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ADDITIONAL OBSERVATIONS ON THE DEVELOPMENT OF THE EYE WORM OF POULTRY

by

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The post-embryonic development of nematodes, which is on the whole insufficiently known, has been divided by Looss (1911) into five periods, four larval and one adult stage, separated by four moults. This division is commonly used, but according to Yokogawa (1922) does not hold good for *Heligmosomum muris*, since he found that there were actually only four stages separated by three moults, the last moult being incomplete, the adult showing a double cuticle. He considers that the entrance of the larvae into the host is comparable to a second moult. The present paper deals with the various stages in the development of the larvae of the eye worm of poultry in the intermediate host, a cockroach *Leucophaea (Pycnocelus) surinamensis* L., in North Queensland.

The Eggs. Ransom (1904) gives the measurements of the mature eggs of *Oxyspirura mansoni* as 0·05 to 0·065 mm. by 0·04 to 0·045 mm. Sweet (1910) states that in the Australian worm, named by her *O. parvovum*, they are much smaller (0·033 to 0·045 mm. by 0·025 to 0·030 mm.). My own measurements of both species, covering a fairly long series, did not show such a divergence in size; those of *O. mansoni* (old, fixed, and mature eggs) averaged 0·043 by 0·031 mm., and of *O. parvovum* (fresh, mature eggs) 0·041 by 0·030 mm. In the vagina and lower portion of the uterus the eggs are seen to contain embryos. When segmentation begins the eggs appear much elongated (0·024 to 0·028 mm. by 0·012 to 0·014 mm.), with almost square ends. On hatching they show a clear line of fracture at one or both poles.

First Stage Larvae. The newly-hatched larvae are very active, but survive only for a very short time in cultures. Sanders succeeded in Florida in keeping the larvae alive for two months, after which time they measured 1·5 mm. long. I am not aware whether he noted any ecdysis during this period. We were unsuccessful when using cultures, but succeeded in rearing the worms by feeding the eggs to the cockroaches *Leucophaea surinamensis*. On hatching in this intermediate host the larvae were very active, and remained so. They were found in the gut from the third to the tenth day, measuring 0·23 to 0·26 mm. in length and 0·012

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to 0·014 mm. in width. From the tenth to the fourteenth day they were observed in the body cavity, generally measuring from 0·32 to 0·36 mm. long by 0·018 to 0·020 mm. wide, but a few individuals were encountered which were only about the size of those seen in the gut, indicating possibly that they were new arrivals in this location. On the seventeenth and eighteenth days the larvae had mostly attained the stage of encapsulation when they measured from 0·485 to 0·6 mm. by 0·026 to 0·028 mm., while the capsules were more or less oval (0·22 to 0·27 mm.). At this stage the larvae are lethargic and difficult to extricate from the capsule; most probably it is at this time that moulting begins, but no definite information could be obtained until the twenty-fifth day, when a few larvae were recovered which were apparently in the final stage of casting their first cuticle. These first stage larvae are, so far as could be seen, only slightly attenuated at both ends, the anterior being rounded and the posterior very bluntly pointed. Anteriorly there is a small cup-like cavity, evidently a rudimentary buccal cavity, from which the filariform oesophagus extends back for approximately one-fifth of the body length. The skin is comparatively thin throughout, the body cavity is extremely small, and the anal opening is not evident. At approximately one-third and two-thirds of the body length from the posterior end are two rounded structures, of which the former is most probably the genital primordium, while the latter is perhaps associated with the oesophageal or cervical glands. Posterior to the genital primordium there are apparently numerous very small cells, extending to the anal region.

On the twenty-fifth day some of the capsules collected were in the same state as those obtained on the seventeenth and eighteenth days, and these larvae were considered to be in the first stage.

Second Stage Larvae. It was considered that by the twenty-fifth day the second stage had been attained. Some had obviously completed their moulting, while others were partly enclosed in an envelope, apparently the remains of the former cuticula. Under experimental conditions it would appear that it is at this stage that the major portion of larval growth occurs. The shape very closely resembles in miniature the adult worm, but the tail is somewhat rounded and not pointed, as in the adult. In some specimens in the early stage there is a covering over the anus. This was at first considered to be an anal button, but as it was not encountered in all specimens, and was not uniform in size and shape, it would appear that its presence is merely accidental. In the later stage larvae there appeared to be present on the tail four small papillae. The capsules measured 0·34 to 0·38 mm. in diameter, the contained larvae being 0·63 to

Fig. 1. First stage larva from body cavity of cockroach.
Fig. 2. Head of early second stage larva.
Fig. 3. Tail of early second stage larva.
Fig. 4. Head of late second stage larva.
Fig. 5. Tail of late second stage larva.
Fig. 6. Head of third stage larva.

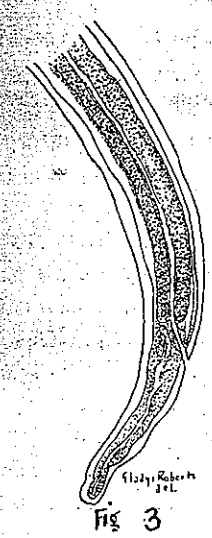
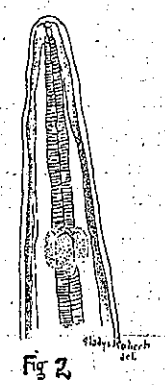


Fig 3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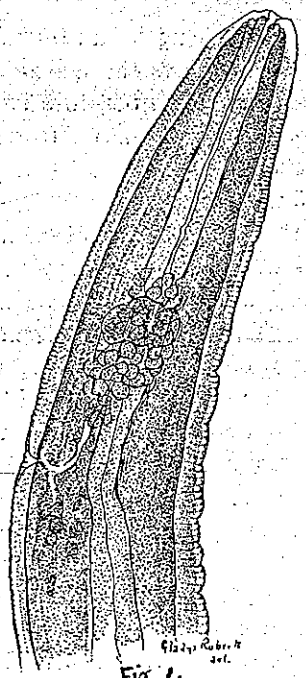
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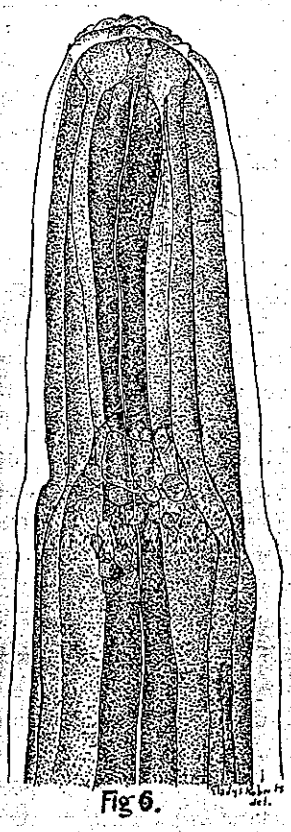
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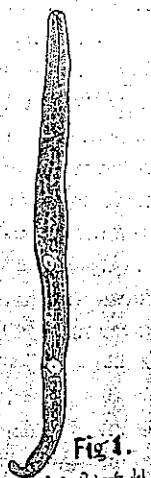
Fig 1.

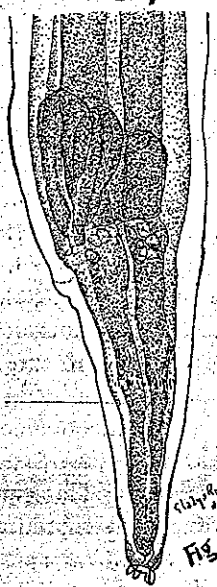
Fig 5.

1·01 mm. in length and 0·028 to 0·035 mm. in width. By the thirty-second and thirty-ninth days the larvae showed a distinct increase in size, measuring 1·91 to 2·09 mm. by 0·073 to 0·082 mm. and 3·5 to 3·8 mm. by 0·118 to 0·127 mm. on the respective days. By the forty-sixth day they were obviously undergoing a further ecdysis, and had attained 4·6 to 4·9 mm. in length by 0·127 to 0·145 mm. in width.

For detailed measurements of this stage a larva, 3·6 mm. long by 0·125 mm. wide, was selected. The skin appears to be distinctly but finely striate transversely, and is about 0·008 mm. thick. Definite papillae were not seen on the head. The pharynx in its anterior portion appears as a small cup, measuring about 0·008 by 0·008 mm., the succeeding part, which extends a distance of 0·078 mm., being only slightly narrower. The nerve ring and excretory pore are situated about 0·156 and 0·24 mm. respectively from the anterior end. There is a slight constriction at about 0·728 mm. from the head end, probably separating the oesophagus from the intestine, which has a very narrow lumen. The anal opening is about 0·195 mm. from the tip of the tail. The rectum is surrounded by three or four fairly large ovoid glands and a number of small cells. Situated about 0·273 mm. from the tip of the tail is a somewhat oval structure, 0·0195 mm. by 0·008 mm., considered to be the genital primordium.

Third Stage Larvae. By the fifty-second day the larvae had obviously undergone a further ecdysis, and were now in the third or infective stage, as was proved by feeding the infected cockroaches to chickens, whose eyes soon showed the presence of larvae. Further examinations of naturally infected cockroaches revealed the fact that third stage larvae can attain a length of 8·5 to 9 mm., this stage thus varying from 4 to 9 mm. On entering the final host they apparently prepare for a further ecdysis. The tail is more elongate, approximating that of the adult worm. The head end appears bossed, due to the presence of papillae; the buccal cavity somewhat resembles that of the adult, but is slightly smaller, the anterior portion measuring about 0·015 mm. long by about 0·02 mm. wide, the somewhat narrower posterior portion being slightly longer than it is wide. The oesophagus is about 1 mm. long by 0·04 mm. anteriorly, and 0·056 mm. posteriorly. The nerve ring is situated from the anterior end between 0·214 to 0·234 mm., with the excretory pore about 0·1 mm. behind it. The anus opens about 0·3 mm. from the tip of the tail; there are four fairly large gland-like structures and a number of small cells in close association with the rectum. The genital primordium, which is much larger than in the previous stage, is situated about 0·9 mm. from the tip of the tail.

Fourth Stage Larvae. On the day after reaching the final host moulting begins, and is completed by the third or fourth day, when the larvae enter their fourth stage. Under experimental conditions they then grow from 4·55 to 8·46 mm. in length, and after about eleven to twelve days apparently begin their final ecdysis. The pharynx is now similar to, but somewhat larger than, that of

the preceding stage; the length of the oesophagus is variable but the diameter is more constant. The nerve ring and associated cells appear very prominent, the former lying at from 0·214 to 0·234 mm. from the anterior end; while the excretory pore is situated 0·08 to 0·1 mm. behind the ring. It is in this stage that the sexes can be distinguished; the uterine tubes can be plainly seen extending a distance of 2·3 to 3·6 mm. from the posterior extremity and ending in a round knob; in some specimens the vulva can be made out plainly at a point between 0·64 and 0·73 mm. from the tip of the tail. The anus is situated at 0·31 to 0·37 mm. from the posterior end.

The Adults. The adults are attenuated at both ends, the anterior being rounded, while the posterior gradually tapers to a point. There appears to be some difference of opinion regarding the cuticula. Magalhaes (1888, 1895) describes it as being finely striated transversely. Megnin states that there is no visible striation. Ransom (1904) mentions that it is very transparent and perfectly smooth; but Sweet states that it has very fine transverse striations and some faint longitudinal ones, which are finer than those due to the polymyarian structure of the muscle layer. After an examination of a great amount of material, both American and Australian, as well as larval worms in the different stages, I have come to the conclusion that there are no striations evident, either transverse or longitudinal, except in the case of the second stage larvae, which have well-marked but fine transverse striations. The muscle fibres of the body wall are plainly evident through the cuticle as coarse longitudinal striations. The mouth may be circular or somewhat oval, the pharyngeal opening being more or less closed by a cuticular membrane, which is referred to by Ransom as the circumoral cuticular ring. The mouth part is surrounded by six small oral papillae, four of them submedian and two lateral, the latter sometimes difficult to see. Posterior to these there are four others, sublateral, and fairly large. The nerve ring varies somewhat in position from 0·27 to 0·32 mm. from the anterior end. The excretory pore is also variable in its position. This variation is not always in conformity with the size of the worm, opening at about 0·32 to 0·38 mm. from the anterior end in medium-sized specimens, and up to 0·45 mm. in some of the larger specimens. In relation to the excretory pore on each side of the body there are small cervical papilla. I have been unable to demonstrate with any degree of certainty the presence of caudal papillae in either the American or the Australian worms. The length of the pharynx is variable, and ranges between 0·036 to 0·056 mm.; the anterior portion is shorter and wider, being from 0·02 to 0·03 mm. long by 0·030 to 0·035 mm. in width; the posterior portion appears more cylindrical, measuring from 0·02 to 0·03 mm. long by 0·016 to 0·024 mm. wide, being slightly wider where it overlaps the oesophagus. The latter is club-shaped, varying in length from 1·0 mm. to 1·547 mm., wider posteriorly than anteriorly, and somewhat bulbous.

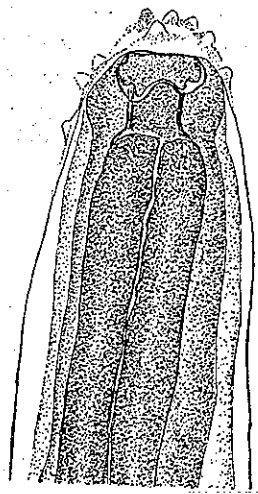

Fig. 10
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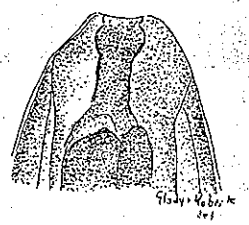


Fig 7

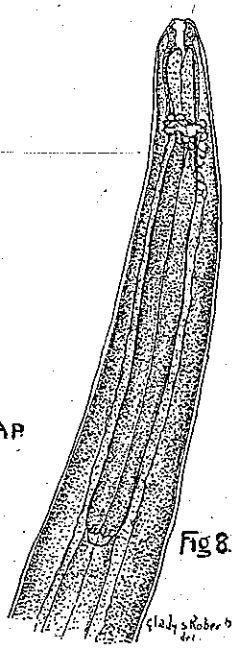
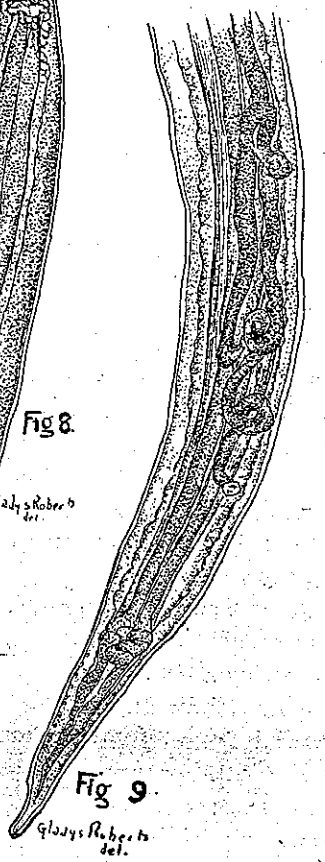


Fig 8.



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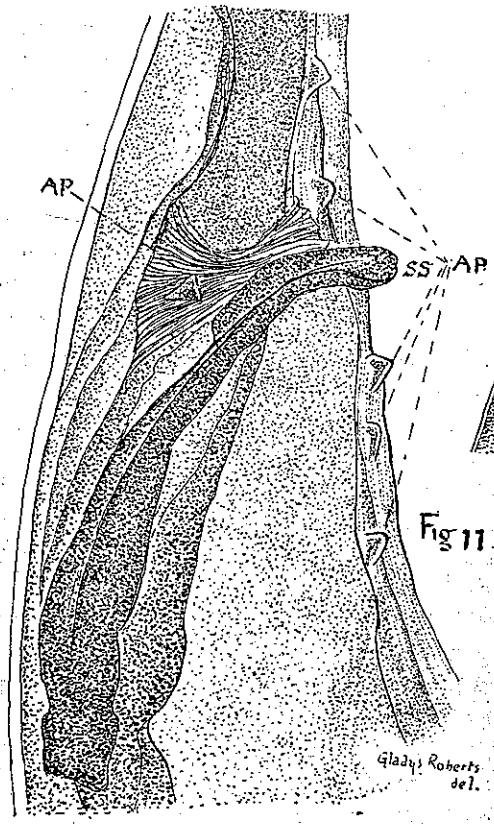

Fig 11.

Male. Cobbold gives the length of the male as 16 mm.; Magalhaes as 14 mm.; Megnin as 12 mm.; Ransom as 10 to 14 mm. by 0·2 to 0·35 mm. in diameter, stating that the smaller worms were immature; while Sweet gives a range between 9·2 and 14·5 mm. in length by 0·26 to 0·33 mm. in diameter. My observations indicate that the extremes are even greater, ranging from 8·2 mm. long in specimens which had just attained maturity to 15·47 mm. in older specimens, the mid-diameter ranging from 0·254 to 0·327 mm. The width in the region of the posterior portion of the pharynx and the cloaca appear more consistent, averaging 0·074 and 0·136 mm. respectively.

The distance from the tail to the cloaca is very variable (0·253 to 0·390 mm.). There appears to be a difference of opinion regarding the number of anal papillae. Magalhaes states that there are eight pairs, three postanal and five preanal. Megnin mentions five pairs, Ransom six pairs (three preanal, two postanal, and one pair adanal and somewhat sublateral. Sweet states that in the Australian worms there are only five pairs, failing to find the adanal or sublateral papillae. After an examination of a large number of specimens I have been unable to see any really definite papillae, except in two or three instances, where they were fairly large structures, considered to be preanal papillae, in the position indicated by Ransom, but the presence of caudal papillae is doubtful. The long spicule measures from 3·64 to 4·55 mm. by about 0·012 mm. in diameter; at its base it is almost twice as wide. The second spicule is much shorter, and measures from 0·214 to 0·235 mm. by 0·03 to 0·043 mm. in maximum thickness, and is somewhat boat-shaped. The testis begins anterior to the middle of the body as a slender cord, gradually increasing in diameter, making some short loops backward and forward, sometimes extending anterior to the junction of the oesophagus and intestines. From the middle of the body it runs in an almost straight line to a point about 1·5 mm. from the anus, where there is a constriction, separating the wide, thin-walled, seminal vesicle from the ejaculatory ducts, which joins the intestine about 0·1 mm. from the anal opening. No evidence of swollen cuticular wings anteriorly (Sweet) was obtained, but numerous specimens were seen in which there was an undoubted constriction near the head.

Female. The measurements of the female worms have been variously given. Magalhaes gives a table of measurements of various organs of five females. The average length of the worm according to him is 16·2 mm. Ransom indicated that they ranged between 12 and 18 mm. in length, with a diameter of 0·4 to

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Fig. 7. Head showing pharynx, fourth stage larva.
Fig. 8. Anterior end, showing oesophagus and nerve ring, fourth stage larva.
Fig. 9. Tail, genital tubes, fourth stage larva.
Fig. 10. Head of recently mature male.
Fig. 11. Tail of recently mature male.

All drawings executed with the camera lucida.

Explanation of lettering: a.p., anal and adanal papillae; s.s., short spicules.

0·43 mm. at the middle of the body; 0·05 mm. at the anterior end; 0·21 to 0·28 mm. at the vulva; and 0·09 to 0·1 mm. at the anus. Sweet gives the range as 13·5 to 20 mm. long by 0·27 to 0·39 mm. in diameter. My measurements of six females ranged between 15·47 and 17·38 mm., averaging 16·64 mm. in length. The minimum observed was much less than that above, one female which had just attained maturity, and had started egg-laying, measuring only 7·6 mm. The diameter ranged between 0·27 to 0·42 mm. at the middle of the length. The vulva is situated in the posterior part of the body, at a variable distance from the tip of the tail: Magalhaes, 1·0 to 1·33 mm.; Ransom, 1·0 to 1·4 mm.; Sweet, 0·78 to 1·07 mm.; Fielding, 0·91 to 1·55 mm. The anus is distant from the tip of the tail between 0·364 to 0·509 mm. (Ransom, 0·40 to 0·53 mm.; Sweet, 0·39 to 0·44 mm.; Magalhaes, 0·4 to 0·53 mm.). The nerve ring lies at about 0·27 to 0·32 mm. from the anterior end (Ransom, 0·25 mm.; Sweet, 0·22 to 0·30 mm.), and closely associated with it are some fairly large cells with prominent nuclei—ganglion cells (Magalhaes).—The excretory pore opens at about 0·05 to 0·13 mm. behind the nerve ring. The two large swollen uteri unite to form the vagina a short distance (0·8 to 1·3 mm.) in front of the vulva.

SUMMARY.

The development of the eye worm of poultry has been followed in detail from egg to adult. Its course is described with observations on the morphology and measurements of the different stages.

ACKNOWLEDGMENTS.

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