The Anatomy and Life-History of the Haemonchus Contortas *(Rud).*

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INTRODUCTION.

THE experimental work forming the subject of this paper was commenced in 1911 at the instigation of Dr. (now Sir Arnold) Theiler, Director of Veterinary Research, with the object of obtaining accurate data on which a scientific prophylactic treatment could be based.

In the first instance, the anatomy, morphology, and cycle of development of *Haemonchus contortus* was studied, and experiments are now being undertaken from the point of view of medicinal treatment.

FREE LIFE.

THE EGG.

The methods of examination applied were as follows—

1. Examination of fresh faeces, placed directly under the microscope.

2. Sedimentation method, in which the faeces are emulsionized with water, allowed to precipitate, and the sediment collected.

3. The sieve method, which is an improvement on method No. 2, consisting in using a sieve of suitable mesh, whereby the coarser particles are retained, and the eggs found in the sediment.

4. The centrifugalizing method, which is another variation of the two last-mentioned methods, consisting in centrifugalizing the sediment whereby the eggs (being heavier than the particles of faeces) remain at the bottom of the tube, and can easily be collected by means of a pipette.

5. The comminution method, consisting in a combination of the above methods suggested by Mr. Maurice Hall. The method can be described briefly in his own words. " . . . After having been broken up, the faeces are poured through a set of six brass sieves. The sieves have a mesh aperture ranging from 3 mm. in the largest to about one-fourth of a millimetre in the smallest. . . . The sieves are nested in the order of mesh aperture, with the coarsest on top, and placed in a large porcelain evaporating dish or in a large crystallization dish. The faeces are poured into the top screen, and pass through the screens to the evaporating dish, particles of different sizes being held by different screens. . . . Tap water or normal salt solution is poured in the upper sieve until the water stands in the evaporating dish at a level above that of the bottom of the upper sieve. This sieve is lifted and shaken a little until the fine matter has passed through. It is then lifted out and put in a large crystallization dish half full of water or salt solution and the matter it contains examined on the screen or washed into the dish. . . . The sediment left in the evaporating dish after removing the finest sieve is poured on to a screen of Miller's silk bolting cloth with a mesh aperture of 0-117 to 0-134 mm., and the finer particles washed through into a tall jar." The sediment is then centrifugalized, and in this way the eggs are obtained in a concentrated residue of faeces which can be poured on a microscopical slide for examination. The material thus obtained is also suitable for preservation after having been fixed by one of the usual methods.

A number of other methods have been suggested by Bass, Garrison, Wellman, and Teleman, in which chemicals are used to dissolve the soluble constituents of the faeces, but these methods are not to be recommended, as the chemicals may be injurious to the eggs, more especially if the eggs are to be used for purposes of cultivation. I tried practically all the above methods, but abandoned them in favour of another one which consisted in (1) breaking up all the faeces by means of a spoon and diluting with enough water to bring the material to a semi-liquid consistency, (2) passing through a brass sieve with a mesh aperature of ·5 mm. to 1 mm.; (3) sedimenting in a jar, removing the supernatant liquor, and washing with water by three successive sedimentations;

(4) centrifugalizing the sediment and collecting the eggs with a pipette. The sediment was found to be particularly suitable for cultural experiments..

For working with one particular worm, collection of material in the way indicated is only of value if the animal contains a pure infection of that species, but under natural conditions this is most frequently not the case, and then recourse has to be taken to the study of eggs freshly obtained from females. For this purpose, live females were obtained, from an animal only recently dead, placed in tepid water, and kept in an incubator. The worms were from time to time transferred to new Petri dishes where they subsequently laid eggs; in the case of *Oesophagostomum columbianum* was found possible to keep the worms alive for twelve to twenty hours. The eggs thus obtained can be used for the purpose of cultivation or for microscopical examination. The method of Looss, which can be summarized as follows, was utilized for the preservation of eggs :—70 per cent, alcohol at a temperature of 60° C. is poured over the egg. The following day the alcohol is changed and replaced by 80 per cent, and later 90 per cent. In order to make the eggs transparent they are removed from the 0 per cent, alcohol to 70 per cent, alcohol plus 5 per cent, glycerine. The alcoholis then allowed to evaporate either at normal temperature or at 6° C. Some more liquid is added and allowed to evaporate until the eggs, re finally in pure glycerine. Eggs can be preserved in this medium.

Morphology of the Egg.

An egg of *Haemonchus contortus* is oval, with one side frequently more curved than the other, the poles being unequal, one being usually less, convex than the other. The average size is 70 - 9 µ x 45 -9µ; a common minimum size is 6 6 - 5 µ x 43 -3 µ and a frequent maximum is 79µ, X 46-6µ..

In fresh droppings the eggs have a transparent shell slightly yellowish in colour with a thickness of about 1 micron and sometimes even less. The yolk of the egg apparently occupies the whole of the shell, but on both poles there are frequently empty spaces noted, 3 to 4 micron diameter. In some cases a polar body is detected in one or both of these spaces. The yolk is surrounded by a hyaline substance. The yolk is segmented, and in faeces freshly examined 24-26 segments or blastomeres can be; counted. These blastomeres are homogenous, measure 12 microns in diameter, are granular in appearance with nuclei appearing as a faint spot in the centre of each blastomere. When an egg is examined from the right-hand side, eight blastomeres are found superficially in one upper layer. Below this is situated another exactly similar layer. Sandwiched between these two, and partly projecting over their margins, is a third layer also with eight blastomeres. Fig. VII may be taken as representing the way in which the intermediate layer shows up below the uppermost eight blastomeres.

The yolk frequently touches the hyaline membrane, whilst in other cases there are empty spaces, varying in size, between the two. An egg freshly laid by the female is mostly found to be in the four-cell stage. It is exceptional to find eggs in three or even two cell stage which, under suitable conditions, continue their development. On examining the female, eggs in the one-cell stage are found in the distal portion of the uterus.

(Fig. 1-2), in the median portion two and three cell stages (Fig. 3-4), and in the proximal one, four-cell stages (Fig. 5).

In the stomach of the host eggs at the six, seven, and eleven cell stages {Fig. 6) may be found and, exceptionally, also at the " morula stage." No eggs beyond the morula stage are found in the intestinal tract even in cases of constipation. The larger majority are in earlier stages. It is possible that oxygen is necessary for a further development from the morula stage, and as oxygen is practically absent in the intestines all eggs are thus found only in stages up to the morula. This opinion is based on the •observation, that if infected faeces are stirred up with water and placed in culture, only the eggs in contact with the air hatch. Hatching is •observed to take place outside the body at a temperature higher than that found in a normal sheep, and the body temperature cannot therefore be held responsible for the inhibition of development.

Formation of the Embryo.

The embryonic development of *Haemonchus contortus* does not differ from that described by other authors in the case of other strongylidae. The duration of the embryonic period varies according to temperature and media, even in eggs obtained from the same source.

The following notes refer to certain investigations undertaken into the development of the embryo in eggs kept at 26° C. in a dark place under favourable conditions of moisture.

Initially.—The eggs used consisted of batches obtained from faeces and found to contain 11-26 blastomeres, together with batches in the morula stage from sources indicated in the table below.

Four Hours Later.—A number of the eggs are in the advanced morula stage (Fig. 9). The hyaline sac is filled with a number of blastomeres, averaging from about 6μ in diameter. On the wider blunter pole of the two, the morula contains a depression, and running through the centre a dark longitudinal stripe seems to indicate the initial stages of the alimentary canal. The whole egg is occupied by the embryo, but on both poles there is an empty space equal to about one-tenth of the length of the egg. In breaking the egg in this particular stage, I was able to count ninety blastomeres. There are only a few eggs to be found so far advanced.

Six Hours Later.—The embryo is now at the tadpole stage (Fig. 10-11), the anterior end being about twice as thick as the rest of the body and the thicker part slightly curved. A depression representing the mouth is now well marked, and it is possible to detect some movements on the part of the embryo.

Eight Hours Later.—The embryo is now twice the length of the egg (Fig. 12), and shows a few structural details. The anterior end is broader, and somewhat conical. A buccal cavity is recognizable by its cylindrical shape. A strand of black cells can be distinguished as the initial stage of the oesophagus and the intestine. The movements of the embryo are now frequent. At this period of observation numbers of eggs are also in the tadpole stage, whilst many others are still in the morula stage.

Ten Hours Later.—In some cases the embryo attains three times the length of the egg (Fig. 13), whilst in a few others it is four times as long. There are still some eggs in the same batch in the morula stage. Embryos, are frequently found to be curled up in the shape of a figure 8 and show frequent, or often constant, movements (Fig. 14). At this stage the shell is still fairly resistant, as is proved by the fact that pressure on the cover glass frequently leaves the shell intact but breaks the embryo inside. The shape of the body is now cylindrical, the head is conical, and the tail long. The mouth cavity is well marked by two longitudinal lines,each terminating posteriorly in a point. The oesophagus has the rhabditoid appearance, and the so-called " teeth apparatus " is distinct. The chyle intestine contains blackish, slightly transparent granules. The lumen, although hardly visible, is narrow and spiral-shaped. The rectum is long and wide. Around the constriction of the oesophagus is a transparent ring which can be recognized as the primordium of the nerve ring. Some embryos obtained from eggs that were broken accidentally were measured, and the length was found to be 280µX 17µthickness, whilst the egg itself measured 73 - 29µ x 43 -27µ. At this period of observation a few free larvae are occasionally found.

Twelve Hours Later.—The movements of the embryo are now more marked, but no further development seems to have taken place.

Fourteen Hours Later.—About 25 per cent of eggs are found to have hatched.

Seventeen Hours Later.—About 50 per cent of the total number of eggs are now found to have hatched. The remainder hatch slowly, and by the 48th hour only a few eggs have not hatched.

In cultures made in a liquid medium the majority of eggs hatched from the 14th to the 17th hours, probably owing to the more uniform conditions prevailing.

Hatching of the Embryo.

I am under the impression that the embryo breaks loose from the egg owing to want of room and food within the shell. The young larva can be seen to persistently move its head against the inner side of the wall after several futile attempts it changes its position and repeats the pushing movement of the head. This process is repeated several times until the embryo finally succeeds in making a hernia-like protuberance which bursts and allows the young larva to escape.

Table to show Period of Hatching in Different Media.

No. of Experiment.	Temperature.	Medium.	Where Eggs were Obtained.	Time in Hours.	REMARKS.
1	27° C.	Faeces	Faeces	9	No larvae.
				14	80 % young larvae hatched.
2	35° C.			13	No larvae.
				15	23 % young larvae hatched.
2a	35° C.			15	No larvae.
				18	30 % young larvae hatched.

No. of Experiment.	Temperature.	Medium.	Where Eggs were Obtained.	Time in Hours.	Remarks.
9	35° C.	Decoction of Faeces	Laid by female :		
			2nd lot....	18	80 per cent. larvae hatched.
13	35° C.	,,	1st lot....	17	Some young larvae hatched.
				19	35 per cent. larvae hatched.
				21	Nearly all larvae hatched.
14	35° C.	,,	2nd lot....	18	60 per cent. of larvae hatched.
				22	Nearly all larvae hatched.
15	35° C.	,,	1st lot....	20	10 per cent. larvae hatched.
			2nd lot....	18	20 per cent. larvae hatched.
16	35° C.	,,	1st lot....	19	50 per cent. larvae hatched.
			2nd lot....	17	50 per cent. larvae hatched.
			3rd lot....	15	5 per cent. larvae hatched.
18	35° C.	,,	1st lot....	22	Few larvae hatched.
			2nd lot....	16	No larvae.
				17	20 per cent. larvae hatched.
19	35° C.	,,	1st lot....	22	2 per cent. larvae hatched.
			2nd lot....	15	60 per cent. larvae hatched.
			3rd lot....	14	60 per cent. larvae hatched.
20	35° C.	,,	1st lot....	24	No larvae.
			2nd lot....	16	2·3 per cent. larvae hatched.
21	35° C.	,,	2nd lot....	16	50 per cent. larvae hatched.
22	35° C.	,,	Faeces	13	Some larvae hatched.
				16	90 per cent. larvae hatched.
23	35° C.	,,	,,	11	No larvae. Eggs in advanced stage.
		Faeces	,,	11	No larvae.
		Decoction of faeces	,,	14	30 per cent. larvae hatched.
		Faeces	,,	14	50 per cent. larvae hatched.
		Decoction of faeces	,,	19	60 per cent. larvae hatched.
		Faeces	,,	19	95 per cent. larvae hatched.
24	35° C.	Decoction of faeces	Stomach contents	19	60 per cent. larvae hatched.
		Faeces	,,	19	90 per cent. larvae hatched.
		Decoction of faeces	,,	21	All larvae hatched.
		Faeces	,,	21	All larvae hatched.
25	35° C.	Decoction of faeces	,,	23	All larvae hatched.
		Faeces	Faeces	23	All larvae hatched.
26	35° C.	Decoction of faeces	Broken females	17	70 per cent. larvae hatched.

NOTE.—The terms first, second, and third lots were used to differentiate between eggs laid by the females after varying intervals from the time they were removed from the sheep and transferred to the artificial medium, thus :—

First lot indicates eggs collected in the first three hours after removal.
Second lot indicates eggs collected between the third and ninth hours after removal.
Third lot indicates eggs collected between the ninth and fifteenth hours after removal.

Conclusion.

1. From the above table it is evident that the interval after which eggs hatch varies in relation to the time that elapses between the removal of the female from the host, a shorter time being necessary for hatching eggs laid by the female three hours after removal, than in the case of eggs laid by females within three hours from removal.

2. Eggs obtained from faeces hatch sooner than eggs that are laid by the female. This is probably due to the fact that whilst the eggs pass through the intestines the evolution of the embryo continues. In cultures obtained from eggs in freshly-passed faeces, young larvae are soon visible: under the optimal conditions of moisture and hatch 14-15 hours after evacuation. The majority of eggs hatch at the 19th hour ; by the 22nd hour few eggs are found unhatched.

THE LARVAE.

Cultivation of Larvae.

Luekart obtained his cultures by placing pregnant females in water, whereupon the eggs escaped and the larvae developed. Parona and Grassi, Perroncito, Leichtenstern, Giles and Looss, used eggs obtained from evacuated faeces, using the latter as a medium, either pure or mixed with charcoal. Major Smith cultivated the eggs in distilled water, whilst Nissle and Wagner used a thin layer of agar on which eggs were spread out. In my experiments I used various methods, which varied with the object in view, viz. :—

(1) Cultivation for diagnostic purposes ; or
(2) cultivation to obtain a stock of larvae for experiments; or
(3) the cultivation of larvae to be studied in their free stage.

(1) *Diagnostic Purposes.*—When microscopical examination of the faeces is not resorted to, the presence of eggs can be determined by allowing them to hatch. This applies chiefly under conditions where the eggs are so rare that they may be overlooked even with a microscope. In such cases faeces were placed in a suitable beaker or dish, just covering the bottom, and were spread out with water when they dried up. The vessel was then covered to prevent evaporation and was either placed in an incubator at 26°-35° C, or kept at room temperature. If development occurred the larvae were seen to crawl up the walls of the vessel four days later, and could be recognized as white masses branching out in various directions. This method was found preferable to microscopical examination in cases where the infection was only a slight one. It allows of the easy detection of the embryo on the walls of the glass, and can be utilized by the layman to diagnose the state of infection in a flock of sheep.

In general examination the following procedure was adopted :—The animal under observation was placed in a box and the faeces were collected every morning. Sometimes they were obtained direct from the rectum by emptying it out with the finger, at other times a bag was placed against the anus and tied to the tail and the droppings thus collected. The faeces were then broken up and moistened. About 10 grammes of the mixture was placed in an ordinary test tube and, by means of a glass rod, distributed on the walls in two longitudinal rows so that the larvae could crawl over the intervening space. The necessary moisture for the hatching of the eggs was present in the faeces placed in the bottom of the tube. The tube was then stoppered with a cork or with cotton-wool plugs. A number of tubes were placed in a suitable receptacle, covered with a lid, and removed to the incubator.

(2) *Obtaining a Stock of Larvae.*—When the object was to obtain a large stock of larvae, consideration had to be given to the consistency of the faeces. Diarrhoeic faeces are a bad medium ; they may be used if charcoal be added, although even then a proportion of failures must be expected. When material is collected from the caecum and colon on post-mortem examination, the cultivation of eggs is not always successful, probably owing to the presence of fermentation. The normal droppings of sheep are the best medium for the cultivation of larvae, and such were generally used.

After placing in a jar measuring 9 c.m. in diameter and 20 c.m. high, the jar was covered with a lid and kept at room temperature. Sufficient air was available inside the jar to permit of the eggs hatching, but when fermentation took place in the faeces all further development stopped.

As stated previously, the larvae appeared on the wall of the jar four days later and then crawled up to the top where they could be easily removed with a piece of blotting-paper. They were in a clean state, free from faeces. (It is necessary to have the vessel closed to prevent larvae escaping from the top.)

(3) *Studying the Larvae—Cultures made in Liquid Medium.*—It occurred to me that it would be advisable to devise means whereby a large number of larvae could be examined simultaneously in the medium, and whereby individual larvae could be removed, for closer examination of their structure. This object was attained by using a transparent medium spread out in a dish and placed under the microscope.

The best results were noted by using a filtered decoction of faeces prepared in the following way :—

Two tablespoonfuls of normal faeces from a sheep were boiled in 100 c.c. of tap water ; the mixture was then filtered, and the decoction poured into a Petri dish in a layer of about 1-3 mm. in thickness to which was added some eggs laid by the females. (The eggs developed if the layer of the medium was not thicker than indicated, otherwise failures were frequent.) The Petri dish was then kept in the incubator, and in order to prevent desiccation new medium was added from time to time. It was found advisable to place such Petri dishes in a covered evaporating dish, the bottom of which contained a layer of cotton-wool soaked in water. A modification of this method consists in using simply tap water as medium to which is added a small quantity of sheep faeces.

For the purpose of examination the Petri dish is placed under the binocular microscope, using ocular No. 2 and objective A2. In this way the hatching of the egg, or any developmental stages, can easily be observed and controlled on a number of larvae at the same time. By means of a pipette individual larvae can be picked out, transferred to a drop of water on a glass slide and covered with a cover-glass, when they can easily be examined under a higher power. The sudden change of temperature from the incubator to that of the room does not hurt the larvae. There seems to be no difference in the rate of development in larvae kept in liquid or in solid media. Sometimes it appears that solid cultures are more reliable than liquid ones. The fine granules in the body of the larvae seem

to be more numerous and of a larger size in solid media. The following; table shows the difference in the development of the larvae in liquid and solid media :—

Date of Culture and Medium used, November 11th, 1913.	Time of Observation—November, 1913.					
	12th—7 a.m.	12th—10 a.m.	12th—1 p,m.	14th—11 a.m.	15th—11 a.m.	16th—11 a.m.
Stomach contents in water	60 per cent. eggs hatched	99 per cent. eggs hatched	5 per cent. larvae in first lethargus	25 per cent. in second stage	50 per cent. in second lethargus	Majority of larvae mature.
Stomach contents in earth	95 per cent. eggs hatched	100 per cent. eggs hatched	25 per cent. larvae in first lethargus	50 per cent. in second stage	50 per cent. mature	Do.
Faeces in water	2 per cent. eggs hatched	40 per cent. eggs hatched	100 per cent. eggs hatched	25 per cent. in second stage	Do.	Do.
Moistened faeces	10 per cent. eggs hatched	60 per cent. eggs hatched	99 per cent. eggs hatched	40 per cent. in second stage	80 per cent. mature	Do.

Cultures Made on Agar.—With slight alterations the agar cultures as suggested by Nissle and Wagner gave satisfactory results. These cultures were prepared and infected with eggs in a manner similar to that described under liquid cultures. It was found that a 0.5 per cent, agar concentration gave a better result than the 1 per cent, suggested by the above authors. Looss' criticisms concerning the unsuitability of agar as a medium (owing to bacterial contamination) were taken into consideration. The heated liquid agar was accordingly poured into the Petri dishes and immediately covered up. When kept at the ordinary temperature of the room they were found to be still useful after the lapse of a month. Agar plates inoculated with eggs obtained from females or from the faeces, and kept moist at 25°-30° C, were still suitable for examination fifteen days later. The growth of moulds at this temperature was quite insignificant and did not interfere with the object in view, always provided that the Petri dishes were kept covered. As an extreme case in point, it may be mentioned that agar plates inoculated on 5th March, 1915, were still suitable for microscopic examination on 27th June, and still provided larvae for further investigation.

It is interesting to note that cultures in faeces with charcoal, kept at a temperature of 40° C, failed, but succeeded when made with agar and faeces. At high temperatures the agar cultures gave better results. On the other hand, at a low temperature the agar cultures were the first to fail. The reason for this may perhaps be that at high temperatures decomposition occurs in the faeces to a greater extent than in agar culture, even if the faeces are mixed with charcoal.

Method of Culture used by Major F. Smith.—Liquid cultures, according to the method of Major Smith, are made by placing eggs in pure water. A number of cultures were made on these lines and the result of the observations on one of them is given hereunder.

The females were collected from the stomach of a freshly-killed sheep, were broken up, and the eggs separated from the fragments ; the cultures with the eggs were inoculated in the following media :—

(1) Decoction of faeces (control culture).
(2) Pure water.
(3) Water containing detritus of the broken-up females.

Twenty-four Hours Later.

The larvae in all cultures were in the first stage.

Forty-eight Hours Later.

Culture 1.—The larvae were in the second stage.
Culture 2.—The larvae were in the first stage, and poor.
Culture 3.—Few larvae were in the second stage.

Seventy-two Hours Later.

Culture 1.—The larvae were in advanced second stage.
Culture 2.—Numerous larvae had died ; the remainder were in the first stage.
Culture 3.—Numerous larvae had died ; the remainder were in the first stage.

One Hundred and Twenty Hours Later.

Culture 1.'—The larvae were all mature.
Culture 2.—All larvae had died in the first stage.
Culture 3.—90 per cent, were dead ; the remainder were poor.

One Hundred and Forty-four Hours Later.

Culture 3.—The remaining 10 per cent, had died.

Conclusion.—Eggs will hatch in pure water, but the larvae do not develop and soon die of starvation. In cultures containing detritus of the mothers, the larvae reach a later stage, but finally die, probably owing both to putrefaction of the medium and to starvation.

DEVELOPMENT OF THE LARVAE.

In their evolutive cycle the larvae of *Haemonchus contortus* pass through five stages separated by structural changes. The two first stages and part of the third one are passed outside the host, as free larvae. The second part of the third stage, the fourth and fifth stages, are passed inside the host as parasites. Each stage can be divided into two sub-stages : the first one in which the larvae move rather actively, feed, and develop ; and a second one in which the larvae are found in a lethargic condition, in which they neither move nor feed, but undergo structural change. Entering the fifth stage the worm is sexually mature and is usually called the *Adult Worm.*

First Stage.

Size of the Larvae.—The naturally hatched larvae vary in size from 340-350μ. in length and from 15-20μ in thickness, the average being 345μ X 17μ respectively (the thickness measured at the base of the oesophagus). Morphologically, the larvae belong to the rhabditoid type. The form of the body is cylindrical, decreasing in thickness from the base of the oesophagus to the tail. This form is typical of the first stage and an accustomed eye can at once distinguish it from the second stage.

Skin : [NOTE.—The longitudinal pad-like formations of the cuticle projecting externally over the body will be called *longitudinal lines.* The similar formations of the sub-cuticle projecting in the coelomic cavity will be called *longitudinal bands* (Fig. 21A). These terms will be used both for the larvae and for the adult worm.]

The skin shows very slight transverse striations. On each side of the body a very fine, sometimes dark and sometimes more transparent " longitudinal line," with a sharp edge, can be discerned, starting some distance from the head and ending a little behind the anus (Fig. 16). Below this lateral line the lateral band can be detected of 6μ in width. The dorsal and ventral bands can only clearly be recognized in cross sections as a simple deviation between the two quadrants of muscles.

Digestive Apparatus.—The mouth aperture is circular and slightly conical, ending suddenly in the buccal cavity. On the edge of the mouth opening can be seen six transparent dots about 3μ in diameter. The external diameter of the mouth aperture is 7μ.

The Buccal Cavity.—The buccal cavity is 14μ in length—excluding the mouth opening, measuring 3μ. Under the microscope the walls appear as two sharp refrangent lines, ending posterially in two dots (Fig. 15).

The (Esophagus.—The oesophagus is rhabditoid in shape, and the constriction slowly passes over into the bulb.

The lumen appears as a clear line, and the " Y-shaped mark " in the centre of the bulb is quite clear. The walls of the oesophagus are thick, transparent and finely transversely striated. The length of the anterior portion is 42μ with a thickness of 9-10μ ; the constricted portion is 28μ long and 5μ thick, and the bulb is 14μ long with a diameter of 13-14μ (Fig. .15).

Chyle Intestine.—This extends throughout the body from the oesophagus to the rectum. When observed laterally, the lumen has a zigzag course, slightly funnel-shaped at its anterior opening. The wall consists, dorsally and ventrally, of one row of eight cells ; they are roughly triangular in shape. The average length of the cells is 18-20μ x 9-10μ. in thickness. The nucleus is large, round or slightly oval. The protoplasm contains fine granulations, increasing in number with the growth of the larvae. The two rows of cells are arranged in an alternating order, the protruding part of one row being opposite the marginal parts of the opposite ones. The connection between the chyle intestine and the oesophagus is effected by two rings, which on longitudinal section appear composed of two cells, each containing a nucleus.

The Rectum.—The rectum is long and runs in a slanting direction, passing through a thick granular mass. The anus lies on the ventral side of the body, the edges slightly protruding. The last part of the rectum has a chitinous lining.

Nervous System.—In specimens stained with borax or hydrochloric acid carmine, or in larvae that have been starved in order to cause the granules to disappear, it is possible to recognize the outlines of the nervous system. At the constriction of the oesophagus the nerve ring appears as a band surrounding the oesophageal wall. The ring is surrounded by a number of cells, of which the majority belong to the nervous system. The cells are arranged in lateral and post-lateral groups, the former lying just behind the nerve ring and extending backwards (ganglion cephalicum laterale) ; another group lies ventrally (ganglion cephalicum ventrale). In the neighbourhood of the rectum a group represents the primordium of the anal ganglion.

Excretory Apparatus.—This is represented by a short canal opening on the ventral side, situated in front of the oesophageal bulbus and entering into a group of cells found in that region,

Genital Primordium.—This can be recognized as a small elongated body, 12μ in length. It apparently consists of two cells. It is placed ventrally between the chyle intestine and the body wall, at a distance of about 25μ in front of the anus.

Biology of the First Stage.—As stated previously, two sub-stages can be recognized, one of activity and one of lethargy. The period of activity is noted subsequent to hatching of the eggs. The young larvae move actively in a series of wriggling movements followed by short periods of rest. After about an hour steadier movements are noted and the larvae commence to feed. In larvae at this stage a contraction of the intestine can be noticed, causing a bulging of the posterior end. The larvae do not travel any distance in water. The movements are somewhat slow and very typical. They serve to distinguish the first from the second stage. Later, when the cells of the chyle intestine are packed with granules, the larvae stop feeding and attempt to enter into the medium. In plate cultures the majority of the larvae entered the agar when their movements slackened down. Finally they became stationary. This represents the beginning of the lethargic stage. Similar observations can be made in larvae kept under natural conditions. This lethargic stage apparently has to be undergone in moist surroundings protected from the direct action of the sunlight. On examining pellets of droppings which have partially dried in the open, only dead larvae and eggs can be detected on the surface ; living larvae are found within or between the faeces, and on the ground on which they are placed. Of these larvae some belong to the second stage, others are still in the first stage and are either at, or near, the first ecdysis. Apparently the newly-hatched larvae, whilst feeding, are attracted towards the superficial layers of the pellet where they pass a period of activity. Afterwards the larvae penetrate into the interior of the faeces, where they undergo the first lethargus, waking up again as soon as structural changes have occurred.

First Lethargus.—The length of the active period varies according to the condition of the culture. In liquid cultures, I found that after eight hours a good number of larvae had reached the first lethargus, and after ten to twelve hours the majority were motionless. This corresponds to twenty-four to twenty-seven hours after the eggs have been passed out by the host. The larvae take a comma-like shape and are rigid. If the liquid culture is shaken or exposed to a bright light for some minutes, the larvae do not react. This fact is so remarkable that when first noticed I thought the larvae were dead, but when the culture was examined later some larvae were found to be in a more advanced stage and further developed. This lethargic stage cannot be detected in a solid medium, because when the larvae are removed and transferred to a liquid medium for the purpose of examination they revive. On account of the unequal development of the larvae in faeces, the larvae of the two sub-stages (activity and lethargus) are usually mixed together, and the appearance of the lethargic stage is not so striking. With the beginning of the lethargus the larvae appear shorter. It can frequently be noted that the constricted part of the oesophagus bulges out in the form of a loop (Fig. 17), the intestinal lumen having lost its regular zigzag shape. The length is now reduced to 350μ as a minimum; in most larvae it averages 400-450μ. It is during this lethargic stage that a separation takes place between the old and the new skin (Fig. 17). This is proceeded by a thickening of the old skin, under which the striation of the new one makes its appearance. When more advanced, a new tail appears within the skin of the old one. At the same time the internal structure undergoes slight modifications, the chief ones being a division of the nuclei in the chyle intestines and the appearance of the lateral lines of the new skin, slightly raised, and with sharp edges. After eight or ten hours when the larvae revive, all these structures are more easily recognizable. It is possible that the surrounding moisture—or rather the penetration of the moisture through the skin into the larvae—has something to do with the awakening. There exists some difference of opinion as to when the second larval stage commences. Previous authors describe the second stage as beginning after the larva has undergone its moulting. From the biological point of view larvae are already in the second stage when the structural changes mentioned above have taken place, that is, from the moment that the larvae awaken again. Casting of the skin is purely mechanical and coincides with the activity of the larvae at the beginning of the second stage. The moulting process is independent of the further development of the larvae. Directly the anterior end of the outer skin has been thrown off the larvae begin to feed, even if the remainder of the skin has not been stripped. Consequently, the first ecdysis will be included in the description of the second stage.

Second Stage.

First Ecdysis.—The first act of the awakening larva is to get rid of the old skin. The mechanism of the first ecdysis was frequently observed in a liquid medium, and the following notes were made :—

When the larvae are first awakened the crawling movements are very prevalent. The larvae seem to prefer to pierce into pieces of faeces that

offer resistance. At this time the old tail is bent in the form of a hook, and the larva is firmly attached by means of this hook to a piece of faeces, as it begins to free itself from the skin (Fig. 18). It can also be noted that when the larva forces itself through particles of the faeces, the anterior part of the skin separates and remains hinged to the old skin in the form of a hood (Fig. 18). The hood is also occasionally separated completely, as if pushed away, probably owing to a sudden increase of pressure inside the skin. This ecdysis is probably also connected somehow with this pressure. In the liquid medium the cast skins are frequently found floating on the surface, sometimes with the hood, which is about 15μ in length, still attached. The skin is frequently well preserved, the lateral line being quite distinct, as well as the transverse striation and the cephalic papillae.

The Period of Activity of the Second Stage.—A marked growth seems to take place soon after ecdysis, as the length of the larvae is now found to reach 500μ. All the new structures can be observed ; the lateral lines with a width of 3μ are conspicuous and sharply set off (Fig. 20-Fig. 21A *l. lat.).* The stage of the worm is thus characterized by the structure of the lateral lines. The larvae are cylindrical in shape and of equal diameter from the bulbus of the oesophagus to the anus. The activity in this stage shows itself in swimming movements. The larvae begin to feed again and the intestinal lumen, which previously had a zigzag appearance, now seems to straighten out and increase in diameter, whilst the cells of the intestinal wall become more flattened. The granules in this wall increase and the cells return again to normal shape. Once the larvae have obtained sufficient food they can be noted to move again, with the idea of hiding in the interior of the medium. A peculiar feature is that in liquid media, the larvae are found at the bottom of the liquid and apparently make no attempt to rise to the surface, whereas once maturity is reached they will do so.

Second Lethargus.—The moving larvae gradually become motionless and assume a bent or stretched appearance. The onset of the second lethargic stage depends either on external influence or individuality, a fact which is borne out in the following table :—

No. of Experiment.	Temperature.	Medium.	Eggs Obtained from.	Time after Hatching.	REMARKS.
19*a*	35° C.	Decoction of faeces	Laid by females	3 days	50 per cent, in second lethargus.
19*b*	35° C.	,,	,,	3 days	70 per cent, in second lethargus. (In some larvae second skin noticeable.)
9	35° C.	,,	,,	3 days	90 per cent, in second lethargus ; some mature.
12	35° C.	,,	,,	3 days	50 per cent, in second lethargus ; a few mature.
20	35° C.	,,	,,	3½ days	80 per cent, in second lethargus ; many mature.
24	30° C.	,,	Stomach contents	2½ days	50 per cent, in second lethargus.
25	30° C.	,,	,,	2½ days	90 per cent, in second lethargus.
		Faeces	,,	2 days	90 per cent, in second lethargus.
		,,	Faeces	2½ days	90 per cent, in second lethargus.

From the above table it can be seen that on the 3rd day a large number of larvae are found in the second lethargus. As a result of a number of observations, I came to the conclusion that the beginning of the second lethargus takes place about forty hours after the first ecdysis, or sixty to sixty-five hours after hatching. The size of the larvae at this stage varies, and this variation in size is connected somewhat with the medium in which they grow. The following table brings this fact out clearly :—

Experiment.	Eggs Obtained from.	Medium.	Time in Hours.	Size. μ	Condition of Capsule.
11	Laid in incubator at 30–35° C.	Decoction of faeces	68	600 × 26·42	Outer skin evident.
15	,,	Faeces	65	651 × 24	Outer skin not apparent (œsoph. rhabditoid).
23	Faeces	,,	64	691 × 23·31	Outer skin not apparent.
23*a*	Stomach contents	Decoction	72	599·80 × 23	Outer skin evident.
24	,,	,,	70	682·65 × 23·31	Outer skin not evident (œsoph. rhabditoid).
24	,,	Faeces	70	632·70 × 23·31	Outer skin evident.
28	Faeces	,,	65	756 × 28	Outer skin evident (œsoph. rhabditoid).
29	,,	,,	65	754 × 28	,, ,,
30	,,	,,	65	756 × 28	,, ,,
31	Stomach contents	Stomach contents and charcoal	72	715 × 27·5	,, ,,

The structural changes which take place during the second lethargus are more pronounced and bring the larvae to the mature stage. These structural changes take place in the following order :—

(1) The mouth aperture loses its conical shape.

(2) The buccal cavity is narrower, and apparently there is no longer any distinction in the entrance to the oesophagus.

(3) The constriction of the oesophagus becomes less marked or the oesophagus itself loses its rhabditoid form, and, as already mentioned in the first lethargus, is frequently found in the form of a loop.

(4) The oesophageal valves are distinct, the granules of the chyle intestines become scarcer and more transparent; the lumen is more distinct.

(5) The skin appears thicker and the body is slightly reduced in size.

The final appearance may be described from the observations made on a particular larva, of which the body measured 715μ with a diameter of 27-5μ, the tail length being 130μ. The outer skin was refrangent and separated from the body, but still adherent with the exception of the head, where for some microns in length the skin was detached. The mouth opening of the outer skin was closed. The striations of the inner skin were well marked. The oral opening of the encysted larva was open. The mouth cavity was reduced to a simple line. The oesophagus was 175μ in length and 120μ in diameter at the base. The three portions of the oesophagus were hardly distinguishable and the whole organ appeared to be claviform in shape, with the posterior end as the base. The intestinal valves were not distinct. The cells of the chyle intestine were sixteen in number, roughly triangular in shape, measuring 50μ at the base and 20μ in thickness ; these cells were finely granulated, showing a nucleus in the centre measuring 2μ in diameter. The anus was closed, but still adherent to the corresponding mark in the old skin. The genital primordium was situated at a distance of 175μ. from the anus, measuring 20μ, in length with a diameter of 7-5μ.

The nervous system is more developed, but appears confused with the surrounding cells.

Biology of the Second Lethargus.—After about twelve hours the development of the lethargic stage is completed, although under optimal conditions a period of even eight hours may be sufficient. With the accomplishment of the structural changes the larvae awaken, but if external conditions are unfavourable awakening is retarded. This lethargic state evidently serves not only for further development, but also represents a latent state for the larvae under unfavourable conditions. Here again the end of the second stage may be recognized when the larvae have undergone structural changes. The succeeding ecdysis would therefore be part of the third stage.

Third Stage.

Mature Larvae.—The larvae awaken from the second lethargic stage as soon as conditions are favourable; in general, three days after hatching. A low temperature and dryness delay awakening. The awakening is succeeded by the coiling up movements, and as a result of this, separation of the inner newly-formed skin takes place from the older one. This detachment shows itself distinctly by the difference in the situation of the two corresponding openings of the anus. Once this detachment has taken place, the larvae seem to have acquired the necessary freedom to enable them to travel about. Their movements are now fast as compared to those noted in the second stage, and they can best be described as " swimming." Another peculiarity is the attempt of the larvae to rise. These two phenomena are typical for larvae in the third stage. Once the larvae have reached maturity, their next object is to reach a suitable locality from which they can find access to the host.

Description of the Mature Larva.

Length of the Larvae.—The following table shows the various lengths of larvae grown in different cultures and for varying lengths of time :—

Experiment.	Eggs Obtained from.	Medium.	Time.	Size. μ
1	Laid by females	Decoction of faeces at 35° C.	20 days	571 × 21
	,,	,, ,, ,,	20 days	630 × 22
2	,,	,, ,, ,,	45 days	614 × 23
3	Stomach	,, ,, ,,	10 days	682 × 23·31
4	Faeces	Faeces	—	680 × 23
5	Stomach	Decoction of faeces at 36° C.	3 days	685·95 × 23·31
6	Faeces	Faeces	38 days	614·20 × 25·5
				632 × 25·2
				620 × 25·2
7	,,	Faeces in the open at 30° C.	2 months	714 × 26·30
8	,,	,, ,, ,,	2 months	784 × 23·80
9	,,	Faeces in the open at 35° C.	1 month	799 × 26·5
10	,.	Faeces in the open at 32° C.	10 days	820 × 26·5

Shape.—In shape they do not differ from that described in the second lethargus (Fig. 21).

Old Skin.—The mouth opening of the outer skin is closed and a small appendix represents the old wall of the mouth. The marks of the six head papillae are distinct, as well as remnants of the rectal walls. The lateral lines are conspicuous, having the base wedged into the cuticle (Fig. 21A, *l. lat.) ;* the transverse striations are visible.

New Skin.—The new skin is also ringed. The distance between each ring measures 1-7μ. The lateral lines have a width of 3-4μ and project from the circumference of the body for 1-5μ, with the margin slightly protruding laterally (Fig. 21A).

Subcuticle: Lateral Bands.—In the whole specimen only the lateral bands can be distinguished. They appear at two sides of the body as two longitudinal strands, about 4μ broad. It is rather easy to distinguish these two strands, because they are more transparent than the two neighbouring muscular quadrants. On cross section the lateral bands appear finely granular and protrude into the coelomic cavity (Fig. 21A, b. lat.). The dorsal and ventral bands appear in cross section as a small granular triangle, wedged between the two neighbouring muscular sectors (Fig. 21A, b. ven. and b. dors.).

Musculature.—The muscular arrangements are divided into four quadrants, two dorsally and two ventrally. The muscle cells are rhomboidal, measuring about 4 5 μ in length. Their longitudinal axis runs parallel to the longitudinal axis of the body. On cross section each muscular quadrant occupies 11μ of the circumference of the body and protrudes for a distance of 5-5μ into the cavity. Each quadrant is composed of seven muscle cells (Fig. 21A-M).

Mouth.—The mouth is closed. The mouth cavity starts with a small globular dilatation, and extends as a fine canal into the oesophageal lumen.

The Oesophagus.—The oesophagus is more or less claviform in shape, but has a slight constriction that can only be detected under a high magnification (Fig. 21). The lumen of the oesophagus appears as a straight stretched line with a slight dilatation at the posterior end, where the oesophageal valves are present as described in the previous stage. In the substance of the walls numerous fine granules are seen arranged in longitudinal strands, representing the primordium of the oesophageal glands. The intestinal valves consist of the two rings described previously. The chyle intestine is composed of sixteen cells, more or less rich in granules according to the state of preservation of the larvae (Fig. 21A, int.). The lumen still retains the zigzag appearance described previously.

Nervous System.—It is difficult to examine the nervous system in living or unstained larvae, but staining with aqueous methylene blue renders examination possible, the nervous system being more conspicuous than other organs. Generally speaking, the nervous system corresponds to what has been described previously, although the outlines are now more distinct. The cerebral commissure appears as a band situated 75-80μ. from the anterior end. It is finely striated and does not show any nuclei. In breadth it measures 5μ. Anteriorly, some longitudinal strands of nuclei surround the first part of the oesophagus, representing the primordium of the papillary nerves. Posteriorly to the nerve ring the two lateral cephalic ganglia slain a deep and distinct blue and appear as two compact masses of nuclei, totalling 30μ in length. The ventral ganglion is represented by a group of nuclei, not so compact as the two just referred to, and extends from the cerebral commissure to the end of the oesophagus and surrounds the excretory canal.

In the whole specimen, the nerves running along the longitudinal bands and connecting the central system with the posterial ganglion, were not sufficiently distinct for identification. In cross section three dots in the coelomic cavity were seen (Fig. 21A), and apparently correspond to the two lateral and the ventral longitudinal nerves.

The genital primordium is represented by about sixteen nuclei, taking the methylene blue stain well, and occupying an oval area of about 9-5μ X 8μ, situated obliquely to the medial axis in the ventral region and about 320μ distant from the tip of the tail.

The excretory canal leads backwards into a mass of nuclei, which represents the primordium of the excretory organ, but which cannot be completely distinguished from the nuclei of the lateral and ventral ganglia. The aperture is on the ventral side, about 80μ from the head.

Larvae under Natural Conditions.—In order to control the biological facts noted from liquid cultures, a series of observations was made in the open in the following manner :—

Faeces from infected sheep were placed around a tuft of grass in a field. The faeces were moistened with water and covered with a glass bell in order to conserve the moisture. The temperature varied between 35° C. in the day time and 12-15° C. at night time. On the 1st day the larvae were found in the first stage and more or less uniformly distributed

throughout the droppings. On the 2nd day the majority of the larvae were in the second stage and were found both in the lower parts of the pellets of faeces or in between the pellets themselves, evidently sheltering from sunlight and trying to obtain the maximum moisture. On the outside of the pellets, unhatched eggs, or dead larvae in the first stage, were found; inside and in between the mass of pellets, larvae in the first stage and in the first lethargus were most frequent. On the 3rd day a very few larvae in the first stage were found in the uppermost pellets, whilst in the pellets touching the ground large numbers of larvae in the second stage were found. During the first three days no young larvae were found crawling up the grass. On the 4th day 80 per cent, of the larvae were found in the lower layers of pellets, the remaining 20 per cent, being on the upper layers. The first of the mature larvae were now found crawling on to the grass. The transition from the lethargic to the mature stage is, biologically, not so distinct under natural conditions as it is in liquid cultures, where it is detected by activity of the larvae, but nevertheless mature larvae can easily be recognized by their morphology. In the course of the following days numbers of larvae were still found to have undergone development and to have reached maturity, whilst a continuous migration could be observed on the grass. During the next seven to fifteen days a few larvae were still observed in the different layers of the faeces, representing the various stages of growth from hatching to maturity. Underneath the pellets and in contact with the ground, a number of larvae in the second lethargic stage were found.

Further Changes of the Mature Larvae.—When larvae have reached the mature stage, a certain contraction of the tissues takes place by which the size of the body sensibly decreases, whilst the outer skin becomes thicker and increases in rigidity. As long as larvae were kept in a moist ambient, and other conditions for their preservation were favourable, the body filled the outer skin, but when unfavourable conditions intervened, the outer skin contracted, with the result that the space in between the skin of the body became distinct (Fig. 22). The second feature found in larvae in the mature stage is the presence of vacuoles in varying numbers, and of different sizes, in the chyle intestinal cells. These vacuoles are frequent in living larvae that have been preserved, or in larvae exposed to unfavourable conditions, such as dryness, light, or heat, and the presence of numbers of them indicates that the larvae are nearing death (Fig. 22-23).

The third observation in the mature larvae was the completion of the second ecdysis. This phenomenon occurred at different times, according to the medium used, and the following observations can be recorded :—

(1) *Hydrochloric Acid Solution,* 1-5 *per cent.*—A few larvae freed themselves of the outer skin shortly before death. The observation with 1 per cent, solution was continued for three days, by which time 60 per cent, of larvae were dead. The observation with 5 per cent, solution was continued for twenty-four hours, by which time all the larvae were dead

(2) *Concentrated Aqueous Solution of Methylene Blue or Trypan Blue.*—Completion of the second ecdysis had not occurred after the larvae had been soaked in the solution for some days.

(3) *Gelatine Solution.*—Dry gelatine was soaked in water for a night. The superfluous water was then removed and the gelatine was liquified in a water bath. The gelatine was then allowed to get cold, and when at a temperature of 30-40° C, it was poured into a Petri dish where mature larvae had been placed in a few drops of water. The temperature of the room was 15° C. Next day the gelatine layer was firm. All larvae still retained the outer skin. Those on the surface were moving, whilst those in the mass of the gelatine were motionless. The Petri dish was then kept at 25° C. Two days later the larvae were partly in the gelatine and partly coiled up on the walls of the Petri dish. Practically all of them still had the outer skin.

In a second experiment a 1 per cent, solution of gelatine was made. In some dishes, cotton-wool flakes were added, whilst in others glass filaments were mixed with the gelatine and distributed over the surface.

Three days later the preparations were examined and all larvae had their outer skins intact.

Of larvae kept in ordinary tap water in a Petri dish at room temperature and in a weak diffused light, some were found dead without the outer skin after four to five months of preservation, whilst the remainder were living and ensheathed in the outer skin.

In a parallel observation mature larvae were placed in water 20 cm. deep and kept under conditions similar to the above. Two and a half months later 90 per cent, of larvae were without their outer skins, and of these only 2-3 per cent, were dead. Three and a half months later very few larvae were found with the outer skin ; 80 per cent, of the larvae which had completed the second ecdysis were living.

On the walls of the glass in jar cultures, dead larvae were frequently found without their outer skins, from the sixth or seventh months of observation. I am inclined to consider the casting of the skin in such cases as a sign of approaching death. There are numerous other cases, however, in which it would appear that mechanical injury or friction is mainly responsible for the second ecdysis. For example, larvae escaping through crevices in the lids of culture jars are in most cases found with their outer skins wholly or partly cast. This effect of mechanical friction is also suggested by certain experiments carried out in connection with the mode of infection of the host. The experiments themselves are discussed more fully in a later section, but at this point it may be incidentally observed that larvae placed upon the shaved skin of sheep and protected by a bandage, rapidly lost their outer envelopes ; also that after subcutaneous injection of an aqueous suspension of larvae the majority were found without their outer skins, but still alive, when examined eight days later.

No explanation can be given for the fact that the larvae kept in water remained alive for several months without their outer skin.

INFLUENCE OF THE AMBIENT ON EGGS AND LARVAE.

In discussing the influence of the ambient on the free life of *Haemonchus contortus,* eggs and larvae will be treated together.

Intoxication of Eggs and Larvae.

The first factor interfering with the evolution of the egg, and even sometimes effecting its destruction, is the toxic substances of the intestine of the host. The previous remarks concerning the use of faeces as a medium for culture apply here as well. The fact that toxic substances may be found in evacuated faeces as a result of abnormal fermentation was mentioned when considering the cultivation of larvae. In the normal course of events the shells of the eggs as well as the cuticle of the larva are impervious to the surrounding liquid. It therefore appears that the toxic substances influence this protective property, rendering the membranes permeable, and thus intoxicating the egg-cells or the larvae.

Poisoning of Eggs and Larvae.

The action of certain chemical compounds upon eggs and upon larvae was investigated. Beta-napthol, thymol, picric acid, and copper sulphate were each tested upon liquid cultures of eggs at the morula stage. After four days at 25-30° C. all the treated eggs were opaque and dead, while those in control cultures developed to mature larvae. The compounds mentioned not only inhibit development, but actually destroy the vitality of the egg. A number of other compounds were also tried and it was found that, in general, those which were injurious to the egg were also injurious to the larvae. This point will be taken up again in considering the medical treatment of infected sheep.

Air.

Eggs and larvae of *Haemonchus contortus* require a certain amount of air for development. The amount needed is very small, but if it falls below a certain minimum., inhibition of growth, and finally death, will occur. As indicating the necessity for air is the fact that cultures of faeces do not develop if made into too thick a paste with water, or if carried out in liquid media more than 3 mm. in depth. The difference in development between larvae found on the surface of pellets of sheep-dung, and those found in the interior, may also be explained upon differences in the oxygen supply. In a subsequent section dealing with the effect of moisture upon eggs and larvae, detailed experiments will be given which illustrate the influence of aeration as affected by the depth of aqueous layer in which the eggs are placed.

That the oxygen requirements are low, however, is suggested by the success of the method already described as used in routine cultivation for diagnostic purposes. The tubes containing the faeces were corked up and stored in a closed jar, and free access of air was therefore limited. Nevertheless the development of the cultures was always satisfactory.

Certain other experiments, carried out on the lines adopted by Looss in his work on Ankylostoma Duodenale, point in the same direction. Infected faeces, with eggs in the morula stage, were collected in small specimen tubes 3 cm. high by 1-5 cm. in diameter. The amount of faeces taken was 0-5 to 1 c.c, mixed or dusted with charcoal, and the tubes were hermetically sealed with wax. After from ten days to one month's storage at 24° C., numerous mature living larvae were found coiled up on the walls of the tube. A number of dead eggs in the morula stage were also found.

I am of opinion that more air is required for developing eggs and larvae than for resting eggs and mature larvae. Mature larvae kept in water at a depth of 3 cm. were still alive at the end of five months. Kept at a depth of 20 cm., 50 per cent, were still found alive at the end of three months. Larvae which attempted to escape from culture jars were found alive four months later in the vaseline jelly used for sealing.

Temperature.

With regard to the action of temperature on growing and maturing larvae, some experiments were carried out under laboratory conditions, and as controls another series was undertaken under natural conditions of the veld. When working with high temperatures special consideration was given to decomposition of the culture media, which had a lethal effect more pronounced with increase of temperature. Consequently, as a medium consisting of faeces alone easily undergoes fermentation, I frequently took recourse to a mixture of faeces and charcoal. In other cases liquid nutrient medium or plain water was used, and later agar plates were utilized. The latter proved the most satisfactory. It may be stated at the outset, that the optimum temperature for the development of eggs and larvae was found to be between 20° C. and 35° C. Accordingly, I call *normal temperature* that lying between 20° and 35° C, *high* temperature that above 35° C, and *low* temperature that below 20° C.

High temperatures acting for a short time.—A number of mature larvae of *Haemonchus contortus* were kept for microscopical examination in glass. Petri dishes of 10 c.c. capacity. The various dishes were filled suddenly with water of varying temperature and then left at laboratory temperature of 20° C. The results are given in the following table :—

Temperature of the Water.	Effect of Warm Water on the Larvae.		
	Immediate.	A Minute Later.	24 Hours Later.
50° and 53° C.	Coiled up or curved	Nearly all moving	Very few dead.
55° C.	,, ,,	Some not moving.	Very few dead.
58° C.	,, ,,	90 per cent. moving	2–3 per cent. dead.
60° C.	,, ,,	70 per cent. moving	3–4 per cent. dead.
63° C.	,, ,,	60 per cent. moving	4 per cent. dead.
65° C.	,, ,, (Few distended)	50 per cent. moving	10 per cent. dead.
68° C.	,, ,,	8–10 per cent. moving	10 per cent. alive.
70° C.	Distended	8–10 per cent. moving	5 per cent. alive.
73° C.	,,	2–3 per cent. moving	2–3 per cent. alive.
75° C.	,,	All distended	2–3 per cent. alive.
78° and 80° C.	,,	,,	1–2 per cent. alive.
85° and 90° C.	,,	,,	All distended, and dead.

Conclusion.—At temperatures between " normal " and 65° C. a very small proportion of larvae were killed, whereas at temperatures between 65-70° C. the majority survived. A temperature of from 70-80° C. is decidedly fatal to the majority of the larvae and very few survive. At 85° C. no live larvae were found. Practically, one may say that immersion in water at a temperature of 85-90° C, kills all the larvae instantaneously.

Eggs and larvae exposed to high temperatures : 60° *C.*—From a fifteen days old culture made in faeces, in which practically all the larvae were alive, a sample of the medium containing a good number of larvae was placed in an oven at 60° C. At the same time larvae were fished from the walls of the jar of the same culture, immersed in water and also placed at 60° C.

Periodic examination under the microscope of both batches gave the following results :—

Larvae exposed for thirty minutes in faeces appeared motionless and stretched out. After one to two minutes of observation 90 per cent, awakened and were swimming.

Exposed for an hour in faeces.—After one to two minutes of observation 30 per cent, awakened and were swimming.

Exposed for two hours in faeces.—After ten minutes of observation some larvae were observed to coil up, occasionally showing movements, the remainder were dead.

Two and a half hours of exposure.—All the larvae kept in water or faeces medium were stretched out and dead.

This experiment was repeated several times, with much the same results. When the temperature was not disturbed by intermittent opening of the incubator, two hours' exposure usually sufficed to kill all the larvae.

Cultures of Eggs and Mature Larvae kept at 50° *C.*—Eggs in the morula stage kept in faeces or on agar media for six hours were killed in the morula or tadpole stage. Kept in liquid media, eggs in the last stage were found to have hatched but the larvae were already dead. Mature larvae in faeces or in agar were found dead and stretched out. Attached to a piece of dry grass a few were found alive.

In reducing the time of exposure to four hours eggs were found dead; the mature larvae were found dead if exposed in liquid medium, but some were still found alive after exposure on dry grass.

Eggs in the morula stage kept for two hours on agar were sometimes found dead, but it was nevertheless not rare to find the eggs still alive. Out of five attempts two gave positive results. The surviving eggs put up for culture reached maturity. Mature larvae kept for two hours in liquid media were usually found alive, very few deaths having occurred. Eggs at the morula stage kept in agar and exposed at 50° C. for two hours daily, and in the intervals kept at 28° C, hatched in two out of eight attempts. The larvae reached the second stage on the second day. After the third exposure the larvae were placed constantly at 28° C- On the seventh day all were dead without having reached the mature stage.

Mature larvae in agar plates were also exposed to 50° C. for two hours daily. After the third exposure 20-30 per cent, were found to have been killed. In the further course of this experiment it was not possible to establish the percentage of deaths, owing to the living larvae crawling on the walls of the dish and escaping, but I was able to find living larvae after the sixth exposure.

In connection with these notes concerning the resistance of larvae at 50° C., the following further experiments are also of interest:—

Some pieces of dry grass, on which were numbers of larvae twelve days old, were kept in an incubator at 50° C. and were divided into different batches. After pre-arranged time of exposure each lot was transferred to water at normal temperature and examined twenty-four hours later. The result was as follows :—After an exposure of—

2 hours, 2 per cent, of the larvae were alive and swimming.
4 " 6 " "
6 " 1 " "

After twelve and twenty-four hours all the larvae were dead.

The apparent discrepancy in the results can be explained in the light of the ascertained distribution of the larvae on the grass. Microscopic examination of the material before placing in the incubator had revealed the presence of numerous larvae uniformly distributed on the grass blades, and of some clusters of larvae measuring about 100μ in thickness. The small percentage of surviving larvae after two hours' exposure probably occurred amongst those which were uniformly distributed on the blades ; the 6 per cent, of surviving larvae after four hours' exposure and the 1 per cent, after six hours' exposure is probably represented by larvae collected in the centre of the clusters, which were thus well protected from the direct action of heat and evaporation. This experiment also throws some light on the striking differences obtained in various experiments as recorded by other authors. We are justified in concluding that larvae kept at a temperature of 50° C. in a dry incubator do not resist longer than two hours if well exposed to the ambient, whilst those present in the centre of clusters can resist for six hours. In the veld, larvae are probably well sheltered and thus able to resist high temperatures. This point was also investigated experimentally on the following lines :—

Mature larvae were collected on blotting paper and placed in the centre of pieces of dry black earth. One piece measured 3 cm. in diameter, and the other 6 cm. The two pieces were then kept in a dry incubator at a temperature of 50° C. The first one was examined twenty-four hours later, when 5 per cent, of the larvae were still alive. These results give a good idea of the resistance shown by larvae when sheltered, even if exposed to the high temperatures found in the summer months.

45° C. —Cultivation of eggs in the morula stage was attempted in faeces on three occasions, and in charcoal-faeces on five occasions, but in each case with negative results. Eggs at the morula or tadpole stage were also maintained at 45° C in agar cultures for three or four days and then removed to 38° C, but all failed to develop. Daily exposure of eggs in charcoal-faeces or in agar, for six hours on three successive days, also resulted in the death of the eggs. In the same media, however, eggs were found to withstand a daily exposure of two hours for four days. In charcoal 50 per cent, and in agar 80 per cent, of mature living larvae were found.

Mature Larvae at 45° C—The various observations can be summarized and are given in the following table :—

Time of Exposure.	In Pure Water.	In Agar Cultures.	In Cultures of Faeces.	In Cultures of Faeces plus Charcoal.	On Dry Grass.
5 hours.....	90 % alive	—	—	—	—
8 ,, ...	—	80 % alive	—	—	—
12 ,, ...	50 % alive	—	50 % alive	60 % alive	—
18 ,, ...	—	70 % alive	—	—	—
24 ,, ...	20 % alive	60 % ,,	—	—	10 % alive
36 ,, ...	—	50 % ,,	—	—	—
48 ,, ...	10 % alive	50 % ,,	2–3 % alive	Few alive	—
60 ,, ...	—	25 % ,,	—	—	All dead
72 ,, ...	Very few alive	10 % ,,	—	—	—

Conclusion.—The large percentage of deaths amongst larvae exposed in cultures of faeces is explained by the rapid decomposition which takes place at so high a temperature, even when charcoal is added in large amount. In some instances the larvae were dead within seven hours, whilst in others they survived for twelve and eighteen hours. The comparatively long resistance of some larvae on dry grass can be explained by the formation of clusters, as mentioned above.

42° *C.*—Cultures with eggs in faeces plus charcoal were attempted on two occasions, but with negative results, whereas control cultures kept at 28° C. showed the presence of mature larvae in three days. In agar medium five attempts were negative, the eggs being found dead in the morula stage within four to six days, whilst the medium itself was in very good condition. In a sixth attempt in agar, in which the eggs were at tadpole and embryo stage, several larvae of the second stage were found moving about on the second day, but they were all dead by the following day. Cultures in agar kept at 42° C. for two hours daily and then removed to a temperature of 28° C. proved positive in several instances.

Mature Larvae.—Mature larvae kept in pure faeces at 42° C. are killed very quickly, owing to decomposition of the medium. The addition of charcoal at this temperature gives better results than at 45° C.

Summarizing the several attempts at preservation in charcoal-faeces at 42° C, I found that-

after 17 hours 85 per cent, of larvae were still living ;
„ 24 „ 80
„ 40 „ 50
„ 48 „ 10
„ 65 „ only a few larvae still survived.

The number had still further decreased three to five days later.

In agar medium the larvae resist much longer. The following are the results of several attempts :—

Examinations at varying intervals showed a very small percentage of deaths during the first two days. On the 3rd day 70 per cent, of the larvae were still alive, on the 4th day 65 per cent. By the 6th day it was

no longer possible to accurately estimate the percentage, owing to disintegration of the larvae previously dead, but, roughly speaking, it seemed as if about 40-50 per cent, survived. Ten days later only about 20 per cent, seemed to have survived. There were still a fair number living on the 14th day, but the survivors had considerably decreased by the 18th day. On the 20th day it was only possible to detect living larvae after a careful search, and on the 26th day it was not possible to find any living larvae on the three different culture plates used.

Biological Remarks.—At 42° C. in agar medium the mature larvae examined under the microscope show a more pronounced motility than those kept at 45° and 37° C. Their movements are particularly noticeable during the first two days, but later about half of the larvae appear lethargic and are very slow to move when awakened. It therefore seems that a temperature of 40°-42° C. gives the maximum stimulation to larvae—for limited periods, at least.

40° *C.*—Cultures with eggs in pure faeces at 40° C. invariably failed, In cultures made with faeces plus charcoal, I obtained three positive results out of five attempts. The cultures with agar succeeded in the majority of cases, but mortality amongst the larvae amounted to about 50-60 per cent., chiefly in the first stage. The remaining larvae reached maturity within three to four days. Mature larvae resisted for a considerable length of time at constant temperature of 40° C, when kept in a dark moist place.

In these cultures, left at 40° C, some of the larvae which had reached maturity remained alive for 24-28 days.

37° *C.*—Several cultures were also kept at 37° C. in faeces alone, but out of six attempts two failed, owing to putrefaction, whilst in the positive cases the number of larvae was small. In faeces to which charcoal was added the result was better, but the failures were still frequent and the mature larvae always scarce In agar cultures the result was nearly always positive. In examining these cultures carefully, I found that larvae of the first stage died to the extent of 40 per cent. Considerable numbers in the second stage also died, but the remainder reached maturity either just previously to, or at, normal time. The mature larvae were more motile than at normal temperatures, and the space between the body and the outer skin was quite conspicuous within four to six days of maturity. In continuing the same agar cultures at 37° C, I observed that after four to six days the mature larvae were still alive, but by the 10th day 60 per cent, had died. Only a few were alive on the 20th day, on which date some of them had lost their outer skin. There were practically none alive after twenty-eight days. It can accordingly be concluded that the maximum time of resistance for larvae at 37° C. is about a month.

Observations in the Field—High Temperatures.

(1) On the 12/3/15 a lot of faeces heavily infected with eggs were placed at the bottom of some stems of grass in the open. During the period of observation they were frequently watered and kept covered with a glass bell 30 cm. high to ensure constant moisture. In the afternoon of the first day, the temperature under the glass bell rose to 56° C,

whilst in the centre of the faeces a temperature of 45° C. was recorded. During the night the temperature decreased to a minimum of 12° C, and during the two following days there was practically no variation outside the limits described. On the 15/3/15 when the faeces were examined, the eggs were found to be black and dead, whilst a few dead larvae of the first stage were also found.

(2) The second experiment was carried out on the 12/3/15 under similar conditions except that a large amount of charcoal was added to the faeces. The temperature conditions remained the same, and three days later, when the culture was examined, the results were identical with those of the previous trial.

In the case of the first experiment the death of the larvae could be explained by the decomposition of the faeces under the conditions of high temperature, but in the second experiment decomposition was not so far advanced. This result would support that obtained in the laboratory, showing that a temperature of 45° C, acting for more than two hours daily, killed both eggs and larvae.

Conclusions.—Temperatures above 36° C. act more or less deleteriously on the cultures of *Haemonchus contortus.* Mature larvae resist temperatures up to 70° C, providing they are only exposed for a short time. Constant temperatures between 42°-50° C. are resisted by mature larvae from about two hours for the higher degree, to a maximum of a month for the lower degree, but cultures of larvae are killed. The temperature of 40° C. seems to be the highest at which the larvae can grow, but even at 37° C. the mortality amongst eggs and mature larvae is very heavy. As Looss and some other authors have previously noted, decomposition of the medium plays a very important role in the negative results obtained with cultures under high temperatures. The addition of charcoal to the faeces acts as a palliative to decomposition, but cultures in agar seem to be more suitable for the growth of larvae exposed to high temperatures. Temperatures of 37°-40° C. first act on young larvae by overstimulation, with the result that a certain number die, and the remainder reach maturity in the minimum of time. Between 40°-50° C. the mortality is still higher and the surviving larvae are weaker, whilst the time required for maturing is prolonged.

Mature larvae react under high temperatures showing increased motility, followed by a period of inactivity due to exhaustion, in which marked destruction of the intestinal granulations is noted. This period ends in the death of the larvae. The laboratory experiments at constant high temperatures, however, are only of minor importance in regard to the behaviour of larvae under natural conditions, since in the field the temperature oscillates between a maximum in the day time and a minimum at night. Furthermore, in the open, other important factors such as light and dryness, exercise considerable influence and vitiate comparison between laboratory and field experiments. Under the natural conditions of the field there is also the fact that some larvae protect themselves against the sun by migrating to places where they are protected from the direct rays. This point will also be dealt with when considering the migration of larvae.

" Normal " Temperatures : 35° *C.*—This temperature was generally adopted when dealing with the evolution of the free larvae, and it was found to be very suitable for their growth. The details of some experiments are :--

(1) On the 14/12/13, at 8 a.m., eggs of *Haemonchus contortus* were transferred to a liquid medium and kept at 35° C. At 10 p.m. (fourteen hours later) 40 per cent, of the eggs had hatched. The following evening at 9 p.m. (23-25 hours after hatching) 30 per cent, of the larvae were in the first ecdysis. On the 17th, at 9 a.m., the majority of the larvae were in the second stage, whilst others were in the second lethargus. By 5 p.m. several larvae had reached the third stage. With cultures in faeces or in gelatine the results were much the same, except that wherever marked decomposition of faeces occurred a number of larvae died in the first or second stage. At this temperature the mortality is very small and the growth of the larvae in liquid medium is so uniform that very few immature specimens are found on the fourth day. After reaching maturity, the majority of the larvae immediately commence " swimming " movements in a very pronounced way, and show the optimum of vitality. The migration up the walls of culture jars can be easily seen by the naked eye on the 4th day.

28°-32° *C.*—In cultures grown at this temperature the larvae reached maturity within three days, frequently to the extent of 90 per cent. In jar cultures 30 per cent, of them were seen to be crawling over the walls, reaching 3-4 cm. above the medium. On the 4th day the wandering of the larvae on the walls was very conspicuous. On examining larvae taken from these cultures under the microscope, the typical swimming movements were clearly seen, showing that the larvae were in the optimum of vitality.

25° 0.—Faeces containing eggs from the morula to the embryo stage showed larvae of the first stage, first lethargus, and a few of the second stage, within fifteen hours. On the 3rd day 20 per cent, of the larvae had reached maturity, but they had not yet commenced crawling up the walls of the glass. By the 4th day, however, a heavy migration was usually noticed.

20°-22° *C.*—Temperatures between these two limits were recorded in the laboratory during the greater part of April, 1915, during which time several cultures in agar or in faeces were left in the room, but exposed to only a weak diffused light. By the 2nd day, a lew larvae in the agar cultures were in the advanced second stage, but were rather poor. In cultures in faeces 80 per cent, of the larvae were in the advanced second stage, and were rich in granulations. After three days a very few mature larvae were occasionally seen in agar, whilst in the faeces 40 per cent, of the larvae were usually mature. On the 4th day many larvae had migrated from the faeces on to the walls of the jar, but they were not so numerous as in the case of cultures kept at 25° C.

From these notes it can be concluded that the larvae of *Haemonchus contortus* can grow without showing any marked variations between temperatures of 22°-35° C. These two figures, however, represent fairly well-defined limits, since at 20° C. the migration of the mature larvae on

to the walls of the culture jar is less rapid and at 37° C. they frequently seem to suffer from over-stimulation and exhaustion by loss of their granulations.

Low Temperatures, 15°-18° *C.*—Temperatures between these figures were frequently recorded in the laboratory during May, 1915. Infected faeces slightly spread out in water and kept in diffused light showed the following progress in development after a lapse of three days—expressed as percentage of uninjured eggs originally present.

Eggs in the tadpole stage 10.
Larvae in the first stage 10.
Larvae just past the first lethargus 40.
Larvae in advanced second stage 40.

After four days no larvae were detected on the walls of the culture glasses, but two days later a few larvae had reached maturity, and were found ascending.

15° *C.*—Infected faeces kept in glass boxes showed the following development after four days :—

20 per cent, eggs in the tadpole stage;
50 per cent, larvae in the first stage ;
30 per cent, larvae in the second stage and rich in granulations.

After eight days numbers of mature larvae were seen crawling on the walls of the glass.

At a temperature of 15° C, occasionally descending to 13° C, a culture of faeces kept in a glass box showed on the 12th day a few larvae crawling up the walls.

8° *C*-—Infected faeces placed in a rectangular glass vessel were stored in a dark cold store-room. *On the 10th day* all the eggs were in the morula and in the tadpole stage. A part of the faeces was put up for culture, and after three days 30 cent, of the eggs were found to be dead, while 60 per cent, developed. *On the 18th day* and again on the *21st day,* examination showed that 70 per cent, of the original eggs were dead, while 30 per cent, had developed to larvae in the second stage. These latter were apparently dormant, but gradually awakened under the microscope. From this time onwards the temperature in the cold storage changed, and the observation was discontinued.

4° *C.*—Infected faeces showing eggs in the morula and tadpole stage were collected, placed in glass boxes, and stored in a cool room, at a constant temperature of 4° *C.* On examining a specimen lot after forty days, eggs were found at the same stage as when stored. Cultivation of these was then attempted, but after ten days of observation no hatching embryos or developing eggs were detected. On the 50th day the remaining faeces were placed in an incubator, but no progress in the development of the eggs could be detected on subsequent observation.

0° *C.*—To test the resistance of eggs at this temperature, some observations were made by putting a layer of infected droppings between two

blocks of ice. After varying intervals the faeces were put up for culture and four days later the following results were noted :—

20 *hours' exposure.* A few eggs were found dead, but the majority developed.

22 *hours' exposure.* Culture showed 90 per cent, mature larvae and 10 per cent, dead eggs.

24 *hours' exposure.* Culture showed 50 per cent, of mature larvae and 50 per cent, of dead eggs.

36 *hours' exposure.* Only two incompletely developed larvae hatched out.

48 *hours' exposure.* Only dead eggs were found.

Mature Larvae. Pieces of blotting paper were soaked in water containing mature larvae and afterwards placed in a Petri dish. The Petri dish was stored in the refrigerator between two blocks of ice.

After 1 *month's exposure.* 80 per cent, of larvae showed movements as soon as they were placed under the microscope. The remainder were dead.

After 3½*months' exposure.* On immediate examination the larvae were motionless. Examination of the same lot 12 hours later showed 60 per cent, to be living.

After 4½ *months' exposure.* An examination made 12 hours after the larvae were picked out showed 30 per cent, to be living. 70 per cent, were dead, all of which had large vacuoles in the cells of the chyle intestine.

After 6 *months' exposure.* An examination made 24 hours after removal showed 5-6 per cent, of living larvae with the cells of the chyle intestine poor in granulations and with large vacuoles.

After 7 months' exposure. The few remaining larvae in the Petri dish were dead.

-2 *to* -3° *C.*—Observations were made by exposing infected faeces in the field during the winter time. During the course of five days' observation, a temperature of -2° C. was registered for three nights and a temperature of -3° C. for the remaining two nights. These temperatures remained fairly constant for six hours. Cultures were made from the faeces after exposure, and kept at 25° C. A fair number of mature larvae were detected three days later.

-9.5° *C.*—This temperature was also recorded in the field during the night time, and presumably lasted for some hours. Cultures made subsequently at 25° C. showed the presence of mature larvae within four days.

Temperatures Alternating from Below Normal to Normal.—In the field the larvae are rarely subjected to constant temperature for any-length of time. During the South African summer the daily maximum

is unduly high, while the night temperature closely approximates that best suited to larval development. During the winter the daily temperature approximates the optimun, but the evening temperature is unfavourably low. A few observations upon the influence of alternating temperatures are therefore of interest.

Larvae at the First and Second Stage Exposed to a Sudden Decrease of Temperature.—A quantity of faeces from one infected sheep was divided into three batches, and each batch cultivated in pure faeces and in agar. These six cultures were placed at 24°-25° C. The following day larvae in the first and second stage were found to be present. One batch was then placed at 0° C, the second one at 4° C, and the third at 10° C. Twenty-four hours later the cultures were removed and again kept at 24°-25° C. The results obtained on the two succeeding days of observation are summarized in the following table :—

Day of Observation.	Cultures at 0° C.	Cultures at 4° C.	Cultures at 10° C.
1st day after exposure	*Agar*—Few larvae in second stage	*Agar*—70 per cent. larvae in second stage, not well developed	*Agar*—70 per cent. larvae in second stage, well developed.
	Faeces—20 per cent. larvae in second stage	*Faeces*—70 per cent. larvae in second stage, well developed	*Faeces*—80 per cent. larvae in second stage, 5 per cent. larvae mature.
	In both cultures numerous larvae dead in first stage	Larvae dead in first stage in both cultures.	
2nd day after exposure	*Agar*—No mature larvae	*Agar*—No mature larvae	*Agar*—Some mature larvae.
	Faeces—Few mature larvae	*Faeces*—Few mature larvae	*Faeces*—Numerous mature larvae.

Conclusions.—No cultures died from exposure. In exceptional cases the larvae reached maturity in the usual time of three days, but on the 4th day larvae were found as usual on the walls of the glass in all three lots. In general, therefore, both eggs and larvae of *Haemonchus contortus* seem to resist sudden decrease of temperature, although larvae in the first stage seem to be peculiarly susceptible and numbers of them die.

Daily Decrease of Temperature during Development of Eggs and Larvae.—A similar experiment with three batches was carried out, but instead of a single continuous exposure of 24 hours, an intermittent exposure of 6 hours

was given on three successive days, with storage at 25° C. in the 18-hour intervals. Results :—

Day after Collection.	Cultures at 0° C.	Cultures at 4° C.	Cultures at 10° C.
1st day	*Faeces*—Few larvae in first stage	*Faeces*—80 per cent. larvae in first stage, very few in second stage	*Faeces*—80 per cent. larvae in first stage, 10 per cent. in second stage.
2nd day	*Agar*—Few larvae in second stage	*Agar*—70 per cent. larvae in second stage	*Agar*—80 per cent. larvae in second stage.
	Faeces—70 per cent. larvae in second stage	*Faeces*—90 per cent. larvae in second stage	*Faeces*—70 per cent. larvae in second stage, 20 per cent. in second lethargus.
3rd day	*Faeces*—Few larvae in second lethargus	*Faeces*—40 per cent. mature	*Faeces*—70 per cent. larvae mature.
4th day	*Faeces*—Some mature larvae	*Faeces*—70 per cent. mature larvae	*Faeces*—90 per cent. mature larvae.

Conclusions.—In cultures kept between 4° C. and 10° C. the departure from normal development is small, but marked differences are noticeable in the cultures kept at 0° C. Young larvae exposed for prolonged periods suffer to a greater extent than those exposed for a short period daily.

Trials were also carried out with agar cultures alternating daily, for six days, between 28° and 10° and between 28° and 0°. In the first case the proportion of larvae subsequently developing at 25° C. was relatively high, but in the second case only two or three larvae were found alive. In consideration of the fact that development at low temperatures is more favourable in faeces than in agar, it is safe to assume that the results would have been better in faecal medium and that even at the lower range the larvae would grow although the percentage reaching maturity might be small. In these experiments a transition period at 15° C. was allowed in transferring cultures from 0°-4° C. to 25° C. In the field the slower and more gradual transition from maximum to the minimum of temperature would favour the easier development of the larvae.

Field Experiments.

Cultures at alternating temperatures were also attempted in the field under different climatical conditions, eliminating as far as possible the action of dryness and light.

(1) Maximum 56° C.—minimum 12° C.

This case has already been detailed under the heading " High Temperatures—Observations in the Field."

(2) Maximum 26° C—minimum 15° C. On the 15/3/15 faeces from the same sheep as used in experiment (1) were placed amongst grass under the shade of a tree. The culture was covered during the day time, but left uncovered during the night time, and was constantly kept moist. In the evening of the 4th day mature larvae were found crawling on the grass. The temperature by day provided optimal conditions, but the low temperature at night retarded slightly the migration of the larvae on to the grass.

(3) Maximum 26° C— minimum 12° C. On the 26/3/15 a culture was placed under conditions similar to (2). The maximum temperature recorded during the next six days was 26° C, whilst at night time the following figures were recorded as minima :—12 -5°, 15°, 14°, 13°, 12.5°, 12°, and 12.3° C. On the 4th day the most advanced larvae were still in the second stage, whilst on the 6th day 10 per cent, had reached maturity, and only on the 7th day were the larvae found crawling on to the grass.

(4) Maximum 26° C—minimum 4° C. On the 15/4/15 a culture was placed under the same conditions as before. The temperature on the first night of exposure fell to a minimum of 8.5° C, and only slight variations were recorded in the following ten nights. The first larvae to hatch were only detected on the evening of the 2nd day ; by the following evening 90 per cent, were found in the beginning of the second stage. On the 10th day 10 per cent, of the larvae were mature, and some were crawling on to the grass. 30 per cent, of the larvae were found dead in the first and second stage. From the 10th to the 15th day the culture was allowed to lie uncovered and not watered, the minimum temperature recorded during the night time being 4° C. Per hundred examined, 50 were now found as dead larvae of the first and second stage, 40 were alive in the first and second stage, and the remaining 10 had reached maturity.

It appears, however, that with a minimum in temperature of 8° C. the development to maturity is retarded until the 10th day, whilst the mortality amongst larvae is fairly high. The minimum of 4° C, combined with the progressive dryness, practically stops development of the larvae, and about one-half of the larvae die. The first portion of this experiment differs noticeably from the results obtained from the laboratory experiments referred to above, where the cultures were exposed daily to a temperature of 4° C. and 10° C.

(5) Maximum temperature 26° C, and minimum temperature —8° to —10° C. On the 11/6/15 infected faeces were placed in the open, amongst dry grass 10 cm. high and not very thick, generally resembling that found in the veld grazed on by cattle during the winter time. The culture was constantly kept moist, covered during the day time by a large glass bell and protected from the direct sunlight. Between sunset and sunrise the culture was uncovered. From the 11th to 13th June, the minimum temperature recorded during the night was — 7° C, whilst the maximum under the glass bell during the day time was 26° C. On the 13th June, the culture showed eggs in the morula and embryo stage. In a sample placed

in culture at 35° C, the larvae reached maturity within three days. From the 13th to 17th June the minimum temperature was — 5° C. to — 7° C. On the 17th June a sample culture showed 50 per cent, of eggs unhatched, the remainder being larvae of the first and second stages. On the same day faeces of the same batch, placed near the above culture, on the 11th June (but left unprotected during the day time and not watered) were found not completely dry, but did not show larvae. A sample of the latter faeces cultivated at 35° C gave negative results during the three days of observation. From the 17th to the 23rd June the minimum night record varied from — 5° C. to — 8° C, the latter only being recorded on one night. On the 23rd June 90 per cent, of eggs had hatched, the larvae having reached the end of the second stage and being rich in granulations. Up to the 28th June the minimum temperature varied from — 6° C. to — 8.5° C, but on this latter date no larvae were found on the grass. On the more exposed pellets a few eggs containing embryos were found dead. 40 per cent, of the lot were dead larvae in the second stage, and 60 per cent, were living larvae in the second stage, but were not rich in granulations. No mature larvae were found. Up to the 5th of July the minimum temperature during the night was about —6° C, except for one night, in which —10° C. was recorded. On the 5th July very lew larvae were found in the faeces, and it may be that the larvae found shelter in the ground. On the 8th July, after a rain of twenty-four hours and with a temperature of 10° C, a number of larvae in the second stage were found in the faeces, and six mature larvae were found on two grass blades. On the 15th July, after a night of rain and with a temperature of 14° C, eighty mature larvae were found on three grass blades.

Temperatures in the Field.—In considering first of all the high temperatures, the maximum recorded in South Africa under the conditions of cloudy, moist weather cannot be considered to either prevent the development or to kill the larvae of *Haemonchus contortus.* In sunny weather when the ground is moist, the first observation to be recorded is that larvae lying in wet earth or in faeces are not necessarily exposed to the same maximum of temperature of the atmosphere. On the 17/3/15 I examined a culture in the field that had been kept covered with a glass bell at a temperature of 52° C, whilst at a depth of 3 cm. between the faeces and the earth the temperature was 44° C. On the 26/6/15 the temperature was 43° C. in the sun, whilst in wet cotton wool kept in a glass box the temperature was 22° C.

The second observation, referring to effect of moisture of the soil in sunny weather, is that in moist surroundings the larvae are able to escape from the direct sunlight and reach shelter in cooler places. I will refer to this point again shortly. It must be added that notwithstanding the above instinct of seeking for shelter, numbers of larvae are killed in the first stage, so that it appears that the migratory instinct at that stage has still only a slight effect. It seems to be justified to deduce that in hot weather and on moist veld a high temperature does not have much effect on the larvae of *Haemonchus contortus* when in the second stage. With regard to the eggs, the hot moist temperature of the veld hastens the hatching of the embryo, but if the temperature of the ground rises above

42° C. and remains at that point for several hours, the eggs are killed. If the air and earth are dry, the eggs are killed by temperatures higher than 42° C. This conclusion is made under the supposition that only the temperature was responsible, but in practice it will be found that complete reliance cannot be placed on this factor. Under natural conditions mature larvae may be exposed in dry weather to direct contact with the atmosphere, or they may be sheltered in faeces or in earth. In the first case individual larvae that have crawled on to grass stems will naturally be-subjected partially to the action of the temperature and to the more direct action of the sunlight. The influence of temperature on larvae is subordinated to the action of sunlight to such an extent that no deduction could be drawn from the experiments just referred to.

For reasons which will be discussed later, I may state here, that when in dry weather the temperature reaches about 42°-45° C, the larvae exposed on the dry grasses are killed within ten days. In the second case where the larvae are sheltered, their resistance increases against heat of the sun. One observation on this point was reported when discussing the resistance of larvae to a temperature of 50° C, but more observations are wanted. In hot, dry weather, however, the temperature causes such marked desiccation of the faeces and of the superficial layers of the ground that deductions as to the action of the temperature alone are not possible. It can only be said that the high temperatures experienced in the veld during the summer time undoubtedly help to kill a large number of larvae, but at the same time many of these larvae can be killed before the maximum temperature has been reached. Finally, it may be stated that the average summer temperature of South Africa is very favourable for the development of the larvae of *Haemonchus contortus*.

Concerning the low temperatures experienced in South Africa, it may be said that the normal spring and winter temperatures do not prevent the development of the eggs or larvae, but prolong the period required before the larvae reach maturity. The resistance to adverse conditions of eggs and larvae and their ability to develop, considered in relation to the effects of temperature, in the winter season of South Africa, depend chiefly on the absence of uninterrupted spells of low temperature ; the minimum, even if occasionally very low, only lasts for a short time during the night, whilst in the day time the average is the optimum temperature required for the growth of larvae. The above considerations apply to an even greater extent to the preservation of mature larvae, as in working with the resistance of eggs and larvae I came to the conclusion that in a moderate, cold ambient the larvae remain in a better state of preservation and for a longer period than in warm weather.

During the three years in which these investigations were undertaken, I could not detect in any instance larvae that were killed as a result of exposure to cold weather in the veld, if they were allowed to find shelter either in faeces or in the soil. Unprotected larvae would not survive the winter season for reasons other than cold, and though they might be sheltered in pellets lying on the surface of the ground, yet this would not be sufficient protection. The possibility of the mature larvae reaching the host is practically excluded in winter time, owing to the dry weather, during which the larvae cannot travel. Observation No. 5 in the field

under the heading of " Low Temperatures," shows that on a rainy day in winter time the infection of sheep is feasible.

Moisture.

The conditions of moisture under which eggs and larvae are found in faeces will be dealt with under the headings of Moist," " Wet," and " Soaked Faeces."

Moist Faeces.—Faeces contain a sufficient amount of moisture to preserve the same consistency as when passed by a healthy sheep. This is the case when fresh droppings are placed in a glass vessel, or when dry droppings are artificially moistened with water, or in the field naturally by rain.

Under any of the above conditions the growth of the larvae is normal. They represent the optimum condition of moisture for the development of larvae.

Wet Faeces.—The moisture reduces the faeces to a pulp. As in the case of diarrhoeic faeces, mentioned previously, the eggs do not hatch or the larvae die shortly after hatching. The failure is due either to the presence of toxines or the density of the medium produced by the moisture. Attempts at cultures in pellets infected with numerous eggs and mixed with a fair amount of water to reduce them to the consistency of pulp always failed. This manipulation reduces or destroys the porosity of the faeces and thus prevents the circulation of air ; consequently the insufficient supply of oxygen and the fermentation of the medium are noxious for the growth of the larvae. In the field these conditions are practically never met with, because even in the case of prolonged rain the necessary porosity and thus the air supply is always maintained, allowing the growth of larvae.

Soaked Faeces.—The eggs or larvae are completely immersed in water In this case also the layer of water necessary to cover the pellets is too thick to allow of either the required air supply on of the escape of the products of fermentation. Consequently the eggs and larvae die as is the case in the pultaceous media. The same may be said of the eggs that are washed out from faeces and transferred to a liquid medium, in which case the thickness of the layer of water must be taken into consideration. The following experiments were carried out in connection therewith.

(1) In a series of six glass tubes, with a diameter of 2 cm., eggs in the morula and tadpole stage obtained from different sheep were placed at the bottom and covered with 2 cm. of liquid medium. The tubes were left in a slightly diffused light, at a temperature of 24° C. to 30° C.

On the 7th day no progress was noted in the eggs, when a number of *eggs* were transferred to agar culture. After three days some larvae were found in the first stage in the agar cultures, and after six days very few larvae had reached maturity. The majority of eggs and larvae at the first stage were dead. The remainder of the eggs in the glass tube did not show any development in further examinations after several days and were found dead.

(2) Another series of cultures in six glass tubes, with a diameter of 2.5 cm. and a depth of water of 2 cm. were kept under conditions similar to those in the previous experiment, and gave the following results :—On the 4th day larvae in the first stage were found ; on the 7th day numerous. larvae in the first stage were found ; on the 10th day 80 per cent, of larvae in the first stage were found dead.

On the supposition that the smallness of the diameter of the tube prevented a sufficient change of air in the culture, I made the following experiments :—

Eggs were placed under the same conditions as before, but the liquid medium was 8 cm. high, with a tube diameter of 9 cm.

On the 4th day nearly all the eggs had hatched, and the larvae had reached the first stage.

On the 7th day the larvae were still in the first stage, and 50 per cent, had died. The remainder were extremely poor in granulations. Some of these larvae were placed in agar cultures, and three days later 50 per cent, had reached maturity; the remainder had died.

On the 10th day the larvae still present in the liquid medium had died.

In further experiments the thickness of the water was reduced to 2 cm. and the diameter increased to 11 cm., but fifteen days later all the larvae were found dead in the first stage, and extremely poor in granulations.

Another series of observations was made with liquid cultures 1.5 cm., 1 cm., and 0.5 cm. deep, with a tube diameter of 11 cm., and kept at a temperature of 25° C. On the 3rd day 20-30 per cent, of eggs were found unhatched, and the remaining 70-80 per cent, were free larvae in the first stage, the majority being motionless. On the 10th day very few larvae had reached maturity in the culture 0.5 cm. deep, and were poor ; the remainder had died in the first stage. When the liquid medium was only 1-3 mm. high, the larvae hatched and grew in the same time as in faeces.

I used these liquid cultures for three years, chiefly in dealing with the stages and structural changes of larvae in the outer world, and the method and results are more extensively dealt with under " Cultures of the Larvae."

If the temperature is lower than " normal," the liquid cultures do not show such rapid progress, and the larvae are poorer than in cultures in faeces made at the same temperature.

Conclusion.—In liquid medium kept immobile and deeper than ½cm., a rather small percentage of the eggs hatch after four days, but the larvae make very little progress, and within twelve to fifteen days death occurs, presumably owing to lack of air. The growth of the larvae is possible and normal when the liquid medium is in a thin layer of a few millimetres.

Mature Larvae in Liquid Medium.—In this connection the following experiments were recorded :—

(1) Tap water, not changed, kept in a diffused light at a temperature of 28°-32° C.

On the 21/10/13 young mature larvae were immersed in water 3 cm. deep in an evaporating dish. All larvae were moving quickly. On the 22/10/13, 50 per cent, of the larvae were coiled up at the bottom of the dish, the remaining 50 per cent, were still swimming. On the 25/10/13,

90 per cent, of the larvae were coiled up ; 10 per cent, were stretched out and dead. On the 25/11/13 the conditions were unchanged, but two minutes after being placed under the binocular microscope, the larvae which were coiled up began swimming.

(2) Larvae kept under similar conditions to those in the previous experiment, but in river water. The average temperature was about 32° C. during the first three months, and 25°-28° C. during the remainder.

On the 9/11/13 the larvae were immersed in water. On the 20/12/13, 5-10 per cent, were stretched out dead, with big vacuoles in the chyle intestinal cells. The remainder were coiled up, and awakened after five seconds' exposure to light under the binocular microscope. On the 9/1/14 there was no change. On the 3/3/14, 80 per cent, were still coiled up, showing slight movements after two to three seconds' examination under the binocular microscope. On the 4/4/14, 50 per cent, were still coiled up, but they awakened when exposed to light. The remainder had undergone the second ecdysis, and were stretched out and dead, but did not show any remarkable internal structural changes. Numerous outer envelopes were present in the water. On the 20/4/14, 5 per cent, were still coiled up; the remaining 95 per cent, had passed the second ecdysis and were dead.

(3) The larvae were placed in a graduated tube, with a diameter of 6 cm., containing tap water 20 cm. deep. The average temperature was 28° C. during the first month, decreasing later to 19°-20° C.

On the 7/4/15 the larvae were placed in water. On the 8/4/15 the majority of larvae were moving at the bottom of the tube. On the 11/4/15 to the naked eye the mass of larvae appeared motionless. On the 11/5/15 about 10 per cent, were dead, with the outer envelope present, and large vacuoles in the chyle intestine; the remainder showed swimming movements. Some of them had passed the second ecdysis, were swimming, and the internal structure had not changed ; they were rich in granulations. On the 18/6/15, 90 per cent, of larvae were without the outer skin, coiled up, and awakened under the light of the microscope. Only 2-3 per cent, of the larvae without capsules were dead ; a few larvae with the outer skins were also dead. On the 15/7/15, 90 per cent, of larvae were without the outer skins ; only 5 per cent, of these were dead. Some larvae were allowed to dry on a glass slide, and after twenty-four hours a little water was added. Six hours later all the larvae without the outer skins were dead, and only a few with the outer skins were alive. On the 20/8/15, 70 per cent, of larvae were alive, and of them 90 per cent, lacked the outer skin. Of the dead larvae more than half were without skins. On the 9/9/15, 50 per cent, of larvae were alive ; 95 per cent, of the living larvae were without the outer skin.

From the above observations, it appears that in water mature larvae of *Haemonchus contortus* find a suitable medium for preservation. The small percentage of deaths occurring during the first few days is also observed in other media. In the following days the larvae are observed to be coiled up ; they keep practically motionless, and are rich in granulations and well preserved. From the first month onwards the larvae undergo the second ecdysis. A large percentage of larvae die during the fourth month of preservation, and a small percentage live up to the fifth month.

Taking into consideration the pools of water in the field, it appears that the mature larvae in these reservoirs find a good ambient for their preservation, and to a certain extent a favourable place for infecting the host. In fact, larvae present in pools 20 cm. deep are preserved when lying at the bottom, or when sheltered under the more superficial particles, where they are not hurt by light. As it will be seen later, in dealing with the action of currents in the water under the effect of temperature, changes take place, by which the larvae are able to reach the surface of the water and can be taken up by the sheep. Infection of sheep also takes place when a flock stirs up the water and brings the larvae to the surface. These points go to show that the infection of sheep by infected pools is feasible.

Behaviour of Mature Larvae in Moist and Liquid Media.—On the 11/7/15 a large number of young mature larvae, in pellets of faeces mixed with charcoal, were heaped up in a glass dish containing water 1 cm. deep and kept in a diffused light. At the same time, by means of a piece of blotting paper, numbers of larvae were collected from the walls of the jar containing the above culture. The blotting paper was placed against the internal walls of a test tube, with the lower portion immersed in the water and the test tube was kept in a cupboard. On the 18/7/15 a large number of living larvae were found in the water, whilst in the pellets only a very few were found.

In the test tube half the larvae were found in the water, the other half coiled up on the blotting paper.

In conclusion, it can be considered that mature larvae prefer pure water to faeces, probably owing to the fact that mature larvae usually leave the faeces when they have reached maturity. From the experiment with the blotting paper, however, it appears that the larvae have no decided preference for water when another moist suitable medium is available. Some similar observations are recorded on page 395.

Desiccation of the Larvae.—The removal of water from the larvae can be performed in an effective manner by submitting the larvae to the action of a desiccator. Personally I used calcium chloride as an absorbent, as this chemical compound removes the moisture in a gradual way without hurting the vitality of the larvae too much. It is admitted in chemistry that after the action of the calcium chloride desiccator 2-3 per cent, of moisture is still present. It stands to reason that a higher percentage of water remains in the tissues of the larvae, which are well protected by a double chitinous envelope.

A second method for the mechanical removal of water from the larvae is to expose them to the free air. This is the method which is most similar to the conditions under which free larvae exist. In order to distinguish between larvae kept in the desiccator and those exposed to free air, the latter will be termed " Dry larvae," while the former will be referred to as " Desiccated larvae."

Some observations were made in the laboratory and some on the veranda, where the conditions were practically the same as in natural conditions under trees or in any other shaded spot. Some observations were also made under trees in the open.

The larvae were exposed on a glass slide in faeces or on grass.

In the following paragraphs are included the experiments in which dryness played a predominant role. It was ascertained in the course of these investigations, that the resistance of larvae to the withdrawal of moisture varies according to whether they are exposed singly or in clusters or whether they are sheltered in or under some solid substance, and these facts have been taken into consideration in the general arrangement of the experiments now detailed.

The Appearance of Dead and Living Larvae under the Process of Desiccation.—I was unable to define clearly the difference between the two above kinds of larvae, notwithstanding that the following peculiarity appeared to be constant: In the living larva the chyle intestine is well-defined and the granulations of the cells have a glistening silvery appearance. In the dead larva the edges of the chyle intestine are rather indistinct, the granulations are very fine and slightly yellowish. If the larvae are again immersed in water, the living ones clearly show the internal structure with a few small vacuoles in the chyle intestinal cells. In the dead larvae the internal structure has nearly disappeared and the chyle intestinal cells contain numerous large vacuoles. It appears that larvae are killed by desiccation as soon as the granulations stored in the cells of the chyle intestine are exhausted.

Mechanism of Desiccation in the Larval Body.—In following the process of desiccation under high magnification, I observed that each layer, anatomically distinct in the larva (outer skin, inner skin, muscular coat, etc.), becomes contracted and collapses on to the inner one, giving the larva a wrinkled appearance. Consequently, during the process of drying the resistance of the tissues increases as each contracting layer becomes more impervious to moisture. The desiccation of the chyle intestinal cells, which seems the most fatal process, is thus prevented by the external contracted layers of tissues, furthermore a certain amount of fat is present in the protoplasm of the intestinal cells which evidently retards the desiccation of the same. This fatty substance is easily seen under the microscope in breaking a fresh larva on a glass slide in a drop of water, when numerous small particles of fat can be seen scattered about. In examining transverse sections of fresh larvae, the fat drops are frequently seen, sometimes being so numerous or so large that they interfere with the examination of the section.

Eggs and Immature Larvae exposed in Faeces of the Host.

(1) On the morning of the 22/6/15, infected faeces collected in a bag during the night were placed in a current of air to dry, and during the following night were kept in a desiccator. Some faeces collected from the rectum of the same sheep were at the same time placed in a room and some on the veranda.

From cultures made from the three lots of faeces in the days hereafter noted, the following results were obtained three days after inoculation :

On the 25/6/15 in faeces from the desiccator 2-3 larvae for every 100 dead eggs were found. On the 26/6/15, in faeces from the desiccator 2-3 larvae for every 100 dead eggs were found. On the 29/6/15, in faeces

from the desiccator 2-3 larvae in the whole culture were found. In faeces in the room and on the veranda all eggs were dead. On the 5/7/15 and 10/7/15, the three lots of faeces gave negative results on culture.

NOTE.—The sheep from which the faeces were collected was sheltered in a stable during the night, and the faeces found in the morning were slightly warm. The temperature during the first day of exposure was from 10°-12° C.

(2) On the morning of the 4/7/15 infected faeces were removed from the rectum of a sheep and placed in a draught to dry. In the evening they were divided into three lots ; one lot being placed in the desiccator, another on the veranda, and the third one left in the room. On the 6/7/15, a culture was made from each lot of faeces, with negative results. Negative results were also obtained from cultures made on the 10/7/15. The temperature during the 1st day after collection was below 15° C, and the air was very dry.

(3) On the 15/4/15 infected faeces dropped during the previous evening and still containing a fair amount of moisture were placed in a desiccator.

A culture, made eight days after, gave a fair positive result, numerous eggs and larvae of the first stage being dead, and some mature larvae were alive.

On the 23rd day a culture was made and very few larvae were found. One month later very few larvae were present in the culture.

NOTE.—The temperature of the room in which the faeces were kept from the 14th to the 15th was about 16° C.

(4) Another experiment on the desiccation of eggs was undertaken, which I will provisionally group with the above one.

On the 8/5/15 infected faeces collected from the rectum were placed in a desiccator without having been previously dried in the air. The temperature in the room was 19° C. For twelve hours the internal walls of the desiccator appeared to be foggy, probably owing to the fact that at that temperature the moisture was not absorbed quickly enough by the calcium chloride.

Cultures were made from this sample of faeces on the 2nd, 8th, and 16th days of desiccation and gave positive results.

(5) Eggs in the embryonic stage.

On the 26/3/15 faeces containing eggs with embryos measuring two to three times the length of the egg were placed on the veranda where the rays of the sun could not reach them. On the 6th day the pellets were quite dry and some of these were placed in water, when they were found to contain a fair amount of dead larvae in the first stage and some in the beginning of the second, which had hatched during the drying up of the faeces. A culture of the same faeces contained 50 per cent mature living larvae. Some more cultures were made in the following days, and on the 10th day 15-20 per cent, of mature larvae were found. By the 15th day there was only 1 per cent, of mature larvae and on the 20th day only 1-2 larvae could be seen in the whole culture. On the 25th and 28th days the culture was negative.

During the twenty days of observation there were only ten fine days-; the remainder were either cloudy or rain fell for a half or whole day. The

minimum temperature was 8°-12° C. at night, and the maximum 35°-40° C. in the sun.

Similar experiments to No. 5 were carried out during the same season by exposing infected faeces on the veranda, the result being that on the 20th day practically all the eggs were killed.

Conclusions.—In the first experiment the eggs in the faeces passed during the night had time to undergo development and some reached the embryonic stage before being exposed to the current of air. The same observation can be made on the eggs used for experiment No. 3. In the fourth experiment the eggs placed in the desiccator had also time to undergo development, as in the first twenty-four hours the action of desiccation was apparently weak. In the fifth experiment, the eggs were allowed to undergo embryonal development before being exposed to dryness. From the figures quoted it appears that in the above cases the eggs showed greater resistance to dryness and desiccation than either the eggs used in the second part of No. 1 experiment or in No. 2 experiment, where the infected faeces were exposed to dryness immediately after being removed from the rectum. In the two last cases the eggs presumably did not undergo development. It can be noted that Looss in working with Sclerostomes and Cylicostomes found that eggs with mature embryos resisted desiccation better than eggs in which the embryos were not completely developed.

Exposure of Mature Larvae dried up on Glass Slide.—The experiment was started on the 7/4/15. Some slides were exposed in the room, and some on the veranda.

Table No. 1.

Date of Observation.	Room. (Number of Larvae still Alive.)	Veranda. (Number of Larvae still Alive.)
9/4/15	Homogeneously spread.....	95 per cent. swimming—rare vacuoles.
11/4/15		0·5 per cent. slow—numerous vacuoles.
12/4/15		All dead, with numerous vacuoles.
13/4/15		,, ,, ,,
14/4/15		,, ,, ,,
15/4/15	20 per cent. slow—small vacuoles present	—
19/4/15	10 per cent. slow—small vacuoles present	(In clusters) 2 per cent. swimming—few vacuoles.
24/4/15	All dead—numerous vacuoles....	—
28/4/15	—	(In clusters) all dead.

NOTES.—(1) In order to find whether the larvae were alive they were removed from the cultures and kept in water for twenty-four hours. (2) The large percentage of larvae still alive after two days' exposure on the veranda is due to the conditions of the weather. There was rain from the 7th to the 8th, and it was cloudy in the morning of the 8th. (3) From the above table it appears that larvae exposed to the open air were nearly all killed in four days, while in a room they were all killed after sixteen days. In the veranda few larvae were still alive after twelve days, being protected against dryness. Consequently in the above conditions, a

single isolated larva is killed by dryness, when clustered larvae are still alive.

Exposure of Larvae that had Crawled up on to dry Grass.—The experiment was started on the 17/4/15. The larvae were exposed in a room and **on** the veranda; at the time of exposure they were ten days old.

Table No. 2.

Date of Exposure.	Larvae still Alive in the Room.	Larvae still Alive on the Veranda.
21/4/15	—	0·5 per cent. swimming—few vacuoles
23/4/15	—	0·5 per cent. slow—numerous vacuoles
25/4/15	Superficial (1) 2 per cent. slow—vacuoles. Clusters, 15 per cent. swimming	—
26/4/15	10 per cent. swimming.....	—
30/4/15	2–3 per cent. slow—vacuoles.....	—
5/5/15	All dead.....	—

A piece of grass blade exposed in the room was immersed in water for a few seconds. Afterwards the grass was placed in another lot of water and left for twenty-four hours. It is likely that in the first lot the superficial larvae only became detached, whereas in the second lot the clusters were freed.

Comparative Result from Exposure of Larvae in a Room, on the Veranda, and in the Desiccator.—The experiment was started on the 24/4/15 with larvae on glass slides and on dry grass.

Table No. 3.

Date of Exposure.	Larvae still Alive in the Room.	Larvae still Alive on the Veranda.	Larvae still Alive in the Desiccator.
25/4/15	*Glass*—2–3 per cent., swimming	*Glass*—1 per cent., few vacuoles present	*Grass*—30 per cent.
	Grass—20 per cent., swimming	*Grass*—1–2 per cent., few vacuoles present	—
26/4/15	*Glass*—6 per cent., vacuoles numerous	*Glass*—0·5 per cent. of scattered larvae	*Grass*—10 per cent., slow; vacuoles.
	Grass—10 per cent., slow; vacuoles numerous	*Grass*—1 per cent.....	—
28/4/15	*Glass*—0 per cent. of scattered larvae	*Glass*—0 per cent. of scattered larvae	—
	Grass—1–2 per cent., slow; vacuoles numerous	*Grass*—0 per cent.....	*Grass*—8–10 per cent., slow; vacuoles.
30/4/15	*Glass*—10 per cent. of larvae in clusters	*Glass*—0 per cent. of larvae in clusters	—
	Grass—0·5 per cent., slow; vacuoles	*Grass*—0 per cent.....	*Grass*—0·5 per cent., slow; vacuoles.
3/5/15	*Glass*—1–2 per cent. of larvae in clusters	*Glass*—0 per cent. of larvae in clusters	—
	Grass—1–2 per cent...	—	*Grass*—0 per cent.

NOTES.—The larvae used were five days old. The increase in the percentage of larvae on grass exposed in the room from 30th April to 5th May is possibly due to some clusters being present in the sample tested. The possibility is supported by the difference in living larvae exposed on glass slides in the room from the 28th to the 30th April, when the kinds were separated. For the daily temperature, see page 421.

Comparative Results from Exposure of Larvae in a Room, on the Veranda, in the Desiccator, and in Sunlight.—The experiment was started on the 24/6/15 with larvae on glass slides, grass blades, and in faeces.

Table No. 4.

Date of Exposure.	Larvae still Alive in the Room.	Larvae still Alive on the Veranda.	Larvae still Alive in the Desiccator.	Larvae still Alive in the Sun.
25/6/15	*Faeces..* 98 %	*Faeces....* 90 %	*Glass.....* 30 % *Grass.....* 90 % *Faeces....* 98 %	*Faeces....* 3 %
26/6/15	*Faeces....* 95 %	*Faeces....* 70 %	*Glass.....* 8 % *Grass.....* 50 % *Faeces....* 50 %	*Faeces....*1–2 %
28/6/15	*Faeces....* 90 %	*Faeces....* 70 %	*Glass....* 0·2 % *Grass....* 10 % *Faeces...* 10 %	*Faeces...* 1–2 %
30/6/15	*Faeces....* 50 %	*Faeces....* 30 %	*Glass....* 0·1 % *Grass....* 3–4 % *Faeces...* 2–3 %	*Faeces...* 1–2 %
8/7/15	*Faeces....* 40 %	*Faeces*, 20–30 %	*Glass....* 1 % *Grass....* 8 % *Faeces...* 0·4 %	*Faeces...* 0.2 %
16/7/15	*Faeces....* 15 %	*Faeces....* 5 %	*Glass....* 0 % *Grass....* 0 % *Faeces...* 0·1 %	*Faeces...* 0·2 %

In comparing the results obtained in experiments Nós. 1 and 2, it appears that larvae resist dryness equally well on glass as on grass, which was not the result in experiment No. 3. This can be explained by the fact that during the second experiment the weather was very dry. (See page 145.)

In comparing the mortality of larvae in the three different experiments, an increase is noted from the first to the third experiment. This mortality seems to correspond to the weather condition prevailing at the time; during the period of the first experiment it rained on the first

two days ; during the second experiment the atmosphere was drier than normal, whilst during the third experiment the first two days, on which the mortality was very high, were warm and dry, with a strong wind blowing.

This observation helps to explain the apparent discrepancy between \arious observations. The resistance is due to the varying conditions of the ambient, to which the larvae are very sensitive.

In the third and fourth experiments the mortality amongst larvae in the desiccator was not as high as on the veranda, and during the first lew days was even lower than in the room. This fact is explained by the strong current of dry air acting on the larvae in the open in the Transvaal. In the desiccator the space is limited, ventilation is absent, calcium chloride acts rather slowly, and there is but little light present. The final result obtained in the desiccator consists of a minimum of moisture, which is never reached in the open air ; thus the big mortality of larvae in No. 3 ai d No. 4 experiments.

In comparing the third and fourth experiments, it is remarkable to note the low mortality of larvae exposed on grass in the desiccator as recorded in the fourth experiment. This observation illustrates the role played by the temperature in the phenomenon of desiccation, as the temperature decreased from the daily average of 25°-26° C. in the third experiment, to 10°-12° C. in the fourth experiment.

Observations on dryness in the field.

(1) On the 3/9/14 infected faeces were placed on the earth under a tree so as to be only reached by the rays of the sun for about two or three hours in the morning, and covered with a glass bell. On the 7/9/14 the majority of the larvae had reached maturity, and were mostly found in the earth immediately below the faeces. The glass bell was then removed, and the culture left exposed to the air. On the 24/6/15 several samples of faeces were examined, but no larvae were seen ; the earth just beneath the faeces was also examined with negative results.

(2) On the 8/9/14 two cultures were made under the same conditions as above, but were not covered by a glass bell; they were watered several times during the day. On the 12/9/14 the majority of larvae had reached maturity, and as before were found in the earth under the faeces. On the 24/6/15 no larvae were detected in the faeces.

(3) OB the 26/3/15 a culture was made under similar conditions to the above. On the 2/4/15 the majority had reached maturity, and the culture was allowed to dry. On the 22/6/15 50 per cent, larvae were found dead in the faeces, with pronounced vacuolation of the chyle intestinal cells. The remaining 50 per cent, were living, with very few or no vacuoles, and with the outer skin intact.

During the above period there was only a slight rainfall on the 7/4/15 and on the 1/5/15 which could not reach the faeces sheltered under the tree. From that date onwards the weather was dry.

(4) On the 15/4/15 another culture was made under similar conditions to the above. On the 30/4/15 10 per cent, of larvae had reached maturity, 40 per cent, having reached the first and second stage.

The results obtained in the above experiments were that a large number of larvae resisted the dry weather for a period of three months when sheltered from the sun, and that no living larvae were found in the same sheltered spot after nine months of exposure.

Do larvae prefer moisture or dryness ?—Bohemian beakers containing infected faeces were kept in specimen jars, at the bottom of which were placed thick layers of cotton wool soaked in water. The edges of the beaker were in contact with the inner walls of the jar. The specimen jar was covered with a glass slide. Some of these cultures were left on the veranda, and on examing them two or three months later a few dead larvae were found in the faeces. On the walls of the beaker and on the inner walls of the jar numerous dead larvae were seen. The greatest number of larvae were usually found in the water and on the cotton-wool, some being dead and some still living, with or without the outer skins.

It appears that a layer of water can act as a reservoir for mature larvae.

In gelatine plates used to expose larvae under high temperatures, the larvae were found in the lower layer, where the moisture was more abundant, when the surface of the gelatine was drying. The fact that larvae prefer moisture is shown by their good preservation in water.

In a number of experiments carried out in this laboratory mature larvae lived for five months in water, whilst in dry faeces in the same room eight days later 50 per cent, were dead, and in the desiccator the majority died within four days. The water appears to act as a protection against both sudden changes of the air and desiccation, and, if the layer of water is fairly thick, it protects the larvae from the influence of the sun.

The action of desiccation on mature larvae.—In regard to the action of desiccation on mature larvae, the following conclusions were arrived at :—

(1) The larvae of *Haemonchus contortus* do not resist complete desiccation, and die before this condition is reached, but under natural conditions they resist, (on account of their peculiar structure) the progress of desiccation for a comparatively long time.

(2) The larvae preferably remain in moist surroundings, and by migrating are in a position to seek shelter where the moisture is more constant.

(3) When dryness sets in the larvae gather in clusters, a peculiarity which can easily be observed under the microscope.

On examining a slide, on which larvae were spread in a thin layer of water, the larvae very soon commenced to collect in clusters. With the progress of evaporation of the water only isolated patches were seen surrounding the clusters of larvae. These clusters play an important role in the preservation of larvae in the field against drought; chiefly when the larvae are surprised on grass by the evaporation of dew or rain.

The laboratory experiments on dryness could not be directly applied in the field, owing to the fact that on the one hand the period of dryness in the field is interrupted during the night by the falling of dew, and on the other hand with dryness other important factors, such as temperature

and light, act on the larvae. Nevertheless, dryness plays an important role in connection with the destruction of eggs and larvae in the field.

Dryness during the summer time will be also alluded to in a series of experiments enumerated under the heading of "Light," these experiments being performed in the open and in which the absence of rain played an important part. In this experiment freshly passed infected faeces were placed amongst grass, during a period of drought, but at a time when dew was abundant. If the grass was not high enough the cultures were found to be negative, containing dead eggs and larvae at the first stage, and the pellets of faeces were found to be hard and dry. It appears consequently, that if there is no rain for two or three days very few mature larvae are found in infected faeces. It was also observed that two or three days of cloudy weather were not long enough for larvae to reach maturity, as the evaporation from the faeces takes place so rapidly that the eggs and young larvae are stopped in their development, and later are killed by the first two days of drought.

In winter, eggs and larvae hatch even at a very low temperature in the field if they are constantly kept moist. In this season eggs and larvae are killed chiefly by dryness, as was proved by control cultures carried out. at the same time both with faeces kept moist and with faeces drying naturally, the result of which was reported in the paragraph "Temperature." With regard to the manner in which the infection of *Haemonchus contortus* is preserved in the field in the winter time, it was seen that eggs in the faeces or mature larvae on the dry grass or on. faeces exposed to the air were killed by dryness in a comparatively short time.

It is therefore necessary to find an ambient in which larvae are able to live through the winter time, or through long periods of drought in summer time, and this ambient is found either in water or in the soil.

Water acting as a reservoir has been mentioned previously. The earth as a reservoir for larvae will be mentioned in the paragraph Geotropism of the Larvae."

Light.

The effect of light will be dealt with under the headings of *darkness, diffused sunlight,* and *direct sunlight.*

Darkness.—Eggs and larvae of *Haemonchus contortus* develop under conditions of light such as are observed during the night time or in an. incubator.

Diffused sunlight.—Cultures in faeces do not seem to be the most suitable for the purpose of studying the effect of diffused light on the growth of larvae. In order to demonstrate this point the following experiments were carried out:—

First Experiment.

On the 12/7/15 three cultures on agar were made with eggs washed out from faeces.

(1) One was placed on the veranda.
(2) One on the window table in a room.
(3) One placed in a cupboard.

(4) Some pellets of the same batch of faeces were placed in a glass dish (in which constant moisture was provided by means of a wet flake of cotton-wool) and kept in the room on the window table.

The average temperature in the room was 18° C, and on the veranda 22° C.

On the 13/7/15 at 5 p.m. the results noted were

Veranda : 30 per cent, of eggs had hatched.

Room : The eggs were in the last stages of development.

Cupboard : Some of the eggs had reached the tadpole and some the embryonic stage.

Faeces : On the surface of the pellets were found about 500 larvae in the second stage and in the centre of the pellets about 300 larvae in the second stage.

Another pellet, in which the larvae were probably as numerous as in the above-mentioned one, was placed in a moist glass dish exposed to the sunlight for half an hour, at the end of which time only 10 larvae were found on the surface.

On the 14/7/15 the results noted were :—

Veranda : The larvae were in the first stage and poor in granulations.

Room : The larvae were in the first stage and were poor in granulations.

Cupboard : Larvae in the first and second stages were found, rich in granulations.

On the 16/7/15 the results were as follows :—

Veranda : Poorly developed larvae in the second stage and poor in granulations were found.

Room : Ditto.

Cupboard : 5 per cent, of larvae completed the second stage, and were one-third longer than those on the veranda.

Faeces : All the larvae were in the second stage and well developed.

On the 21/7/15 the results were :—

Veranda : 70 per cent, of the larvae were dead.

Room : Ditto.

Cupboard : 90 per cent, of well developed larvae were at the end of the second stage, 5 per cent, were mature and living, and 5 per cent, were dead.

Faeces : Numerous larvae had reached maturity.

On the 25/7/15 the results were :—

Veranda : All larvae were dead.

Room : Ditto.

Cupboard: 20 per cent, were dead; the remainder had reached maturity. Some were crawling on the walls of the culture.

In the latter part of the experiment the average temperature in the room was 12°-14° C. at night and 16°-18° C. in the day time.

NOTES.—The culture exposed on the veranda was transferred to the room each night.

The number of larvae on the surface of the pellets was determined by soaking the pellet in water and afterwards counting the larvae remaining in the water.

The experiments were repeated several times, with similar results. The following is worthy of note :—

Second Experiment.

On the 4/8/15 a series of agar cultures was exposed, one culture on the veranda, one on the window table, one in a corner of the room where the light could be compared with that of a rainy day, and one on the window table covered with a porcelain dish.

On the 9/8/15 the results were :—

Veranda : All larvae were found dead in the first and second stages.

Window : 30 per cent, of the larvae were found in the first stage and 70 per cent, in the second stage in poor condition.

Corner of room : The larvae were all in the advanced second stage,, and were one-third longer than those in the window.

Under porcelain dish : Ditto.

NOTES.—In the first experiment the larvae on the veranda lived longer than those in the second experiment, probably owing to the fact that it was cloudy and raining for four days.

From the above experiment it appears that the larvae of *Hatmonchus contortus* do not reach maturity if exposed to a diffused bright light all day. If the diffused light is weak as is noticed in rainy weather, the larvae reach maturity equally as well as they do in darkness.

In faeces exposed to strong diffused light the larvae grow as usual.

It seems that the larvae pass a certain part of their developmental period on the surface of the pellets, when in diffused light.

Direct sunlight.—The action of the sun is a complex one, temperature, dryness, and light all coming into consideration. Some experiments were carried out, excluding as far as possible the action of the first two factors, whilst others were undertaken in the open under natural conditions.

The Action of Sunlight on Eggs and young larvae.—In order to remove every possibility the larvae had of finding shelter from the direct rays of the sun, the following experiment was carried out :—

27/6/15, 11 a.m.—Eggs in the morula stage, obtained by washing out infected faeces, were placed into four Petri dishes, two of them containing a thin layer of agar-agar medium, and two a layer of liquid medium (faeces decoction) a few mm. thick. An evaporating dish was filled with cotton-wool, soaked in water, and the four cultures were placed on the cotton-wool. The dish was exposed to direct sunlight and covered with a large glass bell to prevent evaporation taking place too quickly. The glass bell was raised a few cm., and by means of a glass tube cool water was circulated round the cultures when the temperature rose too high. A maximum and minimum thermometer was placed under the glass bell and a water thermometer immersed in the cotton wool.

Four control cultures, prepared in the same way, were placed in an incubator at 35° C. The maximum temperature of 43° C. under the glass bell was noted at 3 p.m., 25° C. was recorded in the cotton-wool and 32° C. in the open. At 5 p.m. the cultures were placed in an incubator.

28/6/15.—At 7 a.m. to note the influence of sunlight for one day only, two cultures were left in the incubator, whilst a gelatine and a liquid culture were exposed to the sunlight as on the previous day.

29/6/15.—At 7 a.m. on examining the different lots of cultures, the following results were noted :—

Culture exposed for two days to the sunlight.

50 per cent, dead eggs.

50 per cent, dead larvae, of which a few were in the second stage.

Culture exposed for one day to the sunlight.

50 per cent, dead eggs.

50 per cent, larvae in the second stage and were well developed.

Control cultures in incubator.

5 per cent, dead eggs.

95 per cent, larvae in the second stage and were well developed.

On the following day the larvae in the control cultures and the surviving larvae in the culture exposed for a day only had reached maturity.

30/6/15.—A second experiment was undertaken similar to the previous one, but to avoid evaporation from the cultures the glass bell was dispensed with, and from time to time small quantities of water were added, having the same temperature as the culture medium. The day was cloudy in the morning, and the temperature was about 20° C.

1/7/15.—The day was sunny; the cultures were placed in the sun, the maximum temperature in the sun being 40° C. and in the cotton-wool 24° C.

2/7/15.—The cultures exposed during the two previous days contained dead eggs and dead larvae in the first stage.

The control cultures contained living and well developed larvae in the second stage.

A number of other experiments with eggs in agar and liquid cultures were carried out in January, 1915.

The cultures were exposed to the sun at about 7 a.m., and supplied with the necessary amount of water from time to time.

Twice, in cultures started with eggs at the embryo stage, free embryos were found at sunset.

In a third instance the eggs hatched during the first night, and in four other instances the eggs were found to be granular and dead at sunset after the first day of exposure.

In the three instances in which living larvae were found after one day's exposure they were found to be dead after the second day of exposure.

In a series of experiments faeces, of sheep were used as a medium. The droppings were placed on moist, black turf, and sprinkled with water from time to time during the day. The experiment was carried out during different seasons of the year and always proved positive. In the summer time a rather large number of young larvae were found dead in the more superficial layers of the pellets, but the remainder reached maturity in the usual time

Observations on the above experiments.—The first two experiments made in winter (June) show that eggs exposed to the sunlight for about half a day during winter time, and kept at a suitable temperature, remain alive, but at the end of the second day of exposure the hatched larvae are invariably killed.

The experiments conducted in summer time show that eggs exposed for about a day to sunlight at a temperature kept artificially at normal are sometimes still living at sunset, but more frequently are dead, and the hatched larvae are invariably killed on the second day of exposure.

The action of sunlight on mature larvae.—On the 8/3/15, and on the 7/4/15, at 7 a.m., mature larvae from 10-12 days old were placed in Petri dishes with a layer of water 5 mm. deep. The Petri dishes were put on soaked cotton-wool, as mentioned previously, and were exposed to direct sunlight for a whole day.

They were examined the following day, and only 1 per cent, of the larvae were moving, the remainder being dead. After the second day of exposure no living larvae were found. The days of exposure were bright and warm, but the temperature in the water did not exceed 32° C.

On the 12/5/15, another lot of faeces was exposed under similar conditions as above for a number of days. The result was as follows :—

After the	1st	day of exposure	20 per cent, of larvae were dead.
	2nd	„	60
,,	3rd	,,	75 ,, ,, ,,
,,	4th	,,	1-2 per cent, were found slightly coiled up, but were not moving about.

The temperature during the day was frequently 46° C. in the sun, but the effect of the sunlight was evidently weaker than in summer time.

The Action of the Sun on the Eggs and Young Larvae naturally exposed in the Field.—Some experiments were carried out with infected faeces placed in the field and exposed to the action of the sun on the bare soil.

(1) On the 4/3/15, at 7 a.m., infected faeces were collected separately from three sheep. A culture was made from each lot of faeces, and the remainder of the pellets were scattered on to black earth and left till sunset. The maximum temperature was 40° C. in the sun. In the evening three cultures were made with the dry faeces. Four days later the control cultures contained numerous larvae, but the three cultures made with the exposed faeces proved negative.

(2) Exposure for part of the day : On the 11/3/15 the faeces were exposed from noon to sunset at a maximum temperature of 53° C. The culture proved negative; the controls were positive.

8/3/15.—The infected faeces were exposed from 7 a.m. to 12 a.m. at a maximum temperature of 53° C. The culture proved to be negative and the control one gave positive results.

(3) Constant exposure during winter time : On the 26/6/15, faeces passed the previous night were placed on black earth at 12 a.m. and exposed to the sun at a temperature of from 35-46° C. A culture was made at sunset and numerous larvae were found within the next three days.

On the following day the temperature was 42° C. in the sun. A culture made from faeces collected at sunset the same day proved negative. Faeces collected from the same lot on the 3rd day at sunset also gave negative results.

A control culture, also exposed to the sun during the same time as the above lot and constantly kept moist, contained well developed larvae in the second stage after the second day of exposure.

Some observations were carried out in the field with infected faeces placed amongst grass.

NOTE.—In the following experiments the atmospheric conditions will not be alluded to, as a daily report for the month of March will be found on page

(1) Faeces deposited in single pellets after sunset amongst short dry grass, during a period of changeable weather :

23/3/15.—At 6 p.m. faeces were placed in grass 6-7 cm. high, partly protected from the sun. The earth was dry.

25/3/15.—In the evening the pellets were examined and were found to be apparently dry, but no larvae were present. A culture made from pellets the same evening only contained dead eggs.

On the 28th and 29th March and 1st of April, pellets of the same lot moistened by rain were examined, but only contained dead eggs.

It appears that the low temperature of the first night did not allow the eggs to reach the embryo stage, and the sun of the following day killed them.

(2) Experiment with faeces deposited in single pellets in high grass in the morning :

26/3/15.—At 8 a.m. infected faeces were placed amongst grass 20 cm. high, well protected from the direct rays of the sun. The ground was dry.

26/3/15.—The faeces were fairly dry at 6 p.m.

1/4/15.—The pellets had been slightly moistened by heavy dew.

4/4/15.—Very few larvae, poor in granulations, were found.

It appears that the tall grass did not prevent the sun and dry wind on the 26/3/15 from killing nearly all the eggs, and only a very few eggs in the centre of the pellets survived.

(3) Experiment with faeces in single pellets deposited amongst grass of medium height. Moist weather ; cold nights.

28/3/15.—At 8 a.m. the faeces were placed in layers amongst grass 8-10 cm. high.

1/4/15.—On microscopical examination moist and well protected faeces gave the following results :—

100 Larvae had hatched and were dead ;
10 Eggs contained living embryos ;
20 Larvae were in the second stage and living ;
2 Larvae in the second stage were dead; and
2 Well-developed mature larvae were alive.

The superficial pellets contained 5 per cent, living larvae, the remainder dead eggs. In subsequent observations only 10 per cent. larvae reached maturity in the well protected faeces.

In consulting the daily atmospheric report for March in the above period, it appears that the conditions were quite favourable for the development of larvae, with the exception of the nights, which were rather cold.

(4) Experiment on the exposure of faeces in single pellets deposited in the field in the morning in tall grass. Variable weather.

30/3/15.—At 8 a.m. faeces in pellets were placed well protected amongst grass about 15-20 cm. high.

3/4/15.—95 per cent, of larvae were dead and had reached the first stage; the remaining 5 per cent, were in the beginning of the second stage and were rather poor.

4/5/15.—Very few larvae had reached maturity and were living.

It appears that the first day was favourable for the hatching of the eggs, but the following cold nights did not allow of development; the afternoon of the 30/3/15 was hot and bright, killing the larvae in the first stage. The low temperature of the following nights was not favourable for the development of larvae presumably in the first stage, and the bright days killed a large number of larvae.

(5) Experiment with faeces in single pellets and clustered pellets deposited in the morning amongst tall grass. The nights were cold.

5/5/15.—Two lots of faeces were placed amongst grass 20 cm. high; one lot in single pellets, the other in clusters.

8/5/15.—The single pellets contained eggs with grown embryos, and the more protected faeces in clusters contained a large number of larvae.

15/5/15.—In the single pellets the eggs were still in the same stage, and apparently dead. In the clusters of faeces only dead eggs and a few living larvae in the second stage were present.

During the first days of exposure the sun was bright and the atmosphere was rather dry; the eggs in the single pellets were killed, whilst the pellets in the centre of the clusters, owing to the sun not being very hot, contained sufficient moisture to allow the larvae to reach the end of the second stage. The moisture during the following days was rather scarce, the nights were cold, and it is presumed that the larvae migrated into the ground.

General Observations on the Experiments carried out in the Veld: Constant Warm, Wet Weather.—When the weather is warm and rain is constantly falling for three days, or the ground is kept moist for the same length of time, with a heavy cloudy sky, the majority of larvae reach maturity.

Constant Warm, Dry Weather.—When the weather is warm and dry, **as** during periods of drought in summer, or dry and sunny as in winter, practically all the eggs and young larvae in scattered pellets are killed in *two* or three days, even if amongst ordinary veld grass.

Variable Weather.—Concerning variable weather, it is not possible to give any definite rule, owing to the action of the ambient being too complex.

In comparing the results of the above experiments with the condition of the weather at the time, it can be concluded that if the first two days are dry and sunny, a very high percentage of eggs and larvae just hatched

are killed. There seems to be no difference if the faeces are deposited at night, as even if the eggs hatch during the night, the larvae in the first stage, which are particularly delicate, are killed during the following day. If infected faeces are placed on dry soil, numerous eggs die, even if the first day is cloudy. On the other hand, if the faeces are dropped after heavy rain, numerous eggs survive in infected faeces situated in grass, even if the first day is sunny and warm.

In the third and fourth experiments it was noticed that the highest mortality of larvae occurred in those in the first stage, thus confirming the results referred to in the laboratory experiments. Further, it was noticed, particularly in experiment No. 3, that notwithstanding a certain amount of moisture being present, the mortality of larvae can be very high.

In supposing that the mortality referred to in experiment No. 3 is due to cold nights, then a difference in the results between the laboratory and field experiments should be very noticeable. As reported in the laboratory experiment on oscillating temperatures, the faeces exposed daily for six hours at 10° C. contained 70 per cent, mature larvae after three days.

Experiment No. 5 shows that if the faeces are deposited in clusters amongst tall grass, some larvae reach maturity, even if sunny days predominate, providing the temperature is not too high.

When infected faeces are passed out on to the veld, a fall of rain, even if prolonged for a day at a time, is not sufficient to allow the majority of larvae to reach maturity, if sunshine is again constant for a few days. Consequently, during periods of drought in South Africa, the majority of eggs and larvae on the veld die. It is only during protracted rainy weather that a large percentage of eggs reach maturity. This fact, connected with the facilities the mature larvae have of reaching the host if the surroundings are moist, explains the heavy infection that is noted in a flock after a heavy rainy season.

In conclusion, even in summer the percentage of larvae reaching maturity is very low, but the number of eggs deposited in a field by infected sheep is very high, as will be shown later. The sun is the chief agent of destruction for eggs and larvae under the natural conditions of the veld, and the mortality increases with increased dryness of the soil and atmosphere.

To comprehend the opportunities the sheep have of becoming infected, in spite of the high wastage of eggs and larvae, the following observation is of interest:—

Single pellets of faeces collected from an infected sheep were placed in a glass dish on a slide, and kept in a suitable room for the development of the eggs. Two days later, in a pellet weighing 0.800 grammes, 3000 developed larvae were found, the unhatched eggs not being taken into consideration.

During the day on which the faeces for the above culture were obtained, the sheep passed 303 pellets, and during the next night 704 pellets. Calculating now on the basis of a thousand pellets being passed during twenty-four hours, the number of larvae would be 3,000,000.

During the following days the counting was repeated, and the same number of larvae was found.