was not fully studied in this investigation, it was noticed that the percentage recovery of the six stages of S. haematobium described was very low thus supporting the above hypothesis. Delay in the

maturation of females of S.haematobium is difficult to explain, but may be due to an insufficient supply of nutrients or other stimuli. This contrasts sharply with S.mansoni the development of which proceeded more rapidly than S.haematobium, and in which females reached sexual maturity immediately after the males were fully mature with a complete set of testes all of which contained spermatozoa (Clegg, 1965).

The present study shows that S.mansoni and S.haematobium cercariae are similarly affected by the factors studied. It also illustrates the importance of the mammalian host skin in determining the subsequent infectivity of the cercariae. Further studies need to be conducted on the host-specificity of S.mansoni and S.haematobium. It would be interesting to carry out qualitative and quantitative analyses of the antigenic stimuli produced by developing schistosomula and adult worms of both species of parasites: it might be possible that S.haematobium worms produce more stimulus than those of S.mansoni thus explaining the limited host-specificity of this parasite; and, in part, the marked apparent drop in age-specific human prevalence rates which occurs after the second decade of life and which is almost certainly due to the development of strong acquired resistance. More studies are to be carried out to determine the role of the mammalian skin in innate immune responses and the degree of protection offered to the host.

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A STUDY OF THE DEATH OF *SCHISTOSOMA MANSONI*
CERCARIAE DURING PENETRATION OF
MAMMALIAN HOST SKIN: THE
INFLUENCE OF THE AGES OF
THE CERCARIAE AND OF
THE HOST

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A STUDY OF THE DEATH OF *SCHISTOSOMA MANSONI* CERCARIAE DURING PENETRATION OF MAMMALIAN HOST SKIN: THE INFLUENCE OF THE AGES OF THE CERCARIAE AND OF THE HOST

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Abstract

GHANDOUR A. M. and WEBBE G. 1973. A study of the death of *Schistosoma mansoni* cercariae during penetration of mammalian host skin: the influence of the ages of the cercariae and of the host. *International Journal for Parasitology* 3: 789-794. The number of cercariae of *S. mansoni* which die during penetration of mouse abdominal skin steadily increases with age following emergence from the snail. An initial mortality level of about 30 per cent is observed for 2-h-old cercariae which rises to 50 per cent at 8 h and 85 per cent at 24 h. These increased losses in the skin are shown to account substantially for the known decrease in infectivity which accompanies ageing of cercariae. The number of cercariae which die in the skin of very young mice (2 days old) is less than one-third of the level in adult mice. Losses in the skin increase with age of the host up to about 28-35 days. This increased mortality in the skin is shown to account for the observed age resistance of mice, where fewer cercariae mature into adult worms in mice of 1 month or more, than in very young mice.

INDEX KEY WORDS: *Schistosoma mansoni*; *Biomphalaria glabrata*; mouse; cercariae; schistosomula; penetration; host skin; ageing; age resistance.

INTRODUCTION

MILLER & EDNEY (1957) first showed that the number of schistosomes (*Schistosomatium douthitti*) recovered as adult worms from the rat was adversely influenced by the age of the cercariae after emergence from the snail intermediate host. A fuller study (Olivier, 1966) demonstrated a similar effect with the cercariae of *Schistosoma mansoni* in mice. The infectivity of the cercariae, in terms of the number surviving to become adult worms, was reduced to half, 8 h after they had emerged from the snail.

The age of the definitive host has also been shown to affect the number of cercariae recovered as adult worms. Young mice exposed to the same batch of cercariae as old mice yielded a higher worm recovery (Stirewalt, 1952). This observation has been greatly extended by Lewert & Mandlowitz (1963) who found that young mice (about 1 month) were more susceptible to *S. mansoni* than old mice (about 1 year). Purnell (1966) showed that 5-day-old mice were particularly susceptible to *S. mansoni*, but this high susceptibility decreased sharply and reached a steady adult level after about 1 month.

Relatively large numbers of *S. mansoni* cercariae die or show severe damage within a few minutes of penetrating the skin of mice, rats and hamsters (Clegg & Smithers, 1968). This initial loss varies considerably in different hosts and is the main factor influencing the higher adult worm recovery from hamsters than from mice or rats.

The objective of the present work was to determine whether losses of *S. mansoni* cercariae during penetration of mammalian host skin are influenced by the ages of the cercariae and of the definitive host.

MATERIALS AND METHODS

A Puerto Rican strain of *S. mansoni* maintained in *Biomphalaria glabrata* (Say), and white mice of the T.O. strain were used in the experiments. Snails were individually exposed to 6–8 miracidia and then maintained in aquaria as described by Webbe & James (1971). Cercariae emerging from a group of 60–80 snails were used throughout the study.

Adult mice were exposed to the cercariae using the ring method of Smithers & Terry (1965). Very young mice, up to 7 days old, were infected by taping each one over a circular well attached to a Petri dish so that the abdominal surface was in contact with a suspension of cercariae in the well.

In order to recover schistosomula from the skin, mice were used in groups of 5, each mouse being exposed to 2000–3000 cercariae within a 10-min period. After an additional 5 min, each animal was killed by breaking its neck and the infected area of the skin was washed with aquarium water and quickly excised. The skin was then chopped into small pieces and incubated in 10 ml Hanks's saline at pH 7.4 for 1–2 h in a water bath maintained at 37°C. The schistosomula were isolated by sieving the suspension through a small wire mesh filter placed in a 15 ml centrifuge tube. They were concentrated in 0.5 ml Hanks's saline and the number of dead or damaged organisms was assessed by their inability to exclude methylene blue (0.03% in Hanks's saline), as described by Clegg & Smithers (1968).

Groups of 10 mice used for worm recovery were each infected with 60–80 cercariae and perfused 6–7 weeks later using the method of Smithers & Terry (1965).

RESULTS

The effect of the age of cercariae on their mortality during penetration of the skin

The cercariae were allowed to age under standard aquarium conditions at a temperature of 25–27°C. Cercariae aged 2, 4, 6, 8, 10, 18 and 24 h were used for the eventual recovery of schistosomula from the skin of adult mice (aged 4–5 weeks).

All the animals were infected on the same day with cercariae collected from a single pool because it has been shown by Evans & Stirewalt (1951) that the infectivity of different batches of cercariae may vary.

Figure 1 summarizes the results of two experiments which demonstrate clearly that the percentage of deaths in the skin rises steadily throughout a 24-h period. The initial level of deaths using 2-h old cercariae, the approximate age at which cercariae are commonly used to infect laboratory hosts, was very similar to that obtained by Clegg & Smithers (1968) and presents a base-line for subsequent experiments.

The percentage of schistosomula dying in the skin (Fig. 1) roughly doubles between the age of 2 and 10 h. Cercariae 2 and 10 h old were therefore chosen to determine whether the increased mortality of cercariae during penetration of the skin due to ageing was related to the percentage of cercariae recovered as adult worms.

Figure 2 shows the combined results of the two experiments. The increase in the percentage of dead schistosomula in the skin, due to ageing of the cercariae, evidently accounts for a reduction in the recovery of adult worms from the hepatic portal system.

The effect of the age of the definitive host on mortality of cercariae during penetration of the skin

Mice varying in age from 2 days to 28–35 days were infected with cercariae within 2 h of emergence and the number of dead schistosomula recovered from the skin was determined. Due to the limited numbers of skin recoveries which can be made at one time all the animals of different ages tested could not be infected from the same batch of cercariae. A group of mice 28–35 days old was used as the control and compared with two other groups of mice of different ages, three replicates of each experiment being made.

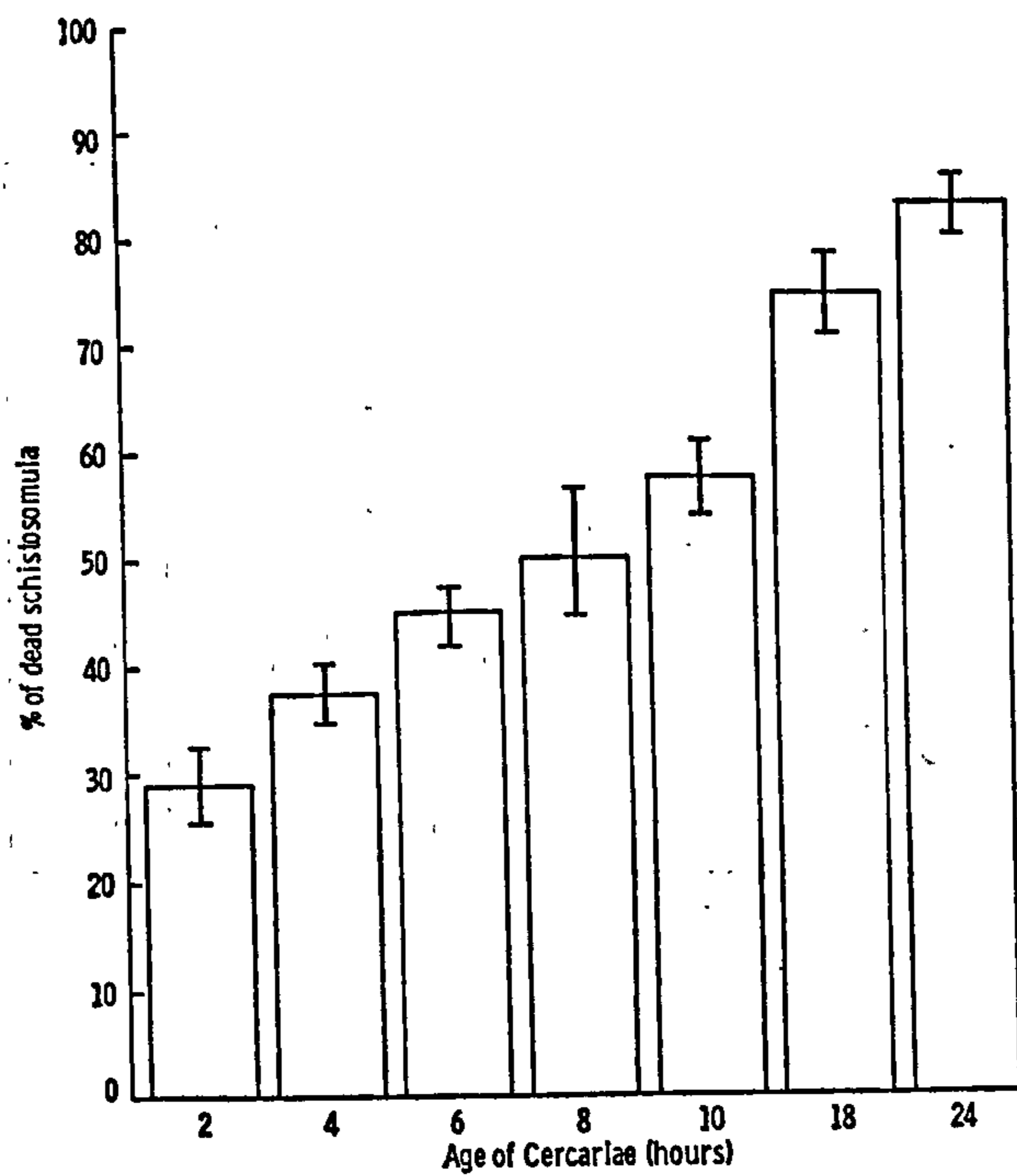


FIG. 1. Percentages of dead schistosomula recovered from the skin of mice infected with cercariae of increasing age. Each column represents the combined results of two experiments.

The results are summarized in Fig. 3 and it is evident that the percentage of dead schistosomula recovered from the skin rises steadily in animals from 2 to 28-35 days old. The level of deaths in 28-35-day-old mice (33.2 per cent) is comparable with results obtained for adult mice infected with 2-h-old cercariae (Figs. 1 & 2) and is roughly three times the level obtained in 2-day-old mice.

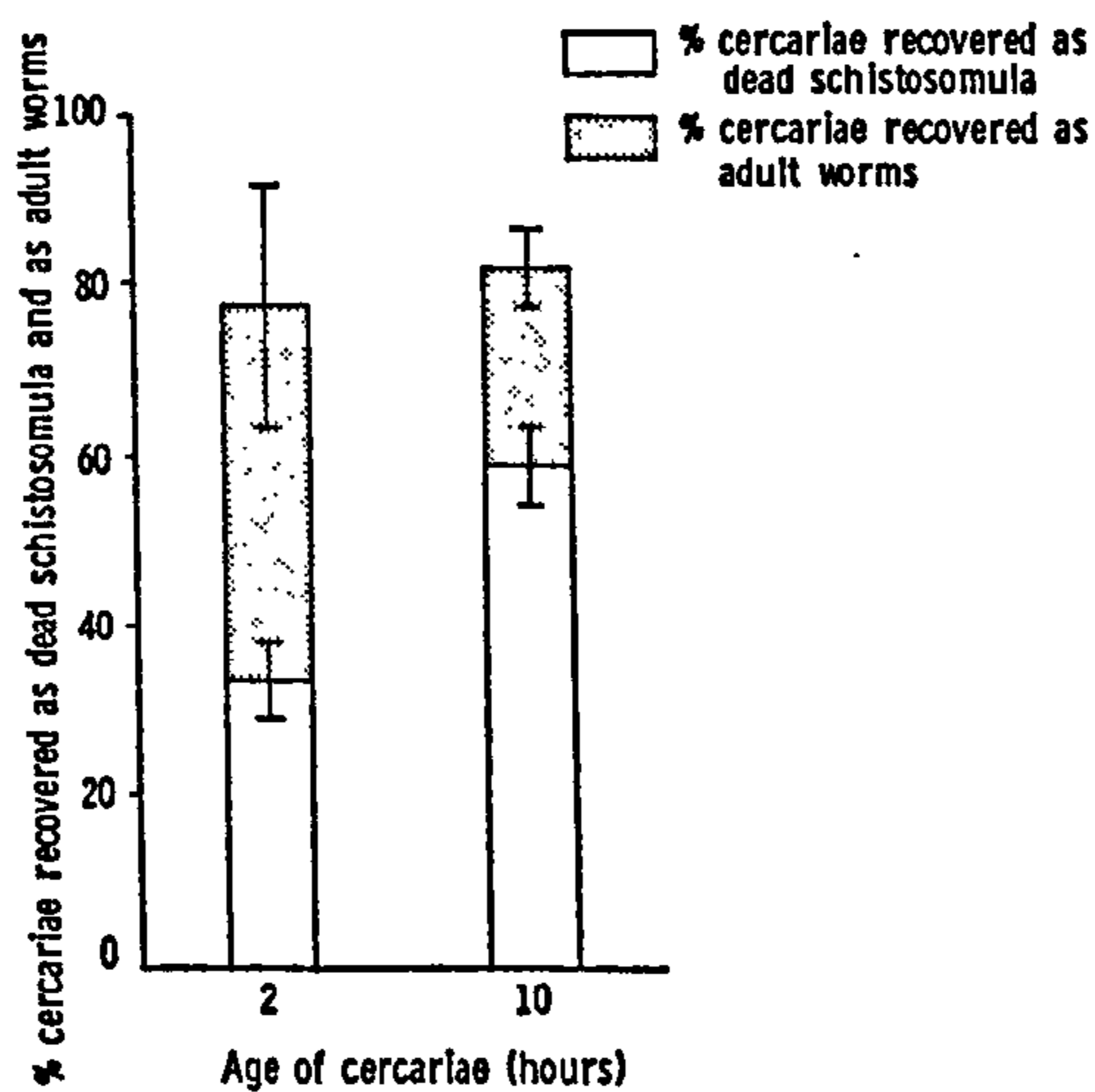


FIG. 2. Comparison of the percentages of fresh and old cercariae (2 and 10 h respectively) recovered as dead schistosomula from the skin of mice with those recovered as adult worms. Each column represents the combined results of two experiments.

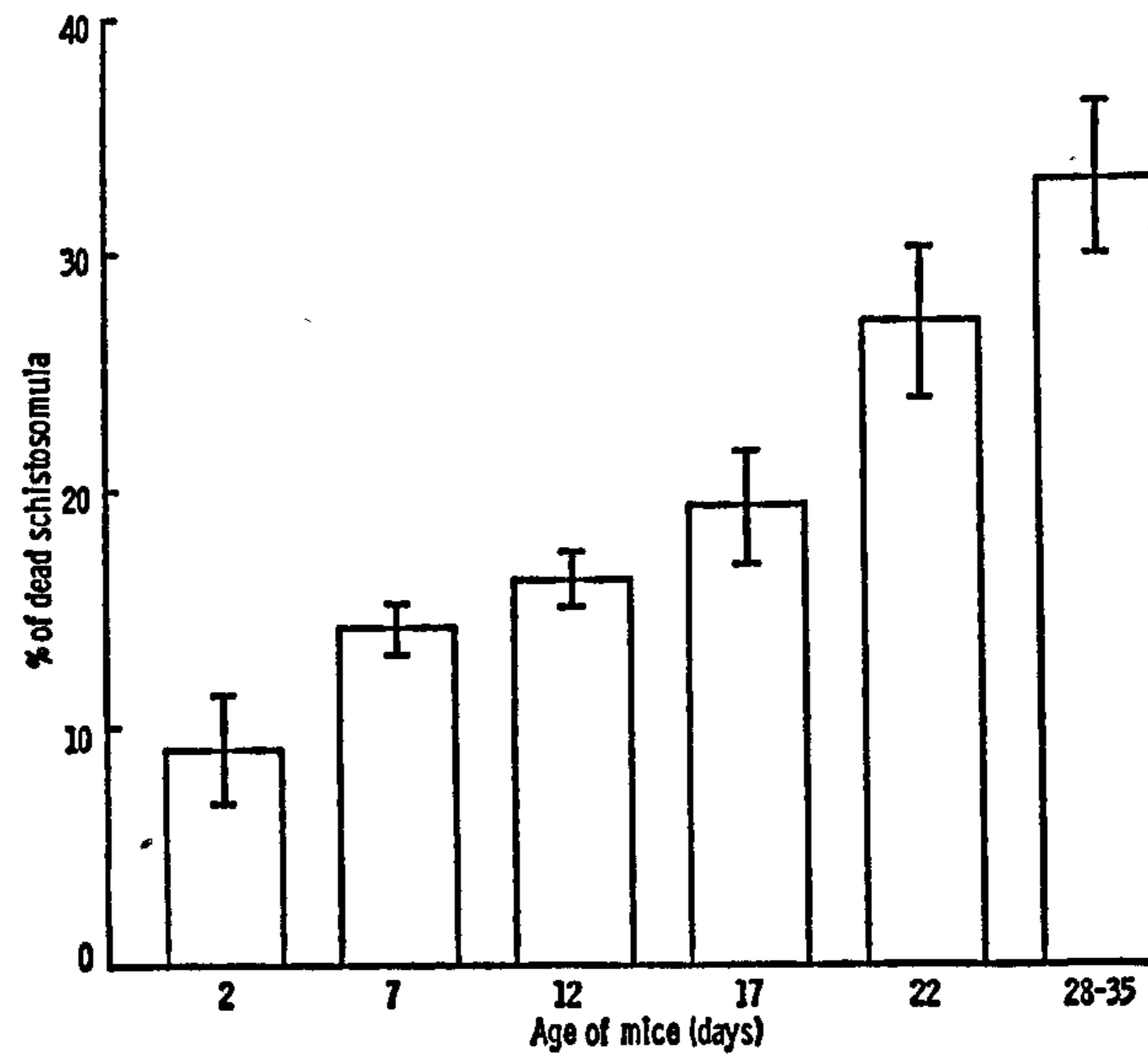


FIG. 3. Percentages of dead schistosomula recovered from the skin of mice of different ages. Each column represents the mean of 15 determinations and the column for 28-35-day-old mice 45 determinations.

On the basis of these findings an experiment was carried out to compare the mortality of schistosomula in the skin with the recovery of adult worms, using 2 and 28-35-day-old mice.

Figure 4 summarizes the combined results of two experiments which clearly demonstrate that the higher recovery of adult worms from young mice (2-day-old) is due to a much lower mortality of schistosomula during penetration of their skin.

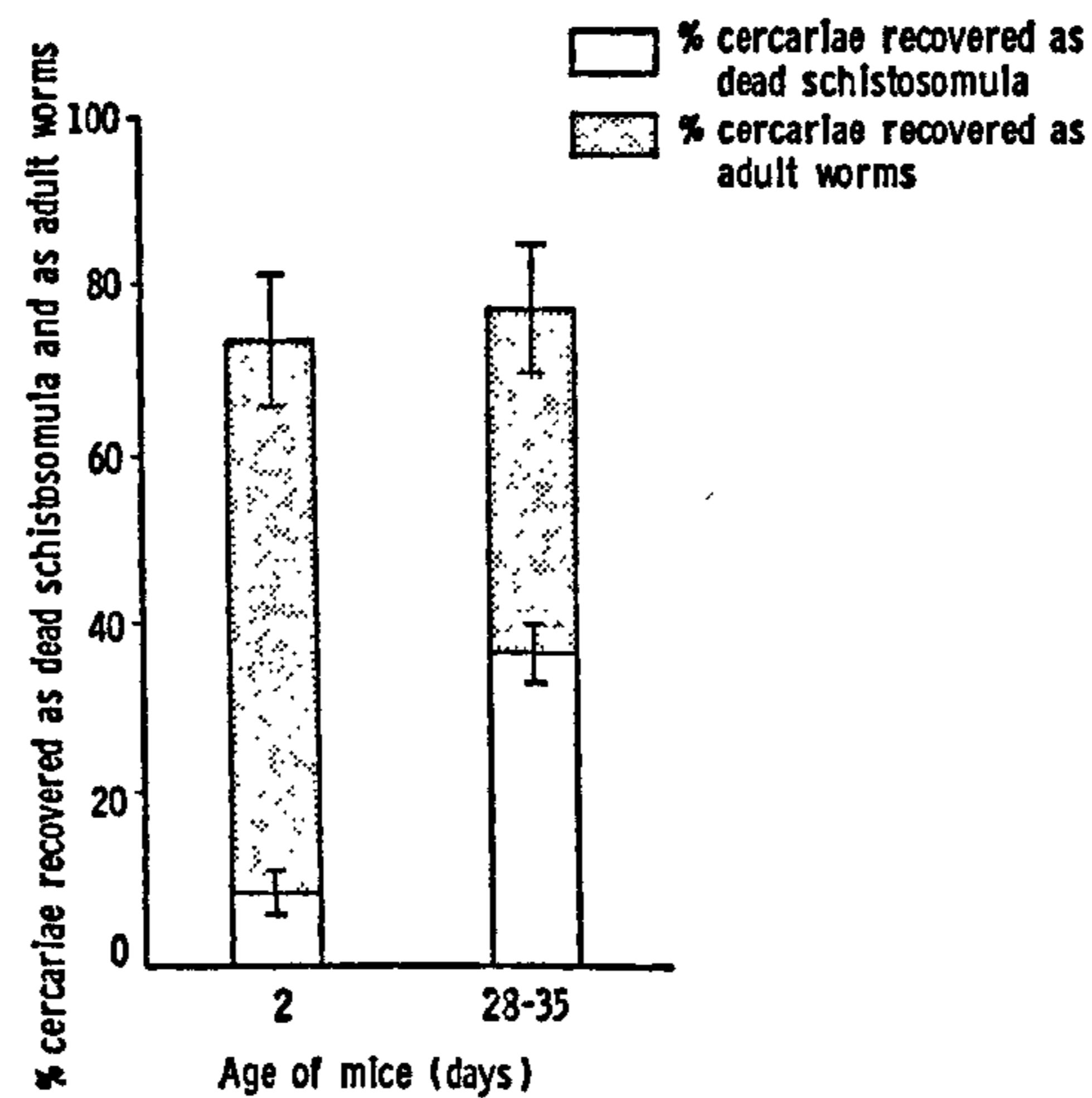


FIG. 4. Comparison of the percentages of cercariae recovered as dead schistosomula with those recovered as adult worms from the skin of young and old mice (2 and 28-35 days respectively). Each column represents the combined results of two experiments

DISCUSSION

This study has established that ageing of *S. mansoni* cercariae after emergence from the snail is directly related to losses in the skin of the definitive host soon after penetration. 2-h-old cercariae show a mortality of approximately 30 per cent in the skin of mice, a figure which falls within the range (27–43 per cent) observed in mice by Clegg & Smithers (1968). As the cercariae age the percentage dying in the skin rises steadily and after 8 and 24 h, respectively, mortality is almost doubled and trebled.

This result correlates well with Olivier's finding (1966) that *S. mansoni* cercariae lose half and almost all their infectivity 8 and 24 h after emerging from the snail, judged by the number which survive to become adult worms in the hepatic portal system. The inference that the loss of infectivity observed by Olivier (1966) is due to increased mortality of older cercariae in the skin was confirmed experimentally (Fig. 2).

The present study has also shown that the age of the mouse host directly affects the proportion of cercariae dying during penetration of the skin. In 2-day-old mice the level of mortality in the skin was less than one-third the 'normal' level found in adult mouse skin. During the first month of life the number of dead schistosomula rises steadily and reaches the adult or normal level at about 28–35 days.

This result correlates well with the finding that in very young mice a much higher number of the infecting cercariae develop into adult worms than in mice 1 month old or older (Stirewalt, 1952; Purnell, 1966). The obvious conclusion is that the higher susceptibility of young mice to infection is due to young mouse skin being a less formidable barrier to invading cercariae. Experiments which demonstrate this connection (Fig. 4) show clearly that a low level of mortality in the skin of 2-day-old mice results in a high worm recovery, whereas the greatly increased losses (more than three times), almost exactly account for the drop in adult worm recovery.

Two factors have therefore been shown to affect the number of cercariae which die during penetration of the definitive host skin, namely the age of the cercariae after emergence from the snail and the age of the host. Olivier (1966) suggested that the depletion of energy reserves of the cercariae during their short non-feeding free-living state could account for decreased infectivity in older cercariae. The level of glycogen is, in fact, reduced to one-fourth of the initial value in cercariae which have been swimming in water for 18 h after emergence from the snail (Bruce *et al.*, 1969). Rai & Clegg (1968) demonstrated a very much reduced level of glycogen in the cercariae of a bird schistosome which died in the skin compared with the level in living ones. These findings suggest that a low level of energy reserves may be, in part, responsible for increased mortality in the skin, but the evidence is circumstantial and experiments to investigate this question are in progress.

The lower mortality of cercariae in the skin of very young mice may be related to the reduced amount of energy needed during penetration. Lewert & Lee (1954) and Lewert & Mandlowitz (1963) have shown that the ground substance of the dermis and basement membrane of the epidermis of very young mice are much less dense and less highly polymerized than in older mice, and they considered this would make penetration of young skin a much easier task for cercariae.

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The effect of ultra-violet irradiation on cercariae of *Schistosoma mansoni*

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STANDEN and FULLER (1959) have shown that ultra-violet irradiation of cercariae of *S. mansoni* inhibits their development to the adult stage. The present work was carried out in an attempt to explain this phenomenon. A Hanovia ultra-violet lamp was used to irradiate freshly shed cercariae (maintained in a 10 ml. suspension) at a distance of 16.5 cm. from the lamp for 5-20 seconds i.e. exposures that had no visible effect on the motility and behaviour of the cercariae.

The demonstration showed that irradiation of the cercariae for intervals as short as 5-20 seconds markedly increased their mortality during penetration of mouse skin. When normal non-irradiated cercariae were used to infect mice only 30% of them died during penetration. However, when cercariae irradiated for 20 seconds were used as many as 68% died.

Migration of schistosomula to the lungs of mice was unaffected by irradiation of cercariae. The recovery of living fully mature schistosomula was relatively high on day 3 postinfection (2.26%) increasing steadily to a peak of 16.9% on day 7. This contrasts sharply with irradiated cercariae the migration of which was drastically affected: very few (0.5-1.0%) dead immature schistosomula were recovered on day 3 postinfection, and none at all from day 4-10.

The percentage recovery of mature adult worms from their usual locations in the liver and mesenteric veins was quite normal in mice following infection with normal non-irradiated cercariae, as high as 33% recovery being observed. However, no mature worms were recovered from mice infected with irradiated cercariae.

The demonstration clearly showed the inimical effects of ultra-violet irradiation on cercariae of *S. mansoni*.

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