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THE MIRACIDIUM OF *TRICHOBILHARZIA INDICA* BAUGH, 1963 AND ITS HATCHING

by

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SUMMARY

Miracidia of *Trichobilharzia indica* Baugh, 1963 have been studied from embryonated eggs obtained from the excreta of naturally infected *Nettion creca.*

Mode of hatching of the miracidium has been studied. Observations show that both intake of water and the activity of the miracidium within the egg-shell are involved in the process.

Miracidium has the usual shape. Body covered with 22 epidermal plates arranged in 4 tiers; the typical epidermal plate formula being 6: 9:4:3. Variations occur in the total number of epidermal plates as well as in the number of plates in each tier.

Miracidium has lateral processes at the sides of the body between the first and second tiers of epidermal plates, but no papillae or stiff hairs could be detected in front of them.

Minute pore-like sensory organs are present in the terebratorium, in the interplatal region between the first and second tiers of epidermal plates, and also in the interplatal regions of the third tier of plates.

Important anatomical features of the miracidium are: apical gland and penetration glands are present as usual and, of these two, the former is longer than the latter; further, besides these two glands, a pair of posterior secretory glands is present; germinal cells form a compact mass and are enclosed in a membranous sac.

It resembles the miracidia of *T. elvae, T. physellae* and *T. stagnicolae* in the relative size of the apical and penetration glands, but differs in this respect from the miracidia of *T. szidati, T. cameroni* and *T. oregonensis.* It also resembles the miracidium of *T. cameroni* in the absence of a stiff hair or a papilla in front of the lateral process on the body, and in the presence of a pair of posterior secretory glands which, however, do not appear to be composite in nature.

Attempts were made to establish the infection of the miracidium in the common snails, but the result was negative.

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INTRODUCTION

The writer (2) had earlier described *Trichobilharzia indica* from the hepatic and renal vessels of the common teal, *Nettion creca,* obtained from a bird catcher who caught the birds from lakes located in the outskirts of the city of Lucknow.

Recently, the writer came across this avian schistosome in *Nettion creca* supplied by a bird catcher who caught these birds from the lake "Chinhut" which is frequented every winter by them. Six birds were obtained and when one of them was killed and examined for helminths, several mature specimens of *Trichobilharzia* and also some eggs of the same were obtained from its intrahepatic vessels, and these on study proved the fluke to be *T. indica.* The present material does not contribute any additional data of specific importance to the description of *T. indica* presented earlier by the writer.

In a routine examination of the excreta of the remaining five birds, three more were found passing mature miracidium-containing eggs in the excreta which were quite identical with those found in the hepatic tissue. This afforded the writer an opportunity of studying te process of hatching and the morphology of the miracidium of *T. indica.*

Previous works

A study of the literature shows only three works to include the complete life histories of *Trichobilharzia* species. These works are - by Neuhaus (16) on the life history of *T. szidati* Neuhaus, by Wu (32) on the life history of *T. cameroni* Wu, and by Macy et al. (13) on the life history of *T. oregonensis* (Macfarlane and Macy). Macy et al. have indeed given a brief account of the miracidium of *T. oregonensis.* Other works on the life history of *Trichobilharzia* species deal only with the accounts of the adults experimentally raised from the cercariae. These works were carried out with a view to knowing the adults of the common dermatitis-producing schistosome cercariae. Of these works may be mentioned that of Brumpt (3) and of McMullen and Beaver (14). Brumpt experimentally raised the adult of *Cercaria ocellata* La Valette which turned out to be a known species viz., *T. kossarewi* Skrjabin and Zakharow,

but, according to the law of priority, the valid name of the adult became *T. ocellata* (La Valette). McMullen and Beaver worked out the life histories of *Cercaria elvae* Miller, *C. physellae* Talbot and *C. stagnicolae* Talbot, and they described the adults that they raised and also their embryonated eggs. Ameel et al. (1) in their studies on the germinal cells in the miracidia of these three species of *Trichobilharzia* viz. *T. elvae, T. physellae* and *T. stagnicolae* made some observations on the morphology of the *miracidia.*

MATERIAL, METHODS AND RESULTS

Freshly passed excreta of the infected birds were collected in Petri dishes from the slide trays attached to the cages in which the birds were individually kept. Slide trays were examined for the excreta of the birds almost every two hours since dawn. Tap water was added to these Petri dishes and the excretory matter contained in each was stirred with a glass rod. Petri dishes were, then, left undisturbed at the room temperature (16°C to 18°C). They were examined under dissecting microscope for miracidia every 10 minutes. A few miracidia were visible in the Petri dishes after about 15 minutes, but a larger number was visible only after the lapse of about an hour. Miracidia continued emerging from the eggs for at least two and a half hours, but these were not many. Apparently these eggs did not contain fully-formed miracidia when they came out with the excreta of the host. Miracidia were removed by means of a fine pipette to cavity blocks. On occasions when a large number of miracidia had hatched in the Petri dishes, some of them were removed to test tubes four-fifths filled with tap water or pond water. This was aimed at knowing the level of their vertical distribution.

For obtaining miracidia, McMullen-Beaver's method (14) was also followed, but the Petri dish method cited above was found to be much easier in the present study.

To determine the longevity of the miracidia under the laboratory conditions, observations were made at intervals of 10 to 15 minutes on freshly-hatched miracidia kept in 2 small Patri dishes, one containing tap water and the other pond water. Conclusion as to the longevity of the miracidia has been

made from observational data taken on four different occa sions.

Two birds were later killed, and adult schistosomes obtai ned from the mesenteric veins and intrahepatic vessels by teasing these in saline were fixed. Eggs were also obtained from the livers of these birds by teasing pieces of the organ in saline.

One infected bird was alive for 30 days and it provided most of the eggs used in the infection experiments.

As eggs voided in the excreta were not all matured, only those eggs which had had fully-formed miracidia were used for studying the process of hatching. These were picked up by means of a micropipette from the saline-treated excreta of the infected birds, washed in saline, and then transferred to a cavity slide with a drop of saline. Later, saline was replaced by tap water or pond water, and the cover-glass was sealed with vaseline (19). Hatching process was studied under a microscope. To determine the overall time taken for hatching eggs were placed in cavity blocks containing tap water and pond water, and watched for the hatched miracidia under a dissecting microscope.

Live as well as fixed and stained miracidia were studied using oil immersion lens and phase contrast microscope whenever necessary. Neutral red, Brilliant cresyl blue, Nile blue sulphate and Methylene blue were used as intra-vitam stains. A combination of Nuetral red and Nile blue sulphate was used: first Neutral red was added and later Nile blue sulphate. For mounting purpose, miracidia were fixed in hot 10% Formalin, Bouin's fluid, Zenker's fluid, and Bensley's fluid as modified by Ozaki (17), and stained with Delafield's haematoxylin. Adults obtained from the birds were fixed either in 70% Alcohol or Bouin's fluid, and stained with Delafield's haematoxylin and Borax carmine.

For the study of the epidermal plates of miracidia, the usual silver nitrate impregnation method was employed.

0.85% saline was always used in the present studies.

For infection experiments, young specimens of the common snails viz., *Melanoides tuberculata* Müller, *Lymnaea acuminata* Lamarck, *L. luteola* Lamarck, *Indoplanorbis exustus* (Deshayes) and *Gyraulus convexiusculus* (Hutton) were collected from nature and were kept under observation for a week, and

only those found free of cercarial infection were used. Three snails of each species were exposed individually to 4 or 5 miracidia, as far as possible on the same day if the required number of miracidia was available, otherwise on the next day. This was performed as follows: the selected snails were individually placed in watch glasses with a few drops of water and miracidia were pipetted into each under a dissecting microscope. They were then left for a couple of hours. Later, the snails were transferred to small beakers, one in each, half-filled with water. The beakers were daily kept outside in the sun after changing the water. Pond water was always used. To provide food, the common aquatic plants, *Hydrilla* sp., which constitute the food of most of the common snails were placed in each beaker. Water from each beaker was daily examined from the seventh day onwards for the emerged cercariae.

Hatching

When a viable egg containing a fully-formed miracidium was examined in saline under a microscope, the larva did not show any activity, nor did the cilia covering its body. The miracidium remained quite quiescent. Indeed occasionally it was hard to believe it to be alive. Only in some eggs, the contained miracidia evinced a nodding tendency. When, however, the saline was replaced by pond water or even tap water i. e., by a hypotonic medium, the same was imbibed and the middle part of the shell appeared swelling, and the chink-like space containing a granular fluid that exists between the shell-wall and the miracidium gradually widened. Swelling of the eggs was noticeable in 2 to 3 minutes, without having a recourse to measurements. With the swelling of the eggs, the miracidium became active; first the cephalic cilia quivered and then gradually started beating, and later followed the cilia on the more posterior part of the body. This activity of the miracidium is to be attributed to the intake of water that permeated through the shell as when the film of water from underneath the cover-glass was withdrawn by a piece of blotting paper, the beating of cilia remarkably slowed down, but it resumed with the addition of water. With the increased activity of the miracidia, the cilia too, started beating vigorously. In about 5 to 8 minu-

tes, the miracidium started performing active movements of extension and contraction of the apical part of its body which is usually, but not always, directed towards the spinose end of the shell. The apical part of the enclosed miracidium was incessantly dashing against the shell-wall apparently in an attempt to break open the shell and escape. Occasionally, the miracidium curled up, twisted or contracted into a spherical or and oval body, then elongated again. The miracidium even turned around to the opposite pole of the shell. Sometimes the feebly flickering flame cells could be seen in the miracidia about to hatch. Eventually the shell gave way and the miracidium escaped into the surrounding medium and swam off. The shell mostly ruptured at the polar region, possibly because the extensile and contractile anterior part of the body by constant striking weakened this point. In one case, however, the shell ruptured at the doubly-convex equatorial region. The writer concludes from his observations on the hatching process that chiefly the absorption of water from outside and, to a certain mesure, the activities of the miracidium inside the shell are involved in the process. This is further accentuated by the fact that in some non-viable eggs, apparently containing moribund miracidia, hatching did not occur although the eggs swelled to a considerable extent.

Rowan (24) studied the mode of hatching of the miracidia in the operculated eggs of *Fasciola hepatica* (Lin.) He adduces experimental evidence that a "hatching enzyme", produced by the enclosed miracidia, plays a dominant role in the process.

Although no attention was paid to study the factors affecting hatching, but it was incidentally observed that temperature as well as light accelerate the process - as whenever Petri dishes containing hydrated excreta of the infected birds were placed outside in the sun, or under electric lamps after sun set, hatching hastened. Likewise when two Petri dishes containing freshly hydrated excreta were kept in darkness or covered with black cloth and later examined at intervals of half an hour, miracidia were found to have hatched after about an hour and a half, but only a few (one or two in each). These miracidia were removed and then the Petri dishes were placed outside in the sun and, when examined after 10 minutes, 3 miracidia were found in one Petri dish and 5 in the other. These miracidia were removed and after the lapse of another ten minutes, two more were found in each Petri dish.

Movements and longevity

The miracidia swim quite fast close to the surface of water in Petri dishes, occasionally descending to the bottom and then, rising again to the surface as observed by Lengy (11) in case of miracidium of *S. bovis*. Further, quite often, they revolve as they swim about. While swimming in a film of water under a cover-glass fixed to the slide by vaseline, if a miracidium comes across any foreing matter, either it changes its course, or it stops and the body makes active movements of contraction and expansion, and the apical papilla is constantly protruded and retracted, apparently in an effort to bore thereinto. It is indeed a fascinating sight to watch a live miracidium protruding and retracting its apical papilla.

Miracidia left in test tubes four-fifths filled with tap water and pond water were found swimming about in the top layer of about an inch in depth, only stray specimens were found below this level to a depth of about an inch, but none further down.

The miracidia were found to live usually for 2 to 3 hours in tap water at room temperature (16°C-18°C) during winter months, but for a longer time, 5 hours or even more, in fresh pond water.

Morphology

Body in a free swimming miracidium is fully extended and it appears rather bullet-shaped with a broad anterior end and a narrow bluntly rounded posterior end, the widest part being the region of the so-called lateral processes. Behind this region, the body gently tapers posteriorly. But, when a miracidium is at rest, the body presents, under cover-glass pressure, an elongate-pyriform shape and it seems to be somewhat dorsoventrally flattened. It is, however, never fully extended at this time. Admittedly it is extremely difficult to determine the exact size of a live miracidium because of its incessant move-

ments. The size of miracidium, measured from fixed specimens, is variable to a certain extent depending upon the fixative used. 15 Bouin-fixed miracidia measure 0.104 to 0.130 mm. in length and 0.033 mm in width at the level of the lateral processes, the average length being 0.118 mm.; while 10 Zenker-fixed specimens measure 0.108 to 0.128 mm. in length and 0.034 mm in maximum width, the average length being 0.116 mm. 10 Formalinfixed miracidia measure 0.108 to 0.134 mm in length and 0.034 mm in maximum width, the average length being 0.120 mm.

None of the previous studies on the miracidia of *Trichobilharzia* species include an account of the epidermal plates. The writer has studied these plates in the miracidia of *T. indica.* A detailed account of these plates based on a study of 30 mounted specimens is being presented here.

TABLE I

Variations found in the number of epidermal plates of four tiers in 30 miracidia.

First tier	Second tier	Third tier	Fourth tier	Total number of epidermal plates	Number of miracidia
5	7	4	3	19	1
5	8	4	3	20	1
5	8	4	4	21	1
6	7	5	3	21	2
6	9	4	3	22	24
6	9	5	3	23	1

There are four tiers of epidermal plates in the miracidium. In 24 specimens, 22 epidermal plates have been found arranged. (Fig. 1) as follows: the first tier includes 6 plates; the second, 9 plates; the third 4 plates, and the fourth, 3 plates. But there are variations in the total number of epidermal plates covering the body as well as in the number of plates of different tiers. As for example, in one specimen 23 plates have been found; in three, 21; in one, 20, and in another one 19. Table I shows the variations in the total number of epidermal plates and also in the number of plates in each tier. Thus the

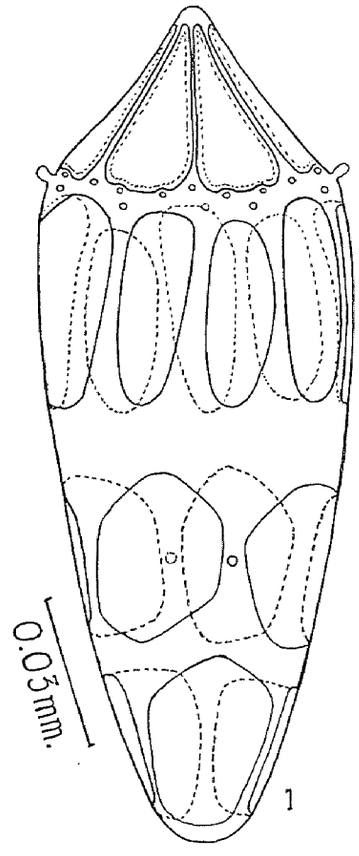

Fig. 1.—Miracidium of *Trichobilharzia indica.* Silver-impregnated specimen showing the epidermal plates and sensory structures.

typical formula of epidermal plates viz., 6: 9: 4: 3 occur in 80% of the miracidia examined for the purpose and the total number of plates varies from 19 to 23 in different individuals. The nuclei of the epidermal plates could not be observed in miracidia by vital staining, even Methylene blue successfully used by Ozaki (17) and Toluidine blue by Pearson (19) for staining these nuclei failed to bring these out.

Further, of the four tiers of epidermal plates, the anterior two tiers are confined to the anterior half of the body, while the posterior two to the posterior half. The gap between the second and third tiers of plates is considerably more than the

gap between any two other adjacent tiers as Nazim (15) has described in the miracidium of *Gigantobilharzia huronensis*. These interplatal regions are, in fact, the exposed subepithelium.

In a typical case, the six plates of the first tier are arranged symmetrically three on each side of the median line. They may be described as being somwhat pyriform or elongate-pyriform in outline, their apices are around the base of the terebratorium and their broad posterior borders lie at the level of the lateral processes. Further, they are placed abutting one another unlike the plates of any other tier. They measure 0.028-0.033 mm in length and 0.013-0.015 mm in width at the base.

The nine plates of the second tier are elongate-oval or rectangular with round corners in outline. They are much longer than the plates of any other tier, and they extend posteriorly almost up to the equatorial line of the body. They measure 0.028-0.036 mm. in length and about 0.013 mm. in width.

The four plates of the third tier are polygonal in shape. They are much broader than the plates of the anterior two tiers - apparently for the reason that they are fewer in number. They measure 0.026 - 0.033 mm. in length and 0.020-0.026 mm. in width.

The three plates of the fourth tier covering the narrow hind part of the body are also broad and polygonal in outline like the plates of the third tier. In surface view, one of them is median, while the other two are ventrolateral in position. They measure 0.026-0.028 mm. in length and 0.024-0.026 mm. in width.

Epidermal plates being ciliated, the cilia, too, are arranged in four annular bands corresponding to the four tiers of epidermal plates. These interruptions in the ciliation of the body are discernible in live miracidia under the high power of a microscope. The cilia immediately behind the terebratorium are the shortest. They gradually increase in length posteriad and attain maximum length just in front of the lateral processes: behind the latter, cilia are all of equal length. The cilia arise from basal granules, one from each, located in the epidermal plates.

Beneath the epidermal plates lies the subepithelium which is exposed to view between the different tiers of epidermal plates and also between adjacent plates of each tier. Cell outlines of the subepithelium could not be discerned, but their nuclei could be observed along the body margin.

The anterior conical part of the body is extremely mobile and is constantly extended and contracted in live specimens. At its tip exists a nipple-like structure, the "terebratorium" of Reisinger (23), or the "apical papilla" of other authors. It is protrusible as well as retractile in nature as is evident in live specimens. The outline of the terebratorium is distinct in silver-impregnated specimens wherein it is left uncovered by the apices of the epidermal plates of the first tier. Some globulets have been occasionally observed exuding from the terebratorium as this structure is protruded and retracted within the body. These exudations appear to be the secretions of glands shortly to be referred to. No opening could be observed in the terebratorium in live miracidia, but in silver-impregnated specimens six or seven pore-like structures whose borders become blackened due to precipitated silver are visible. Some of them appear to be the outlets of the glands present inside, while others to be sensory structures as found by Dönges (5) in the miracidia of *Posthodiplostomum cuticola* (v. Nordmann).

As in the miracidia of other species of *Trichobilharzia,* here, too, no eye spots are present.

A number of circular structures, pore-like in appearance and of varying sizes (0.7 to 1.5 micron in diameter) are visible in the interplatal region of the body between the first and second tiers of epidermal plates. They form a ring in this region of the body. They are hardly visible in live specimens even under a phase contrast microscope, but distinctly appear in silver-nitrate treated specimens as minute dark rings as their borders become impregnated with precipitated silver. These structures have been described in the miracidia of different trematodes, particularly of strigeids and schistosomes, as "papillae" or "anterior papillae", or "processes", or "bristle patches", or even as "pores" by different workers (23; 12; 18; 15; 6; 19; 11; 29; 28; 7). These appear to be sensory organs although no bristle or bristles, or even a process could be observed associated with them in the present case. Their distribution is

subject to variations. Two of them regularly occur in close contact with the posterior border of each epidermal plate of the first tier, often in identations at the posterior border of these plates, and one occurs opposite the line separating two adjacent epidermal plates. Beside these, there are some more whose number and position are variable. Two more similar structures are visible in the interplatal regions of the third tier of plates: one between two plates of one side and the other at the corresponding place on the opposite side.

Two fairly large and prominent peg-like hyaline structures called the "lateral processes" by some authors but "lateral papillae", or "sensory papillae", or even "anterior ducts" by others, are located, one on each side of the body, between the first and second tiers of epidermal plates. They measure about 0.0052 mm. in length and 0.0026 mm. in breadth. These processes are also known to occur in the miracidia of other flukes viz., clinostomatids, strigeids, spirorchiids, and echinostomatids. Some workers (16) regard the lateral processes as being sensory structures, while others (20; 22 y 25) have observed ducts of a glandular mass associated with them. As stated subsequently, the writer's observations also give an indication of the existence of a connection of the lateral proceses with the ducts of a pair of glands.

Neuhaus described a stiff hair in front of the lateral process on each side of the body in the miracidium of *T. szidati*. Wu made no reference to the occurrence of a similar structure in the miracidium of *T. cameroni*. Ameel et al. in their preliminary study on the miracidia of three species of avian schistosomes cited before observed the lateral processes, but not any other structure in front of them. Though Macy et al. did not mention in the text, but their figure of the miracidium of *T. oregonensis* gives an indication of the presence of a stiff filamentous structure on each side of the body in front of the lateral process. In the miracidia of many schistosomes, small papillae called "lateral papillae" or "anterior papillae" by different workers (20; 26; 27; 15; 25; 6; 29 y 9), have been described in place of stiff hairs in front of the lateral processes. These papillae have been also reported in the miracidia of other flukes. The writer, however, has not been able to detect stiff

hairs or papillae in front of the lateral processes in the miracidium of *T. indica*.

A median flask-shaped structure (Fig. 2) is located within the body of the miracidium. It extends posteriorly from the tip

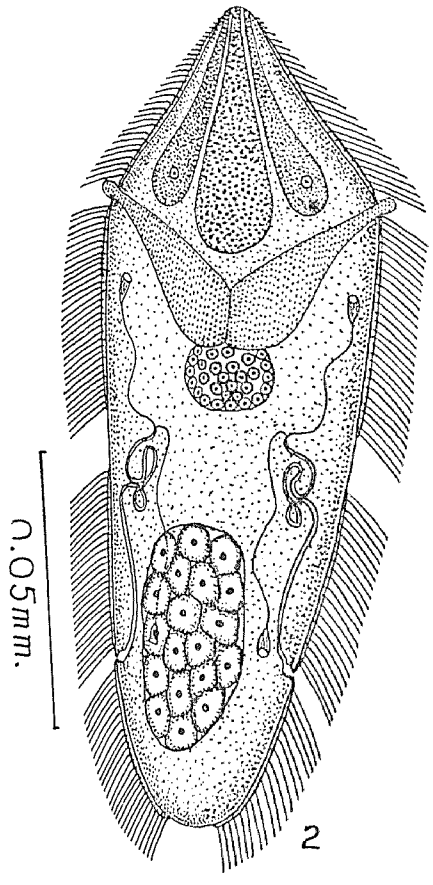

Fig. 2.—Miracidium of *Trichobilharzia indica* showing various structures. Drawn from a live specimen after intravital staining.

of the terebratorium, where in all probability it opens, up to the posterior secretory glands against which it abuts. This is the so-called primitive gut of old workers. In fact, it is a glandular structure and, following Lynch (12), hereinafter referred to as the apical gland. It is packed with coarse granules which are only visible in live miracidia, and are stainable with Neutral red. In fixed and stained specimens, these granules

TABLE II

Chief features of the miracidia of different species of *Trichobilharzia* Skrjabin and Zakharow, 1920

Species	Size *			Epidermal plates	Relative size of apical gland and penetration glands	Number of germinal cells
	Live	Fixed	Fixative used			
T. szidati Neuhaus, 1952	0.18 × 0.05	?	?	?	Apical gland shorter than penetration glands	13 (figured)
T. cameroni Wu, 1953	0.175 — 0.190 × 0.035 — 0.042	0.110 — 0.146 × 0.038 — 0.044 0.112 — 0.129 × 0.038 — 0.045	Bouin Gilson	?	Apical gland shorter than penetration glands	28 (figured)
T. elvae (Miller, 1923)	?	0.102 — 0.153 × 0.044 — 0.057	Heat killed	?	Apical gland longer than penetration glands	10-18 av. 13
T. physellae (Talbot, 1936)	0.121	?		?	Apical gland longer than penetration glands	20-30 av. 26
T. stagnicolae (Talbot, 1936)	?	0.102 — 0.166 × 0.044 — 0.069	Heat killed	?	Apical gland longer than penetration glands	21-30 av. 22
T. oregonensis (Macfarlane and Macy, 1946	0.12 — 0.16 × 0.05	?	?	?	Apical gland shorter than penetration glands	14-16
T. indica Baugh, 1963		0.104 — 0.130 × 0.033 0.108 — 0.128 × 0.034 0.108 — 0.134 × 0.034	Bouin Zenker Formalin	22	Apical gland longer than penetration glands	15-20 av. 18

All measurements in millimeters. Length is followed by breadth.

are lost from view. Several workers (8; 30; 26; 25; 29 y 9), have described four nuclei in this gglandular structure in the miracidia of human and animal schistosomes, but no nucleus has been observed in the present miracidium, nor Wu has reported to have observed any in the apical gland of the miracidia of *T. cameroni*. According to the findings of Wootton (31; 4; 10), the apical gland is concerned with the secretion of a hystolytic substance that is used during penetration of the miracidia into the snail hosts.

The apical gland is closely flanked on either side by a club-shaped structure with a long duct. These are the "penetration glands" or the "cephalic glands" of some workers (12; 25; 9). They appear as two homogeneous bodies each with a distinct nucleus. The writer's observation regarding the staining properties of these glands is in accord with that of Wu inasmuch as these glands do not stain intravitally with any of the dyes employed in the present study. Regarding the relative size of the penetration glands and the apical gland, it is worth mentioning that the former are roughly three-fourths the length of the latter. In tis point, the miracidium of *T. indica* resembles those of *T. elvae, T. physellae* and *T. stagnicolae,* but differs from those of *T. cameroni, T. szidati* and *T. oregonensis* wherein the penetration glands extend posteriorly beyond the apical gland. The accompanying table (Table II) collates, amongst other features, the relative length of the penetration glands and apical gland in the miracidia, if known, of different species of *Trichobilharizia*. Regarding the role of the penetration glands, while most of the workers treat them as constituting the penetration apparatus of the miracidium - at least a part of it; Wootton (31) in his work on the life history of *Allocreadium alloneotenicum* Wootton observed the process of miracidial penetration and found the secretions of these glands do not aid in penetration but form the cuticle of the sporocyst in the molluscan host. As far as the writer is aware, Wootton's view has not been substantiated by later workers.

Besides the aforesaid glands described as being present in the miracidium of *T. indica,* there is yet another pair of glandular structures which have been previously reported by workers (21,22; 32; 25), as the "posterior secretory glands" or "lateral glands" in the miracidia of some avian and mammalian

schistosomes. Price (20) and Nazim (15) also observed a similar structure in the miracidia of *S. douthitti* and *G. huronensis* that they respectively studied, but they did not designate it. Surprising enough that these glands have not been reported, as far as the writer is aware, in the miracidia of the closely related flukes viz., clinostomes and strigeids. These glands, referred, to in the present work as the "posterior secretory glands" with their ducts appear as two closely adpressed retort-shaped structures mesially placed just behind the apical gland. Their close proximity hardly reveals the line of demarcation. Posteriorly, these glands partly overlap the central nerve mass, but in well-extended specimens they are quite apart. These glands continue into ducts which run forward and outward, and appear to terminate at or near the lateral processes on the body surface. The writer, however, could not observe any orifice in the lateral processes which could be referred to the ducts of the posterior secretory glands, nor did he observe any exudation issuing from the lateral processes. The contents of these glands appear as fine granules and they do not stain with any of the dyes used in the present study. Wu described these glands in the miracidium of *T. cameroni* as being composed of clusters of unicellular glands. Rao (21, 22), too, observed these glands in the miracidia of animal schistosomes viz., *S. spindalis* Montgomery, *S. nasalis* Rao and *S. suis* Rao and Ayyar as being similarly constituted, The writer, however, failed to ascertain the composite nature of these glands in the present miracidium.

A large median nerve mass constituting the brain is visible close behind the posterior secretory glands by which it is usually partly overlapped. It is roughly circular in outline, but in contracted specimens it presents a transversely oval appearance. It is composed of a mass of scattered nerve cells bounded peripherally by closely placed nerve cells. In live specimens, it is distinguishable as a translucent structure; but in permanent preparations, the nerve cells with their large nuclei appear quite distinct.

The excretory system consists of two independent tubules, one on each side of the body, each connected with two flame-cells. Thus there are two pairs of flame-cells - a figure characteristic of schistosome miracidia as well as of strigeid and

clinostome miracidia. The anterior pair of flame-cells is located laterally a short distance behind the level of the lateral processes: the vibratile cilia of these flame-cells are directed backward. The posterior pair of flame-cells is located a short distance in front of the hind border of the third tier of epidermal plates. The vibratile cilia of these lame-cells are directed forward. Often one of these flame-cells is masked by the mass of germinal cells and hence not easily visible. An anterior and a posterior collecting tubule arise from the respective flame-cells of each side of the body. These are capillary tubules and, after following a short winding course, they join to form a common collecting tubule which gently windens to about double the width of the capillary tubule as it runs posteriorly. Eventually this common collecting tubule appears to end in a small vesicular enlargement before opening to the outside by the excretory pore located marginally in the interplatal region between the third and the fourth tiers of epidermal plates. Excretory pores are clearly delineated in silver-impregnated specimens.

A mass of germinal cells is present in the posterior half of the body cavity of the miracidium for which Lengy's term "germinal cavity" is adopted here. The mass comprises 15 to 20 cells in different specimens with an average of 18; the counts being made in 25 live miracidia. The large nuclei of these cells could be distinguished. The germinal cells in the miracidia of *T. oregonensis* range from 14 to 16. The number of germinal cells in the miracidia of *T. szidati* and *T. cameroni* has not been mentioned by the authors (16; 32); but from their figures, the former appears to possess 13 while the latter 28 germinal cells. Further, the germinal cells are not discrete in the germinal cavity, but they form a compact oval mass which does not abut against the central nerve mass as it has been described by Ameel et al. (1) to do in the miracidia of *T. elvae, T. physellae* and *T. stagnicolae.* On the contrary, a distinct gap sets apart the mass of germinal cells from the central nerve mass. In this repect, the present miracidium bears a resemblance to that of *T. cameroni.* Since, the germinal cells lie close abutting against one another, they appear polygonal in outline. During the contractile and entensile movements of the miracidia, the mass of germinal cells shifts

back and forth as one unit but remains at the same locus. The writer is strongly inclined to believe that the germinal cells have fine cytoplasmic processes as described by Price (20), Singh (26), Dutt and Srivastava (6) and Srivastava and Dutt (29) in the miracidia of other schistosomes. These processes firmly bind the germinal cells into a compact mass and also with the subepithelial layer. Thus the mass of germinal cells is held in the same position. These processes could not be seen on account of the closeness of the germinal cells. A fine membrane surrounds the mass of germinal cells.

Infection experiments

3 specimens of the common snails cited before were exposed on different occasions to the miracidia in the manner already stated. Mortalities occurred in the experimental snails. Till the 10th day of exposure of the snails to the miracidia, there was no mortality; but thereafter death ensued. After 15 days of exposure, only 2 specimens of *M. tuberculata,* 2 of *L. acuminata,* 2 of *L. luteola* and 3 of *I. exustus* survived. All specimens of *G. convexiusculus* died by the 15th day. Snails that died during the period were dissected and examined for the parthenitae, but negative results were obtained. By the 20th day of exposure, more snails died and these, when dissected, were found to be devoid of parthenitae. Only three snais survived viz., one *M. tuberculata,* one *L. acuminata* and one *I. exustus* These survivors lived for 35 days but none emitted cercariae. They were later destroyed and examined for the parthenitae but none had any.

The writer feels that it is rather too premature for him to make a conclusion from these infection experiments as to the probable intermediate host of this avian schistosome. These infection experiments need repetition on a more elaborate plan before any conclusion can be made therefrom. Since the bird had meantime died, no more infection experiments could be performed.

RESUMEN

Se estudian los miracidios de *Trichobilharzia indica* Baugh, 1963 de huevos embrionados obtenidos de las excretas de *Nettion creca* naturalmente infectado.

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