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Observations on the Morphology and Life-history of *Gaigeria pachyscelis* Raill. and Henry, 1910: A Hookworm Parasite of Sheep and Goats.*

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INTRODUCTION.

THE genus Gaigeria was created by Railliet and Henry in 1910 to accommodate a hookworm parasite recovered from the small intestine of Indian and African sheep. This remained the only record of the occurrence of this parasite in India, until Cameron (1924) had the opportunity of examining some material obtained from an Indian goat. Since Cameron's article appeared, the literature dealing with Indian helminths is strikingly lacking in further information on the occurrence, bionomics and pathogenicity of this parasite: Even such a recent work as Bhalerao's (1935) text book on the helminths found in Indian domesticated animals simply records the occurrence of this parasite. From this lack of information one is tempted to conclude that this parasite is comparatively rare in India and that it does not play a serious economic role. The writer has recently been able to find this parasite in a recently imported Indian antelope—*Boselaphus tragocemalus*. The faeces of this animal were examined a few days after its arrival at the Johannesburg zoological gardens, and this examination revealed the presence of helminth eggs indistinguishable from typical Gaigeria eggs. On culture typical Gaigeria larvae were obtained and on infecting sheep with these, the adult *Gaigeria pachyscelis* were recovered on post-mortem. This finding showed that the animal had brought with it from India a natural infection with this parasite. As far as the writer is aware this is the first record of the occurrence of this parasite in wild game.

The first record of the occurdence of this parasite in Africa is by Railliet and Henry (1910), who examined some specimens recovered from a sheep at Leopoldville, Belgian Congo. Gedoelst (1911) also records this parasite from the lower Congo. The same author (1916) further mentions the presence of this parasite in the Belgian Congo and he states that it appears to be a fairly common parasite.

* Read before Section D of the South African Association for the Advancement of Science on October 9, 1936.

In South Africa this parasite has only come into prominence during the last ten years, and to-day it is one of the most serious parasites affecting the sheep industry. It is quite possible that before this period it had been mistaken for the common sheep hookworm *Bunostomum trigonocephalum*, otherwise it would be difficult to explain how this parasite could have spread over such a large area in so short a time.

Distribution in South Africa.

The distribution of this parasite in South Africa is somewhat peculiar. One generally associates the presence of hookworms, whether in man or animals, with a humid climate, but this parasite in South Africa is found, except for a few instances, in the more arid regions, namely the areas extending from South West Africa, through the North Western portions of the Cape Colony to the Bechuanaland Protectorate. Outside these areas the writer has once obtained specimens from a goat born and bred in Zululand, Natal, and Bevan and Lawrence (1930) and le Roux (1932) have also recorded its presence in the Rhodesias.

Occurrence.

Up to the present this helminth is known to infect only our domestic sheep and goats. There have been some records of its occurrence in cattle, but the correctness of these records is open to grave doubts. The writer has examined the faeces of numerous cattle running on veld carrying infected sheep, but in no instance was he able to find the characteristic Gaigeria eggs; he has also repeatedly tried to infect calves but always without success. From these findings the writer is definitely of the opinion that cattle are not suitable hosts for this parasite. It would appear that our wild antelopes, either, are not able to harbour this parasite, because examinations of our antelopes have so far not yet revealed their presence, notwithstanding that in some cases the antelopes had been running in areas where gaigeriasis is now endemic. This would tend to show that this parasite is not a natural parasite of our antelopes, and, as at present it is only known from our domesticated animals, one tends to the view that it was at one time or other introduced into South Africa by domesticated animals from elsewhere, probably the East, where, as recorded above, it has also been found naturally infecting an Indian antelope. That this parasite probably has a wide distribution in the East is shown by the record of Noto-Soediro (1928) who described this parasite as G. *smiti* sp. nov. from Java; this parasite was later shown by the author and Ihle (1929) to be the same as *G. pachyscelis*.

MORPHOLOGY.

It is not the intention of the writer to give a detailed morphological description of this parasite; this has recently been done by Cameron (1924), Noto-Soediro (1928) and Noto-Soediro and Ihle (1929), and the writer only wishes to amplify these authors' descriptions.

Cephalic glands (Figs. 1 and 2).—At the apex of each lateral papilla, on the sides of the buccal capsule, there is a small aperture which represents the anterior opening of the cephalic glands. These are conspicuous structures, easily seen in adolescent specimens when they are cleared in glycerine. There are two of these glands, one on

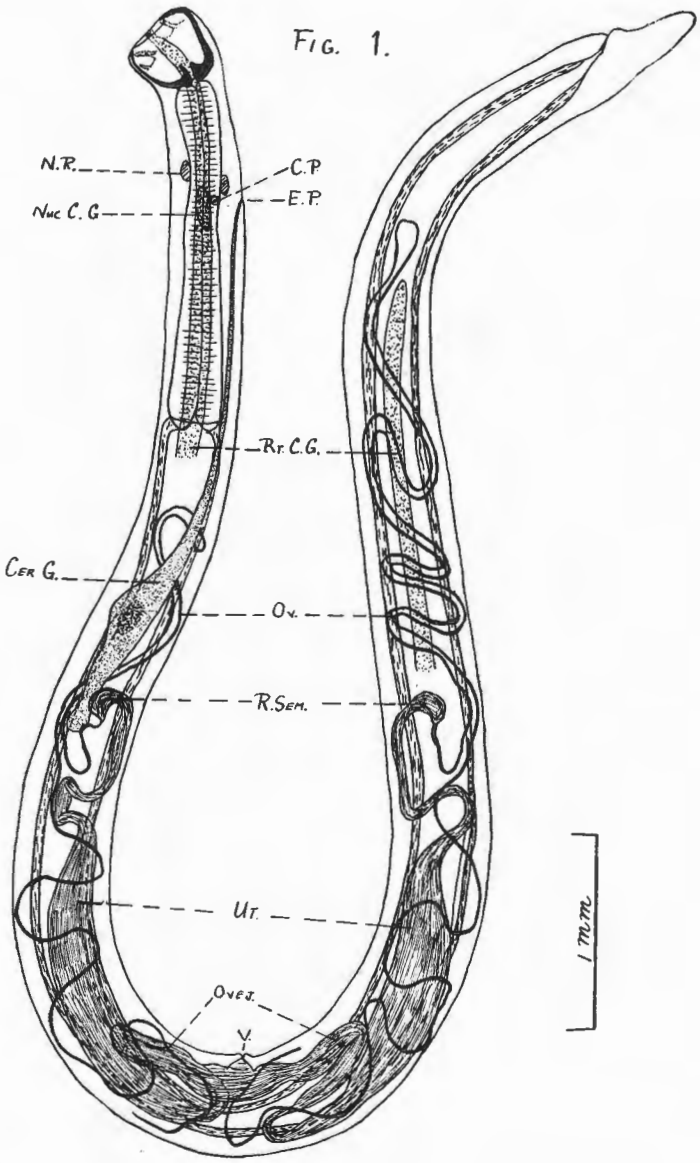


Fig. 1. Adolescent female, 8 weeks old.

(C.P. = cervical papilla; Cer.G. = cervical gland; E.P. = excretory pore; N.R. =nerve ring; Nuc.C.G. = nucleus of cephalic gland; Ov. = ovary; Ovej. = ovejector; Rt.C.G. = right cephalic gland; R.Sem. = receptaculum seminis; Ut. = uterus; V. = vulva).

either side of the body, extending down the length of the body about 5/6th of its length, on each side of the intestine; they are ribbon-like, having a granular texture, each possessing a large oval nucleus a about the level of the cervical papillae.

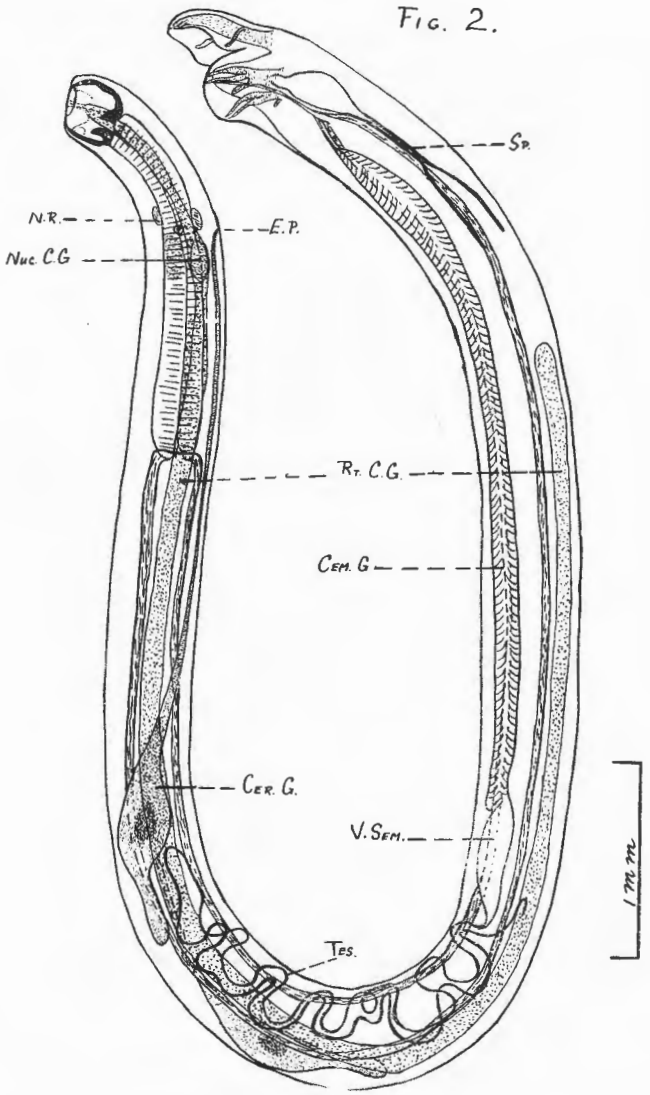


Fig. 2. Adolescent male, 8 weeks old.

(Cem.G. = cement gland; Sp. = spicules; Tes. = testis; V.Sem. = vesicula seminalis; other lettering as in fig. 1).

Cervical glands.—The excretory pore, situated just posterior of the level of the cervical papillae, gives exit to two large cervical glands whose ducts join each other forming a common duct just prior to their opening to the exterior. Each gland is an elongate

structure which becomes wider posteriorly until just before its posterior termination it swells out considerably, after which it tapers again. It is thus somewhat club-shaped, the thickened portion of the club carrying the large oval nucleus. Its internal structure is also granular, but the granules are coarser than those found in the cervical glands. In the males these glands extend to about the junction of the first and second body thirds, but in the females they are only about one-quarter of the body length. Posteriorly they are lateral in position, but as they pass forwards they first enlarge to accommodate the nuclei, after which they quickly become thinner and passing ventralwards occupy a ventral position from the posterior end of the oesophagus to the excretory pore.

Female genitalia.—The vulva is a transverse slit, situated just anterior to the middle of the body; it leads by means of a short vertical vagina into the two ovejectors which occupy an anterior and posterior position respectively, more or less in a straight line ventral to and parallel to the intestine; at about half its length each is sharply bent on itself, and in young females, in which the uteri have not yet become filled with eggs, each limb of the bend only continues as far as the level of the vulva, but in older females, when the uterus is expanded by the contained eggs, each limb, as Cameron has shown, passes beyond the level of the vulva, and only then does it again bend back on itself to join the much enlarged uterus. Each uterus consists of an enlarged and straight bag-like proximal portion and a thinner tubular distal portion which makes a characteristic S-shaped dorso-ventral bend, the second bend of which forms a large ventral sweep, with its tip tilted dorsalwards and slightly enlarged to form a receptaculum seminis; the anterior receptaculum seminis is found at about the level of the posterior end of the cervical glands, whereas the posterior is situated at about the junction of the third and last body quarters. Each reeceptaculum seminis is directed towards the middle of the body; it narrows suddenly and a short oviduct joins it to the ovary; these form several more or less dorso-ventral loops lateral of the intestine. The anterior ovary passes forwards to just behind the level of the posterior end of the oesophagus, where it is bent back sharply on itself and passes backwards parallel to itself to about the level of the vulva where it curves forwards again to end in this vicinity. The posterior ovary passes backwards until just beyond the posterior termination of the cephalic glands, where it also becomes sharply bent on itself and passing forwards parallel to itself terminates in the vicinity of the vulva in a way similar to that of the anterior ovary.

Male genitalia.—There is nothing to add to the existing descriptions of the caudal bursa, except that in most specimens there is a tendency for the origins of the externo-dorsal rays from the dorsal ray to be slightly asymmetrical. The internal organs are very similar to those described by Looss (1905) for *Ancylostoma duodenale*. The cloacal aperture is situated near the tip of the genital cone on its dorsal aspect; it passes inwards for about ½ m.m. when it joins on to the short rectum which passes into the intestine. At the posterior end of the rectum the ductus ejaculatoris joins it on its ventral side and just posterior to this point the opening of the spicular canal is found on its dorsal side. The ductus ejaculatoris passes forward and

occupies a position ventral to the intestine, and in young males is about $\frac{1}{3}$ of the body length. Except for its posterior terminal portion it is enveloped for its whole length by the cement gland which gives out two lateral wings, passing slightly outwards and dorsalwards, thus forming a groove in which the intestine lies. At its anterior end the ductus ejaculatoris bends dorsalwards and leaves the cement gland on its antero-dorsal face to join the straight and spindle-shaped vesicula seminalis. Anteriorly the cement gland forms a short prolongation lying ventral of the vesicula. The narrower anterior termination of the vesicula is bent downwards and joins the testis which passes forwards to the level of the swollen portion of the cervical glands making several more or less dorsoventral loops; here it bends backwards forming a conspicuous oval after which it proceeds backwards more or less parallel to itself to end in the vicinity of the vesicula seminalis.

LIFE HISTORY.

In a preliminary communication (1934) the writer gave an account of the salient points connected with the life history of this parasite. The writer wishes now to treat these with greater detail and also to incorporate additional facts observed since the appearance of this note.

(*a*) Development of the Eggs up to Hatching.

Gaigeria paschycelis possesses one of the most characteristic eggs passed by helminths infecting our domesticated animals. When laid it is somewhat brownish in colour and can, because of its shape and size, be easily recognised when the faeces of the host are examined. The eggs are elongate with bluntly rounded extremities, having a smooth thin shell, about 0·002 thick; when viewed in certain positions it appears that one long side of the shell is somewhat flattened, whereas that opposite is slightly arched; this is also seen in oxyurid eggs, but in these helminths this characteristic is more accentuated, besides the extremities of their eggs are more drawn out and pointed than in Gaigeria eggs. The size of the eggs, excepting those of Nematodirus, are the largest nematode eggs found in the faeces of domesticated animals; they have been found to vary in length from 0·108 to 0·115 m.m. by 0·058 to 0·061 m.m. in breadth. On deposition in the intestine the contents of the eggs have already started to develop, the ovum having divided and sub-divided until the formation of a 16 or 32 celled morula. Each cell of the morula is finely granular and contains a centrally placed nucleus. All the cells appear to have a similar structure and the whole morula, when examined under the microscope, is seen to be dense and opaque. The morula does not occupy the whole cavity of the egg-shell; generally it lies flush against one of the long sides of the egg leaving a clear, empty space opposite and at the two extremities of the egg. A very delicate vitelline membrane is present between the morula and the shell.

Under suitable conditions of warmth and moisture the eggs undergo further development immediately. The routine method employed by the writer is to collect the faeces of an infected sheep in a canvas bag, place these in a jam bottle, and lightly screwing down the lid after breaking up the faeces; the whole is then placed in an incubator room kept at about 26° C. Samples of faeces may now be removed from the bottle at different intervals and the developing larvae examined. Where there is no possibility of the sheep having been exposed to any other type of hookworm, except that of *Gaigeria pachycelis*, the identification of the second and third stage larvae offers no difficulty, it being comparatively easy to distinguish these from those of other nematodes whose eggs may also have been present in the faeces culture. In order to follow the development of the egg up to the hatching of the first stage larva, the only satisfactory method is either to remove the mature eggs from the uteri of washed females or collect the eggs individually from sieved, washed and sedemented faeces; this latter is a laborious process and was only utilised when fresh mature females were not available. Having obtained the clean eggs these may now be incubated either by placing them directly in a little water or else mixing them in a little moistened animal charcoal or sterilised sheep faeces, and spreading out the culture in a thin layer on the bottom of a petri dish; the culture is kept moist by fitting a moist piece of blotting paper into the lid of the petri dish, a method, as far as the author is aware, devised by Prof. R. T. Leper, F.R.S., and which the writer used in his London laboratory.

By examining the cultures 12 hours after incubation it was noted that most of the eggs had undergone considerable development, the morula now consisting of numerous very small cells with densely granular contents; after 18 hours the morula has become elongated and one end has developed a thinner appendage which tends to curve under the rest of the larva; after 24 hours the tadpole stage has been reached and the larva shows signs of movement. The digestive tract is also now clearly indicated. Further development is now comparatively much quicker, the larva elongating considerably and becoming coiled within the egg shell; the larval buccal capsule becomes distinct and the mouth and anal openings appear. By the 30th hour the larvae may begin to hatch, but the majority of the larvae do not begin before the 36th to 48th hour. If the cultures are kept round about 30° C. the larvae develop much quicker than at 26° C and some of the larvae may be found to hatch after 24 hours. Temperatures lower than 26° C. caused slower development, with consequent later hatching. In some cultures which were kept just under 20° C. the eggs, although containing fully developed larvae, had not hatched after five days, and after that appeared to hatch only with difficulty. When the larva is ready to hatch it curls about actively within the egg shell, thrusting its anterior extremity against the shell in various places: by this time the egg shell has become softer, elastic and weaker so that a slight bulge is formed on its outer surface where the larva is pushing from within. Eventually the larva succeeds in rupturing the shell, when it wriggles out and then swims about in the water.

(*b*) First Stage Larva. (Figs. 3 and 4.)

The first stage larva, which emerges from the egg, is very similar to other first stage larvae of the Strongylidae. Just after hatching it is from 0·237 mm. to 0·262 mm. long with a maximum thickness of 0·016 to 0·017 mm. and its cuticle is very delicately annulated; if food is present it actively feeds and grows in length so that when

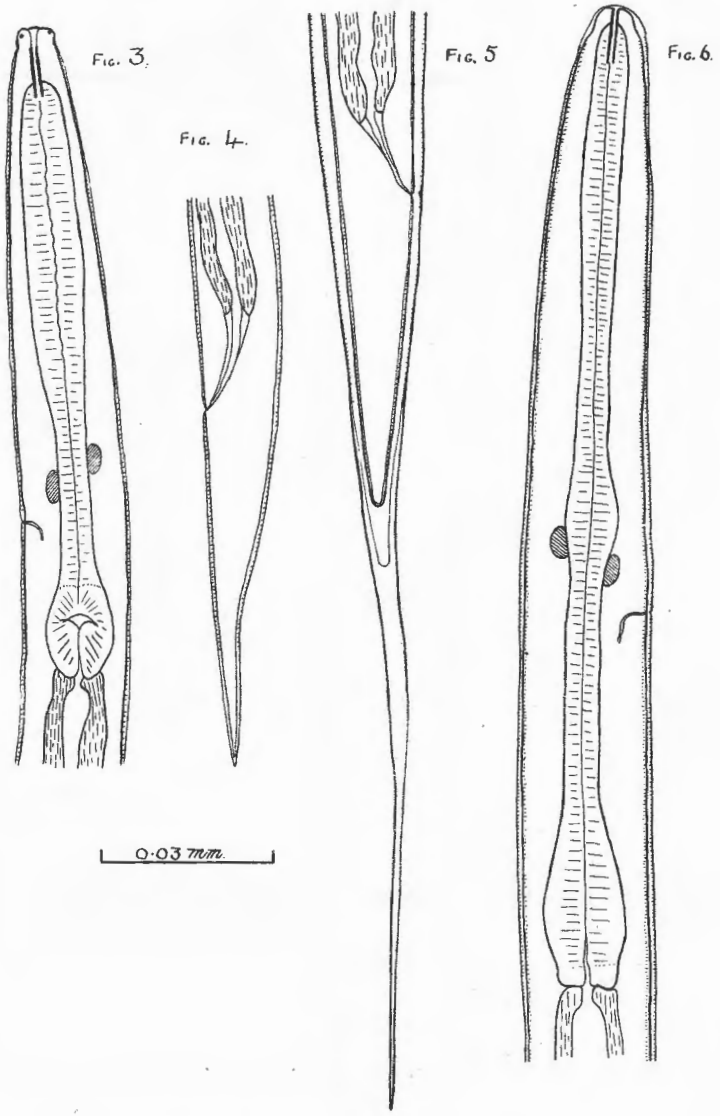


Fig. 3. Anterior extremity of 1st stage larva.
Fig. 4. Posterior extremity of 1st stage larva.
Fig. 5. Posterior extremity of 3rd stage larva.
Fig. 6. Anterior extremity of 3rd stage larva.

it is ready to undergo its first moult and pass into the second stage it may attain a length of 0·464 mm. and a thickness of 0·021 mm. It is provided with three minute lips, about 0·003 mm. high, each carrying a single minute papilla; a slight constriction separates them from the rest of the body. The body itself becomes thicker posteriorly, attaining its maximum thickness about midway between the posterior limit of the oesophagus and the genital primordium. The posterior portion tapers gradually until reaching the anus it narrows considerably to terminate the body in a straight and pointed tail which varies in length from 0·05 to 0·074 mm. according to the length of the larva. The mouth leads into an elongate larval mouth tube, bounded by thin chitinous walls which under the microscope appear as two straight bright refringent lines; this tube is from 0·008 to 0·0095 mm. long with an internal diameter of about 0·001 mm. The anterior end of the oesophagus envelopes the posterior quarter of this tube. The oesophagus is typically rhabditiform and is from 0·071 to 0·095 mm. long, its posterior end is slightly swollen and contains a valvular apparatus. The nerve ring encircles the oesophagus at about two-thirds of its length from the anterior end. Just posterior of this level is situated the very small excretory pore, with the delicate excretory duct passing inwards and backwards.

The genital primordium is a small lens-shaped structure, 0·006 mm. long by 0·005 mm. broad situated just behind the middle of the body which it divides into the ratio of 6:5. It appears to consist of only 2 or 3 cells.

The larva moves about actively and feeds, and the intestinal cells become more granular and opaque. After about 24 hours it becomes somewhat quiescent, and goes into the lethargus preparatory to passing over to the second stage. This transition is gone through relatively quickly because some six hours later it is found that most of the larvae in the culture have already completed their ecdysis. During the lethargus the most striking change is the development of a tail which is long and whiplike; otherwise the other larval parts duplicate those present in the first stage larva. The second stage larva breaks through the cuticle of the first stage by forcibly pushing off the anterior end of the cuticle, which comes off like a cap; the larva now wriggles out and commences to feed again and grow.

(*c*) Second Stage Larva.

The second stage larva, like that of the first stage, remains in the culture medium on which it actively feeds. It grows considerably in length during the next three to four days and may become 0·66 mm. long. It now goes into the second lethargus during which period a new cuticle is formed, and considerable internal changes take place, the most striking of which are the displacement of the old oesophagus by one which now assumes a filariform shape without a posterior valvular apparatus, and the development of a short stumpy tail. The new oesophagus differs from that usually found in third stage strongylid larvae in that it possesses a slight swelling at about its middle, just anterior to the nerve ring; in larvae, which

have accidentally lost their sheath, this swelling serves as an easy characteristic to distinguish the Gaigeria larvae from other strongylid larvae which might have lost their sheath.

(*d*) Third Stage Larvae (Figs. 5 and 6).

The third stage larva does not undergo an ecdysis, but remains encased in the separated cuticle of the previous stage. This, as in all strongylid larvae, forms a protective covering to the larva and is only thrown off after the larva enters its host. The third stage larva creep about among the culture medium, increasing slightly in length, but does not feed. In about two days it creeps upwards and leaves the culture medium. Mature larvae may thus be obtained from the sides of the culture bottle or lid of the petri dish.

The larvae have now reached their infestive stage, and such larvae have been found to vary in length from 0·58 mm. to 0·67 mm., the average length being between 0·62 and 0·64 mm.; the body thickness varies from 0·022 to 0·024 mm. The following dimensions, taken from a larva 0·647 mm. long would represent the average dimensions of the different larval parts:—

Total length, including tail sheath	0·647 mm.
Total length, excluding tail sheath	0·533 mm.
Diameter of body posterior of oesophagus	0·022 mm.
Length of oesophagus	0·161 mm.
Middle of 1st bulb to anterior tip of oesophagus	0·072 mm.
Length of 1st oesophageal swelling	0·024 mm.
Diameter of 1st oesophageal swelling	0·0084 mm.
Diameter of oesophagus anterior of 1st swelling	0·005 mm.
Diameter of oesophagus posterior of 1st swelling	0·006 mm.
Length of posterior oesophageal swelling	0·029 mm.
Diameter of posterior oesophageal swelling ...	0·014 mm.
Length of buccal tube	0·0056 mm.
Genital primordium	0·013 by 0·006 mm.
Genital primordium from anterior end	0·277 mm.
Excretory pore from anterior end	0·102 mm.
Nerve ring from anterior end	0·083 mm.
Length tail sheath from anus	0·176 mm.
Length tail	0·062 mm.

There are no distinct lips as seen in the 1st and 2nd stage larvae, but six small circum-oval refringent dots, representing circum-oval papillae, suggest the incipient presence of three lips. The mouth is a small aperture leading directly from the exterior to the larval mouth tube.

In the above account the development has been given of larvae growing under favourable conditions. It was mentioned that where the temperature was lowered the rate of development was retarded

and that the larvae were not prone to hatch. In cultures which were kept below 20° C., development took place but haching only took place after five days. The remarkable fact which was observed was, that although hatching was held back, yet development of the larvae continued and that the larvae which had evenutally hatched from the sixth day onwards had already passed through their 1st stage and were now 2nd stage larvae still encased in the sheath of the 1st stage. Unhatched larvae from the sixth day onwards, when liberated artificially from their shells under the microscope, were also all found to be in the 2nd stage and all still retained the sheath of the 1st stage. When once hatched those 2nd stage larvae rapidly develop and pass into the 3rd stage within 48 hours, when placed in an incubator at 26° C., and such mature larvae were also used with success to bring about infection of the host.

(*e*) Biology of Larvae.

The first stage larvae are very delicate and show very little resistance towards extremes of heat and dessication and they soon die if proper food is not available. It was noted that whereas larvae were active at 30° C. they became sluggish at 35° C. and at 40° C. they coiled up and showed no movements. Such larvae, however, could be revived again when the temperature was lowered. Should the temperature be allowed to go to 45° C. these larvae are killed as seen by the fact that they do not recover when the temperature was lowered. When placed on a slide in water and the water allowed to evaporate off, the larvae do not revive if water is again added after larvae have been dry for ten minutes; they remain shrivelled.

Second stage larvae show the same reactions to drying and heat as do those of the first stage. They can, however, remain alive longer without food; larvae have been kept alive in water at 26° C. for a maximum of 5 days without food. During this period the intestinal cells lose their granules and become hyaline and the larvae themselves become more and more sluggish.

Third stage larvae show reactions quite different to those of the previous two stages. The most striking is their negatively *geotropic* response; they are active climbers and can be collected in great numbers from the sides of the culture vessels; in tall jam bottles they have been found to climb up to the top and in some cases washings of the lid have revealed their presence. Their responses to *light* are also different; whereas those of the previous two stages always remain inside the dark centre of the culture; these creep out towards the light. This is easily seen in cultures which have been kept in the dark for about a week; here the larvae will be found irregularly scattered on the sides of the vessel just above the culture; should the culture now be placed on a table facing but a little distance away from a window the larvae will congregate on the side of the vessel facing the window and will migrate further upwards along this side. If the vessel be now placed against the window facing bright sunlight, the larvae will at first scatter to different parts of the vessel and will eventually creep downwards and seek

shelter in the culture itself. Although the larvae are encased in the protective sheath of the second stage they show very little power to withstand *desiccation*. This is a characteristic it shares with all other hookworm larvae which have been studied. Still, one would not expect such to be the case in a parasite which, as in South Africa, is practically confined to arid regions. Larvae placed in moist earth, and placed in the shade outside to dry, were found to be dead within a week. Those placed on a glass slide with water were found to be dead within an hour after the water had evaporated. The larvae were all shrivelled up and glistening. A mixture of larvae, consisting of wireworms, nodular worms, trichostrongyles, strongyloides and Gaigeria was placed in a shallow watch glass with a little water and the whole placed in a large petri dish containing water and covered up and allowed to stand on the bench overnight. Next morning all the water from the watch glass had evaporated and all the larvae were shrivelled up although they had been in a moist atmosphere overnight. On the addition of water all the larvae, except those of Gaigeria and Strongyloides, began to revive and these became quite active after two hours. Those of Gaigeria and Strongyloides failed to revive even after two days. Their response to *heating* is also typical for hookworms, being actively attracted by the source of mild heat. Should larvae be placed in water on a slide and a heated penny be placed on the slide, it will be noted that the larvae become far more active and eventually line up near the edge of the water facing the source of the heat. If a warmed glass rod be pressed against the slide directly underneath the water, it will be noted that the larvae congregate round this area, and, if the rod be not too warm, the larvae can be made to follow the movements of the glass rod. On a warmed stage the activities of the larvae can also be followed. For this purpose a boxed-in microscope was used, and by warming the atmosphere surrounding the microscope the reaction of the larvae to increases of temperature were noted. It was seen that from 22° C. the activity of the larvae increases until a maximum is reached at 35° to 40° C. The larvae now vigorously lash their way through the water while some were seen to press their heads against the sides of the vessels in what appeared to be an attempt to pierce it. After 5 to 10 minutes at this temperature the atmosphere was further warmed and at 45° C. the larvae began to show jerky movements and some were beginning to coil up; at 50° C. most of the larvae had coiled up, and those which had not only performed very sluggish movements. A further rise of temperature to 60° C. apparently killed all the larvae, as no further movements were seen, neither did any revive on allowing the temperature to drop to those of the room. A decrease in temperature also has a retarding effect on the movements of the larvae; larvae in water, allowed to stand on a cold rock slab overnight in winter, when the room temperature dropped to about 10° C., were found to be far less active than those kept in the incubator at 26° C. When placed in a refrigerator and forzen, all the larvae were killed, none reviving after allowing the ice to melt. It appeared that mere freezing was sufficient to kill the larvae, because larvae which were recovered from the ice very soon after freezing were not able to revive.

Observations on *skin penetration*, using the Goodey baby mouse skin method, were also made. Although repeated attempts were made, with the water at varying temperatures from 35° C. to 40° C., and allowing the larvae to remain on the skin until all the water on the skin had dried off, the writer was only once able to recover larvae from the water on which the cork and skin had floated. In this case 15 active ensheathed larvae were placed in a drop of water on the skin; after 3 hours at 40° C. the drop of water had evaporated, and on flooding the skin seven still ensheathed larvae were recovered. An examination of the water below revealed the presence of five larvae, all of which had lost their sheaths. As there was no possibility for the larvae to have crept over the surface of the skin and so reach the water below, it became obvious that they must have penetrated the intact skin and in doing so had lost their sheaths. A careful examination of the skin revealed no larvae which were in the process of penetrating it.

In all cases where the larvae were placed on the mouse skin it was noted that the larvae became very active, and thrushing their heads against the skin seem to be making attempts to penetrate it. Larvae were also placed on the shoulder and abdomen of live mice after the hair had been cut short. In most cases it was observed that the mice began to scratch these areas after about five minutes. The mice were killed after 12 to 18 hours, but a careful examination of the liver, lungs, intestine and blood from the heart and general circulation failed to reveal any larvae; neither did the skin, where the larvae had been placed, show any larvae when cleared or in sections. From these results the writer concluded that these larvae can only penetrate the skin with difficulty and that they would normally set up an infection through the oral route and not through the skin. Yet, as will be shown later, this is not the case.

(*f*) Infection of the Host.

The view generally held that infection with *Bunostonum trigonocephalum* only takes place through the oral route, coupled with the writer's findings that the infective larvae of *Gaigeria pachyscelis* only penetrate the skin under exceptional circumstances, led the writer to assume that the probable course taken by the larvae of this latter parasite would also be through the mouth. In consequence 18 lambs, varying in age from 3 to 15 months, which had been born and reared on the station where gaigeriasis is absent, were used. Five of these were each given a little water containing about 100 active larvae on the 19th October, 1931, and this procedure was repeated three times at daily intervals on three sheep and eight times on the remaining two, these receiving larvae twice every week. One of these latter died a fortnight after the last dosing, i.e. six weeks after the first exposure, but a careful search of the intestines was entirely negative for young hookworms, neither were any larvae recovered from the blood, liver, lungs and body cavity wash. Weekly examinations of the faeces of all, from the 4th week onwards after the 1st infection, always gave negative results until 3 months after the last infection, when they were discharged. This negative result made the writer conclude that the good condition of the lambs

might be an inhibitory factor, and also that it was not advisable to allow the lambs to swallow the larvae on dosing. Consequently nine lambs in poor condition, also reared on the station, were next used. On the 27th January, 1932, each had about 100 active larvae squirted into the sides of the mouth in a minimum quantity of water; this was repeated seven times at weekly intervals. One died six weeks after the initial infection but a careful search of the organs and digestive tract proved negative. Weekly faeces examinations from the 4th week onwards also showed that all the remaining lambs had not taken up the infection even after 4 months. Unfortunately the two stock sheep, from whose faeces the larvae had been reared, died before another infection could be set up on the station; consequently it was not before the 15th August, 1932, that the writer was able to continue, other infected sheep having in the meantime been procured from the Vryburg Area. On this date four lambs in poor condition were given a mash to which active larvae had been added; this was repeated twice every week until each lamb had received nine lots of larvae. Examinations of the faeces up to three months after their last exposure did not show any Gaigeria eggs to be present and a post mortem examination held on one of the lambs on 17th April, 1933, showed no hookworms in the intestine.

At the same time that these above-mentioned four sheep were being exposed to infection, 9 other lambs in poor condition were taken; these were divided into 3 lots, one of 5 lambs and the remaining of 2 each. The lot of 5 had larvae in water applied to the skin between the hoofs, and this was done twice a week until nine applications had been made. One lot of 2 lambs had active larvae injected under the skin behind the foreleg on four occasions, and the remaining lot of 2 lambs had the larvae injected into the jugular vein on four occasions. Of the 5 lambs which were cutaneously infected one died nine days after the first infection and another 16 days after. In both cases no larval hookworms were found in the intestine, and, due to a misunderstanding, the lungs were destroyed before a careful examination could be made. The remaining three of this lot continued to live, and weekly examinations of their faeces eventually gave positive results for Gaigeria eggs 11, 12 and 17 weeks respectively after the last exposure. Of the two sheep which had been infected subcutaneously, one did not become infected, while the other began to pass hookworm eggs 15 weeks after the last exposure. The two lambs injected intra-jugularly did not contract the infection.

The above experiments show that of five sheep infected cutaneously, a positive result was obtained in all three on which a satisfactory examination was made; the remaining two, which had died, were doubtfully negative as the lungs were not examined for larval stages. This observation showed that infection was probably brought about *via* the cutaneous route, but the possibility that the lambs had licked themselves at the sites of infection and so picked up an oral infection was not excluded.

An attempt was now made to set up a pure infection by infecting four lambs cutaneously. These lambs had been removed from their mothers immediately after birth and placed in a concrete

box which had previously been thoroughly scrubbed. They were hand-reared on cow's milk. Two had active larvae applied round the mouth and two had the larvae applied between the hoofs. During the ensuing five weeks, from 15.12.32 to 16.2.33, each lamb received ten applications of larvae. All the lambs eventually became infected and passed eggs of Gaigeria 11, 12, 17 and 17 weeks after the initial exposure and 2, 3, 8 and 8 weeks after the last exposure respectively. Except for an occasional strongyloides egg, the faeces of all these lambs contained only Gaigeria eggs.

As in previous experiments no infection had resulted after feeding larvae to lambs, and as in the two experiments mentioned above infections had resulted after applying larvae to the skin, the writer was now fairly satisfied that these larvae had entered the host by piercing the skin. However, to exclude any doubt, the hoofs of the sheep in the next experiment were encased in stout canvas bags for at least a day after each application of the larvae, after which the hoofs were thoroughly washed in disinfectant in order to remove any larvae which might still be present and alive. Nine lambs in medium condition were used for this experiment; they had been reared on the station and examinations of their faeces had proved negative for Gaigeria eggs. Each received 17 applications of larvae between the hoofs from the 26th April, 1933 to the 5th June, 1933. Unfortunately seven died, one at eight weeks, four at ten weeks and two at eleven weeks after the initial infection, but on examination of their intestines adolescent to fully grown hookworms were recovered from all. One sheep began to pass Gaigeria eggs 11 weeks after the initial infection and the remaining sheep failed to pass eggs and was killed after 15 weeks; one adult parasite was, however, recovered from its intestine.

Although this experiment proved fairly conclusively that cutaneous infection had taken place, yet there were still sources of doubt, firstly that the larvae might not all have been removed after each washing; and secondly, although stout canvas bags were used, the larvae might have penetrated them; if these two possibilities did occur it was just possible that the lambs might have picked up larvae when biting their hoofs. In order to overcome this objection the larvae were in all future infections placed behind the ears; it was felt that by placing the larvae on these sites any possibility of the larvae reaching the mouth could be ruled out. Only a minimal amount of water containing larvae was used in all cases, just sufficient to wet the wool down to the skin. Five lambs were now used, bred on the station and negative to Gaigeria. Each of these received ten applications of larvae behind their ears from 12.8.33 to 7.9.33. Three began to pass Gaigeria eggs ten weeks after the first infection and seven weeks after the last and two passed eggs eleven weeks after the first and eight weeks after the last infection. All five lambs died or were killed during December, 1933 and January, 1934, and on post-mortem all had adult hookworms in their intestines.

This experiment conclusively showed that infection could be brought about by the larvae penetrating the skin, and also that infection by this route, being comparatively easy, was probably the normal way in which sheep became infected in the field. All subsequent sheep were infected by having larvae placed behind their ears,

and of 51 sheep of different ages and in different conditions, of which full records were kept, 46 contracted the infection and 5 remained negative even after repeated attempts at infection.

Another attempt was made in December, 1934 to bring about an oral infection. Six young lambs were used and each had about 10 c.c. of water containing numerous larvae distributed round the inside of the mouth on each of five occasions from 11.12.34 to 18.12.34. All, however, on post-mortem examinations, carried out 8 to 10 weeks after the initial infection, did not reveal any immature hookworms in the intestine.

In summarising the results thus far obtained we find that 24 sheep which had been given larvae orally failed to develop an infection, whereas of 74 sheep which had had larvae applied to the intact skin, 69 developed an infection and 5 remained negative. The reason why these last remained clean is not clear, but individual predisposition may possibly be responsible.

The next point to be cleared was the length of time required by the parasite to reach maturity after entering the host. Early in 1933, from the results of the above described experiments then done, it became noticeable that the period was about 10 to 11 weeks. To make quite sure, the sheep in the next experiment were exposed to only one massive infection behind the ears. Ten young clean lambs were used and were infected on 11.12.34. Faeces from each were collected regularly every week from the 6th week onwards and examined for Gaigeria eggs. After the 10th week six of the sheep showed a few eggs and after the 11th week the remaining four also began to pass Gaigeria eggs. From the 14th week onwards the number of eggs in the faeces were fairly plentiful in all the samples and it appeared as if the females had now reached their maximum stage of egg production. This experiment amply supported the conclusion to which the writer had already come namely that his hookworm takes just over 10 weeks to attain maturity in its host.

(*g*) Development of Larvae in the Host. (Figs. 7-13.)

For this purpose young lambs reared on the station were used. They were all infected behind the ears and were killed at various periods after exposure. The experiments were carried out simultaneously with, but independent of, those described above, after cutaneous infection had been established. The object of these experiments was to trace the route taken by the larvae from the skin to the intestine, and to follow the different developmental stages. All the blood from the slaughtered lambs was carefully collected and haemolysed immediately before clotting had set in; it was then allowed to stand for about an hour in a tall urine glass, after which the supernatant liquid was carefully drawn off and the last inch of liquid carefully examined under a binocular microscope. Although blood was examined from lambs from 12 hours to 7 days after infection, not once were any larvae detected.

The lungs were also carefully examined by making squash preparations, and by finely chopping up this organ; the finely chopped parts were placed in a tall urine glass in normal saline at about

40° C., and the whole placed in a sink filled with water at the same temperature. The contents of the glass were stirred from time to time with a glass rod; after about 2 hours the contents of the urine glass were passed through a fine meshed sieve, so that all the coarser lung particles were retained; the saline was then allowed to stand in a urine glass and after about an hour the supernatant liquid was carefully decanted off and the sediment examined under the binocular. By using this method the larvae were recovered from the lungs from the 4th to the 14th day after the infection, but squash preparations never revealed the presence of larvae.

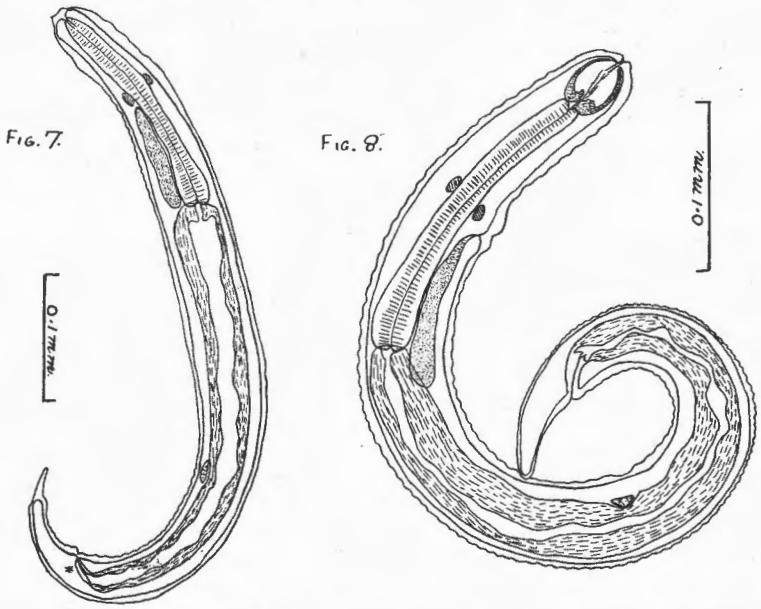


Fig. 7. Third stage larva from lung, 7 days after infection; in lethargus and cuticle separated.
Fig. 8. Ensheathed early fourth stage larva from lung.

Although the 4th day after infection was the earliest date on which larvae were recovered from the lungs, these larvae were not the smallest, thus showing that they had been some time in the lungs and had grown in length. The smallest larva was 0·604 mm. long and was recovered 13 days after infection together with larvae which were already in their 4th stage. This difference in the stages of development may be explained by assuming that some larvae take a longer period to reach the lungs than others, and consequently would not have developed so far as those which had reached the lungs earlier. This small larva appeared to have undergone little or no further development and showed all the characteristics of an exsheathed infective larva. In the lungs the larvae continue to grow and, feeding on the blood, pass into their 4th stage in this organ. The first noticeable character in the further development of the 3rd stage larva is that the body becomes very much thicker, the oesophagus also becomes thicker and loses its central swelling, and the cervical glands become more prominent and their contents become denser. (Fig. 7.) Third stage parasitic larvae recovered

from the lungs from the 4th to the 13th day after infection were found to vary in length from 0·604 mm. to 0·72 mm. The following table gives the principal measurements in mm. of four larvae recovered 8 days after infection:—

	1.	2.	3.	4.
Length	0·65	0·67	0·67	0·7
Maximum breadth	0·058	0·052	0·053	0·054
Breadth, head	0·032	0·032	0·032	0·032
Length, oesophagus	0·179	0·179	0·180	0·182
E.P. from anterior end	0·120	0·122	0·117	0·128
N.R. from anterior end	0·114	0·096	0·096	0·102
G. Prim. from posterior end	0·22	0·225	0·23	0·25
Length, tail	0·074	0·061	0·064	0·064

(*h*) Fourth Stage Larvae.

From the 7th day after infection some of the larvae appear to be preparing themselves to pass into the next or 4th stage: whereas other larvae move and twist in the warm saline, these do not perform any movements. When carefully examined it was noted that the cuticle was becoming separated and a new one was forming underneath; in some, which were more advanced a clear space appears round the base of the larval mouth tube; this enlarges and its outer wall becomes hardened until eventually we have formed the oval provisional buccal capsule of the 4th stage larva (Fig. 8). By this time the old cuticle has become completely separated from the body and at the anterior end it has attached to it the small larval mouth tube together with the cuticular line of the oesophagus. Ecdysis now takes place in the lung and the 4th stage larva continues to feed and grow. Except for the provisional buccal capsule the anatomical characters of the early fourth stage larva are similar to those of the third. The sexes have not yet become differentiated, but the cells of the genital primordium have sub-divided so that there are now a lens-shaped group of about 10 cells. The mouth opening is rounded and simple, and does not show any signs of cutting plates; at first it is anteriorly directed ,but as growth proceeds it eventually points antero-dorsally. The provisional buccal capsule, which causes the head end of the larva to be more rounded, is oval and contains at its base a pointed dorsal tooth whose tip may be slightly tilted dorsalwards, and two small sub-ventral triangular teeth. The writer was not able to make out whether a dorsal oesophageal and cephalic glands were present or not, neither was he able to see any rib-like structures over the bucctl capsule which might represent the head papillae of the adult.

Soon after ecdysis, on the 13th or 14th day after infection, the 4th stage larvae make their way to the intestine. This is probably *via* the trachea, mouth and oesophagus. Repeated scrapings of the inner surface of these organs failed to reveal any 4th stage larvae, except on one occasion when two larvae were recovered from the trachea. This was in a lamb which had been infected 14 days previously, and these larvae were 0·79 and 0·87 mm. long respectively,

showing all the characters of the fourth stage larvae described above from the lungs except that they were slightly larger. As in other ancylostomes and in ascarids the passage from the lungs to the intestine appear to be very rapid, and consequently only exceptional luck would catch them during this migration.

From the 13th day after infection fourth stage larvae may be found in the intestine. The smallest larva, which was recovered on the 13th day, was only 0·721 mm. long with a maximum thickness of 0·054 mm.; it had all the characteristics of the lung form, and was attached to the villi of the small intestine just below the duodenum. The larvae feed by sucking a portion of the villus into its buccal capsule and lacerating it with their teeth causes it to bleed. Growth now proceeds fairly rapidly and during this stage several organs, which were not detected during the lung stage, become visible and the sexes also become differentiated. The first indication of sex differentiation was seen on the 18th day after infection, when the males could be identified in that their tails had become stumpier, whereas those of the females remained elongated and pointed (Figs. 9 and 10). The males were now about 1 mm. long and the females

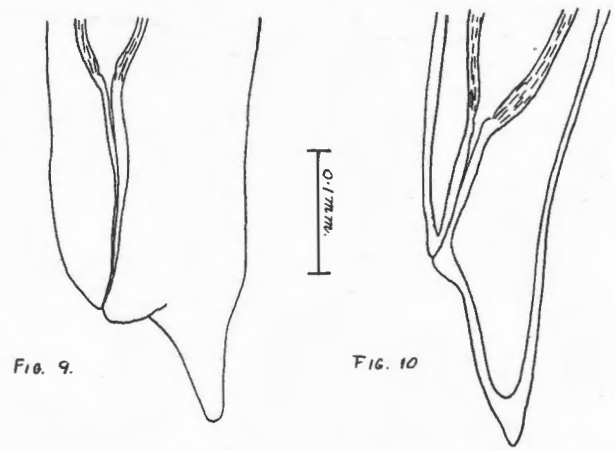
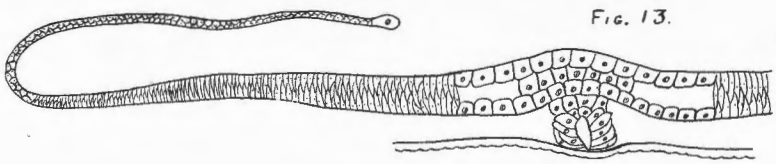


Fig. 9. Outline of posterior extremity of 4th stage male larva.
Fig. 10. Outline of posterior extremity of 4th stage female larva.

just longer. By the 21st day the females had attained a length of 3·56 mm. and the males 3·25 mm. The ribs on the outer surface of the provisional buccal capsule have become quite distinct, three being found on either side, of which the central one representing the lateral head papilla is the more conspicuous. The cephalic glands have also become differentiated, and extend down nearly the whole body length as thin ribbon-like structures; in the oesophagul region they are connected to the tissues of the lateral lines, but further down they appear to lie free in the body cavity. The cervial glands have also increased considerably and have assumed the shape described for the adult; they now extend about half-way down the body. By this time the genital primordium has also undergone considerable development. In the youngest females 1 mm. long it proliferates in

an antero-posterior direction (Fig. 11), so that eventually its middle portion consists of two rows of superimposed cells and its ends of a few cells in a single row (Fig. 12); in addition three cells have appeared between the centre of the middle portion and the cuticle, the central cell being hollow and representing the beginnings of the vagina. As the female grows older the double row of cells of the genital rudiment divide further and become separated from each other, forming a central tube, representing the beginning of the ovejectors (Fig. 13); also the single row of cells at either end have increased considerably in number and may now be recognised as forming two distinct regions; these are firstly the portions joining the primitive ovejectors and extending anteriorly and posteriorly, and secondly the terminal portions which are reflexed dorsally over the first portions. The first portions consist of numerous spindle-shaped cells, which will eventually become separated and give rise to the uteri; the second portions are much thinner and consist of a solid rod composed of hexagonal cells with prominent nuclei; these



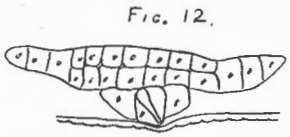


Fig. 11. Genital primordium of early 4th stage larva.
Fig. 12. Early development of vagina and ovejectors in later 4th stage larva.
Fig. 13. Female genitalia in 2.4 m.m. long 4th stage larva, 3 weeks old.

portions are destined to form the ovaries of the adult. The cells surrounding the primitive vagina have also increased in number and have caused the cuticle to bulge outwards; the vagina, however, has not yet acquired openings into the ovejectors and to the exterior. By this time most of these females are from 3 to 3·5 mm. long. In the males the genital primordium has also increased considerably, but most of the growth has been in a posteriorly direction where a single string of cells has thrust its way down to eventually reach the rectum. When the males are about 2·8 mm. long this string of cells is just over 1 mm. long, and anteriorly they already form one or two small twists.

In the following table the principal measurements in mm. are given for four males and four females which had reached the stage of development described above in 21 days after infection.

	Males.				Females.			
	1.	2.	3.	4.	1.	2.	3.	4.
Length.....	2·4	2·53	2·54	3·25	1·85	2·83	3·51	3·56
Breadth.....	0·122	0·122	0·115	0·141	0·096	0·128	0·141	0·154
Buccal capsule.....	0·065 × 0·064	0·07 × 0·069	0·07 × 0·07	0·07 × 0·077	0·064 × 0·054	0·07 × 0·071	0·083 × 0·084	0·077 × 0·077
Oesophagus.....	0·47 × 0·058	0·48 × 0·064	0·45 × 0·064	0·59 × 0·07	0·4 × 0·057	0·55 × 0·061	0·62 × 0·077	0·64 × 0·064
N.R. from front....	0·24	0·25	0·25	0·029	0·22	0·26	0·32	0·31
E.P. from front....	0·25	0·26	0·27	0·3	0·24	0·27	0·34	0·35
Valva from fromt..	—	—	—	—	0·98	1·53	1·83	1·9
Length of tail.....	0·077	0·079	0·085	0·09	0·096	0·122	0·131	0·141

LATE FOURTH AND FIFTH OR ADULT STAGE (FIGS. 14-18).

As the parasite grows older it increases in length and thickness During the fifth week after infection the larva begins to undergo changes preparatory to passing over to the fifth and last stage. By this time the genital tubules have increased considerably in size, and their different parts have become distinctly differentiated. The vagina has, however, not opened to the exterior and into the ovejectors.

The most striking character of the larvae now is its much swollen anterior end, and swollen caudal extremity in the male. This is due to the fact that the adult buccal capsule is now beginning to be formed, and the bursa of the male is developing. During the sixth week the tissues round the base of the provisional buccal capsule disappear and large cavities are formed surrounding the anterior end of the oesophagus (Figs. 14 and 15). These eventually

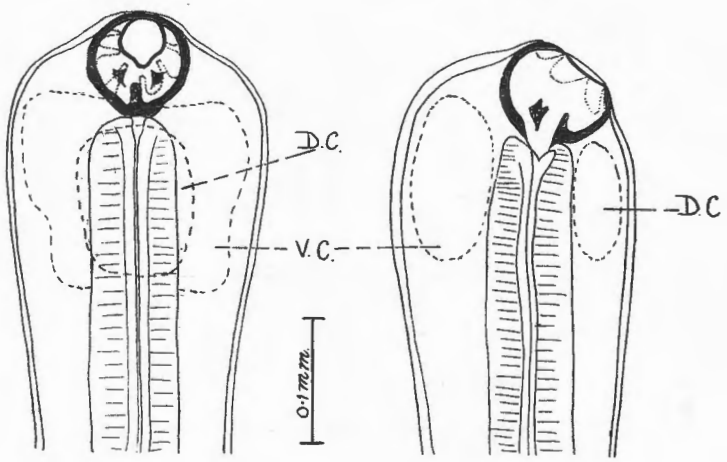


FIG. 14. FIG. 15.

Figs. 14 and 15. Ventral and lateral views respectively of anterior end of mature 4th stage larva showing appearance of dorsal and ventral cavities preparatory to formation of adult buccal capsule. (D.C. = dorsal cavity; V.C. = ventral cavity).

unite and their outer wall becomes strengthened by chitin which forms the beginning of the adult buccal capsule, and as development proceeds this is pushed forwards, away from the front of the oesophagus dragging with it the cuticular lining of the oesophageal lumen (Figs. 16 and 17). By this time a new cuticle is forming and the old one becomes separated off. Just before ecdysis which takes place during the 7th or 8th weeks after infection the small larval buccal capsule may be seen dangling at the anterior end on to one side of the separated cuticle.

The development of the male bursa is more difficult to follow. The posterior extremity becomes much enlarged, and the cells at the posterior ends of the lateral lines multiply rapidly making these ends to appear knobbed. Eventually each splits into a main dorsal and a main ventral branch (Fig. 18). The main dorsal branches

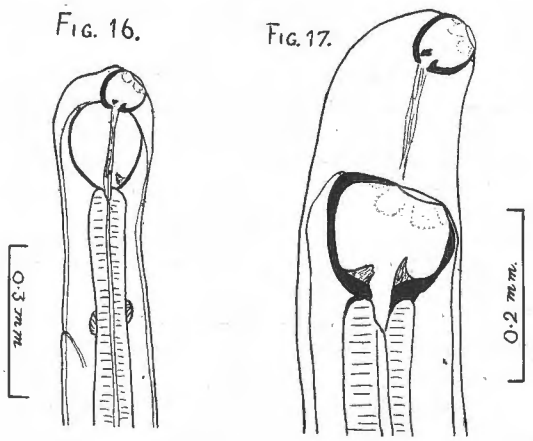


Fig. 16. 4th stage larva showing further development of buccal capsule.

Fig. 17. Early 5th stage larva with adult mouth capsule well formed and provisional buccal capsule separated off.

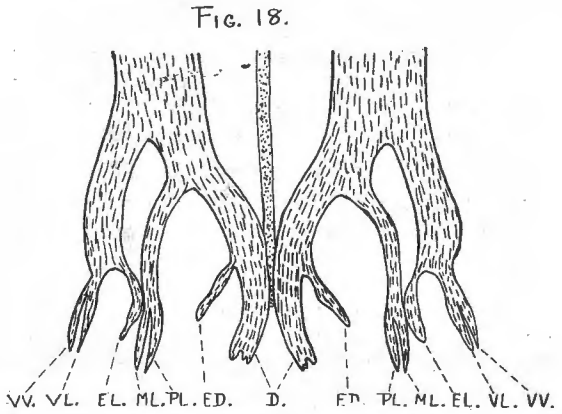


Fig. 18. Sketch showing formation of bursal rays from lateral bands. (D = dorsal ray; E.D. = externo dorsal ray; E.L. = externo-lateral ray; ML = medio-lateral ray; PL. = postero-lateral ray; VL = ventro lateral ray; VV = ventro-ventral ray.)

of the two sides converge towards each other and eventually become fused, the tissues at the posterior end of the dorsal line also being involved. These two fused branches develop and produce the dorsal ray; a ventro-lateral branch from each forms the externo-dorsal rays. The postero-lateral and medio-lateral rays of each side are derived from a secondary branch which arises by a splitting of the

main dorsal branch near its base. The externo-lateral ray is formed from a small dorsal branch arising from the main ventral branch; it curves upwards and backwards and its base eventually fuses with the base of the other two lateral rays; eventually the whole of the main ventral branch become fused to the elongate secondary branch of the main dorsal branch. The ventral rays are formed by the main ventral branch and they take their origin just posterior of the externo-lateral ray.

By the 7th or 8th week after infection the larvae have undergone all the above described developments. The cuticle is now shed and it is now an adolescent organism, all the organs as seen in the adult are now present, except that the genital organs are still immature and the parasite is much smaller. The only organs still to develop are the spicules. At moulting they are not yet recognisable, but about a week later they are just visible as two pale cuticular lines dorso-lateral of the intestine. As the parasite grows older they become darker and firmer and by the tenth week they have acquired their full-grown characteristics. For the rest the parasite simply feeds and grows and becomes sexually mature from the 10th week onwards.

DISCUSSION.

Hatching.

Looss (1911), in discussing the hatching of the larvae of *Ancylostoma duodenale*, says that the embryo takes no part in this process and that the shell bursts of its own accord by the absorption of water, thus liberating the larva, as is easily seen when Schistosoma eggs are placed in water. The writer has not been able to see this spontaneous breaking of the shell in his material, and his impressions, as stated above, are that the embryo, by pushing against the softened and weakened shell, eventually bursts it.

As to the time required for the hatching of the larvae, the writer finds that in his material most of the larvae have hatched by 48 hours when incubated at 26° C. Schwartz (1925), working with *Bunostomum phlebotomum* from cattle, found that it took about 96 hours for the larvae to hatch at 70 to 80° F. (21 to 27° C.). Unfortunately, we have no definite records of the time required by *Bunostomum trigonocephalum* of sheep. Beller (1928) gives the time as one to two days. Hesse (1923) states that 24 hours at 22° C. is sufficient for this parasite, but, as Cameron (1923) has pointed out, Hesse did not make his observations on this parasite, and judging from his figures B and C of the second and third stage larvae, the writer thinks that he was probably dealing with Trichostrongylus larvae, which consequently invalidates Hesse's observations on his supposedly hookworm larvae. Vevers (1921) found that the eggs of *Ancylostoma ceylanicum* (=*A. braziliense*) hatched after 24 hours at 20° C. The eggs of *Ancylostoma duodenale* and *A. caninum* have been found by the writer to hatch after 36 hours at 25° C. Although these times appear to vary for the different hookworms, one cannot interpret them too literally, as, apart from

temperature, other factors such as consistency of the faeces culture, humidity, and aeration were probably different in the respective cultures. A true comparison would only be possible when all the factors necessary for development are identical in the different cultures.

Mature Larvae.

It is a well established fact that in all hookworms, the biology of whose infective larvae has been followed, one of the characteristics of their mature larvae is their ability to penetrate intact skin. The only exceptions appear to be the infective larvae of *Bunostomum phlebotomum* and *B. trigonocephalum* of cattle and sheep respectively, where it is generally accepted, that infection only takes place *per os*. Recent observations, however, tend to show that these hookworms can also set up an infection cutaneously. In experiments, using the Goodey rat skin method, Cameron (1923) was not able to get the larvae of *B. trigonocephalum* to penetrate the skin whereas under similar condition those of *Ancylostoma braziliense* easily did; he, therefore, concludes that they are not skin penetrators, and from this one infers that he maintains that infection is only through the mouth. Bellar (1928) thinks that this parasite can bring about infection either through the skin or through the mouth and he claims to have infected a sheep and a goat cutaneously. He claims, however, to have recovered the eggs of this hookworm 17 days after infection; this claim makes one hesitate before attaching too much weight to the value of his experiments, because it is known that hookworms are slow developers in comparison with other strongyloidea, and the time required to reach maturity has been found to be at least five weeks in those hookworms whose life cycle has been worked out. A time interval of between 2 to 3 weeks suggests that Bellar's animals had been infected prior to experimentation either by hookworms or other nematodes possessing eggs very similar to those of this hookworm, notwithstanding that prior to infection the animals had shown no strongyle eggs in their faeces. Bellar was also able to find larvae in sections of skin excised from a sheep which had received larvae on this particular area 30 minutes previously. The writer, however, has been able to set up a cutaneous infection. One of four merino lambs, which had had infective larvae of *B. trigonocephalum* applied behind their ears, died 2 months after exposure and on post mortem a very heavy hookworm infection was seen; all the hookworms were adolescent adults, no female being seen which had reached the egg-producing stage.*

That the cattle hookworm can possibly also go through the skin is shown by the observations of Reisinger (1916) and Sigetwary (1931). Both observed as a typical symptom of stabled animals

* Since submitting the above for publication the writer has been able to set up an infection in 25 out of 27 sheep by the simple application of mature larvae of *B. trigonocephalum* behind the ears. In addition the writer has recovered 3rd and 4th stage larvae from the lungs 8 to 11 days after infection and 4th stage larvae from the intestine from the 11th day after infection. The egg laying stage was reached in 10 weeks after infection as determined by the appearance of hookworm larvae in faeces culture.

that an itching eczema developed, mostly on the feet, and that the animals were very restless on their feet. This is very suggestive of larvae penetrating the skin, and the writer has also observed a somewhat similar irrigation being produced on sheep which had had Gaigeria larvae applied between the hoofs.

In order to obtain infective larvae the writer always collected larvae from the sides of his culture vessels as in about 8 or 9 days they had crept well out of the culture. Cameron (1923) on the other hand hand was not able to get the larvae of *Bunostonum trigonocephalum* to climb from a semi-fluid culture, although he observed that some larvae showed a tendency to just leave the culture. Later (1927) he found that they do climb, especially when the culture was more solid. In egg cultures of this same parasite the writer had no difficulty in collecting mature larvae from the sides of the vessel, in fact some larvae were collected about four inches above the culture level. These observations would lend support to the view that climbing is a general characteristics of hookworm larvae. Another general characteristic would also appear to be the inability of mature larvae to withstand drying; this characteristic it shares with Strongyloides and Synganus (Ortlepp, 1923), as against mature larvae of Haemonchus (Veglia, 1916) and Triodontophorus (Ortlepp, 1925), whose larvae are very resistant to drying.

Development of Buccal Capsules.

In the development of the fourth stage provisional mouth capsule, the writer's observations tally very closely with those seen by the writer (1923 and 1925) in *Syngamus trachealis* and *Triodontophorus tenuicollis;* they also agree with Looss' (1897) observations on *Ancylostoma duodenale* and Vevers' (1912) observations on *Ancylostoma braziliense.* The way in which the adult mouth capsule is formed is also similar in all these helminths.

Origin of Bursal Rays.

In describing the development of the bursal rays of *Bunostomum trigonocephalum* Cameron (1927) states that the main ventral branch of the lateral bands forms the ventral, externo-lateral and part of the medio-lateral rays. The writers' observations on *Gaigeria pachyscelis* agree very closely with those of Cameron, except that the main ventral branch only forms the ventral and externo-lateral rays, whereas the medio-lateral ray, was, together with the postero-lateral ray, formed by a secondary branch from the main dorsal branch.

Nomenclature.

The status of the genus Gaigeria has been the subject of discussion within recent years by Travassos (1928, 1929), Ihle (1930) and McIntosh (1935). Travassos and McIntosh think that the genera Monodontus, Monodontella and Gaigeria are congeneric, and that all fall in the genus Monodontus (type *M. semicircularis*), the chief distinguishing characters being the large dorsal lobe of the bursa, the smaller lateral bursal lobes, and the asymmetrical arrangement

of the externo-dorsal rays. Ihle, on the other hand, holds that Gaigeria and Monodontus are distinct genera in that, although they are closely related, they differ in the absence of sub-dorsal buccal lancets in Gaigeria, and in this genus the dorsal lobe is also distinctly cut off from the lateral bursal lobes. When opened and flattened out the outline of the bursa in Monodontus is somewhat circular, whereas that of Gaigeria has a distinct shamrockleaf-like appearance of which the lateral leaflets are smaller than the central one. The writer does not agree with McIntosh that these differences are only of minor importance and that they have not a generic value, and he therefore associates himself with Ihle in maintaining that Gaigeria merits generic rank.

Relationships.

The presence of cutting plates at the entrance of the mouth places the genus Gaigeria in the sub-family Bunostominae of the family Ancylostomidae. In this sub-family it appears to be most closely related to Monodontus, from which genus it may be separated, however, by the characters enumerated above, and in addition by its eggs which are quite distinct, being larger than any of those described for other hookworms.

Symptoms, Prophylaxis and Treatment.

As these have already been given by the writer (1934, 1935) in previous publications, it is not the intention to recapitulate them here. Suffice it to add that reports since received from farmers, who have applied the prophylactic measures suggested and the treatment recommended, show that their adoption have been crowned with very beneficial results, some farmers even stating that with these measures sheep farming in gaigeriasis area can now be successfully carried on.

SUMMARY.

1. The hookworm, *Gaigeria pachyscelis*, is a very common parasite of sheep in South Africa; it is also found in the Congo, India and Java. In South Africa it is practically confined to the more arid regions, namely South West Africa, N.-W. Cape and Bechuanaland, where it is one of the most serious parasites affecting the sheep industry.

2. In South Africa the hosts are sheep and goats. It has been reported from cattle in other countries, but this is doubtful; the writer has not found it in cattle, neither was he able to infect calves artificially. Its presence as a natural infection in an Indian antelope is reported.

3. The larvae during their free life pass through three stages, each of which is separated by a moult. The cuticle of the first stage is shed, but that of the second stage is retained by the third stage larva as a protective sheath.

4. Under suitable conditions of aeration, humidity, food and temperature, mature larvae are developed in eight days. These larvae are climbers, skin penetrators, positively photo- and thermotropic, but their resistance to drying is very weak.

5. Infection of the host is shown to be through the skin, and all attempts at bringing about an infection through the mouth have been unsuccessful.

6. After entering the skin, the larvae proceed to the lungs, presumably *via* the blood stream and heart. In the lungs they remain about 14 days during which time the third stage larvae grow, moult and pass into the fourth stage; this larva possesses a globular provisional mouth capsule provided with a dorsal tooth and two subventral lancets. At first the sexes are not differentiated, but later the females may be recognised by their long pointed tails and the males by their short and stumpy tails.

7. The larvae leave the lungs from the 13th day onwards, and travelling up the bronchi and trachea reach the mouth where they are swallowed and so reach the small intestine. Here the larvae attach themselves to the villi and suck blood. The larvae grow and in about a week prepare to undergo another moult and pass into the final or 5th stage. During this transition the provisional buccal capsule is replaced by that of the adult worm and the details of the male caudal bursa also become differentiated.

8. After moulting, the adolescent parasites continue to grow, the sex organs become mature and the worms become fully grown and begin to pass eggs from about the 10th week after infection.

9. The development of the male and female genital organs is followed, as well as that of the male caudal bursa.

10. A comparison is made with related hookworms of the hatching of the egg, the biology of the infective larva, and the development of the mouth capsules.

11. The morphology of the Cephalic and Cervical glands and of the male and female Genitalia is described.

12. The status of the genus Gaigeria is maintained.

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