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Observations on the Life-history of *Bunostomum trigonocephalum*, a Hookworm of Sheep and Goats.

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In a footnote of a previous communication (1937) the writer very briefly outlined the salient features of the parasitic stages in the life history of this parasite. In the ensuing pages these are dealt with in greater detail, and information, which has since become available, is also incorporated.

The fullest and most trustworthy accounts of the biology of certain stages in the life history of this parasite are two communications by Cameron (1923 and 1927); in the former the author deals with the biology of the infective larva and in the second larval parasitic stages are described. Hesse (1923) supposedly gives the morphology and biology of the free living larval stages, but Cameron (1923) has shown that Hesse's cultures must have become contaminated and that his (Hesse's) observations of the 3rd stage larvae were not made on the larvae of this hookworm but possibly on those of a *Nematodirus* sp. The writer (1937) agreed with Cameron in so far that Hesse did not make his observations of the latter stage on hookworm larvae, but differs from Cameron in thinking that it was not *Nematodirus* sp. which Hesse observed, but probably larvae of some *Trichostrongylus* sp.; second stage larvae of both *Nematodirus* sp. and *Bunostomum* sp. have relatively very long tails and this long tail is retained as the sheath of the third stage infective larvae. On the other hand second stage larvae of *Trichostrongylus* spp. have relatively much shorter tails, and an examination of Hesse's figures of the second stage larvae, shows it to possess such a short tail, whose sheath is retained in the third stage as figured by Hesse in his figure of the 3rd stage larva.

In the common strongyloid parasites of sheep Mönnig has found the following to be the average lengths in m.m. of the body and of the tail sheaths of 3rd stage larva.

	O.c.*	H.c.	Tr. col.	B.t.	N. sp.
Average body lengths.....	0·79	0·69	0·39	0·57	1·1
Tail sheath.....	0·214	0·142	0·094	0·14	0·326

* O.c. = *Oesophagostomum columbianum*. H.c. = *Haemonchus contortus*.
Tr. col. = *Trichostrongylus colubriformis*. B.t. = *Bunostomum trigonocephalum*.
N. sp. = *Nematodirus spathiger*.

Hesse gives the body length of his 3rd stage larvae as 0·45 to 0·7 m.m. and from his figure of the 3rd stage larvae the tail sheath is about 0·9 m.m. long; these measurements agree fairly closely with those of a *Trichostrongylus* larva.

The writer is able to confirm Cameron's observations on the biology of the infective larvae and wishes only to make the following remarks. Eggs removed from gravid females and cultured at 26° C. in a fairly firm medium of sterilised sheep's faeces were found to hatch from the 24th hour onwards, the majority having hatched 36 hours after commencement of incubation. The mature larvae began to creep out of the medium from the fifth day onwards. For his experiments the writer collected larvae from the sides of the culture bottles from the 8th day onwards.

The writer also tried the Goodey skin penetration technique, using the skin of very young mice and rats, but in no instance was he able to observe any larvae attempting to penetrate the skin, and in all cases the larvae were again all recovered from the surface of the skin and none were obtained from the water on which the skin had been floated. The only response of the larvae which was noted was an increased activity when the skin became warmer as a result of its contact with the warm water below.

With regard to the morphology and biology of the various free living larval stages, the writer found these to be practically identical with those recorded by the writer (1937) for *Gaigeria pachyscelis* and the reader is referred to this account for the necessary details; the only difference was a slight morphological one in that the average length of the larvae, and consequently also its internal organs, was slightly smaller in *B. trigonocephalum* than in *G. pachyscelis*. The following measurements were obtained by the writer by measuring numerous ensheathed third stage larva which had been killed by gentle heat.

Length 0·563 to 0·632 m.m. Average 0·606 m.m.
Breadth 0·022 to 0·024 m.m.
Length buccal tube 0·015 to 0·016 m.m.
Length oesophagus 0·135 to0·142 m.m.
Length tail sheath 0·15 to 0·158 m.m.
Genital primordium from anterior end 0·268 to 0·275 m.m.
Excretory pore from anterior end 0·099 to 0·102 m.m.
Nerve ring from anterior end 0·085 to 0·087 m.m.

Without the sheath the total body length varied from 0·464 to 0·545 m.m. the majority of the larvae being from 0·505 to 0·510 m.m. long; the tail varied in length from 0·062 to 0·064 m.m. The above measurements are slightly in excess of those given by Mönnig.

Parasitic Stages.

The youngest forms obtained by Cameron (1927) were intestinal forms which had reached a length of 2·3 m.m. These were 4th stage larvae provided with a provisional buccal capsule. He failed to observe any forms showing the transition from the 3rd to the 4th

stage larvae. The writer suggests that Cameron would have observed this stage had he examined the lungs, because, as is shown below, the writer was able to obtain this stage from the lungs and never from the intestine.

Beller (1928) appears to have been the first to conclude that infection could be produced either through the skin or *per os*. He based his conclusion on the following experiment. Two young sheep (one and two year old) and two one year old goats were used, and one of each was infected through the skin and the other two received larvae, suspended in water, with a pipette through the mouth. Proper precautions were taken to prevent oral infection taking place in those sheep infected cutaneously. Prior to the experiment the faeces of the sheep and goats had been examined on several occasions and helminths eggs were found to be absent in all. The one year old sheep had about 100 larvae applied in moist cotton wool on the left side of the chest; portions of the skin were excised after 15, 30, 45 and 60 minutes and immediately fixed and later sectioned; he found that after 30 minutes larvae were already present in the subcutis. After 17 days hookworm eggs were diagnosed in the faeces. The sheep died after 36 days and 42 hookworms were recovered from the small intestine of which only a small proportion were mature. The second sheep (two year old) received about 200 larvae into the mouth; hookworms eggs made their appearance in the faeces after 24 days. The sheep was slaughtered after 71 days and 112 mature hookworms were collected. Of the two goats, one had about 200 larvae applied to its skin and the other received about 200 larvae into its mouth. Hookworm eggs were found to be present in the faeces of both goats after 17 days. In the cutaneously infected goat, slaughtered 71 days after infection, one female hookworm, 22 mm. long was recovered; the other goat was also slaughtered at the same time and two female worms were found. The faeces of both goats were now negative for hookworm eggs

There can be no doubt that Beller set up a cutaneous infection in one of the sheep and his finding of larvae in the skin, 30 minutes after exposure, definitely shows that they had penetrated the skin. The writer, however, doubts whether the eggs seen in the faeces after 17 and 24 days originated from this exposure; although no strongylid eggs were present in faeces prior to the experiment, the possibility that these animals were infected prior to the experiment is not excluded as hookworms may have been present which had not yet reached the egg laying stage. The eggs observed after 17 days in one sheep and the two goats and after 24 days in the other sheep most probably originated from such worms which had now become mature. That the one sheep harboured hookworms from two different infections is shown by the worms recovered from it at post mortem 36 days after the cutaneous infection; Beller states that only a small proportion of these worms were mature, the remainder being smaller. The mature worms probably laid those eggs which were seen on the 17th day and the remaining worms were the result of the cutaneous infection. At post mortem of the other sheep and two goats, 71 days after infection, only mature worms were found to be present which is what one would expect as, according to the writer's findings, the larvae become mature from the 10th week after infection.

The writer carried out his experiments on lambs, reared from ewes which were known to be free of this parasite, and which lived in a paddock where the parasite had never been present. The original material was obtained from a sheep brought in from the Bethal District; this sheep was killed and post mortemed on the same day of its arrival and never came into contact with any of the sheep kept at the Onderstepoort station; these latter sheep being known to be free of this hookworm. The Bethal sheep carried a heavy *Bunostomum* infection and faeces obtained from the rectum were mixed with sterile sheep faeces and cultured in jam bottles in the usual way at 26° C. Numerous hookworm larvae were recovered from the sides of the bottle from the 8th day onwards and these larvae were used to set up an infection. Seven lambs and three young goats were used in the first experiment: These were divided into two groups and treated as follows; four lambs and two goats had active larvae applied behind their ears on six consecutive days and three lambs and one goat were on the same days drenched with a minimum amount of water containing active larvae in suspension, care being taken that no moisture with suspended larvae escaped from the mouth.

Faeces from all these sheep were collected weekly from the sixth week onwards and cultured at 26° C. and the resultant larvae examined for hookworm larvae. Nine weeks after the commencement of the experiment one of the sheep, which had received active larvae behind the ears, died, and on post mortem about two hundred almost full-grown *Bunostomum trigonocephalum* were found. The females contained no eggs in their uteri. The parasites occupied the whole of the ileum posterior to the duodenum. The sheep was very anaemic and showed degenerative (fatty) changes of the liver and marked gelatinization of the fatty tissues. As all possibility of an oral infection had been excluded it followed that this severe infection could only have originated from the larvae applied behind the ears, penetrating the intact skin.

A week later, i.e. 10 weeks after the initial infection, one of the cutaneously infected sheep began to pass hookworm eggs in its faeces as revealed by the presence of hookworm larvae after culture. The following week, i.e. 11 weeks after the initial infectioin, the remaining two sheep which were cutaneously infected also began to pass hookworm eggs. Up to this time all the goats and the two orally infected sheep remained negative. The two orally infected sheep, however, began to pass hookworm eggs 14 weeks after the initial infection and the orally infected goat also passed hookworm eggs two weeks later. The two goats infected cutaneously remained uninfected and were eventually discharged from the experiment as negative, after they had been observed for 18 weeks.

From the above results it was quite clear that infection could be brought about either through the skin or through the mouth; whether larvae, which are admitted through the mouth, pass direct to the intestine and there complete their development, or whether they first have to migrate through the lungs, has not yet been established.

Having now established the fact that larva of *B. trigonocephalum* can penetrate the intact skin and eventually reach the intestine to complete their development, the next step was to ascertain the route followed by the larvae.

Three lambs were used in the next experiment and each received a single application of numerous 3rd stage larvae behind the ears. On the 8th day after infection one of the lambs was killed; eighteen 3rd and early 4th stage larvae, still ensheathed in the sheath of the 3rd stage, were recovered from the lungs; most of these latter had already developed the provisional mouth capsule, but in some only the beginnings of the capsule were evident. A minute examination of all the scrapings from the whole of the trachea, oesophagus and small intestine failed to reveal any hookworm larvae. This lamb thus showed that larvae had travelled from the skin and had reached the lungs, but had not yet reached the intestine; further that the larvae had undergone development during their sojourn in the lungs as shown by the different stages of development of the larvae recovered. The second lamb was slaughtered 11 days after infection and its trachea, oesophagus, lungs and intestine thoroughly examined. Only one early 4th stage larva was recovered from the lungs and this larva appeared to have just finished the development of its provisional mouth capsule. Two 4th stage larvae were recovered from scrapings of the intestine; they were exsheathed and the provisional buccal capsules were provided with dorsal and ventral lancets. Sex differentiation had not yet become evident. Scrapings from the trachea and oesophagus were negative. The third lamb was slaughtered 15 days after infection and the organs thoroughly examined. Larvae were absent from the lungs and none were found in scrapings of the trachea and oesophagus. In the intestine 45 4th stage larvae were recovered, all showing sex differentiation but no development of the adult mouth capsule.

The lungs of all three lambs showed punctate haemorrhagic markings on their outer surface; these markings were numerous in the first lamb slaughtered, less in the second lamb, and only five were seen in the last lamb. On section the lungs of the first lamb showed extensive bleedings into the alveoli.

From the above experiment it is evident that in travelling from the skin to the intestine, the larvae passed through the lungs where they passed from the 3rd to the 4th stage. Although no larvae were recovered from the trachea and oesophagus one is justified in assuming, from analogy of the route followed by other hookworm larvae, that they had travelled via the blood stream to the lungs and from there via the trachea, mouth and oesophagus to the intestine. Also that the time taken to reach the lungs was less than 8 days after infection and to reach the intestine between 8 and 11 days after infection.

The next experiment was undertaken in order to ascertain the shortest time in which the larvae travelled from the skin to the lungs, the length of time in which larvae could still be recovered from the lungs, and whether larvae could be recovered from the blood, trachea and oesophagus en route from the skin to the intestine.

In this experiment nine lambs were used and each received a single application of numerous active 3rd stage larvae behind the ears. The first lamb was slaughtered three days after infection; the blood was collected, hydrolised, sedimented and centrifuged and then carefully examined; the lungs, trachea, oesophagus and intestines were also carefully examined, but in no case was any larva recovered. The second and third lambs were killed four and five days after infection respectively and similarly examined but with negative results. The fourth lamb was killed six days after infection and similarly examined and about 20 third stage larvae were recovered from the lungs; blood, trachea and oesophagus were negative. The fifth lamb was killed 8 days after infection; trachea, oesophagus and intestine were negative for larvae, but about 400 3rd and 4th stage larvae were recovered from the lungs; the 4th stage larvae were encysted in the 3rd stage cuticle and the provisional mouth capsule was present. The sixth lamb was slaughtered 10 days after infection; trachea, oesophagus and intestine were negative for larvae, but about 50 3rd and 4th stage larvae were recovered from the lungs. The next lamb (7th) was killed 13 days after infection; unfortunately, while the lungs were being examined, the oesophagus and intestine were destroyed and consequently were not examined; the blood and trachea were negative for larvae; only one 3rd and one 4th stage larva were recovered from the lungs. The eighth lamb was killed 17 days after infection; blood, lungs, trachea and oesophagus were negative for larvae, but 35 4th stage larvae were recovered from the intestine, all of which showed sex differentiation: The last lamb (9th) was killed 20 days after infection; no larvae were recovered from the blood, lungs, trachea and oesophagus; about 100 fourth stage larvae were recovered from the small intestine all of which showed sex differentiation; the largest males were 3·17 mm. long and the largest females 3·39 mm. long.

The following conclusion can be drawn from the above experiment. Firstly, the larvae are able to reach the lungs in six days; although no larvae were recovered prior to six days the possibility of their reaching this site in a shorter period is not excluded because among the larvae recovered after six days, some showed no further development, showing that they had just recently reached the lungs, whereas some had proceeded to develop and had reached the lethargus stage, showing that they had reached the lungs some time previously, Secondly, the larvae continue to develop in the lungs and pass over to the fourth stage in this organ. They can remain in this site until the 13th day and possibly up to the 16th day, but as shown by the previous experiment, they may reach the intestine on the 11th day. The writer believes that all the larvae do not reach the lungs at the same time, the interval between those first reaching the lungs and the last to do so being due to whether the larvae enter a blood vessel sooner or later after penetrating the skin. The first larvae to reach the lungs would continue their development and also be the first to migrate from this organ, whereas those reaching the lungs later would only leave this organ and reach the intestine after an interval of time equal to that separating the first and last arrival of the larva in the lungs. Further, it would appear that the time spent by the larvae in the lungs is about five days, because there

was an interval of five days between the first appearance of the larvae in the lungs and their first appearance in the intestine. If this is true then it follows that no larvae reach the lungs after 12 days after infection because from the 17th day onwards no larvae were recovered from the lungs and only intestinal forms were present.

The next experiment was carried out in order to follow the developmental stages of the intestinal forms of this parasite. Six lambs were used for this experiment. All were once exposed to a massive infection of active 3rd stage larvae applied behind the ears. A lamb was then slaughtered at 3, 4, 5, 6, 7 and 9 weeks after exposure. The lamb slaughtered after 3 weeks had only 4th stage larvae in the intestine, some of which were passing into the next stage. The larvae recovered from the lamb slaughtered after four weeks had the adult mouth capsule fully developed, but all were still enclosed in the separated cuticle of the fourth stage.

Of the remaining four lambs, three, slaughtered 5, 6 and 7 weeks after infection, only had adolescent males and females present all of which had undergone ecdysis; in the lamb slaughtered 9 weeks after infection mature worms were collected whose females had segmented eggs in their ovejectors.

The result of all the foregoing experiments may be summarised as follows. Eggs are able to hatch within 24 hours under suitable conditions of temperature and moisture. The first stage larva which emerges passes through the second stage and reaches the infective 3rd stage in five days. These larvae can set up an infection either through the mouth or through the skin. When applied to the skin they penetrate it and presumably enter a blood vessel and are then carried by the blood to the lungs which they reach within six days. In this organ they remain for about five days during which period they grow and pass into the next or fourth stage provided with a provisional buccal capsule. They now undergo an ecdysis and presumably creep up the trachea to the mouth and are then swallowed. They reach the intestine from the 11th day onwards as young 4th stage larvae; here they grow and on the 15th day, and perhaps earlier, sex differentiation becomes apparent. They continue to feed and grow and in three weeks after infection they begin to pass over to the next or final stage. In four weeks the adult stage is completed but the larvae are still enclosed in the cuticle of the previous or 4th stage. During the fifth week the cuticle is shed and the emerging forms from now onward continue to feed and grow until the 9th week, when they are fully mature and the females contain segmented eggs.

Morphology of the Larvae.

The morphology of the free living, first, second and third stage larvae (Fig. 1) is identical to that described for the corresponding stages of *Gaigeria pachyscelis*, except that the third stage larvae is slightly smaller. The morphology of the parasitic fourth and fifth stages have been adequately described by Cameron (1927). An examination of his descriptions and drawings shows that the earliest

forms which he was able to study correspond to those found by the writer from the third week onwards after infection. Below the writer will in consequence confine himself to the parasitic portion of the third stage and to the fourth stage larva.

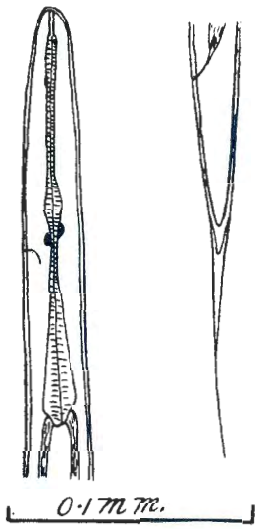

Fig. 1.—Anterior and posterior extremities of third stage larva.

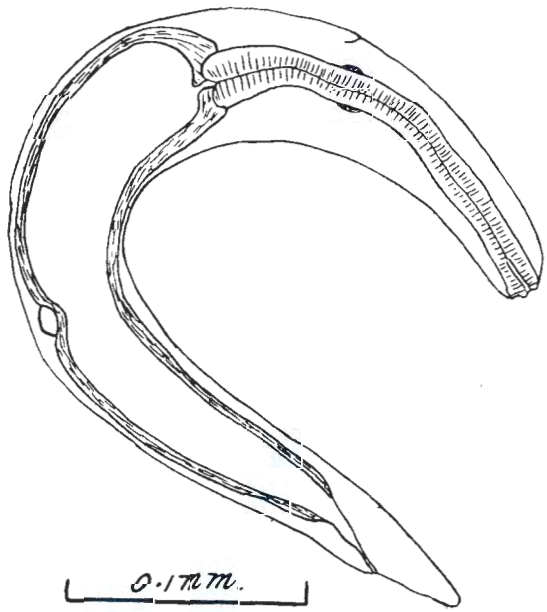

Fig. 2.—Late third stage larva from the lungs just prior to onset of lethargus.

When the infective larva penetrates the skin it presumably also casts its enveloping cuticle, all larvae which were recovered from the lungs being exsheathed. The youngest larvae recovered from the lungs, six days after infection, appear to have undergone very little or no development; their size and shape of oesophagus was similar to that of the infective stage. Once in the lungs they proceed to grow and this growth is very marked in the lateral direction in that a considerable thickening takes place accompanied by practically no increase in length. This thickness is due to changes which take place in the oesophagus and intestine. The accompanying figure (Fig. 2) shows a late third stage larva which is on the point of passing into the lethargus stage. It will be noted that the oesophagus and intestine are much robuster than similar organs found in the infective stage; the lengths of these organs have also undergone a slight change, the oesophagus increasing slightly and the intestine decreasing slightly in length. The following table gives the chief measurements of five late third stage larvae just prior to lethargus (measurement in mm.).

	1.	2.	3.	4.	5.
Body length	0·522	0·510	0·505	0·5	0·522
Body breadth	0·046	0·043	0·044	0·058	0·055
Length oesophagus	0·177	0·165	0·165	0·17	0·151
Max. thickness oesophagus	0·02	0·018	0·018	0·02	0·019
Length of tail	0·046	0·049	0·049	0·045	0·058
Length buccal tube	0·016	0·015	0·015	0·015	0·016
Nerve ring from front	0·116	0·119	0·112	0·115	0·121
Exc. pore from front	0·123	0·133	0·119	0·12	0·13
Genit. primordium from tail tip	0·216	0·232	0·223	0·213	0·22

When comparing these larvae with similar stage larvae of *Gaigeria pachyscelis* it will be noted that morphologically these two are very similar, the only apparent difference being that the larvae of *B. trigonocephalum* are slightly smaller. They have the same reddish colour and show the same type of movement prior to lethargus.

During lethargus the same type of morphological changes set in, the most marked of which is the development of the provisional buccal capsule. The first indications of this capsule is a breaking up of the body tissues between the anterior and posterior levels of the buccal tube; at first the oesophagus is not involved, but as development proceeds the breaking up of the tissue also encroaches on to the outer limits of the oesophagus. A cavity round the anterior tip of the oesophagus is thus formed whose outer border becomes cuticularized. Eventually the whole of the anterior tip of the oesophagus disappears, except its cuticular lining which remains as a thin strand joining the old mouth of the third stage larva to the base of the buccal capsule of the fourth stage. Figures 3 A and B show the anterior extremity of a larva in which the buccal capsule is partially developed and Figure 3 C shows a larva with the capsule

completely formed. By this time a new cuticle has been formed and the larva is now surrounded by the detached cuticle of the third stage. No sex differentiation has yet taken place and no teeth or lancets are present at the base of the buccal capsule. Further development in size takes place in the lungs and the larvae are now ready to migrate to the intestine. Five ensheathed fourth stage larvae, recovered from the lungs eight days after infection, gave the following principle measurements in mm.

	1.	2.	3.	4.	5.
Length of body including sheath	0·591	0·539	0·551	0·592	0·557
Length of body without sheath	0·57	0·511	0·53	0·58	0·537
Breadth of body	0·06	0·055	0·058	0·055	0·055
Length oesophagus	0·151	0·153	0·145	0·16	0·148
Mouth capsule, depth	0·029	0·027	0·026	0·029	0·029
Mouth capsule, breadth	0·031	0·028	0·029	0·026	0·029
Exc. pore from front	0·127	?	0·122	0·131	0·127
Nerve ring from front	0·116	0·112	0·11	0·116	0·112
Length of tail and sheath	0·058	0·056	0·058	0·06	0·058
Length of tail without sheath	0·045	0·038	0·042	0·05	0·043
Gen. primordium from tail tip (excluding sheath)	0·275	0·249	0·247	0·246	0·252

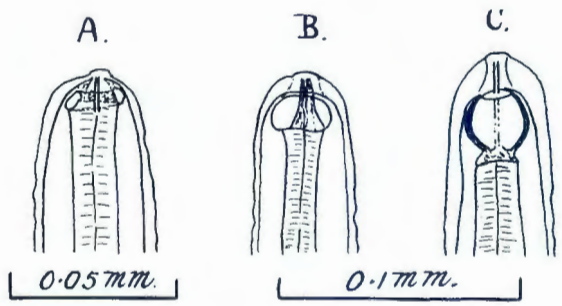

Fig. 3, A, B, and C.—Anterior extremities of fourth stage larvae showing development of provisional buccal capsule.

The smallest ensheathed fourth stage larva encountered was 0·518 mm. long with a maximum thickness of 0·046 mm. and the largest larva of this stage 0·595 mm. long with a maximum thickness of 0·052, the sheath excluded in all measurements.

When the larvae reach the intestine from the 11th day onwards after infection they have already lost their sheaths. The writer has not been able to recover any larvae in the process of casting their skin and he is in consequence not able to state whether ecdysis takes place in the lungs, during migration to the intestine or after reaching the intestine.

After reaching the intestine, development proceeds more rapidly, the youngest larvae recovered from the intestine were two larvae 0·816 and 0·828 mm. long, which had lost their sheaths and in which sex differentiation had not yet taken place. The principal measurements in mm. of these two larvae are given below:—

	1.	2.
Length of body	0·816	0·828
Breadth of body	0·056	0·058
Length of oesophagus	0·234	0·228
Maximum thickness of oesophagus	0·033	0·036
Buccal capsule, depth	0·031	0·033
Buccal capsule, breadth	0·03	0·032
Exc. pore from front	0·168	0·150
Nerve ring from front	0·150	0·135
Length of tail	0·078	0·058

A striking feature of these larvae as compared with fourth stage larvae recovered from the lungs was that in the intestinal form a dorsal and two subventral lancets were present in the base of the capsule (Fig. 4 A) whereas these were absent in the lung forms.

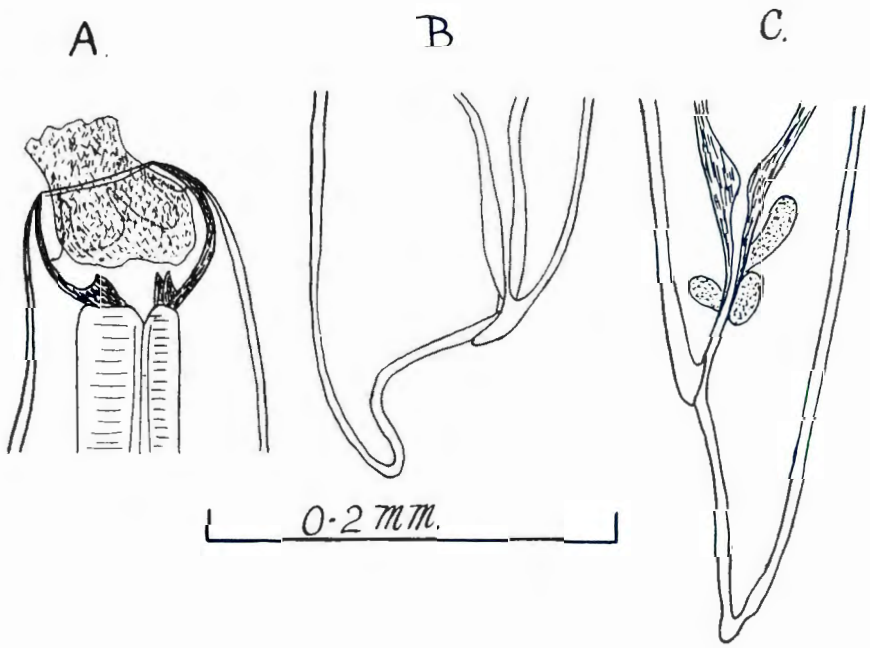

Fig. 4.—A. Anterior extremity of fourth stage larva showing fully developed provisional buccal capsule with portion of an intestinal villus.

B. Posterior extremity of male fourth stage larva.

C. Posterior extremity of female fourth stage larva.

The next lot of larvae recovered from the intestine were from a lamb which had been cutaneously infected 15 days previously. These larvae all showed sex differentiation, the most marked external feature being the stumpy tails in the males and elongate tails of the females (Fig. 4 B and C); whereas in the larvae recovered 11 days after infection the buccal capsule was in a straight line with the longitudinal axis of the body and the mouth aperture faced directly forwards, in these latter larvae the buccal capsule was tilted dorsalwards and consequently the mouth faced antero-dorsally.

In the following table the principal measurements in mm. of the largest three males and the largest three females are given:—

	Males.			Females.		
	1.	2.	3.	1.	2.	3.
Body length	2·76	2·69	2·04	2·27	2·83	2·59
Body thickness	0·12	0·116	0·102	0·12	0·168	0·15
Length oesophagus	0·47	0·468	0·396	0·48	0·54	0·456
Thickness of oesophagus—						
Ant. end	0·04	0·04	0·036	0·04	0·044	0·042
Post. end	0·06	0·057	0·05	0·048	0·063	0·057
Buccal capsule—						
Depth	0·054	0·058	0·048	0·054	0·06	0·058
Breadth	0·06	0·06	0·058	0·06	0·072	0·066
Exc. pore from front	0·03	0·302	0·264	0·305	0·312	0·3
Nerve ring from front	0·282	0·3	0·24	0·27	0·3	0·264
Cervical pap. from front	0·295	0·288	0·257	0·29	0·31	0·285
Vulva from tail tip	—	—	—	1·16	1·46	1·2
Length tail	0·09	0·072	0·06	0·108	0·12	0·126

These larvae showed no sign of a developing adult mouth capsule. The genital primordium of the males has segmented and shifted and consists of a row of cells towards the posterior end of the body. In the female the vulva has become demarcated and the genital cells have segmented to form an anterior and posterior row of cells, which will give rise to the uteri and ovaries, and a central mass of cells connecting these two rows and the vulva; this central mass of cells is destined to form the vagina.

Larvae recovered 17 days after infection showed the same morphological details as those described above, except that the body had become larger and the genitalia had continued to grow. The largest female was 2·8 mm. long and 0·156 mm. thick and the largest male 2·35 mm. long and 0·12 mm. thick. There was no sign of a developing adult mouth capsule.

Larvae recovered 20 days after infection also showed no signs of a developing adult mouth capsule. The largest female was 3·54 mm. long and 0·192 mm. thick and the largest male was 3·36 mm. long and 0·19 mm. thick.

On the 21st day after infection larvae were recovered in which the beginnings of the adult mouth capsule were just noticeable. These, however, were not the largest larvae; the largest female was

3·8 mm. long and 0·194 mm. thick and the largest male 3·1 mm. long and 0·156 mm. thick. The female larvae, where development of the adult mouth capsule had commenced, varied in length from 3·02 mm. by 0·14 mm. thick to 3·7 mm. long by 0·168 mm. thick. No male larvae showed a developing adult mouth capsule.

Larvae recovered from the intestine 28 days after infection showed a considerable further development. The adult mouth capsule was now fully formed and the provisional buccal capsule was separated off; a new skin had been formed and the larvae were ensheathed in the cuticle of the fourth stage. The larvae had thus reached their final or fifth stage, the genitalia had increased in size, those of the male were extending from the posterior extremity of the body to about midway, where the future testicular portion was recurved backwards and made a few loops. The female genitalia have not yet acquired an opening to the exterior, but the vaginal cavity has made its appearance. The cavity of the future ovejectors can just be made out in some larvae. The rest of the genital apparatus is still solid and the future ovaries are recurved and wavy. The females have now reached a length of 4·02 mm. by 0·198 mm. thick and the males 3·93 mm. long by 0·19 mm. thick.

The development of the adult mouth capsule and of the genital organs have been clearly described and figured by Cameron (1927) with whose findings the writer agrees. These appear to be identical to those observations made by the writer on the larvae of *Gaigeria pachyscelis*.

With regard to the development of the bursal rays the writer can corroberate Cameron's (1927) observations, namely that the main ventral branch of the lateral bands gives rise to the ventral, antero-lateral and part of the medio-lateral rays. This development differs from that found by the writer to occur in *Gaigeria pachyscelis*; in this hookworm the medio-lateral ray does not arise from the ventral branch of the lateral bands but, together with the postero-lateral ray, is formed from a secondary branch arising ventrally near the base of the dorsal brauch of the lateral bands.

From this stage onwards the larvae moult during the fifth week after infection and then feed and grow to attain sexual maturity in from nine to ten weeks after infection.

Biology of the Parasitic Third and Fourth Stage Larvae.

The lung forms of the third stage larvae live on blood, they have a red colour, probably derived from the haemoglobin of the blood. Prior to lethargus they are active, negatively photo- and geotropic and show an increased activity when gentle heat is applied. The fourth stage larvae attach themselves to the villi of the small intestine, by drawing portions of the villi through the mouth into the buccal capsule, these portions are lacerated by the buccal lancets and the larvae feed on the liberated blood. Both these stages cannot withstand any drying either in the ensheathed or exsheathed condition.

SUMMARY.

1. Eggs passed in the faeces of sheep or goats or obtained from the uteri or gravid females are able to hatch in from 24 to 36 hours under suitable conditions of aeration, temperature and moisture.

2. The larvae have three preparasitic stages each separated by an ecdysis. The cuticle of the second stage is retained as a protective sheath for the third or infective stage which is reached in five to eight days after hatching.

3. Morphologically and biologically the different larvae are practically identical to those of *Gaigeria pachyscelis*.

4. Infection of the host takes place either by penetration of the skin or via the mouth.

5. After penetrating the skin the larvae proceed to the lungs which they reach within six days; the route taken is presumably via the blood stream.

6. In the lungs the larvae remain for about five days during which period they feed, grow and pass into the fourth stage provided with a provisional buccal capsule.

7. The larvae now migrate to the intestine presumably via the trachea, mouth and oesophages. When first seen in the intestine they have already shed their sheath, but no sex differentiation is yet evident; sex differentiation sets in about four days later. Buccal lancets also appear.

8. The larvae attach themselves to the intestinal villi and feed on the liberated blood. They grow and begin to pass into the final or fifth stage in about a week after reaching the intestine.

9. Final ecdysis takes place about 10 days later.

10. Growth continues and the egg laying stage is reached in nine to ten weeks after infection.

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(References to additional literature may be found in the last-named publication.)