ON THE DEVELOPMENT OF *ASCARIS LUMBRICOIDES* AND *A. MYSTAX* IN THE MOUSE.

PART II.

BY F. H. STEWART, M.A., D.Sc., M.B., MAJOR I.M.S.

(With Plate IV.)

IN previous papers¹ the migration of *Ascaris lumbricoides* in the rat and mouse has been traced to the point at which the larvae of the worm reach the trachea of these animals on the 8th day after infection. As the result of further experiments it is now possible to trace the migration through a further stage, namely that occurring from the 9th to the 15th days after infection. My experiments have not as yet been carried beyond the 15th day and in the following summary the statement that larvae occur in a given situation up to the 15th day is not intended to imply that they do not persist therein after that date.

Larvae are found in the mouths of infected mice on the 8th day, on which day they are also, as previously stated, abundant in the lungs and trachea. They persist in the lungs up to the 15th day. On the 9th day they begin to travel down the alimentary canal and may be found in small numbers in the stomach, small intestine and caecum. On the 10th day this stage is fully established, the larvae travel with some rapidity through the stomach and small intestine and accumulate in the caecum and upper colon where as many as 60-70 may occur. On this day they also commence to pass out in the faeces. The passage from the lungs to the caecum continues up to the 15th day and larvae occur in the faeces on the 16th day.

Between the 9th and 15th days the larvae increase in length. They measure from 1.3-2 mm. on the 10th day and 1.75-2.37 mm. on the 15th.

¹ *Brit. Med. Journ.* (July 1st and Oct. 7th, 1916); *Parasitology* (Feb. 26th, 1917), ix. 213-227, Pl. I. and 9 text-figs. The subject dealt with in the present paper has already been partly described in the *Brit. Med. Journ.* Dec. 2nd, 1916.

The above facts were ascertained from the following experiments:

(1) Experiments on Mice (*k*), (*l*), (*m*), (*p*) and (*r*) recorded in the *British Medical Journal*, December 2nd, 1916. In these experiments *Ascaris* larvae were found in the large intestine in four out of the five mice used, on the 9th, 10th, 11th and 12th days after infection. Larvae were also found in the faeces in two cases, on the 10th and 12th days.

(2) The following additional experiments are now recorded for the first time:

Mice (*q*), (*s*), (*u*) received doses of eggs 23 to 32 days old and were killed between the 10th and 13th days thereafter. No larvae were found in the lungs, intestine or faeces.

Mouse (*v*) received a dose from the same culture as (*u*) on September 4th. Not appearing ill, it received a second dose on October 21st and a third dose of another culture on November 13th. On the 16th day after the third infection one *Ascaris* larva was found in the faeces. The mouse was killed on the 18th day, but no larvae were found in the trachea or large intestine.

Mice (*w*) and (*x*) were killed as controls without infection. No nematodes were found in the lungs or trachea. Oxyurids were found in the colon. No forms resembling *Ascaris* larvae in any part of the intestine.

Mice (*y*) and (*z*) received doses of eggs of *A. suilla* aged 140 days and 41 days on October 13th. On the 10th day no larvae were found in the faeces. On the 11th day 5 larvae in the faeces, of which 2 moved languidly in salt solution. On the 12th, 13th, (and as regards mouse *z*) 14th and 15th days larvae were found in the faeces. Mouse (*y*) was killed on the 13th day. No larvae found in the lungs, 10 in the trachea, none in the mouth, stomach, or small intestine, 7 in the caecum, none in the colon. Mouse (*z*), killed on the 15th day, contained 2 larvae in the lungs, 10 in the caecum.

Thirteen mice (*Aa* to *Am*) died of cold or excessive infection; but 1 was examined. Larvae were found in the faeces of *Ai* on the 12th day.

Mouse *An* received a dose of eggs 31 days old on November 18th. Larvae were found in the faeces on the 9th, 10th, 11th and 12th days thereafter, not on the 14th and 16th days. The mouse was killed on the 17th day. No *Ascaris* larvae were found in the trachea or large intestine.

Mice *Ao*, *Ap* received doses of eggs 35 days old. Larvae were found in their faeces on the 9th and 12th days; they were killed on the 13th day. In *Ao* no larvae were found in the large intestine. In *Ap* three larvae were found in the caecum.

Mice *Aq*, *Ar* received doses of eggs 37 days old. *Ar* died on the 1st day. *Aq* was killed on the 11th day. Larvae were found in the lungs, none in the large intestine.

Mice *As*, *At*, received doses of eggs 39 days old. They were killed on the 11th day. Nine larvae were found in the caecum of *As*. No examination was made of *At*, its entire large intestine being given to Pig 10.

Mice *Au*, *Av* received doses of eggs 52 days old. They were killed on the 10th day. Six larvae were found in the tip of the caecum of *Au*. The intestine of *Av* was given unopened to Pig 10.

The anatomy of larval Ascaris lumbricoides L. (A. suilla Duj.) from the large intestine and faeces of the mouse between the 10th and 15th days after infection.

The general shape (Pl. IV, fig. 1) is cylindrical, tapering slightly to the truncated head, sharply to the pointed tail. The head (Fig. 3) bears the three mamilliform lips characteristic of the genus. The shape of the tail is well shown in Fig. 11. The tip is curved toward the dorsum. The resemblance of this outline to the figures given by Leuckart of the tails of a young *Ascaris lumbricoides* (85 mm.) from man (*Menschl. Parasiten*, II, pt. 1, p. 217) and of young *A. mystax* from the dog (*ibid.*, pt. 2, p. 283) is worthy of note.

The cuticle is not ringed. In sections a fine sharply-marked and densely-staining outer membrane can be distinguished (Pl. IV, figs. 6, 7, 8) which is not present in the larvae found in the trachea or mouth (Figs. 9, 10) and is doubtless formed by reaction to the gastric and intestinal juices. Lateral membranes are present extending from the head to behind the anus (Figs. 6, 7, 8, 9, 10). These structures are first formed when the larvae are passing through the bronchi and trachea and have attained to a length of one millimetre. In a specimen measuring 1.5 mm. from the mouth, the membrane extends from the nerve-ring to the anus. The process of development of the membrane can be made clear by comparison of sections of larvae from the trachea and caecum. In the former the cuticle is raised along the lateral lines enclosing a space, triangular in cross section, the base of which is formed by the lateral lines. The cuticle forming each side of this triangle contains a slightly thickened and strongly staining lamina. The unspecialised and feebly staining cuticle of the body surfaces passes over the outer surface of these laminae. In the larvae from the caecum and faeces the laminae have become more thick and rigid and have partially coalesced, producing a sharp keel-like ridge with a flat base. The lateral membranes are curved toward the dorsum. Lateral membranes do not of course occur in the adult. They are doubtless formed to assist in the active swimming movements of the larvae.

Longitudinal lines. The lateral lines contain as a rule three nuclei in a cross section, dorsal and ventral lines one or two.

Muscle-fields. If the sections are counterstained with eosine the muscle cells can be seen to have assumed the folded form characteristic of the majority of adult nematodes.

The oesophagus (Pl. IV, figs. 2, 3) is club-shaped, anterior extremity slightly thickened, posterior extremity markedly so. The latter pro-

jects into the anterior end of the intestine. The external surface of the oesophagus is, as in the adult, covered with a sharply staining cuticle, a structure which is acquired in the passage along the alimentary canal of the rodent since it is not present in specimens from the trachea or mouth. In correspondence with the formation of this cuticular coat is the reduction of the cellular investment of the oesophagus, which is at its greatest development on the 5th or 6th day after infection. The body of the oesophagus is distinctly muscular.

The intestine is patent and is composed of a tessellated cylinder of hexagonal cells (Pl. IV, fig. 5). The anal canal is curved, expanded at the anterior extremity, and lined with cuticle.

The nerve ring is large and obvious (Figs. 2, 9) and possesses a cellular sheath. It lies immediately in front of the excretory aperture. An anal ganglion is formed by the ingrowth of the dorsal and lateral lines (Fig. 8).

The body of the unicellular excretory gland lies mainly between the left lateral line and the oesophagus (Figs. 2, 3, 4, 6, 10); a process however extends across the body and comes into contact with the right lateral line (Fig. 10). Its anterior extremity also passes toward the ventral line and meets the group of cells which contain the anterior portion of its duct. A considerable portion of the cell is occupied by the nucleus which is furnished with six chromatin masses of varying size. The excretory pore is situated in the ventral line immediately behind the nerve ring. From it the excretory duct (Figs. 2, 3, 4) passes backward through the group of cells of the ventral line referred to above (Figs. 2, 4), inclines to the left and enters the body of the gland in the left lateral line. It does not send a branch toward the right lateral line.

In younger larvae of 1 mm. or less in length the excretory gland appears to be confined to the left side of the body and not to send a process to the right lateral line. It is however also in contact with the ventral line and it appears probable that it originates from the ventral line and is homologous with the ventral gland of free-living nematodes. It is interesting to observe the manner in which the left-sided gland of the larva under consideration fits into the scheme of evolution of the excretory organ of nematodes as worked out by Jaegerskiöld (Jaegerskiöld, 'Beitr. zur Kennt. der Nematoden,' *Zool. Jahrb. Anat. Ontog.* VII. 449; Stewart, *Quart. Journ. Micr. Sci.*, L. 141). The unilateral organ of the younger larvae corresponds with that of *Ascaris decipiens*, the unequally bilateral organ of the older larvae with that of *A. rotundata*.

The rudiment of the gonads is situated about the junction of the

middle and posterior thirds of the body between the intestine and the ventral line (Fig. 5). It consists of a clearly defined lens-shaped group of cells.

The early life history of Ascaris mystax Zeder (A. marginata Rud.).

The development of the eggs of the *Ascaris* of the cat and dog has been studied by Nelson, Verloren, Davaine, Baillet and Grassi (Railliet, *Traité de Zoologie Méd. et Agric.*) and by Leuckart (*Menschl. Parasiten*, II. pt. 2, p. 275). I regret that at present I have not access to the works of these authors with the exception of the last-named.

Segmentation and the formation of an active embryo have been found to take place in water and damp earth. Davaine (quoted by Leuckart) states that development takes place also in a dry condition. Leuckart gives the period necessary for the formation of an active embryo in summer as four to ten weeks and in winter several months. The fully formed embryo differs from that of *A. lumbricoides* in being larger, 0.36–0.42 mm. in length as compared with 0.3–0.38 mm. in the latter, and in bearing a more distinct boring tooth on the ventral aspect of the mouth. There are no signs of the oesophageal bulb of the adult with the exception of a slight swelling of the posterior end of the oesophagus. He gives a figure of the embryo.

This investigator did not succeed by any means in bringing about the hatching of the eggs, nor did he produce any further development by feeding experiments. At one time he considered that he had empirically caused direct infection since he found young worms 3–4 mm. in length in the intestines of dogs which had been kept a long time in a kennel on the floor of which he found embryo-containing eggs. Repeated attempts at artificial infection in more than two dozen cats and dogs gave however negative results. He then attempted to find an intermediate host. He administered eggs of the worms to rabbits and mice and examined these animals after several weeks hoping to find encapsuled nematodes. No such nematodes were found. He states that the eggs passed unaltered through the alimentary canal of the mice.

In a later experiment he placed a number of young cats in a particular dwelling in which a number of animals had recently been kept. The latter had been found to harbour numerous young *Ascarids*. The cats were fed on bread only. After six to eight days the cats were killed. They proved to be infected with *Ascarids* 4–8 mm. long. In the stomach of one of them, a kitten eight weeks old, forty to sixty nematode larvae

were found which measured 0.4–0.6 mm. The stomach also contained fragments of straw and potato. No explanation could be given of the mode of entry of these larvae. No animal remains were found in the stomach or intestine to point to an intermediate host.

Grassi (I quote from Railliet's text-book) reared young puppies in a kennel soiled with egg-containing excrement. Their faeces contained *Ascaris* eggs on the 28th day and numerous Ascarids were found on section. A control experiment in sanitary surroundings proved negative. Grassi concluded that infection was direct.

Segmentation of the egg and development of the embryo.

I made cultures of the eggs of *A. mystax* in a damp atmosphere at a temperature between 25° and 30° C. Under these conditions development proceeded as follows: 1st day, unsegmented to three-cell stage; 2nd day, advanced segmentation; 4th and 5th days curved vermicules (the stage figured by Leuckart, *Menschl. Parasit.*, II. pt. 2, p. 213, fig. 8); 7th day, plump vermiform embryos; 8th day, active embryos.

The intermediate host of A. mystax.

Ripe eggs were administered to mice in their food. The details of the experiments are given below. Active *Ascaris* larvae were found in the liver between the 1st and 3rd days after infection. In Experiment 1 the eggs given on the 9th July were probably not old enough to be infective. The material at my disposal was unfortunately not large.

Expt. 1. 9th July. Mouse *N* given eggs of *A. marginata* 6 days old containing vermiform embryos.

12th July. Eggs of *A. marginata* 9 days old and of *A. mystax* 12 days old.

13th July. Eggs of *A. marginata* 10 days old.

14th July. Mouse *N* killed. No larvae in lungs or liver.

Expt. 2. 14th July. Mouse *P* given eggs of *A. marginata* 11 days old.

15th July. Mouse died 20 hours after infection. Eight larvae found in a portion of the liver, none in the lungs.

Expt. 3. 29th July. Mouse *U* given eggs of *A. marginata* 26 days old.

30th July. Killed. Larvae in the liver, none in the lungs.

Expt. 4. 29th July. Mouse *V* given eggs of *A. marginata* 26 days old.

1st August. Killed. One larva found in the liver, none in the lungs.

Expt. 5. 4th August. Mouse *Z* given a small dose of eggs of *A. marginata* 32 days old.

9th August. No larvae in lungs or liver.

Measurements of larvae of Ascaris marginata from the liver of the mouse. The specimens were killed by heat in salt solution. Larva 20 hours after infection. Total length, 0.38 mm.; oesophagus length, 0.122 mm.; relation of length of oesophagus to total length, 1 to 3.11; maximum breadth, 0.017 mm. The length and breadth are practically the same as in the embryo, the oesophagus has shortened markedly.

Larva on the third day after infection. Total length 0.42 mm.; oesophagus length, 0.130 mm.; length of oesophagus to total length, 1 to 3.23.

EXPLANATION OF PLATE IV.

- Fig. 1. *Ascaris suilla*, larva from the caecum of the mouse, 12th day after infection. $\times 80$.
- Fig. 2. Anterior portion of larva from the small intestine of the mouse, 10th day. $\times 800$.
- Fig. 3. Anterior portion of larva from the faeces of the mouse, 11th day. $\times 800$.
- Fig. 4. Excretory gland and duct of larva from the large intestine of the mouse, 11th day. Seen from a point on the prolongation of the radius midway between the dorsal and left lateral radii. $\times 1360$.
- Fig. 5. The rudimentary gonad in a larva from the bronchi of the mouse, 9th day. $\times 1360$.
- Fig. 6. Transverse section through a larva from the caecum of the mouse, 11th day, level of the excretory gland. $\times 1360$.
- Fig. 7. Transverse section of a larva from the faeces of the mouse, 11th day, level of anterior end of intestine. $\times 1360$.
- Fig. 8. Transverse section of a larva from the caecum of the mouse, 11th day, immediately in front of the anus. $\times 1360$.
- Fig. 9. Transverse section of a larva from the trachea of the mouse, 9th day, level of the nerve-ring. $\times 1360$.
- Fig. 10. Transverse section of the same larva, level of the excretory gland. $\times 1360$.
- Fig. 11. *Ascaris suilla*, larva from the faeces of the mouse 10th to 11th day after infection. Outline of the tail from the left side. $\times 689$.

KEY TO LETTERING OF ILLUSTRATIONS.

a.c. anal canal; an. anus; d.l. dorsal line; e.ap. excretory aperture; e.c. excretory cell; e.d. excretory duct; int. intestine; l.l. lateral line; l.l.l. left lateral line; l.m. lateral membrane; m. muscle cell; m.f. muscle field; n.e.c. nucleus of excretory cell; n.l.e.c. nucleolus of excretory cell; n.r. nerve ring; oes. oesophagus; r.l.l. right lateral line.

TABLE OF MEASUREMENTS.

(Unless otherwise stated specimens were measured in normal salt solution.)

				Total Length	Maximum Breadth	Length of Oesophagus	Length of Oesophagus Total Length	Head to Nerve Ring	Anus to Tail	Post-anal Length Total Length
Embryo 26 days in water				·28	—	·087	$\frac{1}{3.3}$	—	—	—
(Following all from Mice)										
Larva from Liver				6th day	·735	·034	·14	$\frac{1}{5.2}$	—	—
"	"	Lung	6	"	·70	·032	·148	$\frac{1}{4.7}$	—	—
"	"	Trachea	11	"	2·10	—	—	—	—	—
"	"	Bronchus	12	"	1·90	—	·269	$\frac{1}{7}$	—	—
"	"	Trachea	13	"	2·07	—	—	—	—	—
"	"	Small Intestine	10	"	1·61	—	·22	$\frac{1}{7.2}$	·06	$\frac{1}{27}$
"	"	Large Intestine	10	"	1·58	—	·23	$\frac{1}{6.8}$	·062	$\frac{1}{25}$
"	"	Caecum	10	"	1·65	—	—	$\frac{1}{7}$	—	—
"	"	Large Intestine	11	"	2·10	—	·32	$\frac{1}{6.6}$	—	—
"	"	Caecum	12	"	2·02	—	·3	$\frac{1}{6.7}$	—	—
"	"	"	13	"	2·00	—	·3	$\frac{1}{6.6}$	—	—
"	"	"	"	"	2·20	—	—	—	—	—
"	"	"	15	"	2·20	—	—	—	—	—
"	"	"	"	"	1·75	—	—	—	—	—
"	"	"	"	"	2·37	—	—	—	—	—
"	"	Faeces	10	"	1·40	—	—	—	—	—
"	"	"	12	"	1·60	—	·19	$\frac{1}{8}$	—	—
"	"	"	"	"	1·15	—	—	—	—	—
"	"	"	13	"	1·47	—	·2	$\frac{1}{7.3}$	—	—
"	"	"	16	"	1·50	—	—	—	—	—
(Following from specimens in Canada Balsam)										
Larva from Trachea				9	"	1·36	—	·17	$\frac{1}{8}$	—
"	"	Caecum	10	"	1·25	—	·163	$\frac{1}{7.6}$	·061	$\frac{1}{20}$
"	"	Small Intestine	"	"	1·30	·052	·175	$\frac{1}{7.4}$	·063	$\frac{1}{26}$
"	"	Large	11	"	1·50	·073	·23	—	—	—