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STUDIES ON SCHISTOSOMIASIS MANSONI IN PUERTO RICO

III. BIOLOGICAL STUDIES. 2. THE MAMMALIAN PHASE OF THE LIFE CYCLE *

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INTRODUCTION

When Leiper [1] first elucidated the mammalian phase of the life cycle of *Schistosoma mansoni*, his primary interest was the differentiation of this species from *S. haematobium*. He demonstrated that apharyngeal fork-tailed cercariae,

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which matured in second-generation sporocysts in *Planorbis boissyi* in the lower Nile Valley, when inoculated into the Indian monkey by mouth, developed into adult schistosomes in the intestinal veins; and that lateral-spined eggs were first recovered from the feces of this animal 42 days after inoculation. However, as early as May, 1908, Leiper [2] had reported to the British Colonial Office his evidence from human autopsy material of the paired "association of the multitesticular male" (*i. e.* male worms having seven to nine small testes) "with the female having lateral-spined eggs". In his life history experiments this investigator was able to confirm and amplify his earlier findings. Fairley's studies [3] were also based on experimental infection of monkeys, but were limited for the most part to the lesions produced in this host and their similarities to the tissue changes previously reported from the human subject. In contrasting the location of the adult worms of *Schistosoma mansoni* and *S. haematobium* in the experimental animal, Fairley (l. c.) found that the former were present mainly in the branches of the portal, superior and inferior mesenteric veins; those of *S. haematobium* were found to some extent in these veins, but in greatest numbers in the pelvic plexuses, especially the prostatic, vesical and uterine branches, which are reached by travelling *via* the inferior mesenteric to the inferior hemorrhoidal plexus, which leads directly or indirectly into the pelvic plexuses.

With the experimental work of Japanese investigators as a background, Faust and Meleney [4], working in China, carried out an extensive study of the migration route and developmental stages of *Schistosoma japonicum,* and concluded, on the basis of their findings, that once the infective-stage larvae of this bloodfluke had reached the peripheral venous circulation, after penetrating the skin, their normal course of migration through the tissues to the portal vessel was entirely within the blood vessels, thus confirming the work of Miyagawa [5] and his colleagues. The solution of the question of the route of predilection taken by *S. japonicum* was not only biologically interesting, but has served as a clue for subsequent pathological and clinical studies. For many years we have felt that a similar intensive experimental

attack on the migration route and successive developmental stages of *S. mansoni* in the organs and tissues of the body, through which this worm passes and in which it matures, might explain some of the clinical anomalies associated with schistosomiasis mansoni. In these investigations we were also anxious to discover whether *Schistosoma mansoni* paralleled *S. japonicum* step by step in its development within the mammalian body, or whether there might be important differences between these two species. As mentioned in previous studies in this series, we have carried on this work in an area where there is an abundance of *Schistosoma mansoni* material, without the slightest possibility of confusion with *S. haematobium, S. bovis, S. spindale,* or other mammalian schistosomes.

SCOPE OF THE PROBLEM

In undertaking this problem, we have found it desirable to study, stage by stage, the course of migration through the tissues and the detailed development of the worm, from the time of inoculation until maturity and the initiation of oviposition. Such a progressive development could then be compared with that of *Schistosoma japonicum.* Following the prepatent period it was important to ascertain the position in the blood vessels of the adult male and female worms, their relative numbers, and the egg-laying capacity of the females in acute (early) and chronic infections. It also seemed desirable to obtain information on blood changes, if any, which took place during and immediately subsequent to the incubation period. This paper is, therefore, a presentation of information dealing with these closely related subjects. Preliminary abstracts of this work have already been published [6, 7].

ANIMALS UTILIZED AND TECHNIC EMPLOYED

For study of the course of migration through the mammalian body and the successive stages of development up to 40 days after inoculation, large white laboratory rats were utilized. Rabbits supplemented the rats, particularly during the later stages of development of the parasites and the early patent period. Daily blood examinations were made on some of the rabbits as well as on monkeys. Rhesus

monkeys, which had been born in captivity in New York City, were imported for the study of the early clinical and pathological stages of the infection, and were autopsied at critical times after completion of the incubation period, in order to obtain data on the numbers, sex ratio and position of the adult worms in the portal vessels and their adnexa, together with information on the egg-laying capacity of the females and the development of the eggs from the intra-uterine stage until they were discharged in the feces or permanently lodged in the tissues of the host.

The cercariae of *Schistosoma mansoni* used for inoculation were obtained from snails of the species *Australorbis glabratus,* naturally or experimentally infected, which were isolated in direct sunlight at 9 o'clock in the morning, and, if positive, began discharging cercariae almost immediately. At 2 p. m. these swarms of cercariae were pooled, in order to ensure the presence of both male- and female-producing larvae (*vide* Cort[8] and Faust[9]) in the inoculum. These larvae were utilized for inoculation between 4 p. m. and 6 p. m. of the same day, at the time of their maximum viability. In the case of the rats and rabbits, the animals were placed in suitable jars, into which the "infected water" had been poured to a depth of one to two inches. They were exposed for not less than one-half hour, after which they were removed, were allowed to dry themselves and were returned to their cages. Since it was impossible to handle the monkeys in the same maner, other methods were employed. Two of the four animals were inoculated by spraying the larvae onto the buccal mucosa; the other two were subjected to infection by placing grains of corn in a jar of "infected water" in their cages. By this latter procedure (which Leiper[1] apparently first employed) both the hands and the buccal mucosa of the animal came in contact with the cercariae. With the exception of one rabbit, all of the animals became infected, and in those animals sacrificed late enough to detect sexual differentiation, all were found to have obtained both male and female worms.

In the hosts sacrificed during the first 20 days of the prepatent period, a very elaborate technic was consistently employed. Two workers coöperated throughout each autopsy. The animal was allowed to die in an ether chamber

and was then removed to an autopsy board. Examination was first made for any external lesions which might have resulted from penetration of the cercariae into the skin, after which the skin was incised and reflected back from the mentum to the symphysis pubis. Cervical lymph glands were then examined and removed temporarily to isotonic sodium citrate solution. The chest plate was next cut away without injury to the diaphragm. After careful superficial examination of the lungs and pleura, the pleural cavity was irrigated with citrate solution and the washing carefully aspirated into a bottle for later examination. The heart and lungs were removed after ligating all afferent and efferent vessels. The heart was then separated from the lungs. The right and left sides of the heart were opened, washed out thoroughly and the contents carefully examined. The lungs were scrutinized more minutely for evidences of hemorrhage or congestion, a small representative portion of each was fixed in Zenker's fluid, and the remaining portions of each sliced into small bits and placed in a jar of citrate solution. The peritoneal cavity was next opened, inspected for lesions, thoroughly washed out, and the washing aspirated into a bottle and saved for later examination.

Following these procedures the diaphragm was removed, compressed between two thin fecal slides and critically examined under the microscope, first on the upper and later on the lower surface, for any evidence of lesions or worms. Portions of each diaphragm from any suspicious area were fixed in Zenker's fluid. Meanwhile we removed the abdominal organs *en masse,* after tying off all adjacent blood vessels. Representative portions of the liver were then cut off and fixed in Zenker's fluid, and the remainder snipped in several places, perfused with citrate solution, first through the portal vessel and later from the inferior vena cava backward, and the perfused fluids carefully saved for microscopic examination. The residual liver mass was next separated from the remaining viscera after ligation of the portal vessel, minced into small pieces and placed in citrate solution. In animals inoculated less than twenty days previously, perfusion of the mesenteric veins was effected by injecting citrate solution through the main mesenteric artery. In more advanced infections, minute examination of all mesen-

teric veins and radicles was made for young worms. The intestinal tract was first examined for external lesions and was then opened from end to end and scrutinized with a × 12 hand lens for injury to the mucosa which might be attributed to migration of the young worms. The spleen was also carefully inspected, but in no case up to the thirtieth day was there any evidence of gross lesions or even of engorgement. The kidneys were taken out, observed for evidence of petechiae or cloudy swelling, both before and after removal of the capsule, and then sectioned longitudinally. A representative portion of each kidney was fixed in Zenker's fluid.

Mesenteric lymph glands were studied grossly to determine their size, appearance and consistency. A portion of each was fixed in Zenker's fluid and the remainder chopped up in citrate solution. The axial, cervical, popliteal and inguinal lymph nodes were similarly treated. In some cases films and fixed portions of bone marrow were preserved for study. In all of the animals sacrificed during the first 8 days after inoculation, representative areas of the skin of the legs and abdomen were removed, scraped and sliced up, and later examined for larvae.

All washings were completely examined microscopically as thin film preparations, in search for young worms. All sliced or minced tissues, after remaining in citrate solution for 1 to 2 hours, were strained through thin cheesecloth to remove gross tissue elements, the strainings centrifuged, and later examined for worms.

All material fixed in Zenker's fluid was carried through the usual technic and sectioned serially. It was stained with Bullard's hematoxylin-eosin, mounted, and later examined carefully for biological and pathological findings.

Preliminary experiments indicated that the lungs ordinarily became free of immature migrating worms before the twentieth day. Following this period after inoculation, these organs in the pleural cavity were examined grossly and in stained sections, but no microscopic examination of concentrates of fresh sliced tissue was attempted. On the other hand, greater care was exercised in examining the mesenteric veins, and worms recovered by perfusion or dissection of

these veins were studied to determine their number, sex and stage of development, for comparison with similar data obtained from study of the adolescent worms in the intrahepatic portion of the portal vessel. From the time of the earliest migration of young worms from the liver back to the mesenteric radicles, information was obtained as to the exact levels of the intestinal tract to which these schistosomula were directed. As they matured and paired, careful notes were also taken on the egg-forming capacity of the females and the sites where eggs were deposited in the wall.

In the rabbits and monkeys, blood examinations included total red and white counts, differential white and Schilling hemograms, and Sia precipitation technic for serum euglobulin.

SCHISTOSOMA MANSONI IN THE MAMMALIAN HOST

Inoculation of the host

The infective-stage cercaria and its method of attack.—In a preceding communication (Faust and Hoffman [10]) the development of the cercaria and its escape from the appropriate host in Puerto Rico (*Australorbis glabratus*) have been described. Experimental tests have indicated that these cercariae are positively photosensitive, that they usually emerge from the molluscan host in swarms as soon as direct morning sunlight strikes them, and that they remain active for 24 to 30 hours, during which period they are more or less equally distributed through the water. On the basis of his observations in Brazil, Lutz [11] stated that "it is next to impossible to find a cercaria early in the morning, though the water may contain infected snails, while in the afternoon, after several hours insolation, they are quite abundant. . . . The dry season, when there is more sunshine and less water, while its temperature is higher, must be the time when most of the infections are acquired". Following this period of maximum viability the cercariae begin to lose their activity, sink to the bottom of the water, and rapidly die. In nature, a single discharge of cercariae would, therefore, apparently render the water infective for at least one full day. Since it has been found (Faust and Hoffman, l. c.) that infected

snails may discharge broods of viable cercariae every day for a period of several weeks or even months, it may be concluded that water in the immediate vicinity of infected snails will be practically continuously a source of infection for the full period of discharge (*i. e.* until the daughter sporocysts have been completely exhausted or earlier, in case the snail dies as a result of unfavorable conditions of the environment or of injury produced by the developing parasites). Lutz (l. c.) concluded that a contamination of ponds with viable *Schistosoma mansoni* eggs every second month would be sufficient to make the water permanently dangerous.

It has been shown (Hoffman and Faust [12]) that the snails live most commonly along the margins of quiet pools or back waters, or along the banks of irrigation ditches. The cercariae discharged by them would be present in greatest numbers in such waters and much less frequently in water flowing rapidly over a shallow gravelly or rocky bottom. Thus, children wading or bathing in quiet waters, or workers wading along irrigation ditches, are most often exposed and have been found to be the commonest groups of the population of an endemic Puerto Rican area to be infected, while washerwomen, who frequent the banks of streams where the water is shallow and stones abound, are less likely to expose themselves to any considerable number of infective-stage cercariae. Nevertheless, we have histories from intelligent patients who have stated that they became infected while taking a shower bath with unfiltered water from a city water supply derived from infected streams. Careful observations and inquiries have demonstrated that cercariae are apparently unable to attack and penetrate the human skin as long as these cercariae are any considerable distance below the surface film. For persons wading in the water the earliest indications of infection are associated with the part of the body at or above the water level, usually the lower extremities, while individuals bathing or swimming in the infected pools more usually experience attack and invasion by the cercariae over the entire body.

In laboratory animals submitted to skin inoculation the indications of invasion are first noted about one-half hour

after the animal has been partly submerged in "infected water". The animal first becomes extremely nervous, tries to raise itself out of the inoculum and shakes its body vigorously in a persistent effort to dislodge the irritating organisms. This state of irritation continues for some time after the animal has been removed from the "infected water", even several hours after the fur has completely dried. As a matter of fact, the greater the effort to dislodge the inoculum, the more likelihood there is that it will be more generally distributed over the body. When the inoculum is introduced into the mouth of the experimental animal, the same nervous condition develops, but the irritation is confined to the buccal mucosa. Monkeys even try to get rid of the irritation by scratching the inside of their mouth with their fingers. With oral inoculation the shaking of the fur is absent. Lutz (l. c.) found that certain ponds in endemic areas in Brazil caused people who bathed in them to suffer from pruritus immediately afterwards.

Simply stated, schistosomiasis mansoni pruritus is the immediate sequel to attack of the skin or buccal mucosa by the cercariae of *Schistosoma mansoni.* This attack apparently occurs only when the cercariae are caught in a thin film of water which begins to evaporate, both as a result of the body's warmth and of the lesser saturation of the surrounding air, particularly in bright sunlight. The sensation by the host is a sharp nettling pain, similar to minute pin pricks. Clinically it is distinguished from the "ground itch" produced by the invasion of hookworm or *Strongyloides* larvae into the skin. It is a sharper, more intense, exquisite pain, much shorter in its duration. Thus the ordinary uneducated person in parts of Puerto Rico, exposed to *S. mansoni* infection refers to the accompanying pruritus as *piquiña,* and that of "ground itch" as *mazamorra,* the latter being technically the lesion produced by the hookworm larva in the skin, although not differentiated by the patient from the pruritus accompanying the penetration of the skin by this larva. Itching of the skin immediately after exposure to infection with *Schistosoma japonicum* (Faust and Meleney, l. c. p. 212) is probably identical with *piquiña.*

Piquiña in man invariably results in scratching the itching site (See text figs. 1 and 2). In order to avoid *piquiña,* some workers in the Guayama irrigation projects smear axle-grease on their arms and legs. Undoubtedly this reduces exposure very considerably. In the laboratory and in the field we have found that *immediate scrubbing of the skin* with 70 per cent alcohol, following accidental skin contact with *S. mansoni* cercariae, provides complete protection, although a delay of even ten or fifteen minutes in applying the sterilizing agent may allow some of the cercariae to get into the skin. At least one accidental infection by one of our laboratory assistants occurred in spite of alcohol applied not more than fifteen minutes after exposure.

Skin penetration.—Actual entry of the skin is undoubtedly associated with the discharges of the six pairs of penetration glands of the cercaria. The outer openings of these glands are through minute duct tips, which are comparable in sharpness of their walls to empty rifle shells. Their intermittent attack on the outer skin layers, together with the glandular secretions emptied through these duct tips, provide the mechanism for penetration. Infection with *S. mansoni* in man may result from not more than fifteen minutes' contact of living cercariae, in a thin water film, with the skin (*vide supra*). Within this brief time the cercariae have dropped their tails and have frequently penetrated through the outer epidermal layer. Usually, however, the period is probably longer, and may depend on (1) the age of the cercaria, (2) the thickness of the epidermal coat and (3) the activation produced by evaporation of the water film surrounding the cercariae. In furred mammals, as in our laboratory series, the earliest indications of skin attack occurred about a half hour after contact with infected water. We conclude that secretions from the cephalic glands are used for penetration, as the glands are almost void of secretion by the time the larvae have arrived in the deeper skin layers.

In commenting on skin penetration Lutz (l. c.) has remarked that "penetration is easy but its observation is difficult . . . However, it is clearly proved by the disappearance of the bodies of the cercariae from the water which was in contact, their presence in sections of the skin, reaction on the point where they penetrated and finally the appearance of adult blood flukes after a month or more". . . . On remov-

TEXTFIGURE I

TEXTFIGURE 1.—Man in irrigation ditch at Colonia Vives, Guayama, scratching his leg where cercariae of *S. mansoni* are entering his skin and producing *piquiña*.
(Photograph by Hoffman and Viguié.)

GRABADO DEL TEXTO No. 1.—Operario en los canales de riego de la Colonia Vives, Guayama, rascándose la pierna en el momento de experimentar la *piquiña* que le produce la penetración de las cercarias mansónicas.
(Fot. Hoffman y Viguié.)

TEXTFIGURE II

TEXTFIGURE 2.—Close-up of portion of textfigure I, showing where cercariae of *S. mansoni* usually enter the epidermis (i. e. near the water level).
(Photograph by Hoffman and Viguié.)

GRABADO DEL TEXTO No. 2. — Parte de la fotografía anterior (tomada en primer plano) de la región de la pierna próxima al nivel alcanzado por el agua, sitio preferente de la penetración de las cercarias.
(Fot. Hoffman y Viguié.)

ing a piece of exposed skin twenty to sixty minutes after an infective bath "the body of the cercariae may be found in sections. . . The Schistosomulum is seen in the *rete Malpighii* (*i. e. rete mucosum*), the head touching the cutis. The glands are empty, as may be found even in specimens which just began penetrating". Upon arrival in the *rete mucosum* (Fig. 1) the larva may be said to have completed its most difficult task and to be all but ready to undergo its migration through the various tissues to its seat of adult development in the portal blood vessels.

The route of migration from the skin to the liver

All investigators are agreed that on reaching the deeper layers of the skin the metacercaria of the human schistosomes eventually enters a peripheral blood or lymph vessel. In the former case it is carried passively through the right heart directly to the lungs; in the latter case it is frequently directed into a peripheral lymph node where it may be permanently side-tracked, or from which it may sooner or later be freed, eventually getting back into the peripheral venous circulation, and thus be carried to the lungs. In studying the migration route of *Schistosoma japonicum* in young mice, Narabayashi [13,14], and later Sueyasu [15], believed they had evidence indicating that the larvae actively penetrated out of the lungs into the pleural cavity, worked their way through the diaphragm, and, once within the peritoneal cavity, penetrated actively into the liver and portal vessels. On the other hand, Miyagawa [16], and Miyagawa and Takemoto [17], on the basis of their experiments, concluded that the metacercariae passed through the pulmonary capillaries into the pulmonary venules and veins, and thence, *via* the left heart, were carried into the systemic circulation. Only those which reached the mesenteric arteries, and were carried through the mesenteric capillaries into the portal vessel, proceeded with development. Faust and Meleney (l. c.) confirmed the work of Miyagawa and Takemoto, but Fujinami [18] believed that sufficient consideration had not been given to the studies of Narabayashi. In addition to their main line of experimental evidence, Faust and Meleney (l. c.) found that metacercariae migrated out of the lungs into the pleural cavity only in very heavy infections, and that the larvae in the

pleural cavity had not only exhausted their penetration-gland secretions and could, therefore, actively penetrate no farther, but actually died in this site in a few days. Furthermore, larvae which got through the lungs into the systemic circulation, but failed to reach the mesenteric arteries, became lodged in terminal capillaries and were eventually disposed of as foreign protein emboli.

In our studies on the migration route of *Schistosoma mansoni* in white rats and rabbits, we have accumulated data which support, without exception, the hypothesis first advanced by Miyagawa, namely that *the normal migration,* from the time the larvae enter the peripheral venules until they arive in the liver *is wholly within the blood stream.*

In the first place, we have found rats and rabbits to be better animals than mice for critical experiments on the migration of schistosome larvae, since their lung capacities are greater and will, therefore, accommodate a greater number of larvae without excessive damage to this organ. In the second place, we did not submit any of our animals to an overwhelming inoculation. Hence the lungs of the animals in our series were usually not seriously embarrassed. In this way the escape of larvae from the lungs of animals in our series was believed to have been normal and not caused by overcrowding of the pulmonary capillaries, which would result in excessive breakdown of the walls and the consequent freeing of large numbers of larvae into the parenchyma of the lungs and later into the pleural cavity.

For our early period of migration, rats were primarily utilized. The animals were sacrificed so that one or more were examined at the following time intervals (in hours) after inoculation: 16, 22, 42, 64, 70, 86, 112, 136, 166, 184, 208, 232, 304, 450, 546, 720 and 960. It was not deemed necessary to utilize rabbits before the 10th day.

The route from the skin to the lungs.—Although most cercariae had probably penetrated the epidermis of our rats well before two hours after the animals had been removed from the "infected water", the 16-hour autopsy showed that they could be recovered from the deeper layers of the skin but from no other site at this time. After 22 hours, however, although larvae could still be found in small numbers in the corium, both direct and indirect evidence indicated that the majority of the larvae had reached, or were

en route to, the lungs. Marked enlargement, injection and petechial hemorrhage were noted in the cervical, axillary, inguinal and popliteal lymph glands, while both lungs also showed evidence of numerous minute petechial hemorrhages. Although no larvae were recovered from concentrates of minced lymph glands and lungs at the 22-hour period, sections of the latter organs demonstrated the presence of numerous elongated metacercariae tightly squeezed into the dilated lumina of the pulmonary capillaries (Figs. 2, 3). The absence of the young worms in the damaged lymph glands suggested that the worms had passed through these nodes without having become lodged there. The hemorrhages in these glands were similar to those observed in the lungs, where small lacunae of extravasated blood were found nearby the sites where worms were lodged. The findings at the 42-hour period were similar to those of the 22-hour stage, except that larvae could no longer be recovered from the skin, while the lymph node lesions were manifestly older. The animals sacrificed at the 64-hour and 70-hour stage showed essentially the same state of progress as the two preceeding ones, except that some of the larvae were slightly more advanced in their development (Fig. 4). The skin was now negative. The cervical, axillary and popliteal lymph glands were enlarged and petechiate, although no larvae were recovered from them, with the exception of a single undeveloped specimen which was found in the concentrate of the minced right popliteal gland. We were fortunate, however, to recover young larvae from the right heart and the inferior vena cava, indicating that worms previously lodged in the peripheral tissues were still *en route* to the lungs. In the lung tissues, both in minced concentrates and in microscopic sections, numerous elongated larvae were found in the pulmonary capillaries. Thus our evidence indicated that up to the 70th hour after inoculation larvae were still coming into the lungs in considerable numbers, or were still on the afferent side of the pulmonary circulation. All of these larvae were in the early stage of development and showed no evidence of having fed (See table 1).

Migration from the lungs to the liver.—From the 22nd to the 70th hour after inoculation larvae were accumulating in the lungs. This was indicated by the increasing number of young worms in the finer ramifications of the blood vessels

and their absence in the efferent circulation. Evidence of progress in the migration was shown for the first time in the rat sacrificed at the 86th hour. Not only were a few larvae recovered from the right heart and many from the lungs, but a few were found in the chamber of the left heart and a single specimen from perfusion of the liver. By the 112th hour an increased number was found in the left heart, although none were obtained in this animal from perfusion of the liver. In spite of numerous petechial hemorrhages in the lungs, no larvae had yet been recovered from the pleural cavity. Within another 24 hours (136 hours after inoculation) the worms were found fairly plentifully in the liver, although they were fewer than might have been expected. Right and left hearts gave moderate yields, and a single larva was obtained from washings of the pleural cavity. Thus far none of the larvae recovered had fed (*i. e.* contained red blood corpuscles in the gut).

The animal sacrificed at the 166th hour provided very interesting information. Eight larvae in a purulent exudate from the pleural cavity, one from the lungs, five from the left heart and one from the liver, were unfed. On the other hand, several larvae from the lungs, all those from the right heart, and the large majority recovered from liver perfusion and chopped liver concentrates, not only had ingested erythrocytes, but had increased appreciably in size, and some were robust. Moreover, at the 184th hour, larvae, now all fed, were still present in large numbers in the right and left hearts and the lungs, in contrast to the small number obtained from the liver. Even ten larvae (all of those recovered), seen in the washings of the pleural cavity, had previously ingested blood. Not until the 450th hour was there definite evidence that the larvae were actually accumulating in numbers in the intrahepatic portion of the portal stream and had practically disappeared from the pulmonary circulation. By this time the larvae were absent in heart washings and the pleural cavity and were recovered for the last time in the lungs. In other words, the infection had now become established in the portal system (See table 1).

During this period of migration from the lungs to the liver (*i. e.* from the 86th to the 450th hour), it was at first thought that, unlike the metacercariae of *Schistosoma japo-*