The Life-histories of *Trichostrongylus instabilis* and *T. rugatus* of Sheep in South Africa.

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I.—Introduction.

The losses arising from infestations of *Haemonchus contortus* and *Oesophagostomum columbianum* in sheep in South Africa have been known for a long time, and elaborate researches have already been made, and are still in progress, for their control and eradication. Infestations of *Trichostrongylus* in sheep have also been known for some time, but these parasites have only in recent years become a serious menace.

The present researches were commenced in the beginning of 1923 with the purpose of elucidating the life-histories and effects of the parasites concerned, and to base on such knowledge methods of prevention and treatment.

In the course of the present researches three species of *Trichostrongylus* have been encountered, viz., *T. extenuatus*, which occurs in the abomasum, and is not very frequent; *T. instabilis* and *T. rugatus*, which occur, usually together, in the first 8-12 ft. of the small intestine, while the former is occasionally also found in the abomasum. These latter two species are responsible for the losses incurred, and form the subject of the present paper.

II.—The Morphology of the Adult Worms.

Trichostrongylus rugatus has recently been described as a new species, and the description is here repeated for comparison with the well-known *T. instabilis* and *T. extenuatus*.

T. rugatus Monnig, 1924.

The worms are small and slender, of a slightly brownish-red colour. The body is gradually attenuated forward in the anterior half; the head is 12μ wide, the mouth opening circular, with three very inconspicuous lips surrounded by small punctiform papillae. The cuticle has fine annular striations, about 3.6μ apart, in the middle of the body. Cervical papillae are absent. The excretory pore lies in a conspicuous ventral depression, 130μ from the anterior end. The nerve ring is situated a short distance behind the level of the excretory pore. There is no clearly differentiated buccal cavity; the oesophagus is long and simple, measuring 900μ in the male and 903μ in the female.

The male is 4.5-6.6 mm. long and about 80μ thick in front of the bursa. The testis is simple and uncoiled. The bursa has large lateral lobes and a very small median lobe, and is supported by six rays in each lateral lobe (fig. 1a). The ventro-ventral ray is slender, and reaches nearly to the edge of the bursa. It is directed ventrad, and is far apart from the latero-ventral ray, which is

directed more laterally and runs together with the lateral ray, and is also much thicker than the ventro-ventral ray. The externo-lateral ray is about as thick as the latero-ventral, but ends abruptly with a small knob-like extremity, and does not quite reach the margin of the bursa. The medio-lateral ray runs close to the externo-lateral, but is thinner, and reaches the edge of the bursa; the tips of these two rays are closer together than those of any of the others. The postero-lateral ray is thin, diverges from the medio-lateral, and also reaches the edge of the bursa. The externo-dorsal ray is thin and short, about half as long as the postero-lateral. The dorsal is relatively long and divided near its end into two branches which each again bifurcate. The spicules are yellowish-brown and conspicuous, differing in size and shape (fig. 2a). The left is 141-152μ long, and has, roughly, the shape of an elongate equilateral triangle with the base anteriorly; the right is 137-145μ long, and has a more rectangular shape. There is an angular projection, slightly bifid, on the ventral surface of each spicule posteriorly, and beyond this each spicule ends in a drawn-out process slightly enlarged at its extremity. This process stands at a smaller angle to the long axis of the spicule in the case of the right than in that of the left. A conspicuous feature of this species is a series of transverse ridges over the ventral surfaces of the spicules, hence also the name " rugatus." Each spicule bears a knob-like process on its anterior end. The gubernaculum is similar in colour to the spicules and measures 86.4μ in length; in shape it resembles roughly a sickle whose anterior part has been bent straight. It is widest in the middle of its length. Prebursal papillae are very small, and can only be seen with difficulty.

The female is 5.8-7.3 mm. long and 79μ wide in the region of the vulva; the latter is placed 1.25 mm. from the tip of the tail, and is a longitudinal slit, 50-55μ long, surrounded by prominent chitinous lips. Sphincters and ovijectors are strongly developed, one lying anteriorly and one posteriorly, their combined lengths averaging about 450μ. The uteri contain usually about twelve eggs each, which measure 80-86μ by 43-46μ, and are laid in the 8-16 celled stage. The ovaries lie in wavy lines, but are not coiled. The body becomes but slightly thinner from the vulva to the anus; behind the latter it narrows suddenly, and ends in a narrow sharp tail, which is sometimes bent slightly dorsalwards (fig. 3). The tail is 55-70μ long.

It is very difficult and usually practically impossible to distinguish the females of *Trichostrongylus rugatus* from those of *T. instabilis* as, with the exception of the differences in size, they are practically alike. The differences in size vary much in each species, and, as only the *average* length of *T. instabilis* females is less than that of *T. rugatus* females, this feature is also of little value for diagnostic purposes. The males can be more easily distinguished. *T. rugatus* can be readily recognized by the ridges on the ventral sides of its spicules. It differs from *T. instabilis* in the greater length of the postero-lateral ray of the bursa, and in the fact that the spicules are dissimilar; from *T. extenuatus* it differs in the shape of the spicules and gubernaculum and in the fact that the postero-lateral ray is more slender, and that its tip is far apart from that of the medio-lateral ray, whereas in *T. extenuatus* the tips of these two rays are relatively close together. *T. rugatus* takes an intermediate position between *T. extenuatus* and *T. instabilis* in a number of points as regards its spicules and also its bursal rays, as can be seen from fig. 1.

In the case of *T. falculatus* Ransom, 1911, there is a closer similarity, and it was considered advisable to make sure that these two species are not identical. Material of *T. rugatus* was accordingly sent to Prof. Ransom, who kindly compared it with his type specimens of *T. falculatus*, and came to the conclusion that "the shape of the spicules as indicated by the drawing of *T. falculatus* is very different in detail from that of *T. rugatus*, including the absence of the ridges, which are conspicuous features of the latter species," while, besides, the spicules of *T. falculatus* are shorter. This material of *T. rugatus* was placed in the United States National Museum Helminthological Collection No. 26112. Comparing Ransom's description and drawing of the bursa of *T. falculatus* * with that of *T. rugatus*, there seems to be very little difference; the two species could, however, be distinguished by the difference in the spicules mentioned above and by the fact that the spicules of *T. rugatus* are not similar in shape and size as in *T. falculatus*.

III.—The Development of the Free-living Larval Stages.

As *T. instabilis* and *T. rugatus* were found separately in sheep only on a few rare occasions, the life-histories of the two species could not be studied separately, and were consequently worked out from mixed infestations. As stated above, there is little or no difference between the females of the two species, and, similarly, their eggs and free larval stages could not be distinguished from each other. The first definite specific difference becomes visible in the parasitic stages when the male spicules and bursal rays develop. From this period onwards the males can be differentiated easily enough—any other differences noted in earlier stages, and also in females of later stages, though they may be specific, could not with certainty be described as such for want of proof.

(*a*) *The Egg.*—The eggs of *T. rugatus* measure 80-86μ by 43-46μ, those of *T. instabilis* 75-80μ by 40-43μ. These measurements were taken from fixed worm specimens obtained from cases where the two species were found separately. In both one finds an occasional egg that surpasses the limits of the given measurements. The eggs are more or less oval, with one pole a little wider than the other and one side slightly flattened.

The eggs are laid normally in the 8-16-celled stage. As noted by Looss and other authors, young females, when they begin to lay, will pass eggs in much earlier stages than normal, and the same happens in older females under irritation, e.g. when they are removed from the host and placed in cold, physiological saline. These facts were frequently observed in the course of the present researches. It was, however, also seen that eggs may be retained longer than normal under the same conditions. Moreover, when females were kept alive in physiological saline in the incubator at 37° C., they would lay all the eggs present in the uteri in a short time, and no more eggs would be formed, although the worms remained alive and fairly vigorous for quite a time.

The eggs are passed by the sheep in the 24-32-celled stage, as was ascertained on several occasions by taking faeces from the rectum of a sheep and examining the eggs present or by examining freshly passed faeces (fig 4).

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* *B. H. Ransom.*—Two New Species of Parasitic Nematodes. Proc. U.S. Nat. Mus., Vol 41 (1911), pp. 363-369.

(*b*) *The formation and hatching of the embryo* does not differ notably from the usual course of development known from other Strongyles, but it is very rapid. In a few cases, female worms taken from a sheep at post-mortem, were placed in physiological saline in the incubator at 37° C., and the eggs laid within definite periods were removed, placed in the same medium or in a filtered infusion of sheep's faeces either in the incubator or kept at room temperature, and their development was followed. These experiments were not as satisfactory as the following method, which was mostly used: Faeces were removed from the rectum of an infested sheep, broken up in a good quantity of water, strained through a set of sieves, and allowed to settle. The supernatant liquid was then poured off, and the fine faecal particles and eggs spread out thinly in petri dishes. It is needless to say that the sheep used as sources of eggs had pure infestations with *Trichostrongylus* only.

In the incubator at 37° C. eggs in the morula stage with about 32 cells, as taken from the rectum of a sheep, may develop into complete embryos within eight hours. At room temperature (18-21° C.) the development is not quite so rapid—after four hours the mass of cells has a more or less rectangular shape, showing in the centre two longitudinal rows of about eight cells each, which probably is the beginning of the intestine. There are now about 50 cells. An hour later there are some 90 cells, and the mass is thicker at one end—the future head end. The embryo now elongates and has to bend double in the egg-shell, the head end becomes much thicker than the rest, and the mouth opening is already discernible—the "tadpole" stage. After about eleven hours the egg contains an apparently completely developed embryo, which is curled up and moves about actively. The larvae, however, do not hatch at once. At room temperature the minimum period observed from the time the eggs were taken from the rectum of a sheep until they hatched was eighteen hours; usually they take about nineteen hours.

(*c*) *The Larva in the First Stage.*—Immediately after hatching, the larvae measure 335-350μ in length and grow rapidly; about three hours later they measure 380μ long by 20μ thick, the oesophagus is 114μ, the intestine 204μ, and the tail 66μ long. At the end of the first stage the larva has reached a length of about 480μ (fig. 5).

The body is more or less cylindrical, tapering more towards the tail end than towards the head, which is fairly blunt. From the anus backwards the body tapers especially rapidly to a thin point. Under a high power of the microscope (immersion) it can be seen that this point is fairly blunt and produced into a thin, cylindrical appendage that ends in a small knob (fig. 5). This appendage is characteristic for the first larval stage, and is very similar to a corresponding structure described by Theiler and Robertson (1916) for the first stage larva of *Ornithostrongylus douglassii*.* The cuticle is thin, and appears smooth with the exception of the two lateral lines which, as has been noted before in other species of Strongyles, present sharp projecting ridges that often follow a wavy line and are difficult to see even under the immersion lens. In the subcuticle, rows of nuclei can further be seen, representing the median

* 1916. Sir Arnold Theiler, K.C.M.G., and W. Robertson: "Investigations into the Life-history of the Wireworm in Ostriches."—3rd and 4th Reports of the Director of Veterinary Research, November, 1915, pp. 293–345.

and lateral lines. The mouth opening is anterior and surrounded by six minute papillae. Lips or other structures are not visible, and the mouth is constantly open in larvae of this stage. The mouth is followed by a cylindrical buccal cavity, 11.8μ deep, lined with chitin, which forms a ring at the posterior margin. The buccal cavity is posteriorly surrounded by the beginning of the oesophagus. The latter has the usual rhabditiform shape, but the three regions are not as sharply marked off as e.g. in the larvae of *Strongyloides papillosus*. In the bulbous part the usual Y-shaped valvular structure is conspicuous. The oesophagus is fairly transparent, and a fine radial striation, as well as a number of nuclei, can be seen in it in the living subject, however, with difficulty. At the end of the oesophagus two pairs of cells can usually be distinguished, which really belong to the intestine and go to form the future intestinal valves. The intestine is composed of eight dorsal and eight ventral cells, the nuclei of which alternate with one another and cause the cells to bulge out into the lumen of the intestine, so that the latter has a wavy appearance when seen from the side. The beginning and the end of the intestinal lumen are much wider than the rest. The cells contain a number of fine granules. They are relatively transparent shortly after hatching, but the granules accumulate subsequently. The rectum is a fine, slit-like canal lined with chitin. Around its origin from the intestine the three cells of the future rectal ligament can be clearly seen. The excretory pore lies ventrally, more or less in the middle between the level of the nerve-ring and the centre of the oesophageal bulb. It is continued inwards by a very narrow canal, which soon bends backwards and becomes lost to view in a group of cells which lies ventrally to the oesophagus.

The nervous system consists of the circum-oesophageal ring and groups of cells, of which some lie anteriorly, but the most posteriorly, to this ring around the oesophagus. There is also a group of cells in the region of the rectum and anus, also extending further back, in which the primordia of the lumbar and anal ganglia would be contained; but these could not be distinguished. The genital primordium lies usually opposite the junction of the fourth and fifth ventral intestinal cells, and is composed of two cells lying side by side, the one a little behind the other. The free abdominal nuclei described by Looss in the larvae of *Ancylostoma duodenale* can be seen also in this species, as well as a number of fine granules lying in the body cavity.

The period of activity and growth which follows upon hatching of the larvae is succeeded by a period of lethargus, during which the larvae become inactive and internal changes take place preparatory to the first ecdysis. There are no great differences between this and the second stage, and consequently no great changes take place. The intestinal cells do not divide as has been described for some other species, but only their shape and the position of their nuclei change. The new cuticle can be seen through the old one in the late stages of lethargus when the latter has been raised off, and it shows the special features that it has in the second stage.

(*d*) *The Larva in the Second Stage.*—The first ecdysis takes place about twenty-five to twenty-eight hours after the hatching of the larvae, with a minimum period of twenty-four hours. The larvae now measure about 500μ, but grow rapidly, and soon attain an average length of 585μ, breadth 22μ, buccal cavity 11.8μ, oesophagus

118μ, intestine 388μ, tail 80μ, and the nerve ring is 84μ from the anterior end. After this they grow more slowly to attain a maximum length of about 600μ.

The head is blunt and the tail tapers out to a fine point. The cuticle has fine transverse striations, and the lateral lines now have a flat projecting edge about 3μ wide, still often wavy in their length. The circumoral papillae are still visible, and the buccal cavity has the same structure as before, but the mouth is, in later stages, not permanently open. The intestinal cells are the same number as before, but the nuclei have shifted away from the lumen and lie against the outer margin of the cells. The shape of the cells has become more sharply angular, and the lines of division between adjacent cells are marked—those between the first four pairs of cells form a typical picture in all specimens: the first line is diagonal, and runs backwards and inwards, the second is transverse, the third diagonal, and running backwards and outwards (fig. 6). The intestinal cells are now much more opaque due to their being filled with greenish-yellow granules. The constriction around the intestine near its end that was described by Looss for larvae of *Ancylostoma duodenale* is more clearly seen in this stage than in the first.

The genital primordium has increased to about 6-8 cells. This period of growth and activity is again followed by a period of lethargus during which morphological changes take place and the old skin is loosened.

(*e*) *The Mature Free Larva.*—At the end of the second lethargic period the larva awakens, but the second ecdysis is not completed by the casting of the skin. The larva has now reached the well-known " mature " or " infective " stage, in which the old skin is retained as a protective sheath. The changes that have taken place during lethargus can be seen from the following description of a mature larva. From the completion of the first ecdysis till the end of the second lethargus about nineteen to twenty hours elapse, with a minimum of eighteen hours. It will therefore be seen that under favourable conditions the minimum period for the development of the larvae from the time the egg is passed by the sheep until the larva has reached the infective stage is sixty hours.

The mature or infective larva (fig. 7) has an initial length of about 600μ, and grows to an average length of about 700μ. The head ends bluntly, and the body does not taper as much anteriorly as posteriorly. The tail ends bluntly with a slight swelling, on which a few irregularly placed projections or tubercles are situated, of which one is usually terminal and a little longer than the others. In this stage the tails of the larvae therefore also bear some resemblance to that of the corresponding stage of *Ornithostrongylus douglassii*. The cuticle presents fine circular striations, and the lateral lines appear similar to those of the second stage. The mouth, which can be opened and closed at will, is still surrounded by six small papillae, although these are now not so easily seen. The buccal cavity as such does not exist any longer, as it has lost its chitinous lining, and the oesophagus has advanced forward, now beginning practically at the mouth opening. The greatest change has occurred in the oesophagus which, through increasing in length, has lost its rhabditiform character, and is not composed of three distinct parts any more. The Y-shaped valvular structure has lost its strong chitinous lining, and is therefore less conspicuous. The elongation of the oesophagus has

brought about a change in its position relative to surrounding structures, so that the excretory pore now lies much nearer to the nerve ring than to the posterior thicker part of the oesophagus. The intestinal cells are still eight pairs, but the typical lines of division anteriorly that were seen in the second stage have changed; the cells do not contain so many granules now and are consequently more transparent. The rectum is still a very narrow canal, and the rectal cells are distinct. The excretory system has changed as concerns the group of cells into which the canal runs; they are now a more clearly circumscribed group. The cervical glands are two cells lying one behind the other in the body cavity between the end of the oesophagus and the genital primordium. They are spindle-shaped, and have long necks directed forward, which could, however, not be followed farther forward than the end of the oesophagus.

The nerve ganglia and some nerves could be readily made out in specimens stained with carmine. Laterally behind the nerve ring are on each side two ganglia—the ganglion cephalicum laterale, and behind it the smaller gangl. ceph. postlaterale; ventrally behind the group of excretory cells is the gangl. ceph. ventrale, and dorsally is a small gangl. ceph. dorsale. Anteriorly to the nerve ring there are six nerve-strands, the papillary nerves, two lateral and four submedian, which can be readily seen in stained specimens, as they each contain a row of nuclei, which are oval, and lie closely together at first, becoming more spindle-shaped and farther apart anteriorly. Of the longitudinal lines, especially the ventral one is conspicuous through its nuclei, an aggregation of which in the anal region forms the anal ganglion; similarly there is a lumbar ganglion in each lateral line. The genital primordium is composed of 12-16 cells, and just in front of it lies a single nucleus with a small amount of faintly staining cytoplasm—a cell as already described by Looss and others. There are besides, other " abdominal cells " and a pair is specially conspicuous lying laterally to the beginning of the intestine.

Numerous specimens of mature larvae were examined alive, killed over the flame, or fixed and stained in carmine in order to detect any possible differences between the larvae of the two species. The cultures from which these larvae were taken usually contained about 60 per cent. *T. instabilis* and 40 per cent. *T. rugatus*, as was shown by the number of males of the two species that developed in sheep drenched with larvae from similar cultures. No differences could, however, be detected excepting differences in size. It was noticed that, though the larvae all measured between 680μ and 730μ in length, a few were regularly found, comprising about 5 per cent., which measured 750μ. There were no morphological differences discernible between these large specimens and the rest, with the exception that their intestinal cells regularly presented a darker appearance, due to the greater number of food particles assembled in them. The lengths of the larvae given above represent the total length of the larva with its sheath. These lengths vary much more than do the lengths of the larvae alone, which approximate 683μ.

IV.—The Development of the Worms in Persian Lambs.

(*a*) *The Second Ecdysis and the Larvae in the Third Stage.*—The act of casting the skin of the second ecdysis has not been observed, as it happens under natural conditions, but it probably takes place in the stomach soon after the larvae have been swallowed by the sheep.

Whether the skin is cast already in the first three stomachs is uncertain; an experiment in which mature larvae were placed in fresh filtrate of the ruminal contents of a sheep and kept at 37° C. gave no result. Mature larvae when placed in a weak solution of hydrochloric acid become very active, especially when kept warm, lashing about vigorously with their tails. They curl around one another—apparently to get some resistance—and cast-off skins can then be seen floating away from these clusters. It appears, therefore, as if the ecdysis would take place under the action of the hydrochloric acid in the abomasum. The rate at which larvae pass through the stomach of a sheep may be seen from the following figures, which were obtained with larvae of *Oesophagostomum columbianum*. The rate will probably be the same for both species:—

	5 hrs. (9 a.m.–2 p.m.).	8 hrs. (6 a.m.–2 p.m.).	12 hrs. (9 p.m.–9 a.m.).
Rumen	70 %	8 %	Rare
Reticulum	2 %	0.2 %	Nil
Omasum	6 %	4 %	0.6 %
Abomasum	16 %	13 %	9 %
Intestine	(6 %)	(74.8 %)	(90.4 %)

A known number of larvae were in each case given to the sheep in moist bran, and the numbers found in the various parts after killing the sheep were expressed as percentages of the number given. The number not found in the stomachs were supposed to be in the intestine, as in this organ the larvae would soon penetrate into the wall, and could, therefore, not be counted.

In studying the development of the parasitic stages, only Persian lambs about one year old were used, since it would appear from observations in the field that these suffer most from the parasites concerned. Mature larvae in known numbers were drenched to the lambs, and the latter killed and examined after various periods as required.

In order to ascertain whether the larvae of these two species of *Trichostrongylus* migrate out of the intestine into other parts of the body during their development, as is known to happen in the case of certain other nematodes, samples of blood were taken from the portal vein, posterior vena cava, pulmonary artery, and left auricle of freshly killed lambs twenty-four hours, forty-eight hours, and four days after infection, as well as scrapings from the trachea and oesophagus and pieces of lung and liver. All these specimens were carefully examined, but proved negative, and this may therefore be taken as one proof that these larvae do not migrate via the blood-stream. Another proof may be found in the fact that the expected number of larvae were always found in the intestine. During earlier experiments, it had been observed that only about 10 per cent. of *Trichostrongylus* larvae administered to a lamb in a drench are successful in establishing themselves in the host. The same number of 50,000 larvae was therefore given to each lamb in the present series of experiments, so as to be able to note the percentage that develops and if there should be differences in susceptibility amongst the lambs used. The latter, it may be stated, was not noticed to be the case during the time these experiments were carried out in summer, except in one case, four days after infection, when the

number of larvae found was relatively low. However, in two other cases, four days after infection, the expected number was found.

Twenty-four hours after infection the larvae are numerous in the first 6-8 ft. of the small intestine, and have cast the skin of the second ecdysis. The new cuticle is definitely ringed at intervals of 2.4μ. The larvae have an average length of 683μ by 19.5μ broad; they have therefore not grown in the host to an appreciable extent yet. The lateral lines have the same appearance externally as in the preceding two stages. The tails correspond to what was seen in the mature larvae, and they have a length of 58μ. At a distance of 35μ from the tail end a pair of lateral caudal papillae can be seen distinctly. The oesophagus is typically filariform, and the intestine is still composed of the original eight pairs of cells.

Forty-eight hours after infection the larvae have reached a length of 710μ by 21.6μ broad. The external prominences of the lateral lines are 4.5μ wide. The nuclei of the intestinal cells have now shifted towards the lumen again, and some of the cells have divided, especially the anterior and the posterior ones, to form in all 12-14 pairs. The genital primordium has grown to a length of 16μ-19μ. The cervical glands are two large, clear, spindle-shaped cells lying just anteriorly to the genital primordium, being especially thick, and displacing the intestine half-way between its beginning and the level of the genital primordium.

Three days after infection the larvae show differences that allow the males and females to be distinguished from one another. The males have an average length of 865μ, and their hind ends are somewhat thickened. The females measure 942μ, and have the usual tapering ends. The average thickness of both sexes is 24.5μ.

Four days after infection the larvae are in the third lethargus, and a few have already passed the third ecdysis. They can now easily be separated into males and females; the latter have a tapering tail, while in the males the hind end of the body around the anus is swollen. This swollen part narrows posteriorly rather suddenly, and ends in a narrow tail, which now stands at a small angle to the long axis of the body. This swelling is produced by the proliferation of certain groups of cells lying around the rectum that will later form the spicular apparatus and the bursal rays. The cervical glands lie just behind the end of the oesophagus, reaching back to the genital primordium and ending bluntly. Anteriorly the necks of the cells can be followed to near the excretory pore, as they contain a number of fine granules that move up and down in them. The excretory pore lies in a conspicuous depression, and the canal runs inwards, becoming lost to view in the group of excretory cells. The necks of the cervical glands seem to end blindly, one on each side of this group of cells; their anterior ends are sometimes distended with granules. The nerve ganglia can be seen fairly distinctly under pressure. From the lumbar ganglia there run two fine nerve strands to the caudal papillae. The abdominal cells can also still be seen as before. The intestine consists of about twenty-eight pairs of cells. In the third lethargus the males measure 952μ by 27.5μ on the average and the females 971μ by 27.5μ.

(*b*) *The Larvae in the Fourth Stage.*—About four and a half days after infection the majority of the larvae have passed the third ecdysis. They now grow very rapidly, so that those that have just passed the ecdysis are considerably shorter than others that have

developed more rapidly. If one measures a large number of males or females and plots these measurements on a curve, a simple curve does not result, but one with a double apex, showing that there are two different strains; but the measurements grade into one another in such a way that the two strains—probably the two species—could not be differentiated in this way. The males have an average length of 1,346μ by 27.5μ and the females measure 1,635μ by 27.6μ. The genital primordium has grown considerably in both sexes. In the females it is 314μ long and its posterior end is 216μ from the tip of the tail. It is an elongate strand of cells, in which the nuclei are arranged in a single row towards the ends and in pairs in the middle [fig. 8 (*a*)], the positions of the nuclei in this central portion already indicating the future ovijectors and the vagina. In the male the genital primordium is composed of three distinct parts: anteriorly the future testis appears as an oval group of cells 19μ long; it is followed by the primordium of the vesicula seminalis, which is 51μ long, granular, and somewhat transparent, and contains only three nuclei; posteriorly to this lies the future ejaculatory duct, a double row of cells with a single cell at each end, measuring 90μ in length—its posterior end is 290μ from the tip of the tail [fig. 8 (*b*)].

Six days after infection the larvae have grown considerably, and especially the genital organs have developed rapidly. The excretory pore has prominent lips, and the cuticular striations are 2.4μ apart. The females are 2.59 mm. long by 43μ thick. Their genital primordium has undergone differentiation, so that the various parts can be recognized. The vagina is composed of a solid group of cells bulging out the body wall. The ovijectors have developed lumina, and are each composed of five pairs of cells and a single one lying next to the vaginal group. The uteri and ovaries are still solid and represented by two long strings of cells, of which the anterior is usually longer than the posterior [fig. 9 (*a*)]. The various parts measure 57μ for each ovijector and an average of 183μ for the uteri and ovaries. The posterior end of the genital primordium is 153μ from the tip of the tail, the future vulva about 400μ, and the tail itself is 90μ long. The males have reached a length of 2 mm. by 35.3μ thick, the anal region is still more swollen, and the narrow tail end stands away from the body at an angle of forty-five degrees to the long axis; it is 35μ long, and the distance from its tip to the anus in a straight line measures 66μ [fig. 9 (*b*)]. The genital primordium has grown backwards to join a group of cells ventrally to the rectum; its three portions have still much the same appearance.

Eight days after infection the larvae are in the fourth lethargus. The females are 2.8 mm. long and the males 2.5 mm. In the females the uteri have now also developed their lumina, which are lined by flat cells; the receptaculum seminis is distinct, and the ovaries have grown so that they are slightly coiled. The vagina has also developed its lumen, and this has joined up with the lumina of the ovijectors. In most of the males the bursal rays can be seen distinctly standing out from the posterior end of the body; the spicules and gubernaculum are also distinct, although not complete in detail (figs. 10 and 11). The old cuticle has separated off from the body around the hind end in the males, and its narrow tail region is still persistent. The old cuticular lining of the rectum can be seen extending from the old cuticle to the anus of the worm inside it. The specific differences between the males of *T. instabilis* and *T. rugatus* now begin to appear

in the spicules and the bursal rays; the latter species seems to be a little behind the former in development, although the difference is not pronounced.

(*c*) *The Adult Stage.*—About 10½ days after infection the majority of the larvae have passed the fourth ecdysis. The males of *T. rugatus* are not quite as far advanced as those of *T. instabilis*. The sizes of the worms vary very much. Females still in lethargus measure 2.9-4.4 m.m., those in the adult stage already up to 5.6 mm.; males in the fourth stage measure 2.9 mm. and in the adult stage up to 3 mm. for *T. rugatus* and 3.8 mm. for *T. instabilis*. The worms now grow steadily, but no marked morphological changes take place as all the internal organs are practically complete. Twelve days after infection eggs can be seen forming in the ovaries of a few females. Two days later an occasional one has an egg in the uterus, and after sixteen days quite a few females were seen with up to four eggs in the uteri. These eggs all consisted of a single cell each, and lay distributed through the whole length of the uterus at short distances from one another; not closely packed together as one sees them in mature worms. There are some females with shorter tails than the others and without eggs in the uteri. These may be females of *T. rugatus*, seeing that the males of this species are also slower in their development than those of *T. instabilis*. The worms are still quite transparent, while in the males the spicules are gradually becoming yellowish, and the posterior region of the body is developing a reddish tint. About the eighteenth day the vesiculae seminales and ejaculatory ducts of the males, as well as the receptacula seminis of the females, become more opaque, while the spicules are light brown in colour; this probably indicates that copulation has taken place. A number of females have eggs in the uteri and ovijectors, all one-celled, and probably these first few eggs are not fertilized. They are readily laid under irritation. On the twentieth day many of the worms have reached the size of full-grown specimens. The females now contain more eggs in the uteri, some of which are developing. On the twenty-first day some females lay eggs in the 2-4-celled stage, but the majority do not start laying before twenty-two days after infection. That this also happens naturally in the sheep cannot, however, be inferred from what is seen *in vitro*, because, as already mentioned, females will lay eggs readily under irritating conditions. Egg-counts in the faeces of infected lambs showed clearly that eggs are normally beginning to be passed by the lamb on the twenty-fifth day after infection.

Conclusions.—The development of *T. instabilis* and *T. rugatus* therefore follows the same general lines as are known from other Strongyles; there is a free-living larval stage divided into three periods by two ecdyses and a parasitic stage in which also two ecdyses take place before the worms become adult. The free-living stages can develop to completion in a minimum of sixty hours, and the parasitic stages take about twenty-five days until their eggs are passed by the host. The life-history is direct and simple, and the larvae do not migrate out of the intestine, although, as will be described later there is a remnant of a migratory process to be seen soon after the larvae enter the intestine, when they enter its wall in order to pass through the third ecdysis there.

V.—The Development of the Worms in other Sheep.

The above experiments on the development of *T. instabilis* and *T. rugatus* were carried out on what seemed to be the most natural host and during that time of the year (January-March) when the sheep would under natural conditions acquire the infestation in the field. It has been noticed previously, also with other worms, that adult sheep do not seem to be as susceptible as lambs, and that the susceptibility of sheep seems to vary at different seasons. Moreover, in the case of *Trichostrongyles*, it had appeared from observations in the field as if Persian sheep were more susceptible than Merinos, although chiefly Persian sheep are bred in the infested areas. A series of experiments was therefore planned to elucidate these questions. We shall first consider the development of the worms in different classes of sheep during the summer months.

(*a*) *The Development in Merino Lambs.*—The method of experimentation, it may be stated, was exactly the same in this series of tests as in the previous series with Persian lambs. Merino lamb 8441 was killed four days after drenching with 50,000 larvae. The anterior portion of the small intestine contains numerous larvae, of which a fair proportion have already passed the third ecdysis. There is no apparent difference between these larvae and those obtained from Persian lambs the same period after infection, except that their development seems to have progressed even more rapidly. The males of the fourth stage have an average length of 1,540μ and the females 1,826μ, which exceeds the lengths previously found for larvae from Persian lambs by about 200μ in both cases.

Merino lamb 8442 was killed 10¼ days after infection. The anterior portion of the small intestine contained numerous larvae, of which the majority had passed the fourth ecdysis, the others being in the third lethargus. There is in this case practically no difference between these worms and those of the same age from Persian lambs, unless the present lengths may, on the average, be slightly shorter. A third lamb had been drenched with larvae at the same time as the above two, and the eggs in samples of its faeces were counted daily from the twenty-first day after infection It was found that also in this case there was no notable difference from the Persian lambs. This lamb was kept alive, and it passed large numbers of eggs for several months while it lost in condition, showing symptoms of heavy worm infestation. Several other Merino lambs were also infected successfully in later experiments. It therefore seems reasonable to conclude that these two species of *Trichostrongylus* will develop equally well in Merino and Persian lambs.

(*b*) *The Development in Older Merino Sheep.*—Merino sheep 7275, 2½ years old, was killed four days after infection. There were no larvae in the abomasum. The intestine contains only a small number of larvae, which are in the third stage, and measure on the average 710μ. There is no sexual differentiation to be seen. These larvae correspond with those found two days after infection in Persian lambs, but are very much fewer in number.

Merino sheep 7359, full mouth, killed eight days after infection No larvae were found in the stomach and intestine.

Merino sheep 7363, full mouth, killed ten days after infection. Altogether fifteen young worms were found, all females, measuring 3.8 mm. average length. They were quite inactive, some apparently

dead. All were in a very poor condition. Their bodies were of a glassy transparency, containing a number of fine granules. Each worm contained a number of eggs already at this age. They were irregularly distributed in the uteri and ovijectors, while some seemed to have broken through the uterine wall, and were lying in the body cavity bulging out the body wall. The worms appeared to be still in the fourth stage.

This experiment seemed to indicate that these worms would not develop in Merino sheep of over 2½ years. Another experiment was made in which two Persian lambs and two adult Merinos were simultaneously drenched with 100,000 larvae each from the same cultures.

One Persian lamb and one adult Merino were killed three days after infection. In the lamb were found very numerous active larvae, amongst which the males and females could be distinguished measuring 865μ and 942μ respectively. In the Merino only about one-tenth as many larvae were found. These measured on the average 673μ, and no sexual differentiation was visible.

The second Persian lamb and adult Merino were killed nine days after infection. In the lamb very numerous larvae were found in the fourth lethargus. In the Merino a few (4) larvae were found after a prolonged search; they looked exactly like those described above from sheep 7363. Another two adult Merinos were infected and observed, but they never passed *Trichostrongylus* eggs in their faeces. From these experiments one may reasonably draw the conclusion that these species of *Trichostrongylus* do not develop in adult Merino sheep. Other ages of Merino sheep were then tried, and it was found that in 2-tooth sheep (about eighteen months old) the worms still develop normally. In 4-tooth sheep (about two years old) four days after infection a moderate number of larvae were found of normal size and appearance, while fourteen days after infection the number was rather small, and the worms seemed to have just passed the fourth ecdysis, which normally takes place after about 10½ days. It therefore appears as if these species of *Trichostrongylus* will infect Merino sheep up to the age of two years, but that above that age infection is improbable.

(*c*) *The Development in Older Persian Sheep.*—Experiments similar to the above with Merinos were carried out with Persian sheep, with the following results:—

Two-tooth Persians (fifteen to eighteen months old) were just as easily infected as lambs.

Four-tooth Persians, four days after infection, showed a fair number of larvae of normal appearance. Ten days after infection a smaller number was found, but they still appeared normal, and were just about to pass the fourth ecdysis.

Six-tooth Persians, four and ten days after infection, showed rather few larvae of smaller dimensions than normal, and not as near to the fourth ecdysis as in the younger sheep.

Full-mouth Persian sheep, four days after infection, showed very rare larvae, about equal in size to those found in lambs two days after infection. After ten days no larvae were found, although one sheep harboured a small number of adult *Trichostrongyles*.

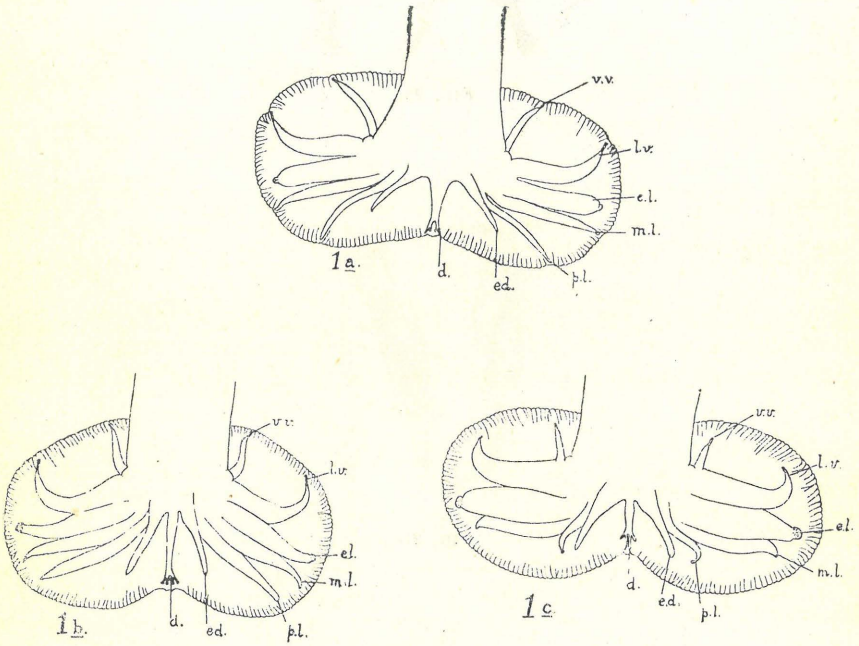
It would therefore appear that these species of *Trichostrongylus* will infect with certainty Persian sheep up to four-tooth, possibly also six-tooth, but not full-mouth sheep, although the latter may still harbour adult worms.

Experiments in connexion with the following points are in progress, and have been partly completed:—

(*a*) Bionomics of the Free-Living Larval Stages.
(*b*) The Development of the Worms in Sheep at different Seasons.
(*c*) The Effects of the Parasites on their Hosts.
(*d*) The Prevention of Infection.
(*e*) The Treatment of Infested Sheep.

EXPLANATION OF FIGURES.

Fig. 1.—(*a*) Male bursa of *Trichostrongylus rugatus*, (*b*) male bursa of *T. extenuatus*, (*c*) male bursa of *T. instabilis*.
Fig. 2.—Spicules and gubernaculum of (*a*) *Trichostrongylus rugatus*, (*b*) *T. extenuatus*, (*c*) *T. instabilis*.
Fig. 3.—*T. rugatus*. Hind end of female.
Fig. 4.—*T. instabilis* and *rugatus* Egg in morula stage as passed in faeces of sheep.
Fig. 5.—*T. instabilis* and *rugatus*. Larva in the first stage.
Fig. 6.—*T. instabilis* and *rugatus*. Larva in the second stage.
Fig. 7.—*T. instabilis* and *rugatus*. Larva in the infective stage (the two cells lying laterally to the beginning of the intestine have been represented as if lying dorsally and ventrally).
Fig. 8.—*T. instabilis* and *rugatus*. Hind end of (*a*) female and (*b*) male just after the third ecdysis.
Fig. 9.—*T. instabilis* and *rugatus*. (*a*) Genital organs of female, (*b*) genital organs and hind end of male six days after infection.
Fig. 10.—*T. instabilis*. Hind end of male ten days after infection and just before the fourth ecdysis.
Fig. 11.—*T. rugatus*. Similar to Fig. 10.



FIGS. 1a, 1b, 1c

Life-histories of Trichostrongylus. | *H. O. Mönnig*

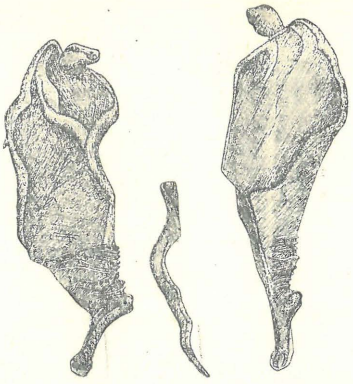


FIG. 2a.

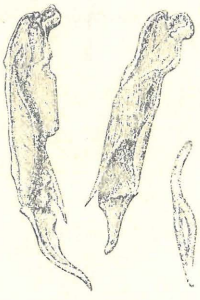


FIG. 2b.

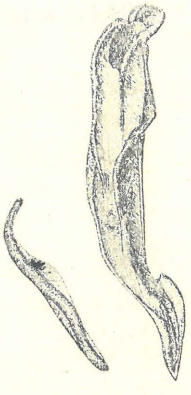


FIG. 2c.

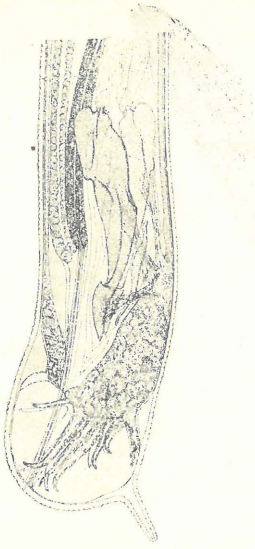


FIG. 10.

FIG 1

FIG. 3.

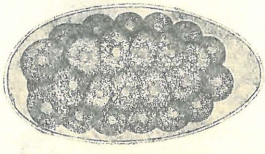


FIG. 4.

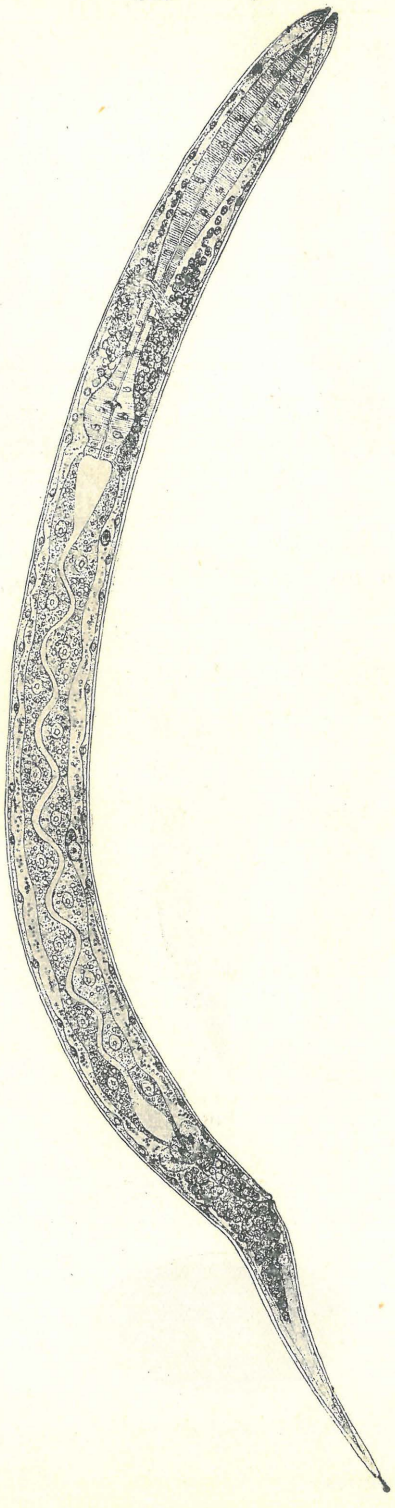


FIG. 5.

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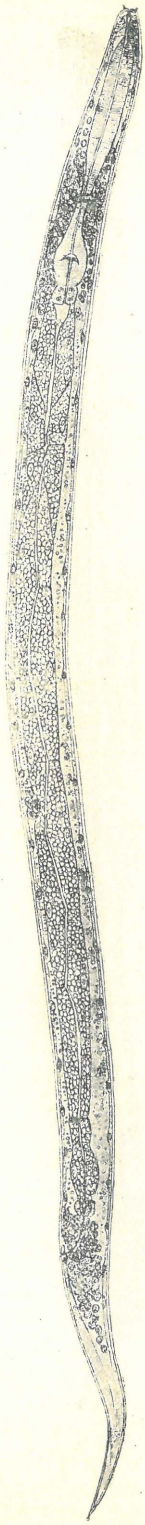

FIG. 6.

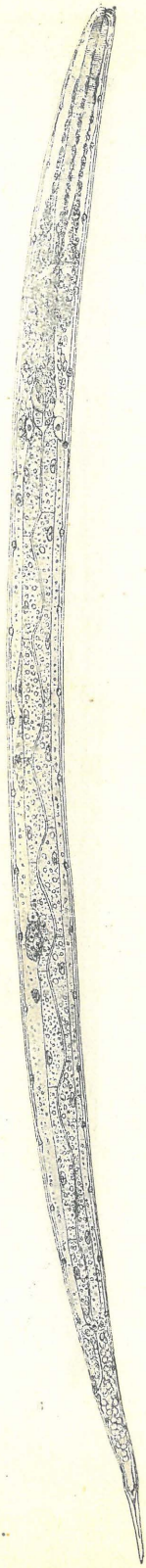

FIG. 7.

FIG. 8.

Life-histories of Trichostrongylus. [*H. O. Mönnig.*

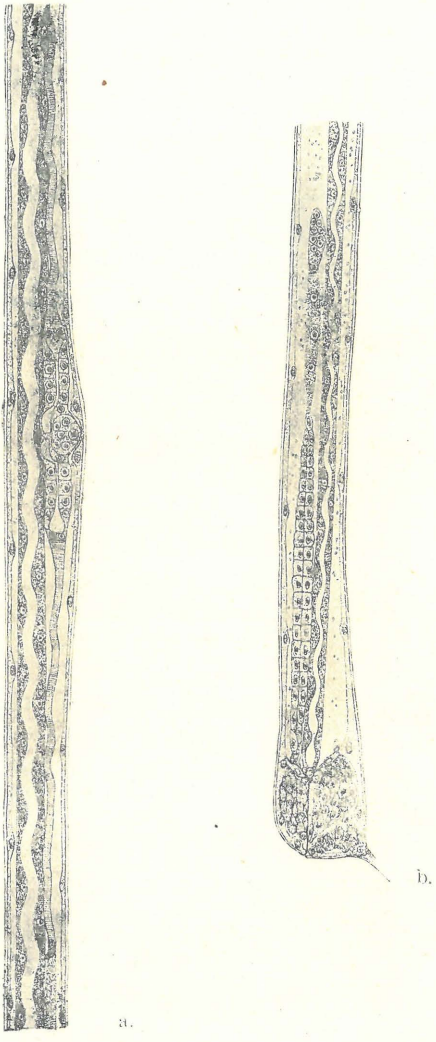

FIG. 9.