A STUDY OF THE LINEAR GROWTH OF COOPERIA CURTICEI IN LAMBS

*

* * *

*

BY

R. I. SOMMERVILLE

Thesis presented in partial fulfilment of the requirements for the degree of M. Agr. Sc.

Massey Agricultural College,
University of New Zealand.

1950

TABLE OF CONTENTS.

Table of Contents, continued.

: : : :

TABLE OF FIGURES.

: : : :

I N T R O D U C T I O N.

GENERAL.

The trend in agricultural parasitology in the past two decades has been to emphasise the importance of studies on the cycles of nematode parasitism in farm animals. However, singularly little work has been directed towards an elucidation of those factors in the environment of the host and free living larval parasites which are important in the acquisition of parasitic infestations.

OBJECT OF THIS INVESTIGATION.

The object of this investigation is to study the growth of a common nematode parasite of sheep in an attempt to determine the "age" of a population. The term "age", as used here, denotes the time from infestation to the death of the worm.

IMPORTANCE OF THE INVESTIGATION.

Data from this work will, it is hoped, be of use in determining the age of populations of parasites acquired by hitherto worm-free lambs placed on contaminated pastures. The age of a population being determined, it follows that its date of acquisition is also known. Environmental conditions pertaining to this period can then be examined in the light of their possible influence on uptake or larval stages of parasites.

ORGANISATION OF THE STUDY.

A series of populations covering all phases of the development period in the host were required for measurements of growth. Artificially reared worm-free lambs were infested with known numbers of infective larvae and killed at suitable intervals. A sample population of known age was collected from each lamb and the length of individual worms measured. These lengths were plotted in frequency distributions, which are reproduced in Part II of this thesis.

Originally it was hoped to study the growth of *Ostertagia circumcincta*, and at the same time determine whether there was any possibility of separating the females of *O. circumcincta* from *O. trifurcata*, which so far are accepted as being morphologically identical.

This work was to take the form of a simple measurement

of the lengths of large numbers of females from a mixed population, with a study of the distributions. An alternative was to study the relative growth of parts of the females and to examine the curves of relative growth, as Sproston (52A) has done for a monogenic trematode.

It will be explained in the text that the numbers of Ostertagia spp. available were too few for an adequate study, and that Cooperia curticei was substituted. The only work on Ostertagia, as far as this experiment is concerned, is an estimation of the length of infective larvae.

As a result of this study it is now possible to determine the age of populations of C. curticei to within approximately one day of the correct value by using the technique described later. It now remains to apply this information in field studies.

: : : :

CHAPTER II

REVIEW OF LITERATURE

Literature relevant to this thesis falls into two divisions. Firstly, work on the estimation of age by the measurement of the size of organisms, and secondly, on the growth of the Nematoda.

Examples of the determination of age by size are very common in biological literature. D'arcy Thompson (66) quotes work on the estimation of the age of fish by measuring the length. "Size grouping" can be recognised if the measurements are plotted in a frequency curve, and these groupings can be identified as representing "age grouping". Thompson points out that the spread or scatter of each group becomes greater with increasing age and that consequently there may be no distinction between groupings in older populations. Estimations of age by this method will therefore be of no use for the later stages of life especially of long-lived animals. He draws attention to the tortoise as an example: a slow growth continues for an unknown time and the animal never appears to reach a final limiting size. Some animals may cease growth altogether for a vast proportion of their life, as does the sea-anenome. Thompson states that these have been kept in captivity for sixty or eighty years and shown no growth, though otherwise functioning normally. Age may even cease to have any real influence at all on growth. Brody (42) quotes work on a planarian which can be made to "grow smaller" and assume an immature form merely by reduction of the feed supply. Increase of size likewise is a function of food supply rather than age.

In spite of these exceptions, it can be said that many animals pass through an immature phase where their growth in size is correlated positively with their increase in age.

So far as is known, this thesis is the first systematic attempt to apply this principle to the parasitic nematoda. Its potential value in connection with nematode studies has been recognised for some time.

Veglia (68) states that by noting the degree of development of Haemonchus contortus in regard to length, colour, and relative growth of the ovaries, he was able to estimate the age of the parasite, and consequently the time at which the parasite entered the host with a reasonable degree of certainty.

He talks in terms of the "previous winter" and the "spring just

ended" as the periods at which the host acquired the parasite. This thesis is aiming at a more precise and shorter time, so that for statements like "the previous winter" it is hoped to substitute "seven days ago" or "ten days ago these parasites entered the host".

Tetley (65) prior to suggesting this study had already used size of the worms and the degree of development of the sex organs as an approximate guide to the age of nematodes in studies in the elimination of the parasites from a lamb. This was not based on a systematic study of populations of known ages as is being done in the present work.

So far as the growth of parasitic nematodes is concerned, while their life histories have been studied closely, comparatively little work has been done on their linear growth.

Probably the most complete account is Ackert's (1) study of the growth curves of the parasitic stages of Ascaridia lineata. He infected 30 four-week old chickens, killed them at intervals over a period of fifty days, and measured the lengths of the parasites recovered. Nematodes were measured alive, or in 5% formalin. No indication is given as to whether there is any difference in length between specimens which might be due to this differential treatment, neither is a description of the method of measuring given.

The newly hatched larva in the host is described in some detail which is of no direct importance here. At 8 days the nematode moults, sex differences can be discerned, and the larvae (mean of eight specimens, the sex of which is not stated) are noted as 4·2 mms. long.

The next moult takes place between 14 and 15 days. Females measured 5·9 mms. on the fourteenth day and males 6·4 mms on the fifteenth day. By the eighteenth day there was a marked variation in development and the third moult followed on the 22nd day. A few female worms measured on the 19th day were 15·5 mms. long, while males were 14·5 mms. long.

The growth curve has been summarised in terms of six eight-day periods from the hatching of the eggs. Average daily growth for these periods was 0·12 mms. for the first; 0·75 mms. for the second; 1·5 mms. for the third, fourth and fifth; and 3·0 mms. for the last.

Though Ackert gives separate measurements for the sexes in the later stages of development, his curve of growth is a composite one for both sexes, and is hardly a satisfactory representation of the

growth of either sex.

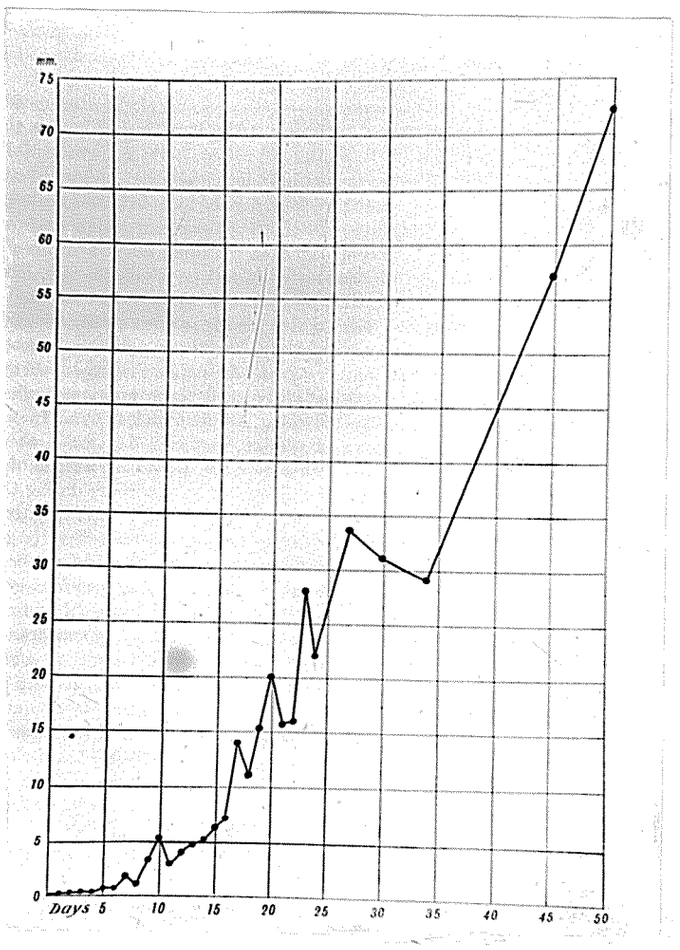


Fig.1. Rate of growth in length of A. lineata. (from Ackert, Jnl Parasitology 23:374 (1931)).

At 14 days, the females are 0·5 mms. shorter than the males on the fifteenth. If this is accepted as true then the males must grow faster than the females at first, and later slow up until they are growing slower than the females, which are longer than the males by the nineteenth day. This is unlikely. The differences may be due to too small a sample - some males are bigger than some females in the same population - or it may be that the females measured on the fourteenth day had not molted, whereas the males measured on the fifteenth day had molted and were growing fast. The growth curve does not show the "step and stair" effect which is expected where there is a lethargus. This again may be due to the very small numbers which were evidently used. Ackert himself admits that the inclusion of more individuals would have modified the curve.

The only other account published of nematode development is Scott's (49) study of the growth of adults of the dog hookworm, Ancylostoma caninum, in the dog and cat.

He demonstrated the existence of two strains of the dog hook-

worm, one normally found in the dog and called by him strain Do, the other from the cat and designated Cl. Studies were made of the growth of these strains in the two hosts and growth curves were constructed along points representing the mean lengths of any number of worms up to 25 from the one host.

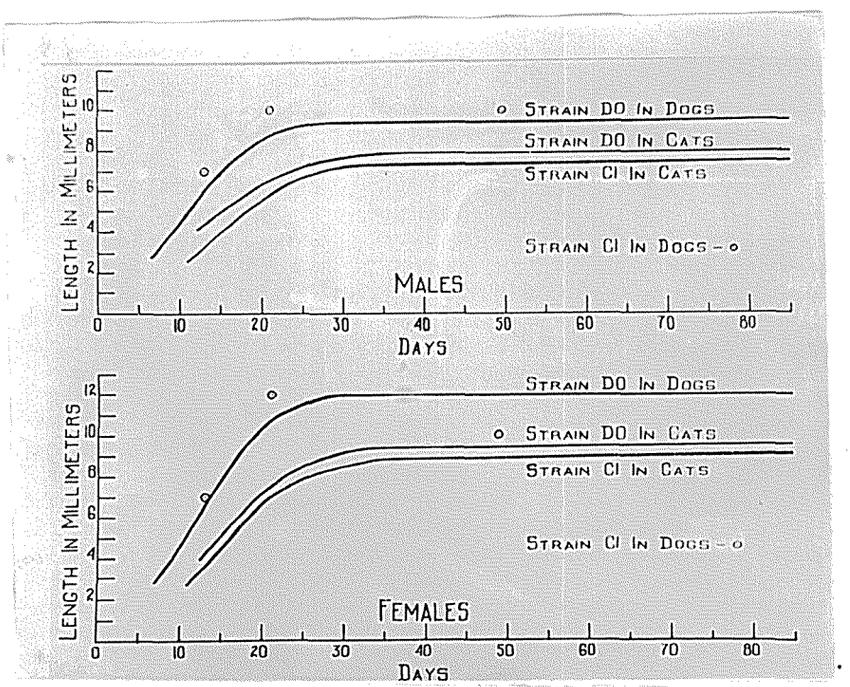


Fig.2. Growth curves of the Dog and Cat strain of A. Caninum. (from Scott, Am. Jnl Hyg. 10:137 (1929)).

Curves were expressed in terms of a logistic type equation, and the constants of the equation were used to compare the growth rates. (In a later section of this work, the use of logistic and other equations in analysis of growth curves will be examined in detail.)

To prepare specimens for measuring, worms were fixed in Carnoy phenol after standing overnight in tap water, or else hot 70% alcohol was used immediately after autopsy.

Previous work (50) had shown that these produced identical results as far as length is concerned. Both fixatives produced worms which were curved in the same plane or else straight.

Specimens were arranged in a flat dish in alcohol and photographed directly dnto a bromide paper. The camera was set to give a constant magnification when the worms were in focus, and reflected light was used to give a silhouette photograph. This method elimir ated the need to print from a negative, and was quite fast. Worms were measured with a map measurer.

Scott found interesting relationships between the hosts and the different strains of the hookworm. Worms of the dog (Do) strain, if they succeeded in establishing in the cat, did not grow as well as in the dog, but grew no better than the cat (Cl) strain in the cat. This latter strain, if able to establish in the dog, grew to the same final size as the Do strain in the dog.

In Scott's own words, "The ability to grow at all in a given host is inherent in the worms but the growth rate and final size are controlled by the host."

It was also found that variation in the mean lengths from host to host was greater than the variation within the host.

Scott extrapolated his curve back through the immeasured third stages and found that at zero the curve had a value of 0·7 mm, which is the length approximately of the infective larvae. This indicates that in spite of the pause in growth at the fourth lethargus, the growth rates remain the same throughout parasitic life. However, Scott made no measurements of growth during the early stages up to seven days, and even later in some cases, mainly on account of mechanical difficulties in collecting representative samples, and because of extreme variations from host to host in the time of various moults.

The failure on Scott's part to study the third larval stage and the fourth or primitive buccal capsule stage makes his growth curve incomplete. Extreme variations from moult to moult would not seem to be an obstacle. With patience, larvae under one millimetre long can be picked out from a mass of ingesta in the sheep, so that the same task in the dog or cat, where a much smaller quantity of ingesta is involved, would presumably be easier.

Scott's reasoning that the growth rate remained the same throughout parasitic life is confirmed by McCoy's (34) work on the free living stages of Ancylostoma caninum. Examining the influence of various environmental factors on the development of eggs and larvae of the hookworm, he found that the growth curve at 23° C. was essentially the same as that recorded by Scott for the parasitic life of A. caninum. McCoy used the same logistic curve as did Scott, and found that the values of the "b" constant in Scott's equation, if divided by 24 to reduce the curves to the same time scale, were not significantly different.

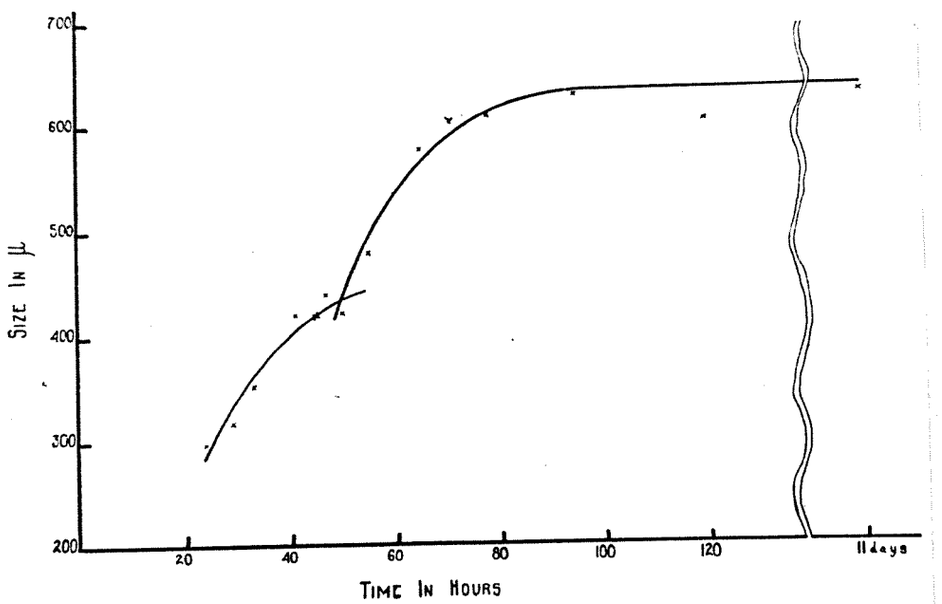


Fig.3. Growth of the larval stages of A. caninum at 23° C. (from McCoy, Am. Jnl Hyg. 11:425 (1930)).

It is generally agreed that comparisons of this nature, based on growth equations, are valid (71). The comparison is thus very interesting in view of the wide disparity between the environments of the free living and parasitic stages. McCoy's curve shows clearly the "step" effect introduced by the pause in growth prior to the first moult, in effect lacking in both Scott's and Ackert's curves.

A review of growth studies in the Nematoda would not be complete without reference to the use of growth as an index of the suitability of different environments for nematodes, and as a means of differentiation between different species, particularly larval forms. The usual method involves a study of the final size reached by the nematode, rather than a study of its complete growth cycle.

Fenwick and Franklin (15) have published work in which they have analysed the difficulties in determining parameters for the larval lengths of different species of Heterodera, with the object of identifying eelworm cysts in a soil sample. The analysis is very thorough and shows the difficulties involved when small objects are to be measured accurately. The problems faced differed somewhat from those in

this experiment; sampling, for example, was complicated by a number of factors.

Using the potato as a host plant they found that, while the mean lengths of larvae from cysts on one plant may differ significantly from the mean lengths of larvae from any other plant, the variety of the potato used was without any significant effect. It was also found that the variations in the mean lengths of larvae from different cysts were greater than the variations found within cysts from a large number of plants and sampling a few larvae from each cyst.

In this thesis an analogous situation does not arise because there is no equivalent to the cyst in the parasitism of sheep by C. curticei. However, Fenwock and Franklin's work does draw attention to the importance of inter-host and intra-host variation in selecting the size of the sample. If the former was greater than the latter, it may be advisable to select as many lambs as possible for any one day, kill, and take a relatively small proportion of the intestinal parasites for measurement. This aspect is discussed later.

Fenwick and Franklin met special problems in measuring larvae which are of no concern in this work. They measured larvae with an ocular micrometer, using a lens combination which made the image of the larvae cover approximately half the scale. This method was applicable because pre-treatment had made all sound, fully developed larvae die lying out straight. The same method was employed in this thesis and will be discussed later.

Lengths of mature Ascaridia galli have been used by Sadun et al (45) in studies on the effect of the host's diet on the host-parasite relationship. Hosts receiving a diet deficient in pteroylglutamic acid were found to harbour significantly more and longer worms than those receiving an adequate diet, while in a further experiment, these workers claim to show that A. galli grew to a greater final size because of the presence of a substance in the diet of the hosts which was derived from dried liver.

Sadun (46) in further work on the antibody basis of immunity of chickens to A. galli used the length of the worms, amongst other factors, as an indication of the degree of resistance conferred by immune serum.

Taylor (57) found that Syngamus trachea from the chicken had (amongst other differences) a different growth rate from the morpho-

logically identical form parasitic in starlings. As a basis for comparison the length of the longest female instead of the mean length of a population was used. This may be justifiable if the standard deviation for each age group of worms is low, but even then the mean would probably be a superior statistic. Where the standard deviation is high and some values lie a long way from the mean, then the use of the extreme value as an index of the length of the population is scarcely justified. Taylor gives no details as to the range in lengths found, and it can only be assumed that the use of the mean value would have shown the same differences in growth rates as did the extreme values.

: : : :

CHAPTER III

SELECTION OF THE GROWTH INDEX

SUMMARY.

The life history and general morphology of C. curticei are reviewed and the reasons presented for the selection of length as an index of growth.

INTRODUCTION.

In order to express the quantitative relationship between growth and time, it is necessary to select some feature of the anatomy which can be measured at successive intervals. The choice is best made in the light of a study of the anatomy and the life history of the parasite. The following aspects of these themes are therefore presented.

THE LIFE HISTORY OF COOPERIA CURTICEI.

This account is taken largely from the work of Andrews (2) who made the first detailed study of the life history of this nematode.

The pre-parasitic stages:

This growth study deals with the parasitic stages of C. curticei only. For the sake of completeness, an outline of the pre-parasitic stages is presented.

The eggs first appear in the faeces of the host about fifteen days after infestation. They have equally rounded ends and the sides are parallel. The range in length is given by Andrews as being from 70μ to 82μ; the width as being from 35μ to 41μ. He gives the average dimensions of one hundred and two eggs as being 76·8μ by 37·8μ. Tetley has data (unpublished) showing the mean width to be almost the same as that given by Andrews. His range in length is slightly greater however, being from 79μ to 87μ.

Curtice (12) gives dimensions of eggs which are certainly not Cooperia species. Ransom (41) gives dimensions for eggs of C. curticei which are much smaller than those obtained by other workers. His figures are 63μ to 70μ long by 30μ to 32μ wide.

At room temperature, the eggs will hatch about twenty hours after being passed in the faeces. The first stage larvae are about 300μ long with a rhabditiform oesophagus. After twelve hours from hatching, in which time Andrews states they have grown an average of 200μ, the larvae enter upon the first lethargus which lasts approx-

imately eighteen hours. They again become active at the end of this period, and cast their sheaths.

The second stage larvae are very similar to the first stage, though larger. The intestinal cells are also more granular. Andrews reports this stage as feeding actively for approximately forty-eight hours before it enters upon the second lethargus, which lasts twelve hours.

At the conclusion of this second lethargus, the larvae again become active in what is called the free-living infective stage. This is reached ninety hours after hatching. The second stage cuticle is retained by this larva until it infects the future host.

It is to be noted that the figures given for the times to reach various stages from hatching, as well as the time for the eggs to hatch, are acquired under laboratory conditions. These may have been such as to hasten development. In any case, they would almost certainly be different in some degree from those experienced in the field, and interpretations from them in regard to field conditions should be made with this in mind.

Andrews does not discuss the effective longevity of the third stage larvae in the field. Baker (3) in a simple trial found that, along with other common nematodes of the alimentary tract of sheep, C. curticei infestation could be acquired by these animals in a paddock which had not held stock for twenty-one months. This indicates that some of the infective larvae were still viable after this period, but it does not give any indication of the proportion of the original larvae population they represent. This latter figure would be of more value. Work by Kates (26) in U.S.A. shows that Cooperia larvae do not survive the winter in rested pastures at Beltsville, Md.

The parasitic stages:

Two days after the infection of experimental lambs, Andrews found that the larvae had cast their second stage sheaths. They were still, however, in the third (parasitic) stage. They had increased in length very little, some being shorter than the infective larvae.

While still in the third stage, three days after infection, the larvae passed into the crypts between the villi of the small intestine. A day later, they underwent the third ecdysis, becoming fourth stage

larvae. They remained in the crypts with their posterior ends projecting into the lumen.

In the fourth stage, sex differentiation was possible. The males had a thicker posterior end than the females, and the genital primordium was further from the posterior end than in the females.

On the fifth day, the larvae returned to the lumen, and marked development of the genitalia took place. In the male, the genital primordium was about 2000μ from the posterior pole. In the female, it was 180μ from the posterior end and the germ cells were at opposite extremities.

On the sixth day, the genital primordium had developed to such an extent that parts of the reproductive organs could be distinguished.

On the eighth day, the cuticle was loosened and the genitalia were well developed, though there were great variations from individual to individual.

On the ninth day, the final ecdysis took place, and the worms were mature adults.

A day later, though a few fourth stage forms were present, the majority were immature adults, as shown by absence of eggs from the uteri, and the size and colour of the genitalia.

On the twelfth day from infection, eggs ~~prepared~~ appeared in the uteri and the spicules darkened. Monnig (37), working on the life history of Trichostrongylus species, took this darkening to be a sign that copulation had taken place. The same may apply to C. curticei.

At fourteen days, Andrews stated that the worms had reached the size of mature individuals. The following day eggs were found in the faeces.

THE MORPHOLOGY OF THE ADULT NEMATODE.*

In general form the nematode is more or less cylindrical and elongate. Length is its main dimension. Relative to the total length, the width is very small. The writer measured the width of ten male worms at a point one hundred microns anteriorally from the anterior end of the spicules. The mean measurement was 90·4 microns.

* This account is derived largely from "An Introduction to Nematology" ed. by B.G. and M.B. Chitwood. Washington, D.C. 1937.

the range being from 74 to 101 microns. The modal length of the male population from which these measurements were taken is 4·9 mm. The measurement of width is thus about one fifty-fourth part of the measurement of length.

In C. curticei the actual length is not so obvious because the nematode curls in the anterior portion, leaving the latter third projecting more or less tangentially from the coiled portion.

The Cuticle.

This is the name given to the non-cellular external covering of nematodes. At the anterior end of C.curticei, the cuticle forms a cephalic inflation, and is marked by transverse striations. The diameter of the cuticle here will vary with the degree of inflation from 25 microns to 50 microns (41). In the fourth stage larvae this striation may be seen clearly beneath the cuticle, especially just prior to the last ecdysis. This fact was used to identify fourth stage females from adult females in the present work.

The cuticle covers the body entirely, and is marked in C. curticei with fourteen to sixteen longitudinal lines or ridges which appear under close inspection to be rows of small dots.

The cuticle itself does not present any feature which could be utilised as an index of growth. Indirectly, it is important in measurements of total length, in that it marks the upper limit of that dimension. Nematodes which have been unduly affected by a fixative will shrink back somewhat from both ends of the cuticle. Immediately prior to ecdysis, the cuticle may often be damaged in fixing and will frequently work loose. In the latter cases, the distance between the extremities of the cuticle does not necessarily represent the true length of the worm. This aspect will be discussed in a later section.

The Caudal Bursa.

In the Strongylina, the caudal bursa is a lateral cuticular modification at the posterior end of the adult male (11, page 26). Signs of its presence may be seen in the thickening of the posterior end of the male in the early part of the fourth stage. The bursa does not show distinct development till the sixth day of parasitic life, or even later (2). The bursa may be divided into one dorsal and two lateral lobes. Each lobe is divided into a number of rays.

the shape, size and relative position of which are used in classification. Baylis (4) gives the following description of the bursa of the genus Cooperia:

> "The bursa of the male has a small dorsal lobe. The lateroventral ray is considerably thicker than the ventro-ventral and is rather widely separated from it, but curves in the same direction towards its tip. The postero-lateral ray is slender. The externo-dorsal rays are long and originate high up on the dorsal stems. The dorsal ray is cleft for from one third to one half of its length, and its main branches may be parallel or curved to form a lyre or horseshoe. From each main branch, usually near its origin, a short branch extends ventrally into a vesicular swelling on the inner surface of the bursa."

In C. curticei, the main branches of the dorsal ray form a lyre-shaped structure.

The bursa would not be a very easy subject to measure from the point of view of development. As with other sexual characters, its growth might be utilised to indicate the age of the worm in the later stages. Andrews (2) however mentions that at eight days there is great variability in bursal development.

The Hypodermis.

The hypodermis is a thin protoplasmic layer underlying the cuticle. From it project internally four longitudinal thickenings dividing the internal areas. The thickenings are known as chords, being termed respectively the dorsal, ventral and lateral (two) chords. Within the chords are the nuclei of the hypodermal cells, the nervous system and the lateral canals. Some reserve food granules are also present.

The Somatic Musculature.

(a) The Unspecialized Muscles.

Though no data relating directly to the musculature of C. curticei was available, information could be obtained on two closely related forms, Ostertagia and Haemonchus. The following is an account of the general plan of nematode musculature.

The muscle cells lie in parallel groups in the antero-posterior

direction, separated by the chords into four primary muscle fields. Each of these in turn is subdivided by smaller thickenings of the hypodermis, so that there are eight fields in all. These are named the subdorsal, dorso-lateral, ventro-lateral, and sub-lateral.

When there are few muscle cells between the chords the musculature is termed meromyarian, while many cells form a condition termed polymyarian.

The muscle fibres are perpendicular to the hypodermis with a contractible substance as a base. In some cells this substance folds perpendicularly from the hypodermis up the sides of the cell almost enclosing the protoplasmic central portion. This type of cell is termed coelomyarian. Where the protoplasm is more or less uncovered by the contractible substance, the cells are called platymyarian. From the protoplasmic surface facing the interior, there run a series of "innervation processes" extending from the vicinity of the muscle cell nucleus to the motor nerves.

In the Trichostrongyloidea such forms as the genus Ostertagia are meromyarian-platymyarian. Chitwood and Chitwood (11, page 42) state that the genus Haemonchus is peculiar in showing in cross section at the mid region of the body four sub-lateral large platymyarian cells and forty to forty-eight sub-median small coelomyarian cells.

(b) The Specialised Muscles.

Besides the muscles of the body wall, there are a number of specialised muscles, some of which are:-

Somato-oesophageal	somato-intestinal
depressor ani	dilator ani
copulatory	bursal
spicular	vulvular.

The Pseudocoelmic Cavity.

In all nematodes there is a body cavity called the pseudocoelome, lined with a membrane originating (in the Phasmidia) from a single cell dorsal to the oesophagus.

Within the cavity are cells which are not a part of the connective tissue and which are known as coelomocytes. Very little is known about their function or indeed their presence in the various forms of nematodes.

Cephalic Structures.

B.G. and M.B. Chitwood believe that nematode lip structure is

based upon a hypothetical primitive pattern represented as having six lips. Two of these are dorsal, two are sub-lateral, and two sub-ventral. On the summit of each lip is one papilla, forming an inner circle of six. On the posterior and lower portions of the sub-dorsal and sub-ventral lips are two papillae, and on the posterior portion of each of the sub-laterals, one papilla, making a total of ten. They believe that all cases can be represented in terms of this basic plan, though fusion and reduction can modify the arrangement in any given species to a marked degree.

The Trichostrongyloidea "... seldom show rudiments of either three or six lips. The oral opening may be of diverse form but is nearly always surrounded by an inconspicuous circumoral membrane. Representatives studied by the writer have an internal circle of six reduced papillae and an external circle of ten simple papillae..." (11, page 57).

With direct reference to C. curticei (using the synonym Strongylus ventricosus) Curtice (12) states:

"No neck or head papillae visible. Mouth terminal, very small and round. The end of the head is furnished with a hemispherical cap-shaped chitinous piece. Other oral armature apparently absent."

Ransom (41) describes the anterior end of C. curticei as

"Head rounded, relatively thick, 25 microns or more in diameter, without well defined lips or papillae... mouth cavity small, indefinite."

There are two points of difference between Ransom's and Curtice's descriptions. First, Curtice states that the head has a hemispherical chitinous piece. This is not mentioned by Ransom. Secondly, Ransom mentions papillae in contra-distinction to Curtice's statement "... oral armature apparently absent."

Ransom believes that Curtice's description is based on more than one species. He cites in support of his contention the obvious differences between Curtice's fig. 2 and fig. 7, both purporting to be Strongylus ventricosus. Though Ransom does not mention it, fig. 2 in Curtice's description appears from its natural size and general appearance to be Nematodirus species. The dimensions for the eggs of S. ventricosus given by Curtice are 0.13 mm. by 0.07 mm. These di-

mensions are very close to those cited by Tetley (62) for N. filicollis. He gives 91·2 % of eggs of N. filicollis as falling within the measurements 133 by 176 microns long, and 71 by 87 microns wide.

Ransom mentions other additional errors made by Curtice in the drawings. The writer has not observed any structure in C. curticei which corresponds to the 'hemispherical cap-shaped chitinous piece.' Probably this is a further error on Curtice's part.

The Stoma and the Oesophagus.

The stoma is the passage between the oesophagus and the oral aperture. So far as Trichostrongylids are concerned, it is very much reduced.

The oesophagi of nematodes present a wide range of form and function. In the Strongylina, Chitwood and Chitwood (11, page 78) state that the oesophagus is "grossly clavate to cylindroid", but in some instances, faint indications of procorpus, metacorpus and bulbar region are observable in the adult.

The oesophagus in the adult C. curticei is given by Ransom (44) as being 20 in diameter at the anterior end, narrowing quickly to 15 , then gradually increasing to about 30 or 35 . This shape is the very reverse of clavate, and barely cylindroid. It does not show indications, however faint, of procorpus and metacorpus. The bulb at the base may be construed as representing the bulbar region of Chitwood and Chitwood's description.

In many trichostrongyloids, the first stage larvae have a distinctly rhabditoid oesophagus. From Andrew's (2) figure 1 b, it would not appear that there is no distinct differentiation between procorpus and metacorpus which is associated with the rhabditoid oesophagus. The second and third stage oesophagi are characterised by the gradual obliteration of the obvious regions.

The third stage larva of C. curticei has an oesophagus with a slightly swollen posterior bulb, which tapers to pass at its narrowest through the nerve ring and then increases in diameter slightly to run to the globular buccal cavity. The oesophagus undergoes further changes involving a thickening of the anterior end in the later part of the parasitic third stage, when it soon becomes recognisable as the adult type.

Ransom (41) gives the length of the oesophagus in the adult male

as being 250μ to 275μ long. In the adult female, the measurements given are 255μ to 290μ.

The oesophagus is readily measured, and being present in all stages of the life cycle develops throughout the developing life of the worm. These characteristics make it an obvious subject to measure in a growth study. This aspect will be discussed later.

The Excretory System.

In nematodes, the excretory system shows great diversity. The Rhabditoid system, as exemplified in Rhabditis strongyloides is described as being (with modifications) the type found in the Strongylina.

M.B. and B.G. Chitwood (11) describe the essentials as including "(a) a terminal elongate cuticular duct, (b) an excretory sinus connected with paired lateral glands, and (c) paired subventral excretory glands also connected with the excretory sinus." The cuticular duct is connected to the exterior by the excretory pore which is a ventral structure.

No direct information is available on the excretory system of C. curticei.

The Intestine and Rectum.

In nematodes, the intestine is a simple tube with an epithelial cell wall. In some species, there are appendages from the anterior portion, but these forms are rare. The cell wall is a single layer with differentiation (mainly in cell height) which enables a histological division into three regions to be made.

The intestine is connected with the hind gut by an intestino-rectal valve. It is lined with cuticle, and rectal glands are usually present. The rectum is modified in the male by the vas deferens which opens on the ventral side of the rectum, forming a cloaca. The opposite, or dorsal wall, is modified and gives rise (11, page 115) to the gubernaculum (not present in C. curticei) and the spicules.

The Spicules.

The spicules of C. curticei are described by Baylis (4) as being "relatively short, broad, and somewhat twisted". Lengths, as measured by Tetley (61) range from 111μ to 165μ. Tetley used spicules as an index of development in studies on the relative suitability of

the environment in different parts of the intestine towards C. curticei. Measurements provided some indication of the differences in the final development of the nematodes. Spicules would not be useful in growth studies over the whole parasitic life since they do not commence development until after the sixth day.

The Nervous System.

The focal point of the nervous system is the circum-oesophageal commisure. From this there are directed forward six nerves. Each of these has three chief distal branches, which innervate certain organs of sense at the anterior extremity. These organs may be tactoreceptors or chemoreceptors. The chemoreceptors at the anterior end of the nematode are called amphids.

In the hypodermis are several nerves posterior to the nerve ring. These are the dorsal, the ventral, two (or three) pairs of laterals and four submedian nerves. Ganglia are present in the ventral and to a lesser extent in the lateral nerves. None are found in the dorsal or submedian nerves.

Other structures which may be associated with the nervous system are various papillae, phasmids (similar to amphids, but situated posterior to the anus), and innervation processes from the somatic muscle cells to the dorsal, ventral, and submedian nerves.

The presence or absence of phasmids is the basis of the difference between the two sub-classes of Nematodes Phasmidia and Aphasmidia, Chitwood and Chitwood, 1933. Phasmidia include plant and soil forms and those parasitic in animals. Aphasmidia include more commonly marine and fresh-water forms.

The Female Reproductive System.

No detailed account of the reproductive organs of C. curticei is available. The Strongylins as a whole, however, have a system based on the following plan, with modifications.

The vulva in C. curticei is a transverse slit, surrounded by chitinous lips and situated in the posterior third of the body. In the Strongylins generally, a very short vagina lined with cuticle (the vagina vera) leads from the vulva to a thickened portion, the ovejectors paired oppositely. Each ovejector may be divided into three distinct parts to which many names have been given. The following is the nomenclature suggested by Ransom (41). That thin-

walled portion common to both ovejectors, into which the vagina opens, is called the ovejector I. At each end of ovejector I is a sphincter muscle, ovejector II. This in turn leads to a thick-walled ovejector III. In several hundreds of females of C. curticei examined by the writer, eggs have been observed in the uterus beyond ovejector III and in ovejector I, but never in ovejector II or III. This is understandable in view of the narrowness of the lumen and the muscular nature of the walls of ovejectors II and III. Ransom (41) however, in his fig.70, page 73, shows an egg in each ovejector III.

The uteri lying beyond ovejectors III have walls of squamous epithelium. The distal portion usually functions as a seminal receptacle.

The thick-walled oviduct sets off the uterus from the ovary. Each egg is fertilised when it reaches this organ, (11, page 175).

Beyond the oviduct lies the ovary, a tubular sac in which can be distinguished two divisions, the Germinal Zone and the Growth Zone. As its name implies, the germinal zone is an area of rapid cell division, while in the growth zone there is a rapid increase in cell size. This zone can be very large, especially in parasites.

In Cooperia the genitalia extend forward in the case of the anterior ovary, while the posterior uterus extends to near the posterior end and then turns back, the ovary running anteriorally past the vulva.

The Male Reproductive System.

The reproductive system of male nematodes has not been studied in the same detail as that of the female. This is due largely to the fact that while the female system shows varied development, being especially adopted for parasitism, the male system is more uniform, especially in so far as accessory structural modifications are concerned. It does however increase in length with parasitism.

Phasmidians, of which division C. curticei is a member, commonly have one testis divisible like the ovary into a germinal zone and a growth zone. In a few forms the testis may be separated from the seminal vesicle by a vas deferens. The seminal vesicle is a simple dilation of the main duct for the storage of sperm. The vas deferens is the terminal portion of the genital system and enters the rectum in nearly all males from the ventral side. It may be divided into a tubular and a glandular region. The vas deferens shows much variation from group to group in its detailed structure.

DESIRABLE ATTRIBUTES OF THE GROWTH INDEX.

It is considered that the factor selected as a growth index should have the following attributes:-

A. The growth of the anatmmical feature should cover as great a period of the parasitic life as possible. It is obvious that certain sex organs which do not differentiate till about the sixth day of parasitic life would be useless as a measure of growth for the whole of the developing period of the parasite. Such features might be of use, however, if they continued to develop when all other growth processes had ceased, and it is not unreasonable when discussing the genitalia to assume that they would do so.

B. Whatever feature of the anatomy is selected, it must grow fast enough to give distinct differences between successive measurements. Since the growth of C. curticei in the host covers not more than about twelve days, it would be desirable to measure an anatomical feature which would grow enough to show daily changes distinctly.

C. It is preferable but not essential that the feature selected be readily measured, especially if a large number of nematodes are to be measured. Another minor point is that the feature should be resistant to changes in fixation. Spicules were used by Tetley (61) to measure relative differences in development of adult male C. curticei because they were not affected by different reagents used in fixing.

THE GROWTH INDEX SELECTED.

From an examination of the life history and morphology of C. curticei in the light of these three points, the following anatomical features were studied as being most likely to give a clear measurement of the growth of the nematode:-

(1) the total length

(2) width

(3) length of the oesophagus

(4) certain sex organs.

With the exception of the latter, these show continuous change throughout the developmental period of the nematode in the host.

Other features could be utilised to show the age of the parasite. The most obvious is the stage of the life cycle. For example, generally any Cooperia in the fourth stage can be placed, on the basis of our knowledge of the life cycle, as having been from four to eight days in the host. Similarly, a third stage larva showing an oesophagus

with a thickened anterior end could be placed in the second or perhaps the third day of parasitic life. The changes may be termed discontinuous. They apply to one special portion of the parasitic life. Their value is therefore limited because they cannot be used to give any idea of the age of C. curticei over big periods of its developmental history.

Of length, width, and oesophagal length, the first is by far the most suitable characteristic to measure. Width is difficult to estimate correctly because slight flattening or distortion of the nematode can alter it to a large degree. Also the absolute change in size is not great. The writer measured ten adult male C. curticei at a point approximately 100μ anteriorally from the proximal ends of the spicules and found the average width to be 90μ. A similar measurement of the width of ten infective larvae, taken across the base of the oesophagus was 23μ, a total increase in width of only 67μ. Similarly, the length of the oesophagus increased from 150μ (14) in the infective larvae to 270μ (4) in the adult female, a total of 120μ. Thus the oesophagus does not even double its length in development from the infective stage.

According to Andrews (2) the adult nematodes cease growth at about fourteen days. Assuming growth to be a regular increase (though this is not so), the oesophagus averages about 9μ a day, and the width less than half this figure.

The overall length of the infective larva on the other hand increases from 750μ (14) to 5,000μ (4) in the adult male; i.e., over six times its original length. The adult female is even longer.

Length is therefore the dominating feature of nematode morphology. It may be said to have in essence one dimension, length, which is the sum total of the growth processes of the parasite. Its relatively large size makes it easy to measure, and ensures that slight variations in development within or between populations could readily be discerned. With the exception of the first two days of parasitic life, the length increases until about twelve days when growth ceases. Its growth thus covers almost the whole of that period, when the nematode is developing in the host.

Scott (50) has shown that Ancylostoma caninum will vary by up to ten per cent. of its total length, depending on the fixative used. So far as C. curticei is concerned, so long as one standard method of

fixation and preservation is adopted, there will be no error from this source.

: : : :

CHAPTER IV

METHODS FOR MEASUREMENT OF LENGTH

SUMMARY.

The use of a micro-projector, camera lucida, and an ocular micrometer for various measurements of C. curticei are described.

THE MICRO-PROJECTOR.

A. The Apparatus.

The greater part of the measuring was done with this instrument, made by Watson Victor Limited of Wellington especially for this work. As its name indicates, it projects an enlarged image of any small object into a piece of paper placed at the base.

The three essential parts are:-

(1) a 100 watt projector lamp,

(2) a condensor made from a single piece of glass, with no diaphragm,

(3) a x20 ocular, fitted to the lower side of a bakelite stage.

These were mounted on a vertical steel tube screwed to the base board, and could be moved up or down either together or independently.

The makers recommended certain distances between lamp, condensor, lens, and base board as giving the best definition. When the apparatus was set up in the laboratory, these distances were used as a basis on which to make some slight adjustments and so obtain the most satisfactory results.

The distance from the base board to the lower edge of the field lens in the ocular was finally set at about 60 cms. The condensor was 5 cms. above the top of the ocular. This combination gave a magnification of 39·4 diameters, calculated with the aid of a stage micrometer according to the formula

$$\text{Magnification} = \frac{\text{size of image}}{\text{size of object.}}$$

Once this position was established, lens, lamp and condensor were screwed tightly into position and the steel column was marked as a check against any movement. A further precaution to prevent any change in magnification involved the use of a stage micrometer. This was placed on the stage and the image of the lines reflected on to a piece of paper at the base. The lines were then drawn in on the paper, which was kept as a standard for future reference.

The base board was made of solid wood. As it was not suitable

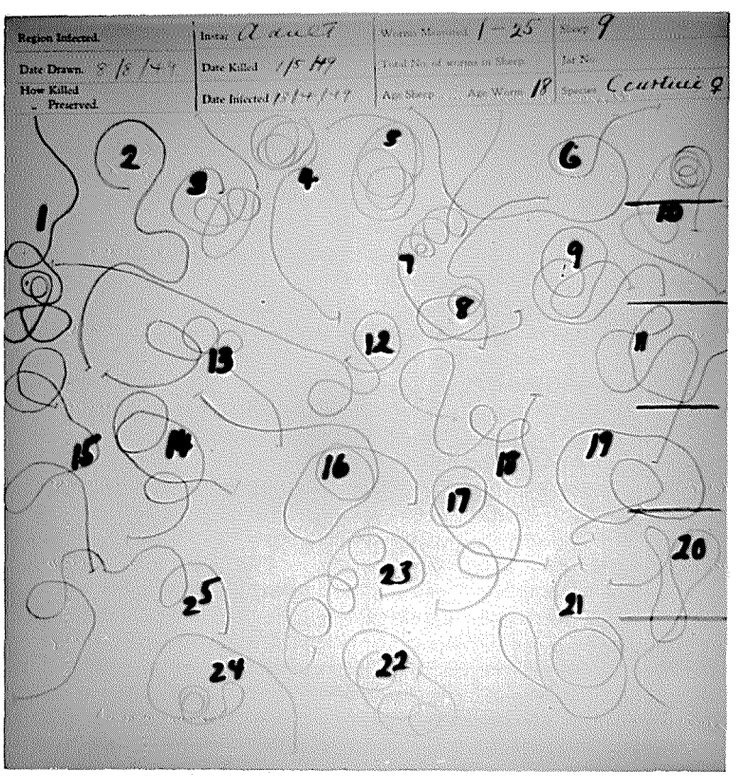


Fig. 4. Worm drawings.

for drawing with paper placed directly on it, all measurements and drawings were made with a special sheet of cardboard between paper and board.

For the drawings, headed paper 11 inches by 11½ inches was used. (See fig. 4). In the heading, space was available for the number of the sheep and the dates at which it was infected and killed. Amongst other details the most important were those referring to the species and to the instar. No space was allocated for the sex of the worm, this being inserted after "species".

Before using a sheet of paper, all the details mentioned above were filled in. In addition, the stage micrometer was used to draw a scale on the paper. This consisted of a series of lines down one side at a distance apart corresponding to the lines on the micrometer 0·1 cm.

Before the instrument could be used, a special screen around three sides and the top had to be constructed, to exclude light In measuring small images, a piece of black velvet was also used on the fourth side from which the operator worked.

B. Operation.

Worms were placed on a slide in a drop of 5% formalin and glycerine which was stained a light brown with Lugol's Iodine stain. The number of worms placed on a slide depended on their size. Adult worms when fully developed could not be handled in numbers greater than fifteen or twenty. With younger worms, fifty or sixty could readily be handled on one slide. Higher numbers than this were not used since they led to confusion.

A cover slip was placed over the drop, and excess formalin removed with a piece of blotting paper. This ensured that the cover slip pressed gently down on the worms and flattened them into one horizontal plane. Errors would of course be introduced in measurement if the worms or a portion of their length were in the vertical plane.

The slide was transferred to the stage of the micro-projector and the images brought into focus. It was then moved about the stage so that a view could be obtained of the general distribution of the worms. It was found that this was a great help in drawing, since the nematodes would be grouped in small clumps forming patterns of irregular shape. If a starting point was selected on the slide,

it was easy to work around amongst the worms, drawing each one with very little chance of missing or repeating.

In drawing, a short line was made at right angles to the longitudinal axis of the nematode at both the head and tail. A pencil mark was then made down the central axis keeping to the centre the whole way and connecting the two short lines marking the anterior and posterior extremities.

In males the measurements were made from the tip of the bursal rays. These could easily be twisted or doubled back but any errors arising in this way would not be significant. In fourth stage larval males the remnant of the larval tail projects at an angle from the swelling of the developing bursal rays. This tip could not be discerned under the micro-projector in most cases, and the tail end was taken from the tip of the developing rays. The actual difference would in this case be insignificant.

In spite of the fact that C. curticei is very often coiled into rather complicated shapes, very little difficulty was experienced in following the central line of the nematode. In a few cases in which there was doubt, due to very tight coiling, the nematode was discarded.

When a sheet of paper was filled, the worms drawn were individually numbered, and the numbers contained on the sheet entered in a space provided in the heading.

C. Measuring the drawings.

(1) Using Dividers. At first it was decided to use a pair of dividers to measure the length of the worms. A pair was obtained with a screw in the centre to hold the points at a set distance. The points were set 0·5 cms. apart, and the dividers were worked along the lines, at the same time counting the number of divisions marked out in this way. Knowing the scale, the numbers of divisions in the length could be readily transferred into units of measurement. This method was found to be slow and not very accurate when the worms were coiled. It would nevertheless be quite satisfactory for worms which were more or less straight.

(2) Using a Toothed Wheel.

(a) The apparatus. It was decided to use a small toothed wheel to run along the pencilled lines representing the length of the nematodes. The number of revolutions and fractions of revolutions could then be transposed with the aid of the scale into stand-

ard units of length.

A toothed wheel was eventually taken from a watch. It was approximately 9 millimetres in diameter and had thirty-five small teeth on the circumference. This wheel was fitted onto a metal handle amd a piece of watch spring soldered to the handle, with one end of the spring engaging in the teeth. This, while allowing the wheel to move backwards or forwards, acted as a brake and made the instrument much easier to handle.

(b) Calibration of the measuring wheel. For this purpose, five lines were drawn on a piece of paper. Along their length, using the reflections on the paper of the millimetre lines on the stage micrometer, were drawn a series of lines. The distance between each of these corresponded to one millimetre as magnified by the projector, and there were six divisions to a line.

The wheel was marked out with a speck of black paint on every fifth tooth; one was marked in a special way to distinguish it as the first. Using this as a starting point, the wheel was run down each line four times, two each way, and the number of revolutions and parts of a revolution recorded, the latter being expressed in terms of "teeth" or one thirty-fifth part of the circumference. Thus, 727 means that the wheel has made seven complete revolutions and twenty-seven thirty-fifths of one revolution.

Figures obtained were as follows:-

1st line	2nd line	3rd line	4th line	5th line
727	727	727	727	727
728	727	728	728	728
727	727	728	728	727
728	727	728	728	728

This averages 7 complete revolutions and $\frac{27.5}{35}$ or 0.8571 of a of a revolution overall. Since this is equivalent to a distance of 6 millimetres, one complete revolution will therefore be equivalent to 0.77 mm.

With this figure as a basis, a table was constructed which gave the values in millimetres of revolutions up to nine, and the values of each thirty-fifth fraction of a revolution. This table is reproduced in Appendix XI and was used in determining the lengths in millimetres of populations from the following sheep: 6, 9, 18, 24, 29, 36, as well as the infective larvae. (Graphs 70, 71; 66, 67; 72, 73; 17, 18; 68, 69; 52, 53 and 1 respectively.)

Shortly afterwards the calibration of the measuring wheel was

checked and it was found that the wheel actually turned a slightly greater distance in covering the equivalent of six millimetres. To determine this variation, ten lines, each representing six millimetres at the magnification employed, were measured twice in terms of revolutions of the measuring wheel. Each was, of course, seven complete revolutions long plus the following thirty-fifth parts of a revolution:-

1st line	2nd line	3rd line	4th line	5th line
30	30	29·75	27·75	28·75
30	30	29·75	27·75	28·75

6th line	7th line	8th line	9th line	10th line
29·5	29	29	29	30
29·5	29	29	29	30

The mean value is 29·28; therefore the average number of revolutions equivalent to the six millimetre line was $7\frac{29.28}{35}$ or 7·8365. The value of one revolution in terms of millimetres was 0·765 mms (compared with 0·770 for the first conversion table) and one thirty-fifth part was equivalent to 0·022 mms, which is the same as the figure for the first conversion table if both figures are corrected to three decimal places.

This correction was small, and the conversion table derived from it (Appendix X) differed from the original table only in the higher values. Thus a worm 119 units, i.e. $1\frac{19}{35}$ of a revolution long would be 1·19 mms in both tables. However a worm 800 units long, i.e. 8 revolutions, would be 6·16 mms long if the original conversion table was used, but only 6·12 mms long if the corrected table was used.

Therefore the measurements already made with the original table were unaltered, since the corrected table would only make some insignificant changes to the longer worms in sheep 6, 9, 18, 24, 29, 36. All other measurements using the micro-projector were made with the corrected conversion table.

When measuring, a sheet of paper was prepared having three columns. The first contained the number of the nematode as set out in the drawing. The second contained the length of the nematode in revolutions. The third contained the actual length in millimetres, read off from the table. The chances of error were reduced by recording the number of revolutions, rather than by reading the length directly from the table.

THE CAMERA LUCIDA.

The micro-projector was not used to measure the lengths of larval stages younger than four days. These forms gave only a very small image, and when this was coiled it became very difficult to measure properly with the measuring wheel. Another disadvantage of the micro-projector lay in its inability to bring up any anatomical details. Work on sex differentiation in very immature forms made it necessary to locate the genital primordium. This could not be done without the microscope.

When the camera lucida was set in the barrel of the microscope, the instrument had to be tilted toward the observer. This brought an image of the drawing paper, placed between the observer and the instrument, into the field of view along with the image of the object.

The angle at which the microscope was tilted was such that the image of the lines 0·1 mm apart in the stage micrometer were superimposed upon the paper at a distance equivalent to one revolution of the measuring wheel.

Therefore every revolution of the wheel corresponded to 100 . Measurements of fractions of a revolution (in terms of the number of teeth) were transformed into microns with the aid of a table based on the relationship, $\frac{1}{35}$ of a revolution $= \frac{100}{35} = 2{\cdot}86$ (see Appendix XII).

The method of measuring length was the same as for the micro-projector. Certain work involved measuring from the genital primordium to the tip of the tail. In this case, larval nematodes were placed one at a time on the slide, and the cover slip moved gently to roll the nematode over until the genital primordium could be seen.

THE OCULAR MICROMETER.

This was used once to measure the length of some infective larvae from a culture with which the experimental sheep had been infected.

Straight larvae were easily measured. It was considered however that the majority of the straight infective larvae in this culture were those which had died prior to fixing the population with formalin. (Cultures of infective larvae often have a number of dead larvae present, generally extended). They had therefore undergone a different treatment from the other larvae, and as this may have had some influence on their length, they were not measured.

Curved larvae were by far the most common, but being difficult to measure with the ocular micrometer, this method was abandoned.

: : : :

CHAPTER V.

THE EXPERIMENTAL FLOCK.

SUMMARY.

The management, feeding, and pre-experimental parasitism of the lambs is described.

TREATMENT PRIOR TO WEANING.

Romney cross ewe and wether lambs born the preceding spring were used for the experiment. These animals were reared under conditions which prevented any possibility of significant parasite infection.

Sired by ordinary flock rams, with typical flock ewes as dams, the lambs at birth were removed to a shed adjacent to the ewes' paddock. All lambs were ear-marked with numbered brass tags at birth. In the shed the lambs were bedded down on straw which was changed twice weekly. At feeding time the ewes were brought in to the lambs from the paddock. At first this was carried out five times a day, but the number of feeds was gradually reduced till at weaning in the middle of December the lambs were fed only once a day. To take the place of the grass with which the lambs would have otherwise supplemented their diet, concentrates and hay were fed from the beginning of October. The concentrate was made up from crushed oats, peas, lucerne chaff and bran. The latter, on account of its laxative effect, was fed in variable proportions as needed. Immediately prior to weaning, a proprietary concentrate 'sheep nut' was fed in small amounts.

TREATMENT AFTER WEANING.

After weaning in mid-December, the lambs were removed to a specially constructed shed. This was divided into pens which held three lambs. The floor was concrete, and over this was a thick layer of straw for bedding. Water was supplied in concrete troughs, and each pen had a wooden trough fixed to the railings for concentrate feeding. Hay was put in wire-netting racks at the back of the pens.

The whole shed was cleaned out once every four days. The straw was replaced, the concrete floors of the pens scrubbed down, and the lower railings hosed and scrubbed clean.

In the shed both lucerne and meadow hay were available at

all times. In addition the 'sheep nuts', fed first just prior to weaning, were fed twice a day at the rate of half a pound per day per head. After three weeks this was increased to three-quarters of a pound until ten days immediately prior to the main infestation on the 13th of April when it was entirely discontinued. After the first month from weaning, the lambs lost interest in their food, so for two weeks they received an additional ration of crushed oats, peas and bran, fed at the rate of three pounds per day per head. A standard commercial lick was available.

The 'sheep nuts' contained copper sulphate, and it was also likely that the lick contained a substance having anthelmintic properties. These were therefore eliminated from the diet before any attempt was made to infect lambs in the Main and Subsidiary experiments, and the lambs received hay only.

The lambs were weighed at weekly intervals. Those lambs used in the main experiment all weighed over eighty pounds just before infestation, the average weight being ninety-one pounds (Appendix IV). The lambs were thus doing very well. For comparison, a field flock reared on the land where the experimental lambs were born weighed on the average seventy-eight pounds at the same time the following year.

PRE-INFESTATION TREATMENT.

Diet.

The reduction of the diet to hay alone has already been mentioned.

Dilution egg counts as an index of parasites present.

Although a few fecal egg counts had been made for odd animals, there had been no attempt to examine all the lambs in a systematic fashion. Therefore, just prior to the Main Experiment, examinations of the faeces of all the lambs were carried out using Lane's Direct Centrifugal Flotation method (28) as applied by Stoll (54) for trichostrongyle eggs. This method is described in Appendix I. Results of the egg counts are in Appendices II and III.

These showed that ova of *Strongyloides papillosus*, *Nematodirus spathiger* and *N. filicollis* were present in nearly all the lambs, but in no instance did they exceed twenty-two eggs per gram of dung.

In the faeces of lambs 10 and 51 Trichuris ovis were found at the rate of 1·25 per gram. In lamb 29, an egg was recovered which measured 106μ long by 48μ wide. This placed it within the range of eggs in the Ostertagia Trichostrongylus group as defined by Tetley (65).

A further dilution count was made on the eight lambs selected for the Subsidiary Experiment on the day on infestation. The results revealed an increase in the numbers of eggs over the first count in the case of lambs 10, 23, 40, 42 and 49. Lamb 25 still recorded a negative count, while lambs 28 and 34 had approximately the same number of eggs per gram of faeces.

There was a marked change in the species present.* Those lambs recording increased egg counts had up to a maximum of 10 eggs per gram. By their measurements these were mainly in the Ostertagia Trichostrongylus group. The exception was lamb 40 which had, in addition to this group, one egg with the dimensions of typical C. curticei ova, and another which, although it fell within the range of Haemonchus contortus eggs (65) was most likely a flattened (and therefore widened) Cooperia egg.

When the ingesta from the lambs in the Subsidiary Experiment was examined, no record was made of the presence or absence of any mature forms of Trichostrongylus species. The abomasa were not examined, so the presence of Ostertagia species and possibly of Haemonchus contortus remained unconfirmed. The presence of C. curticei was confirmed in lamb 40 by the finding of two adult female C. curticei in the intestinal ingesta, whose uteri were full of unsegmented eggs. In the half of the intestinal ingesta from lamb 10 which was examined, one adult female C. curticei was found, the uteri containing segmented eggs.

: : : :

CHAPTER VI.

THE DEVELOPMENT OF A PURE INFESTATION.

SUMMARY.

The use of live adult Ostertagia and cultures of mixed species of infective larvae to infect a stock culture animal is described.

PREPARATION OF STOCK CULTURE ANIMALS.

The preliminary step in obtaining a sufficient number of infective larvae for the experimental infestations was to build up a pure infection in what was called a "stock culture animal".

Since the experiment was designed for Ostertagia spp., the first attempt to produce a pure culture centred round this worm.

DEVELOPMENT OF A STOCK CULTURE USING LIVE ADULT WORMS.

Initial attempt.

Collection of worms. A visit was made to a local abbatoirs late in January 1949. The abomasa were collected from a number of lambs and taken back to the laboratory. Here they were cut open, and by gentle washing the ingesta and most of the worms were removed and placed in a sieve. A quick flow of water through the sieve removed most of the ingesta and the material so obtained was washed into a beaker of water. The mucosa was then examined closely and any remaining worms removed with a dissecting needle, and transferred to the beaker.

Sorting, counting and identification of the population. This population was then examined with the aid of a low power (x12) binoculars in a special dish (Appendix VI). Males and females were separated and the male population was divided into the two species of Ostertagia - O. circumcincta and O. trifurcata.

The males of these species can readily be told apart by the shape of the spicules. In O. circumcincta, the spicules are long and comparatively narrow. Baylis (4) gives the dimensions as 0·28 to 0·32 mm. In O. trifurcata, they are short and broad. Baylis (4) gives 0·18 mm as an average length. This difference can be picked very easily with low power binoculars.

Ransom (41) states that the female of O. trifurcata is unknown. It is presumably morphologically identical with that of O. circumcincta. Therefore the only way to obtain a pure infection

of any one of these species is to use adult males of one species with a female population of both Ostertagia species. Since it was desirable to have the worms back into their normal habitat as soon as possible, only 200 O. circumcincta female were removed. 800 male Ostertagia were available.

Selection of the potential host. Lamb no. 1 was selected as a potential host because it was a wether. A wether is far more suitable as a stock culture animal when a bag has to be fixed to collect dung in reasonable quantities. With a ewe, the dung and bag become contaminated with the urine, making conditions unfavourable for the development of eggs to the infective larval stage.

An egg count made of the faeces of this animal on the day of infection (27/1/49) did not reveal the presence of any eggs.

Administration of the nematodes. The parasites were given to lamb no. 1 with the aid of a funnel and a piece of stout rubber tubing about sixteen inches long. This was placed in the side of the mouth and pushed down on the back of the tongue. Care was taken not to push the lamb's head back too far and thus hinder swallowing. 20 ccs of physiological salt solution (0·85 gms NaCl in 100 ml H_2O) were given first of all. This was an attempt to utilise the oesophagal groove reflex action to prevent the worms from becoming lost in the paunch and dying before reaching the abomasum. The 200 male O. circumcincta and 800 female Ostertagia spp. were then administered in 30 ccs of water. To wash the funnel and tube clear, a further 20ccs of salt solution was then used.

At the time of administration to lamb 1, the worms had been 5½ hours away from their host. No change was made in the diet of hay and concentrates.

Faecal egg counts. Examinations of the faeces for Ostertagia spp. eggs (using the D.C.F. method) were made as follows from the time of administration:-

9 hours	2 (segmented) eggs	93	x 45
		85	x 45
15 "	1 egg	85·5	x 48
18 "	1 egg	81	x 48
22 "	no eggs		
24 "	1 egg	94	x 51 .

Counts made 5 and 7 days later showed no eggs.

The dimensions given above place these eggs within the range of the Ostertagia-Trichostrongylus egg group, as determined by Tetley (65).

Subsequent attempts.

(a) Using Lamb 3 as a host. This was also a wether.

Numbers of worms administered. On 31/1/49, further material was collected as before. Ostertagia species females and O. circumcincta males were administered as follows:-

After 4½ hours away from a host.

200 female Ostertagia
100 male O. circumcincta.

After 10 hours away from a host.

250 female Ostertagia
100 male O.circumcincta.

One day later, (1/2/49) more parasites were obtained and administered as follows:-

After 4 hours away from a host.

400 female Ostertagia
50 male O. circumcincta.

After 5½ hours away from a host.

300 female Ostertagia
200 male O. circumcincta.

After 7 hours away from a host.

250 female Ostertagia
60 male O. circumcincta.

Total for the day.

950 females and 310 males.

On 3/2/49 (two days later) further larvae were administered as follows:-

After 3 hours away from a host.

200 female Ostertagia
150 male O. circumcincta.

After 6½ hours away from a host.

160 female Ostertagia
150 male O. circumcincta.

Total for the day.

360 females and 300 males.

Egg counts. Counts were made of the eggs present in the dung of lamb 3 on 2/2/49, two days after the initial infestation. This showed 2·4 eggs per gram of faeces. Dimensions of these

eggs were:-

88 by 41
90 " 46
96 " 46

and from these dimensions, as well as their general appearance, they were judged to be Ostertagia eggs.

The following day (3/2/49) an egg count revealed no eggs in the faeces, and the same result was derived on 4/2/49.

On 6/2/49, the egg count rose to 3 eggs per gram of faeces, and 2·2 eggs per gram on 8/2/49.

After twenty-five days from the final administration of worms to lamb 3 (i.e. on 1/3/49), the count was just over one egg per gram of faeces.

(b) Using Lamb 22 as a host. When it became apparent that Ostertagia were not holding in either lamb 1 or lamb 3, it was decided to attempt to infest a third animal. Both lambs 1 and 3 were big healthy animals. Acting on a belief that a smaller animal of poor appearance may be more readily parasitised than these bigger animals, lamb 22 was chosen. It weighed 60 lbs at this time.

Numbers of worms administered. On 4/2/49:-

After 3 hours away from a host.

300 female Ostertagia
150 male O. circumcincta.

After 6 hours away from a host.

160 female Ostertagia
35 male O. circumcincta.

(a few drops of this were lost in administration.)

Total for the day.

460 females and 185 males.

Only one further dose of adult worms was made, this being given on 9/2/49, as follows:-

After 2½ hours away from a host.

200 female Ostertagia
42 male O. circumcincta.

After 5 hours away from a host.

300 female Ostertagia
100 male O. circumcincta.

Total for the day.

500 females and 142 males.

Egg counts. On 6/2/49, two days after the first worms were given to this lamb, just under one egg per gram of faeces was found.

On 8/2/49, there were 2·4 eggs per gram of dung. Nematodirus species eggs were also present to the same extent.

A count made on 11/2/49, just after the final administration of adult worms to this lamb, showed one egg per gram of faeces.

These repeated failures to obtain a reasonable infestation using live worms led to the abandonment of this method.

DEVELOPMENT OF A STOCK CULTURE FROM INFECTIVE LARVAE.

Sources of Infective Larvae.

(a) Viable eggs from female Ostertagia. Lambs' stomachs were obtained from the Longburn Freezing Works. Eight hundred and fifty Ostertagia females were removed from these, and placed on a slide in convenient sized lumps. Using two needles sharpened like knives, these worms were cut up into small sections to break open the uteri and release as many eggs as possible. The low power binocular (x 12) was used for this work.

Many of the eggs were completely released by this treatment, but a number remained within short lengths of uteri; these in turn were often still encased within portions of the worms.

About four hundred grams of dung were obtained from comparatively worm-free lambs in the animal house and sterilised over a water bath at boiling point for one hour. The dung was then removed from the bath and thoroughly mixed with finely powdered animal charcoal. A little water was added in this process and the whole well mixed until it was damp and rather 'doughy'. Enough of this charcoal-dung mixture was placed on the bottom of a mason jar to make up to about two inches in depth. The surface was not smoothed, but left uneven just as the material dropped in.

When this mixture had cooled down to about 80°F, the crushed worms, together with the eggs, were mixed with a little water and poured into the mixture. It was necessary to ensure that only a little water was used, otherwise the water balance was upset. A lid was screwed on and the jar was labelled both on the top and side with the following details:-

(1) Source of Culture.

(2) Date collected

(3) Date at which the culture was due to be removed from incubation.

The jar was then placed in a "Warm Box" for nine days. This latter was a simple wooden box with a lid, the whole being lined with felt. Warmth was supplied by a 25-watt lamp, and the temperature control obtained by moving the lid to adjust the opening.

When a large quantity of dung had to be incubated for the Main Experiment, a second box was made of ten cubic feet capacity. It was lined with newspaper to keep as even a temperature as possible. A typical series of readings over a period of five days was as follows:-

Original Warm Box	74°F	72°F	72°F	74°F	70°F
New Warm Box	80°F	72°F	70°F	62°F	65°F

After removal from the box, if the culture was not required immediately, it was placed in a refrigerator at 45°F. It was considered that the room temperature of approximately 60°F would make the larvae very active, and much reserve energy be wasted. The larvae would thus be weakened when the time came to infect the lambs.

When examined after incubation, this culture failed to produce any larvae.

(b) From the Dung of an Infected Animal.

(1) Using the Stock Culture Lamb. This lamb was voiding faeces at the time with an egg count of 2·5 eggs per gram. It was decided to use this dung for cultures, which would be administered back to the lamb.

i. Collection of Faeces. For the collection of the faeces, a rubber bag was fastened to the lamb with the aid of leather harness especially made for the purpose. About one hundred and seventy grams were collected and placed in two mason jars. These were labelled in the same fashion as those used for the culture made from eggs of live worms, and were placed in the warm box for nine days.

ii. Collection of larvae. The larvae were washed off the sides of the jars with a gentle stream of tepid water. In all, one hundred and forty-three larvae were recovered from this sample. They were all dark in colour and active. A few free

living female stages of Strongyloides papillosus were present.

(2) Using an Undrenched Flock Lamb. It was realised that re-infection of lamb 22 from its own faeces was likely to be a slow process. Therefore a search was made for a field animal which had not been drenched and which would therefore be likely to have a good population of Ostertagia. A lamb was located and brought into the experiment. This animal was known as "the lame ewe lamb".

i. Species present in this Lamb as shown by egg counts. The total count was 750 eggs per gram of dung. From their appearance they were judged to be mainly the Ostertagia-Trichostrongylus group (65).

A few Cooperia curticei and Haemonchus contortus eggs were also present as well as Nematodirus spathiger and N. filicollis. Numerous coccidia (Eimeria spp.) were also observed.

ii. Species present as shown by Post Mortem. Early in April the lame ewe lamb was killed. No count was made of the number of worms present, a qualitative analysis only being made. Yorke and Maplestone's (72) classification was used. Species present were:-

Large Intestine

Oesophagostomum venulosum, (Rudolphi, 1809).

Small Intestine

Bunostomum trigonocephalum (Rudolphi 1808).

Nematodirus filicollis (Rudolphi 1802)

N. spathiger (Railliet, 1896)

Trichostrongylus vitrinus Looss, 1905

T. colubriformis (Giles, 1892)

Strongyloides papillosus (Wedl 1856) Ransom, 1911

Cooperia curticei (Railliet, 1893)

Abomasum

Ostertagia circumcincta (Stadelmann, 1894)

O. trifurcata Ransom, 1907

Haemonchus contortus (Rudolphi, 1803)

It should be noted that only one species of Cooperia was present.

On 2/4/49, an egg count was made of both the stock culture animals, and the eggs seen classified on their shape as Ostertagia

or Cooperia. It was soon apparent that C. curticei eggs were much more abundant than Ostertagia species.

The eggs of C. curticei are readily differentiated from those of O. circumcincta and O. trifurcata. The former are both narrower and shorter than the latter, averaging 37 wide by 80 long, as opposed to O. circumcincta and O. trifurcata which are approximately 95μ by 45μ (5). C. curticei eggs have parallel sides and the actual shape is more different from the Ostertagia eggs than the dimensions suggest.

This diagnosis was later confirmed when the relative numbers of worms in the experimental lambs were examined. Lamb 2, for example, killed on 22/4/49 when the population had been developing nine days, gave 290 Ostertagia species in the abomasum whereas the number of C. curticei in the small intestine was 4050. (These figures are from data collected by Tetley).

As detailed on page 39, worms of other spedies were present in the C. curticei population but at no time in any significant numbers.

It is therefore obvious that the mixed populations in the Lame ewe lamb were not a serious factor, in spite of the multiplicity of species present.

In later discussion on egg counts, where the population is definitely known to be Ostertagia, the noun is used without inverted commas. Where the population is actually mixed, including Cooperia and Ostertagia spp. "Ostertagia" - in inverted commas - is used.

iii. Collection of faeces. Since the lame lamb was a ewe, it was not possible to use a bag to collect the faeces. Cultures had therefore to be made from small amounts of dung collected from the rectum with the finger. This eventually became a difficult task, since the lamb got into the habit of voiding its faeces as soon as it saw the collector. Dung which was thus voided was not used unless it was actually seen to land on the grass and there was absolutely no doubt as to its origin. The presence of other sheep in the paddock made this important. If it landed on bare earth or amongst faeces from other sheep, it would be likely to become contaminated with either free living nematodes or those from other sheep.

iv. Collection of Larvae. Larvae were washed from the sides of the jar with tepid water as mentioned above and examined on a small watch glass under the microscope. Many Strongyloides were seen and approximately two hundred "Ostertagia" larvae.

The presence of many Strongyloides in cultures from this lamb led later on to the idea of eliminating them by gentle heating. An electric fan was used to blow warm air onto a filter paper on which the larvae were lying. As soon as the paper had dried, it was placed in water and the larvae washed off. Many of the Strongyloides failed to recover after this treatment, but a smaller proportion of "Ostertagia" larvae also succumbed. The treatment was not attempted again.

ADMINISTRATION OF THE INFECTIVE LARVAE.

Apparatus and Method.

The larvae were administered with the aid of the rubber tube and glass funnel used previously when dosing lambs with adult worms. The larvae were suspended in about thirty ccs of water and this was poured slowly into the funnel. A similar volume of water was used to wash out the funnel and tube, and to make quite sure that the larvae were swallowed.

Administration to Lamb 22.

Date.	No. of Larvae ("Ostertagia").	Source of Larvae.
16/2/49	143	Lamb 22
26/2/49	80	Lamb 22
	200	Lame ewe lamb
	280	
27/2/49	47	Lamb 22
	155	Lame ewe lamb
	202	
28/2/49	2000	Lame ewe lamb

Administration to Lamb 3.

1/3/49	2550 "Ostertagia"	Lame ewe lamb.

EGG COUNTS OF STOCK CULTURE ANIMALS.

Lamb 22.

Immediately prior to administration of the first dose of infective larvae on 16/2/49, the egg count was less than one "Ostertagia" spp. egg per gram.

Counts of eggs in the faeces four weeks later were as shown in the table on the following page:-

Egg counts for Lamb 22.

Date.	No. of eggs per gram. ("Ostertagia" spp.)
13/3/49.. ..	..just over one
21/3/49.. ..	..56
22/3/49.. ..	.268
24/3/49.. ..	.272
26/3/49.. ..	.228
27/3/49.. ..	..16
29/3/49.. ..	.416
31/3/49.. ..	.176
1/4/49.. ..	.356
2/4/49.. ..	.424
28/4/49.. ..	.363

The count for 2/4/49 was analysed as follows:-

Ostertagia species - 144 e.p.gm.

Cooperia curticei - 280 e.p.gm.

Lamb 3.

When the infective larvae were given to this animal on 1/3/49, the "Ostertagia" egg count was just over one egg per gram of faeces. The following egg counts indicate the growth of the population of "Ostertagia" in this lamb:-

Egg counts for Lamb 3.

Date.	No. of eggs per gram. ("Ostertagia" spp.)
21/3/49..	23
22/3/49..	36
24/3/49..	72
26/3/49..	36
27/3/49..	56
28/3/49..	68
30/3/49..	160
31/3/49..	264
1/4/49..	112
2/4/49..	120

Enough eggs were now available on the dung of lambs 3 and 22 to ensure the development of sufficient infective larvae for the experimental infestation in the Main Experiment.

: : : :

CHAPTER VII.

THE TRIAL EXPERIMENT.

SUMMARY.

A pilot experiment, carried out to determine what was a suitable number of infective larvae for administration, and to gain some information on the development of Ostertagia, failed because only small numbers of larvae were recovered. Suggestions are made as to the numbers of infective larvae required for reasonable infestation, and possible reasons for the failure of this experiment are examined.

OBJECTS AND SCOPE.

The objects of this experiment were threefold:-

(a) To establish a procedure for the administration, collection and measurement of the parasites.

(b) To determine the number of larvae to be administered to each sheep.

(c) To gain some preliminary knowledge as to the size and relative development of Ostertagia circumcincta and O. trifurcata in the early stages.

PLAN OF THE TRIAL EXPERIMENT.

(1) Number of larvae to be administered. The relevant literature on life history studies was consulted before a figure was decided upon. Threlkeld (67) working with Ostertagia circumcincta used doses of infective larvae warying from fifteen hundred to one hundred thousand larvae for each lamb. He reports that one lamb which had received fifteen hundred larvae was killed five days later and only five worms were recovered. No mention is made of the number recovered when five thousand were administered to a lamb which was killed ninety hours later, but apparently they were present in "workable" numbers. The same applies to the lambs receiving six thousand larvae, and killed two and seven days later respectively. The other doses he employed were: three at twenty thousand, one each at fifty thousand and seventy-five thousand, and one at the hundred thousand level.

In all these examples, numerous larvae were apparently recovered.

Andrews (2) studying the resistance of lambs to superinfection with Cooperia curticei used varying doses from two hundred and

fifty to one million. His data is of little use for determining the size of dose for the proposed experiments, since, in all except one case, several doses were given to the one lamb over a period of several days. The largest dose of one million is the only case he cites of a single dose being given, and in this example, 9·6% of the larvae administered were recovered at five days.

After considering the above data, it was decided to use five thousand infective larvae as a dose. If ten per cent of these survived, there would be ample for measurement.

(2) Number of lambs to be killed. It was decided to kill three lambs in all, at intervals of three, five and seven days respectively from the date of infestation.

PREPARATION OF THE CULTURES.

When the infection of the three sheep used in this experiment was carried out, the only source of infective larvae in large numbers was the lame ewe lamb. This was the only occasion on which this lamb was used as a direct source of experimental material.

Owing to the difficulties of collection already explained, only small quantities of dung, amounting to ten or twelve grams, could be collected at any one time. A number of these small cultures were developed in the warm box and kept in the refrigerator until 4/3/49 when the infection was made.

COLLECTION, ESTIMATION OF THE NUMBERS, AND ADMINISTRATION OF THE INFECTIVE LARVAE.

The methods employed are treated more fully in a later section describing the Main and Subsidiary Experiments.

The total number of larvae recovered amounted to 15,400 "Ostertagia" and 236,200 Strongyloides spp. These were divided into three lots each containing about 5,000 "Ostertagia" larvae. (The term "Ostertagia" refers to Trichostrongylid larvae generally. Of these, true Ostertagia larvae were probably in the majority, but many Cooperia and Trichostrongylus spp. would be present.)

Three lambs were each dosed with 40ccs of water containing the larvae between 2 p.m. and 2·15 p.m. on March 4th, 1949. These lambs were then killed as follows:-

Lamb 31	killed	7/3/49,	infestation	3	days	old
Lamb 17	"	9/3/49,	"	5	"	"
Lamb 12	"	11/3/49,	"	7	"	".

EXAMINATION OF THE MATERIAL.

The abomasum was collected from lamb 31, killed three days after infestation. The ingesta was removed and the pyloric region washed very carefully twice. The washings were stained in Lugol's Iodine and examined under the low power binocular microscope (x12).

From the 340 grams of the ingesta, a series of 5 gm samples were taken and 75 ccs of water added to each. From these samples, 12 cc aliquots were collected and centrifuged, as in the technique for egg counts already described. Following centrifuging, the cover slips were removed, placed on slides and examined for larval forms. A total of twenty-eight slides were prepared in this way.

Lamb 17 was killed five days after infestation. The abomasum was collected and the ingesta washed out. Portions of this were centrifuged, a total of twenty-six slides being prepared. Portions of the ingesta in one or two gram aliquots were pressed between glass plates and examined under the low power microscope. The pyloric region was washed and the washings examined. The surface of the pyloric region was scraped, and the scrapings stained in Lugol's Iodine and compressed gently between two slides.

Lamb 12 was killed seven days after infestation. The ingesta was removed from the abomasum and portions were centrifuged. The walls of the abomasum were washed twice and the washings examined.

Small white elevations on the walls of the fundic region of the abomasum of this lamb were dissected under the microscope. Others were subjected to pressure and watched under the microscope for the presence of larvae.

RESULTS.

From the three lambs killed in this experiment, a total of six larvae were recovered as follows. From lamb 31, three larvae were found upon centrifuging. None were found in lamb 17, but in lamb 12, killed seven days after infestation, three larvae were found after centrifuging the ingesta, two of which were damaged.

All the specimens were mounted, but they were damaged before measurements were taken. They were all approximately the same size, and were either Haemonchus contortus or Ostertagia species. The writer was unable to establish identity definitely.

The fundic region of the abomasum of lamb 12 was covered with a series of round white raised areas, varying in diameter from five to less than one millimetre, and present to the extent of seven or eight to the square inch approximately. None of these elevations were found in the pyloric region of the abomasum. No larvae were found in those elevations which were examined.

DISCUSSION.

(a) Methods.

Size of infecting dose. The fact that so few larvae were recovered may be partly ascribed to the size of the infecting dose. Other possible causes are set out below. However, in the absence of any very definite indications to the contrary, it was considered that five thousand larvae are too small a number to administer under the conditions of this experiment, if measurements of several hundred adult parasites are required.

Reasons for the failure to find parasites. While no positive answer can be given a number of factors are set out which may have some bearing on this problem.

(1) The size of the infecting dose. This has already been considered above.

(2) The species present in the infecting dose. It has already been shown that the lame ewe lamb, from which the culture of infective larvae came, harboured a wide range of parasites. The significance of this was not fully understood when infestations were made. It is possible that the actual numbers of parasites of the abomasum (H. contortus and Ostertagia species) which were present at that time did not form the bulk of the five thousand larvae, but only a small proportion.

(3) Viability of the larvae. So far as is known, the larvae were healthy. The vast majority were active when counted. Between the conclusion of the counting at 11 a.m. and administration at 2 p.m., they were stored in three lots each of 40 ccs in a refrigerator at 40°F. It is not known whether their viability was affected in this period.

(4) The method of administration. This was the same as that used in later successful experiments. The writer is unable to list any factor which might have caused a loss of larvae in this process.

(5) Feed of the lambs. The lambs were receiving the concentrate sheep nuts at the rate of three quarters of a pound per day, as well as hay ad lib., at the time of infestation. The manufacturer's analysis of the sheep nuts indicated that considerably less than 1·5% of copper

sulphate was present. It is unlikely that this would have any anthelmintic effect on the infective larvae. The writer is aware of Taylor's (58a) work wherein he showed that ram lambs receiving a 'concentrate cake ration' had a lighter incidence of parasitism compared with those receiving a smaller ration. Taylor points out that this may have been due to the shorter grazing time for those animals receiving large amounts of the concentrate. In the absence of more definite information, it cannot be said that the apparent failure of the larvae to infect the lambs was definitely due to the concentrate sheep nuts.

(6) The technique of collection. Whatever criticisms there are which can be levelled at the methods employed in searching for the parasites, the writer is certain that, had there been several hundred larvae present in the abomasum, the technique would not have failed to locate a fairly high proportion.

In the light of later experience it is realised that a more critical examination of the ingesta would have been made. By taking one fifth of the ingesta, dividing it into fine and coarse particles by sieving, adding Lugol's Iodine stain and diluting with water, the ingesta could be examined thoroughly fibre by fibre if necessary. This method was used with success in later work where very small forms of C. curticei had to be found. Though painfully slow, it is very efficient.

As an additional check, the abomasum itself should have been washed in copious quantities of water, paying special attention to the folded fundic region. The water, left to stand so that particles of ingesta, epithelium and worms might settle, could then be poured off and the solid material added to that already collected.

The examination of the raised white areas was considered adequate; though no sectioning was carried out, the methods used would not have failed to locate at least some of the larvae, had they been present to any significant degree.

The plan of the experiment. The plan would have been improved if three separate levels of infective larvae had been administered, e.g. 5,000; 10,000; and 15,000. Assuming that the actual size of the dose was responsible for the small numbers found, then it is possible that this method would have yielded some parasites at the higher levels.

(b) Results.

The three objectives of this experiment were set out in the section on the Plan of the experiment. It remains to be seen to what extent these objectives were realised.

(1) The results showed that the centrifuging of the ingesta was not satisfactory, mainly because only small amounts could be studied.

(2) The dose rate for infective larvae has already been discussed.

(3) Nothing was learnt about the size and development of Ostertagia at different ages. Those forms recovered seven days after infection were approximately the same length as those recov-

ered three days after infection.

This lack of development may be the clue to the apparent absence of larvae. It is possible that the larvae were unable to develop past the third parasitic stage, due to the feed of the lambs or some other factors - though the actual cause cannot be stated with any degree of accuracy. The larvae would then be likely to be eliminated.

The round white areas in the fundic region of the abomasum were reported by Threlkeld (67) as appearing 90 hours after the commencement of parasitism. Embedded in these he found from one to four coiled larvae, without sheaths and presumably in the fourth instar. After 120 hours from the commencement of parasitism, Threlkeld reported that the walls of the abomasum were macroscopically normal except for a few elevated areas which contained no larvae.

In this work the writer did not find any raised white areas until the seventh day of parasitism. This would seem to indicate that the development of the larvae was slower than reported by Threlkeld, at least in lambs 17 and 12, killed on the 5th and 7th day respectively.

It has been pointed out that the raised white areas were numerous in the fundic region of lamb 12. This indicates that many larvae must have been present at some time to cause this upset to the epithelium. Why were they not found within these elevations? It may be that they were eliminated while trying to penetrate the wall of the abomasum, but had already caused sufficient damage to the tissue to produce the raised areas. Alternatively they may have already left the shelter of the epithelium in the normal (but delayed) course of their life, and were then eliminated. Threlkeld says that on the fifth day of parasitism only a few elevated areas were left, and they contained no larvae. It can be suggested that, had lamb 12 been killed a few hours later, fewer elevations would have been seen.

: : : :

CHAPTER VIII.

THE MAIN AND SUBSIDIARY EXPERIMENTS - METHODS.

SUMMARY.

The experimental plans are described. Details of the collection of larvae, the estimation of their numbers, administration to and slaughter of the sheep, and the collection of the parasites are given.

INTRODUCTION.

Although infection and killing of the lambs was carried out at different times for these two experiments, the methods are very similar. So far as the results are concerned, they are combined, and the methods will therefore be discussed as one rather than separately.

THE EXPERIMENTAL DESIGN.

(a) The Main Experiment.

Objects and scope. This experiment was originally designed to cover the growth to maturity of Ostertagia circumcincta in the sheep. As explained elsewhere, it later changed to a growth study of Cooperia curticei. Since the life histories of these worms are very similar, no change was made in the programme as originally conceived.

In designing the programme, three factors had to be considered:-

First, the plan must cover the total period of growth of the worm, and allow also for any individuals or populations which are slower in reaching maturity than the norm;

Secondly, as many populations as possible must be obtained at the times when the parasite is making the most rapid changes in morphology; and the intervals between the observations must be close enough to ensure that these changes can be followed accurately;

Thirdly, since individual sheep vary in their suitability as hosts for parasites, with some showing more susceptibility than others, as many sheep as possible must be slaughtered at each killing in order that some measure of the individual variation can be obtained.

When the time came for the administration of the larvae, two separate dose rates were employed, in view of the numbers

available. The object of this was to determine the effect, if any, of size of population upon the growth of individuals within that population. This necessitated some modification of the original programme, which is explained in a later section.

Plan of the Main Experiment. It was decided that two killings at intervals of twenty-four hours would be adequate to give populations at measurable stages of development.

The following programme of killings was therefore made out:-

Days from administration of infective larvae.	No. of lambs to be killed.
1 day	1
2 - 12 days	2 each day
14 days	2 lambs
16 "	2 "
18 "	2 "
20 "	2 "
22 "	2 "
Total No. of lambs	35

Only one lamb was to be killed on the first day. Larvae, after twenty-four hours in the host, would be very little changed and it was thought that the one animal would suffice.

From two to twelve days, Cooperia and Ostertagia pass through a period of rapid growth, including two molts. Accordingly, two sheep were killed each day.

After twelve days, the interval for killings was changed to two days. Reasons for this were:- first, twenty-three sheep were already included in the programme. It was necessary to keep sheep numbers within reasonable limits, not only because the number available was not great, but also because too large a bulk of material would take too long to measure. Secondly, the worms were nearly mature at twelve days and the greater part of their growth was finished. Thirdly, the number of infective larvae available would not be great.

On the fourteenth, sixteenth, eighteenth, twentieth and twenty-second days, two sheep were to be killed. Though worms would be fully mature at fifteen or sixteen days, the killings were planned to extend to twenty-two days in order to make quite sure, as already mentioned, that any worms which were slow in reaching maturity would have a chance to finish their growth.

Number of Larvae in Each Dose. After the failure of the Trial Experiment using five thousand infective larvae, it was de-

cided to use ten thousand for each dose.

Later in the same day, when further infective larvae were collected by Baermann's apparatus, and it was realised that more were available than were expected, a second level of infestation was selected - 22,000 larvae per dose.

(b) The Subsidiary Experiment.

Objects and scope. As an outcome of work done on the total populations in sheep killed in the main experiment (Tetley conducting another experiment concurrently, data unpublished), it was found that there was a distinct fall in the numbers of worms recovered on the seventh day. With the primary object of investigating this phenomenon, eight sheep were made available for further study. From the point of view of this thesis, these sheep were to serve the following purposes:-

(a) To provide additional data for the period covering the final ecdysis of Cooperia curticei.

(b) To study further the effects of very heavy infestations compared with moderate or light infestations.

(c) By providing additional data for the age groups already covered, to give further insight into the question of variation between hosts.

Plan of the Subsidiary Experiment. The eight sheep were to be killed two at a time on the sixth, seventh, eighth and ninth days after infestation.

Number of Larvae in Each Dose. The infestation was to be at two levels:-

(1) In the vicinity of twenty thousand larvae

(2) A very heavy infestation, the number of larvae used being dependent upon the number available.

THE COLLECTION AND COMPOSITION OF THE INFECTIVE MATERIAL.

(a) The Main Experiment.

Stock culture animals used. As soon as the stock culture lambs 3 and 22 commenced to pass large numbers of eggs in their faeces, dung was saved as a source of infective larvae for the Main Experiment. The Lame ewe lamb was not used as a source of infective material for either this or the Subsidiary Experiment.

The procedure with the cultures was the same as that pre-

viously explained. After incubation they were stored in a refrigerator.

Species present in the cultures. Egg counts were made of the faeces of the stock culture animals while the cultures were being prepared.

As explained in a previous section, eggs from C. curticei soon outnumbered those from Ostertagia species.

It was also possible that eggs of other species were present which would confuse the early parasitic stages. Tetley (5) has shown that the dimensions of Trichostrongylus colubriformis and T. vitrinus are practically the same as O. circumcincta and O. trifurcata.

In order to make sure of the species present in the cultures, it was decided to kill stock culture lamb 22. This was done as soon as sufficient faeces for culturing purposes had been collected.

Technique for collection of the parasites and their removal from the ingesta was the same as with the Lame ewe lamb killed earlier. Species present were:-

Ostertagia species (mainly O. circumcincta, a few O. trifurcata)	10
Cooperia curticei	180
Trichostrongylus spp.	15
Nematodirus spp.	33

In the large intestine were found a few Trichuris ovis and Oesophagostomum venulosum. No count was made of these.

It was therefore considered that those species present in the cultures other than C. curticei and Ostertagia spp. would not be an important foreign element in the final populations of the infected sheep.

(b) The Subsidiary Experiment.

Stock culture animals used. For this purpose lambs 3 and 5 were used as a source of infective material. Lamb 5 had been drenched with 22,000 infective larvae in the Main Experiment. When lamb 22 was killed, it was decided to retain lamb 5 as a stock culture animal. The faeces of these lambs were collected and cultured in the warm box, and stored in the refrigerator

until required.

Species present in the cultures. The same conclusions were reached as for the Main Experiment.

THE ISOLATION OF INFECTIVE LARVAE FROM THE CULTURES.

Two methods for collecting infective larvae from the cultures were employed:-

(1) Washing larvae off the sides of the jar with a gentle stream of warm water.

(2) Placing the faeces in Baermann's apparatus.

(With the exception of the Trial Experiment, both these methods were employed. In the Trial Experiment, method (1) only was used.)

(a) Collection of Larvae from the sides of the jar.

In most cultures, the larvae collected in great numbers on the sides of the jar. They formed large aggregations which could readily be seen with the naked eye. To wash these larvae out, the jar was held with the mouth pointed slightly downward and the sides were washed with a gentle stream of tap water at a temperature of approximately 70°F. The jar was revolved several times so that the water came in contact with the entire inner surface. The suspension of larvae was then poured off into a petrie dish. This process was then repeated a second time.

Care was necessary to ensure that the faeces did not work loose and fall out into the water when the jar was tilted.

(b) Collection of Larvae from the faeces. Baermann's apparatus was used for this purpose (See Appendix V.

The dung was removed from the culture jars and placed in the apparatus. The larvae soon became visible as they moved downwards to the bottom of the funnel. When the majority had reached the bottom (in about 30 to 60 minutes) the first few ccs of water containing the larvae were run off into a petrie dish.

ESTIMATION OF THE NUMBERS OF LARVAE.

Procedure.

The suspension of larvae was poured into a measuring cylinder and made up to the nearest convenient whole number of ccs with water.

For counting, a series of samples, each of 0·1 cc were

taken. Prior to withdrawing the sample, the measuring cylinder was inverted several times in order to ensure that the larvae were distributed as evenly as possible throughout the water. A pipette fitted with a rubber bulb was inserted to approximately the same depth at each sampling. It was allowed to half fill with suspension. Upon withdrawing, the rubber bulb was squeezed and the liquid forced out until the bottom of the meniscus reached the 0·1 cc. mark. The bottom of the pipette was held against the side of the measuring cylinder during this operation. This aided in the removal of excess liquid, and prevented any drops from adhering to the end of the pipette. The suspension was then spread over a piece of glass ruled into small squares.

Counting was done under low power binoculars (x 12) with the aid of a hand counter. The ruled squares facilitated accurate work. The following are the details of the counting for the Main and Subsidiary Experiments:-

(1) The Main Experiment.

(a) Larvae washed from the sides of the jars.

Counts made on four samples were as follows:-

1st	sample,	volume 0·1 cc.	28	larvae
2nd	"	" "	37	"
3rd	"	" "	39	"
4th	"	" "	38	"
		Mean	35·5	"

Total volume of suspension - 540 ccs.

The total population was therefore estimated to be $\frac{540 \times 35{\cdot}5}{0{\cdot}1}$, or 191700 larvae.

The dosage rate already decided upon was 10,000 larvae for each of 16 lambs. To obtain this number, $\frac{540 \times 10{,}000}{191{,}700}$, or 28·2 ccs would be needed for each lamb.

This left 90 ccs or 31,950 larvae. This portion was concentrated by standing for a short time, the larvae fixed by the addition of a little formalin, and kept for future study.

A number of 2 oz. screw top jars were used for each sample. A measuring cylinder was used to determine the 28 ccs, and a pipette for the 0·2 ccs. The suspension was thoroughly agitated before measuring each aliquot.

(b) Larvae collected with Baermann's apparatus.

Counts were made on five samples as follows:-

1st sample, volume 0·1 cc.		57 larvae
2nd	" " "	66 "
3rd	" " "	57 "
4th	" " "	50 "
5th	" " "	46 "
	Mean	55·2 "

Total volume of suspension - 600 ccs.

The total population was therefore estimated to be

$\frac{600 \times 55{\cdot}2}{0{\cdot}1}$, or 330,000 larvae.

The dosage level was selected as being 22,000 larvae. With 15 lambs, 40 ccs of suspension was used.

This was measured with the aid of a measuring cylinder and placed in screw top jars.

(2) The Subsidiary Experiment.

Larvae from the sides of the jar and from Baermann's apparatus were bulked together. The suspension was made up to 500 ccs. Samples of 0·05 cc were taken for measurement.

Sample No.	Larvae counted.
1	157
2	175
3	174
4	234
5	190
6	188
7	198
8	172
9	157
10	166
11	165
12	185
13	186
14	186
Mean	181·6

Therefore Larvae in 500 ccs totalled 1,820,000.

A slide on which was placed a drop of the suspension was examined under the microscope and larvae present were identified as "Strongyloides" or "Cooperia-Ostertagia" since these latter were likely to be present in large numbers compared with other Trichostrongylid larvae.

Of 86 larvae examined at random, 56 were Strongyloides, both free living female forms and parasitic forms.

Four doses of 15·7 ccs volume were made up. These each contained 57,148 infective larvae of all types. Of these approximately 19,900 would be "Cooperia-Ostertagia".

The remainder of the suspension was divided into four parts of 109 ccs volume each, and each portion contained 396,700 larvae.

of which approximately 138,000 larvae were "Cooperia-Ostertagia".

ADMINISTRATION OF THE LARVAE.

Procedure.

The larvae were administered in all experiments with the aid of a glass funnel and a short length of pressure tubing. This was the same apparatus as had been used previously in administering both live worms and infective larvae to lambs.

The suspension of water containing the larvae was poured into the funnel a few drops at a time, until the full dose had been given. A "chaser" of water of approximately 30 ccs volume was then poured slowly into the funnel in order to wash the larvae as far as possible down the throat of the lamb.

Details for each experiment.

(a) The Main Experiment. The first 16 lambs dosed received 10,000 larvae each. There was some selection in this group since only those lambs with the lowest numbers of Strongyloides and Nematodirus eggs in the faeces were dosed.

The following lambs received 28·2 ccs of suspension containing ten thousand infective "Ostertagia" larvae on April 13th, 1949, between 10·30 and 11·00 a.m.

	Lamb No. (as on ear tag)
1	2
2	3
3	8
4	9
5	16
6	18
7	26
8	27
9	32
10	33
11	36
12	37
13	44
14	46
15	48
16	52

The remaining 15 lambs received 40 ccs of suspension in water of 22,000 "Ostertagia" larvae on April 13th, 1949, between 4·30 and 5·00 p.m.

	Lamb No. (as on ear tag)
1	5
2	6
3	7
4	14
5	19
6	24
7	29

(continued over...

Lamb No. (as on ear tag)	
8	31
9	35
10	38
11	39
12	41
13	43
14	47
15	51

(b) The Subsidiary Experiment. For this purpose, eight lambs, selected at random from those left in the animal house, were used.

The following received 19,900 "Ostertagia" larvae in 15·7 ccs of water between 3·00 and 3·45 p.m. on June 16th, 1949:-

Lamb No. (as on ear tag)	
1	10
2	25
3	40
4	42

The following received 396,700 larvae in 109 ccs of water between 3·00 and 3·45 p.m. on June 16th, 1949:-

Lamb No. (as on ear tag)	
5	23
6	28
7	34
8	49

THE KILLING PROGRAMME.

Modifications to the Original Plan of the Main Experiment.

A dose of ten thousand larvae was given to sixteen lambs. Later in the same day, when the remaining larvae had been recovered from the dung by the Baermann's apparatus, it was decided to reduce the number of lambs from nineteen to fifteen, thus enabling a dose to be given which would be over double that administered in the morning. This difference in population, it was hoped, would bring out any possible differences in growth due to level of population. Each day would see 2 lambs of different population levels killed. (The obvious difficulties which this introduces by confounding population level differences and individual host variation will be discussed later.)

The final plan adopted for both experiments, together with the individual numbers of the lambs killed, is shown in the following table.

Table ____.

Age of Worm in Days	MAIN EXPERIMENT		SUBSIDIARY EXPERIMENT	
	Sheep receiving 10,000 larvae. Sheep no.	Sheep receiving 22,000 larvae. Sheep no.	Sheep receiving 20,000 larvae. Sheep no.	Sheep receiving 138,000 larvae. Sheep no.
1	46			
2	32	47		
3	44	39		
4	16	14		
5	4	35		
6	37	24	40	28
7	-	40 & 43	10	23
8	8	19	42	34
9	2	7	25	49
10	48	-		
11	36	38		
12	26	-		
14	52	51		
16	33	30		
18	9	29		
20	-	6		
22	28 & 18			
	N.B. Numbers in these columns are those on ear tags of the sheep.			

Table of Main and Subsidiary Experiments, showing numbers of lambs killed.

The programme is unchanged up to nine days, except that at seven days two lambs (nos. 41 and 43) which had received the same dose of infective larvae were killed. This was due to an error in a field note book used when lambs were selected for killing.

At ten and twelve days, one lamb (instead of two as originally proposed) was killed. The same applied at twenty days. On the twenty-second or final day of the experiment, two were killed, both being of the same dosage group. This was unavoidable, owing to the previous error on the seventh day.

The Subsidiary Experiment.

This experiment did not deviate from its original plan.

THE COLLECTION OF THE INGESTA.

The following procedure was adopted for all killings in all experiments, with the exception of one lamb, as mentioned later.

The stomach and intestines were available for treatment about twenty minutes after the animals were killed. The mesenteric fat was removed as much as possible from the abomasum. An incision was made with a knife between the reticulum and the omasum and through the duodenum immediately posterior to the

pyloric valve. This kept the abomasum free with both ends blocked.

A mason jar 6½ inches high and 3½ inches internal diameter was placed in a large pie dish. The latter was intended to catch any ingesta which failed to enter the jar. The abomasum was then cut free and the fundic end inserted into the jar into which most of the ingesta then flowed. A slit was made down one side of the abomasum and the pyloric region close around the valve everted to expose it as much as possible to the fixative and preservative.

Formalin (40%) was then added at the rate of approximately 50 ccs to 500 ccs or 600 ccs of water making a 3% or 4% solution.

The small intestine was then run out in three-foot lengths until the thirty-ninth foot mark was reached. It was cut at this point and run into a mason jar similar to the one used for the abomasum. The left hand was placed over the top of the jar with one end of the intestine protruding from between the fingers which were held tightly together. The intestine was then pulled gently so that it ran through the fingers and the ingesta was thus held back and forced out into the jar. Fifty ccs of 40% formalin was added to this, while the intestine itself was discarded.

The second half of the small intestine was cut free from the large intestine and accorded the same treatment.

Each jar had a one-inch strip of brown paper completely encircling the exterior. On this was written the number of the lamb, the date killed and the region from which the contents were obtained. The jars were also numbered consecutively from one for the abomasum of the first sheep killed through to one hundred and twenty-four for the second part of the small intestine of the last sheep killed. This was a useful guide in storing the jars.

All jars had screw tops. These were removed on arrival at the laboratory and a check made on the presence of formalin. In addition, a piece of white cardboard one inch by three was inscribed with the same information as on the label of each jar, written in Indian ink, and placed inside the jar.

In addition to the contents of the gut, a sample of faeces was removed from the rectum at the same time. This was not

preserved, being handled in the laboratory immediately.

This procedure differed for the sheep killed on the first and second days of the main experiment. The sheep killed on the first day had a tenth portion of the contents of the rumen collected, as well as the reticulum and omasum. The reticula of the two animals killed on the second day were kept. These organs were handled on arrival in the laboratory.

COLLECTION AND SORTING OF THE NEMATODES.

(a) From the Intestines.

Ingesta was poured into a large glass bottle graduated in five hundred cubic millilitres up to the five thousand mark. It was thoroughly stirred and then poured into a fine sieve, after which a fine stream of water was directed onto it. This forced the finer debris through, leaving the coarse material and all but the very immature nematodes. It was found that those forms which had been in the host for only three or four days would sometimes wash through the sieve, so the "filtrate" was always examined for their presence.

It was easier in all cases to sieve and search the fine and coarse fraction separately, rather than examine the undivided mass.

The coarser residue in the sieve was washed into a beaker and a little of Lugol's iodine stain added. It was then diluted with a little water and a small quantity poured into the special container mentioned in Appendix VI. This portion was then searched for nematodes, using a low power dissecting microscope. Worms were readily removed with a dissecting needle. Only Cooperia curticei were collected, odd individuals of other species being ignored. The "filtrate" was treated similarly except that, in those cases where all the nematodes in the coarse portion had been fairly mature forms, it was considered sufficient to examine a part, rather than the whole. In order to ensure that a good representative sample was collected, it was the custom to collect nematodes from at least half of the ingesta. In some cases, where numbers were low, all were removed. Nematodes were preserved in small jars containing a five per cent solution of formalin and glycerine.

(b) From the Omasum, Reticulum and the Faeces.

In lamb 46, one fifth of the rumen contents was saved for examination.

In this lamb, and in lambs 32 and 44, the reticulum was also saved. For all sheep, the faeces from the rectum were collected.

All this material was examined for larval forms by using Baermann's apparatus. The larvae were preserved in test tubes containing a dilute solution of formalin.

: : : :

CHAPTER IX.

THE MAIN AND SUBSIDIARY EXPERIMENTS - RESULTS.

Though the Main and Subsidiary Experiments have received separate treatment in the discussion on methods, it is more convenient to regard them as one experiment for the analysis of results. The subdivision will therefore be discarded.

A. THE LINEAR MEASUREMENT OF AGE GROUPS.

In Appendix ~~VI~~, the ~~results of the~~ Tables of measurements of lengths of *C. curticei* of different ages are shown as histograms [Graphs 1-75]. The data from which these were derived is contained in Appendix X, ~~Graphs 1 to 75~~. As a convenient pictorial summary, the results are presented in Figs. 5 and 6, which in outline follow the Dice - Leras method for the comparison of samples (quoted by Hubbs and Perlmutter (24)).

The results are discussed in terms of populations of *C. curticei* from day to day. They will be easier to follow if read in conjunction with the plan of killings on pages ~~50 and 57~~ 58.

Day One. (That is, populations 24 hours old). Sheep 46 was killed and 87 larvae were recovered from one fifth of the contents of the rumen. The distribution of lengths is illustrated in graph 3. The mean and standard deviation was 759 ± 40μ. measurements being made with the ocular micrometer. All larvae were in the third (infective) stage, shown by the retention of the second stage sheath.

The abomasum was not examined.

From the first 39 feet of the small intestine, one larva in the third (parasitic) stage was recovered.

Day Two. Sheep 32 and 47 were killed. The length distribution of the population from lamb 32 is shown in graph 4. The mean length of 98 larvae measured with the camera lucida was 727μ and the standard deviation 41μ. All larvae were in the third (parasitic) stage; this is readily distinguished by its blunted tail.

The frequency distribution for lamb 47 is shown in graph 5. The mean and standard deviation for 87 larvae was 725 ± 61μ. All larvae recovered were in the third (parasitic) stage.

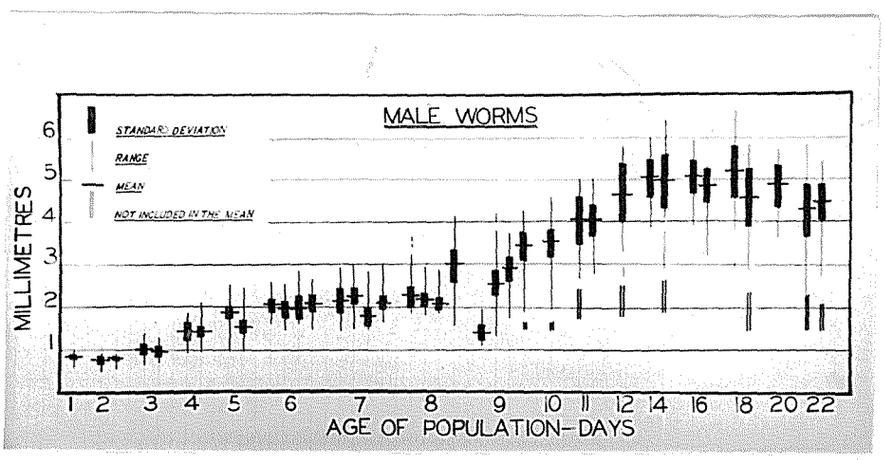


Fig. 5. Comparison of Range, Mean and Standard deviations for male populations.

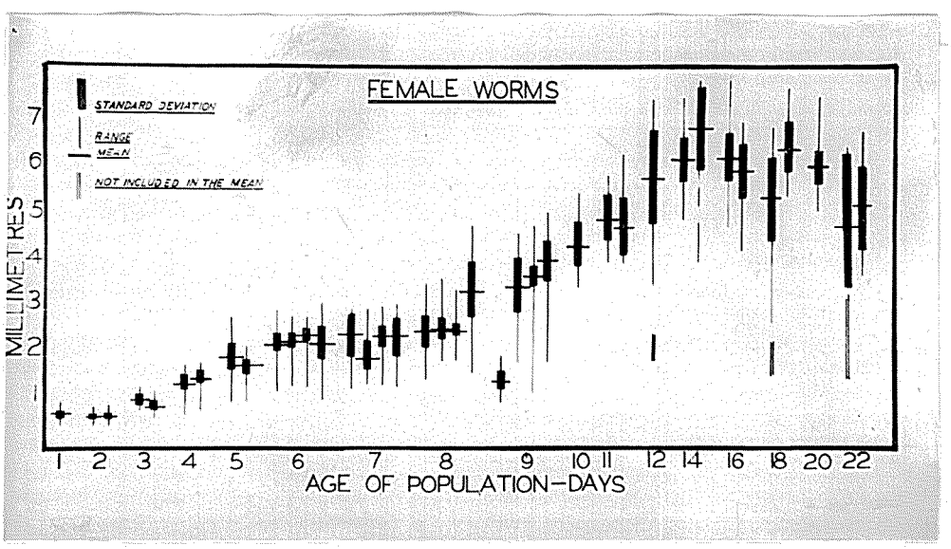


Fig. 6. Comparison of Range, Mean and Standard deviations for female populations.

Day Three. Sheep 39 and 44 were killed after three days of parasitism.

The distribution for lamb 39 is shown in graphs 6 and 7, being two different presentations of the same data. Two types of larvae were found:-

(1) Third Stage (Parasitic) Larvae: Mean length and standard deviation for 31 of these was 870 ± 140 μ. These were nearly all close to the third ecdysis, because in many cases the oesophagus at the anterior end had assumed the characteristic swollen shape of the fourth stage form. The sheath in some cases was loose, especially around the head, and in one or two specimens it was broken. Distinguishing features were:-

(a) The oesophagus had a shape very similar to that seen in infective larvae, and like that found in two-day old populations. In some cases, it was more like an early fourth stage oesophagus (see below) but a stoma, or elongate mouth cavity could always be found.

(b) The tail tip was blunt compared with the fourth stage larva, and within it in most specimens the outline of the fourth stage tail could be seen.

(2) Fourth Stage Larvae: Relevant figures for the mean and standard deviation were 996 ± 90 for 117 larvae. These were readily differentiated from third stage (parasitic) larvae by the following characters:-

(a) The anterior end of the oesophagus was wider and thicker than the corresponding portion in the third stage, and the elongate stoma was lacking.

(b) The tail was more pointed than in the third (parasitic) stage. The cuticle was finely wrinkled or crumpled, an effect lacking in the third stage larvae.

The distribution for lamb 44 is given in graph 8. Again fourth stage and third (parasitic) stage appeared together. The mean and standard deviation for 22 third stage larvae was 754 ± 161 μ. Corresponding figures for fourth stage larvae were 1·05 mms ? . The third stage larvae shown here are much shorter on the whole than those in lamb 39.

NOTE: Unless otherwise mentioned, in the following data 200 ± 2 or 3 Cooperia curticei have been measured for each sex.

Day Four. Sheep 14 and 16 were killed on the fourth day of parasitism. Corresponding graphs are numbers 9:10 and 11:12. Only fourth stage larvae were found.

Sex differentiation was possible, males having a distinctly blunter tail than females.

Mean lengths in millimetres, and standard deviations were:-

	Males	Females
Sheep 14	1·35 ± ·11 mms	1·38 ± ·16 mms
Sheep 16	1·45 ± ·16 mms	1·49 ± ·13 mms

Day Five. Two sheep were killed, numbers 4 (graphs 13:14) and 35 (graphs 15:16). All larvae were in the fourth stage. Means and standard deviation were:-

	Males	Females
Sheep 4	1·79 ± ·15 mms	1·99 ± ·24 mms
Sheep 35	1·61 ± ·17 mms	1·69 ± ·22 mms

Day Six. Four sheep were killed, numbers 24, 28, 37 and 40. Graphs for these are numbers 17 to 24. The distributions for sheep 28 (graphs 19:20) differ from others of the same and preceding days in the positive skew of the male frequency curve and the flat topped effect or negative kurtosis of the female curve. All larvae were in the fourth stage. Means and standard deviations were:-

	Males	Females
Sheep 24	2·10 ± ·14 mms	2·30 ± ·25 mms
Sheep 28	1·97 ± ·25 mms	2·26 ± ·37 mms
Sheep 37	1·99 ± ·13 mms	2·27 ± ·21 mms
Sheep 40	2·14 ± ·17 mms	2·38 ± ·22 mms

Day Seven. Sheep 10, 23, 41 and 43 were slaughtered and frequency distributions for the lengths of Cooperia recovered are in graphs 25 to 32.

From sheep 10 (graph 25) one adult male was recovered, all other worms from this and the other sheep being in the fourth stage. Adults of C. curticei were readily differentiated from the fourth stage larvae by the presence of the cephalic inflation, and (in the male) by the expanded bursa and absence of the projecting rudiment of the early larval tail. In the larvae, the cephalic inflation was visible through the fourth stage cuticle, and in some females close scrutiny was needed to determine whether or not the fourth ecdysis had taken place.

The distributions for sheep 23 (graphs 27 and 28) show a pronounced positive skew; as mentioned above (sheep 28) this is exceptional.

The entire population of sheep 43 was measured, giving 84

males and 142 females. The female distribution (graph 32) is bimodal. The male (graph 31) has a pronounced negative skew. Means and standard deviations were:-

	Males	Females
Sheep 10	2·11 ± ·19 mms	2·45 ± ·27 mms
Sheep 23	1·77 ± ·22 mms	1·91 ± ·34 mms
Sheep 41	2·22 ± ·21 mms	2·40 ± ·39 mms
Sheep 43	2·46 ± ·27 mms	2·43 ± ·51 mms

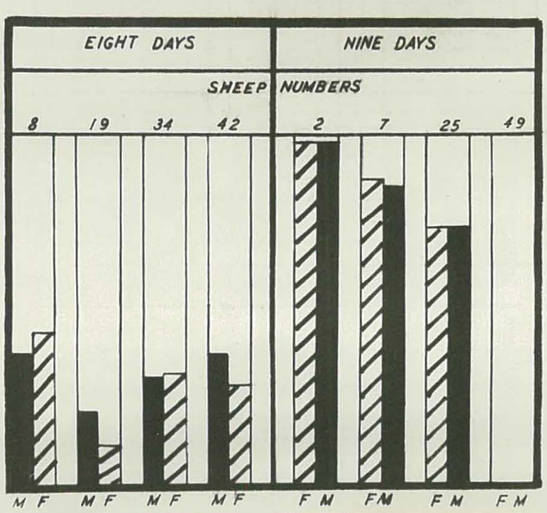
Day Eight. The frequency distributions for the lengths of populations of sheep 8, 19, 34 and 42 are recorded in graphs 33 to 40.

Both fourth stage and adult forms were present, the adults forming less than half the population in all cases (see Fig. 6A). These formed separate frequency distributions, with the mean length for the adults always greater than the corresponding figures for the fourth instar. Means and standard deviations are given below:-

	Males		Females	
	Fourth instar	Adult	Fourth Instar	Adult
Sheep 8	2·19 ± ·19 mms	2·38 ± ·26	2·49 ± ·25 mms	2·12 ± ·41mms
" 19	2·16 ± ·13 mms	2·38 ± ·19	2·52 ± ·25 mms	2·91 ± ·23mms
" 34	2·77 ± ·25 mms	3·46 ± ·29	3·26 ± ·60 mms	4·01 ± ·43mms
" 42	2·14 ± ·15 mms	2·33 ± ·19	2·43 ± ·23 mms	2·79 ± ·36mms

Day Nine. Sheep 2, 7, 25 and 49 were killed. The distributions of lengths of the Cooperia populations are shown in graphs 41 to 49. In 2, 7 and 25 the proportion of fourth stage larvae was less than half the total, but in sheep 49, the population was markedly undeveloped and all larvae were still in the early fourth stage (Fig. 6A.).

Two samples were collected from sheep 25. Graphs 45 and 46 represent populations recovered from the intestine in the normal manner, described in Chapter 8. Graphs 47 and 48 are distributions of populations left behind by this method, and recovered by flushing the intestine with water. They have therefore been termed "washings". Shorter fourth stage larvae were present in these distributions. Means and standard deviations (in millimetres) were as follows:-



Nos. of larvae
Fig. 6A. Fourth stage and adult ~~larvae~~ at 8 and 9 days.
recovered from sheep 8, 19, 34, 42 and 2, 7, 25, 49.

M = male
F = female
Blank sections represent Nos. of larvae.

		Males		Females	
		Fourth Instar	Adults	Fourth Instar	Adults
Washings	Sheep 2	2·12 ± ·26 mms	3·41 ± ·38 mms	2·40 ± ·28 mms	4·19 ± ·46mms
	" 7	2·24 ± ·23 mms	3·00 ± ·36 mms	2·74 ± ·52 mms	3·69 ± ·43mms
	" 25	1·99 ± ·18 mms	2·63 ± ·34 mms	1·93 ± ·36 mms	3·17 ± ·38mms
	" 49	----sexes undifferentiated.....1·30 ± ·24			

Day Ten. Only sheep 48 was killed on this day. The population was mainly adult, only isolated fourth stage forms being measured. The distributions of length are shown in graphs 50 and 51. Means and standard deviations were 3·5 ± ·39 mms for males, and 4·45 ± ·51 mms for females.

Day Eleven. Sheep 36 and 38 (graphs 52 to 55) were slaughtered. Isolated fourth stage larvae were found in all populations except the males of sheep 38 (graph 54). Means and standard deviations were:-

	Males	Females
Sheep 36	3·92 ± ·51 mms	4·76 ± ·63 mms
Sheep 38	4·09 ± ·35 mms	5·02 ± ·47 mms

In fifty female worms examined from sheep 38, only two contained ova. A similar number from sheep 36 were all without eggs.

Day Twelve. Sheep 26 (graphs 56 and 57) was killed. Only a few fourth stage larvae were found. The female distribution (graph 57) exhibits an extreme negative skew, and has a tendency towards a bimodal curve. Males (graph 56) are less skewed. Means and standard deviations for males were 4·60 ± ·68 mms, and 5·83 ± 1·02 mms for females.

Approximately one third of the females contained eggs.

(The presence of ova has been mentioned here in order to draw attention to the attainment of sexual maturity. Their presence or absence will be discussed more fully in a later section.)

Day Fourteen. On this day, sheep 51 and 52 were killed. Frequency distributions of length are given in graphs 58 to 61. Isolated fourth stage larvae were still present, except in the female distribution of sheep 52 (graph 61). Means and standard deviations were:-

	Males	Females
Sheep 51	5·00 ± ·65 mms	6·81 ± ·85 mms
Sheep 52	4·96 ± ·39 mms	6·26 ± ·47 mms

Day Sixteen. In graphs 62 to 65 the frequency distributions of the lengths of C. curticei recovered from sheep 30 and 33 are shown. No fourth stage larvae were found. Means and standard deviations were:-

	Males	Females
Sheep 30	4·85 ± ·39 mms	6·02 ± ·51 mms
Sheep 33	5·16 ± ·37 mms	6·35 ± ·47 mms

Day Eighteen. Sheep 9 and 29 were killed. Distributions of length are illustrated in graphs 66 to 69.

In sheep 9, no fourth stage larvae were found, though some very short adults were measured.

In sheep 29, (graphs 68 and 69) fourth stage larvae were present in greater numbers than found for any mature populations previously examined. Means and standard deviations were:-

	Males		Females	
	Fourth stage	Adults	Fourth stage	Adults
Sheep 9	-	5·20 ± ·51 mms	-	6·50 ± ·63 mms
" 29	1·93 ± ·19 mms	4·61 ± ·66 mms	1·63 ± ·75 mms	5·43 ± ·93 mms

Day Twenty. Only sheep 6 was killed on the twentieth day of parasitism; no fourth stage larvae were recovered. Distributions which are drawn in graphs 70 and 71 have means of 4·88 ± ·43 mms for males and 6·13 ± ·43 mms for females respectively.

Day Twenty-two. The final killings of the experiment were sheep 18 and 27 (see graphs 72 to 75). In all cases, fourth stage larvae were found in numbers comparable to those in sheep 29 killed at 18 days, and the adult distributions show wider ranges.

Especially noticeable is the bimodal distribution in graph 73, of the females from sheep 18. In later work, this distribution was analysed into its components. The mean value for the entire adult female distribution is given below, along with the means and standard deviations (in millimetres) for associated distributions:-

		Males		Females	
		Fourth stage	Adults	Fourth stage	Adults
Sheep	18	1·92 ± ·22	4·31 ± ·61	1·80 ± ·31	4·75 ± ·91
"	27	1·82 ± ·14	4·48 ± ·59	1·93 ± ·18	5·30 ± ·86

B. THE STATISTICAL ANALYSIS OF THE LINEAR MEASUREMENTS.

I. OBJECTS OF THE ANALYSIS.

(a) A test is necessary to determine whether there are any significant differences between mean lengths of populations from different-sized infestations.

(b) A similar test is necessary on the mean lengths of successive daily populations.

(c) An estimate of between-host variation is desirable.

(d) A determination of the significance of the differences in length between sexes is also necessary.

II. THE ANALYSIS.

(A) COMPARISON BETWEEN MEANS FOR FOUR LEVELS OF INFESTATION AND LENGTHS OVER SIX, SEVEN, EIGHT AND NINE DAYS.

MALE WORMS

Mean Lengths in millimetres.

	Dose rate (No. of Larvae)			
	10,000	22,000	20,000	138,000
6 days	1·99	2·11	2·14	1·97
7 "	x= 2·31	2·19	2·11	1·77
8 "	2·26	2·21	2·20	2·99
9 "	3·40	2·88	2·58	1·31

Note:- (a) The value at 7 days, x = 2·31 mms, has been determined by minimising the error sum of squares. This procedure was necessary because there was no value in the original data corresponding to this cell. The appropriate formula, set out below, is taken from page 268 of the 4th edition of Snedecor's "Statistical Methods", (1946).

$$x = \frac{tT + bB - S}{(t-1)(b-1)}$$

where x = missing value
t = number of treatments
b = number of blocks
T = sum of items with the same treatment as missing items
B = sum of items in same block as missing items
S = sum of all the observed items.

Substituting, $x = \frac{4(6\cdot07) + 4(7\cdot65) - 34\cdot11}{(4-1)(4-1)}$

$= 2\cdot31$ mms.

In order to keep an unbiased estimate of error, one degree of freedom is dropped in the analysis of variance set out below.

(b) The means for eight days are means for both fourth stage larvae and adults. The mean for nine days (3·40 mms) is the mean of the adult population only. Reference to graph 41 in the Appendix will show that the few fourth stage larvae present are abnormally small and for this reason they are not included in the mean. The two following values for nine days (2·88 mms and 2·58 mms) are means for both fourth stage larvae and adults. Reference to graphs 43 and 47 will show that the larval and adult stages cannot be separated.

ANALYSIS OF VARIANCE. *

Source	Sum of Squares	Degrees of Freedom	Mean Square	5% Point
Dose Rate	·4881	3	·1627	4·07
Days	·6923	3	·2308	4·07
Error	2·5072	8	·3134	
Total	3·6876	14	-	-

There are no significant differences between the daily means, or between means for the different dose rates, because the F ratio would obviously be less than the value required for significance at the 5% point.

The coefficient of variation is 24·59%, which is high. Therefore, it is possible that significant differences do exist, but the experiment was not precise enough to detect them. Later it will be shown that this is actually the case.

FEMALE WORMS

Mean Lengths in millimetres.

	Dose rate (No. of Larvae)			
	10,000	22,000	20,000	138,000
6 days	2·27	2·30	2·38	2·26
7 "	x = 2·67	2·42	2·45	1·91
8 "	2·59	2·55	2·53	3·49
9 "	4·19	3·55	3·07	1·31

The missing value, x, was estimated as described above. The means have been selected on the same basis as for the males, so

* Details of the analysis are to be found in Snedecor's "Statistical Methods", 4th ed., 1946, chapters 10 and 11.

far as inclusion or exclusion of the fourth stage larvae is concerned.

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	5% Point
Dose Rate	·9480	3	·3280	4·07
Days	1·4565	3	·4895	4·07
Error	4·5663	8	·5708	
Total	7·0068	14	-	-

As with the males, there are no significant differences between daily means or between means for different dose rates. The value of 28·82% for the coefficient of variation is likewise high.

It was believed that if the variability in the group receiving 138,000 larvae was reduced, some significant differences would become apparent.

To test this an analysis of variance was made on the data excluding the low value for 9 days (1·31 mms). The expected value (calculated from the formula already described) was inserted. Significant differences were found between the 8th and 9th days for females, but not for males. No significant differences between dose rates were found, and details are therefore omitted.

A further analysis was made, leaving out the 138,000 dosage group. The details are given below.

MALE WORMS

Mean Lengths in millimetres.

	Dose rate (No. of Larvae)			Mean
	10,000	22,000	20,000	
6 days	1·99	2·11	2·14	2·08
7 "	x = 2·35	2·19	2·11	2·22
8 "	2·26	2·21	2·20	2·22
9 "	3·40	2·88	2·58	2·95

'x' represents a value fitted by the formula described above.

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	5% Point
Dose Rate	·1203	2	·0602	1·121	5·79
Days	1·4082	3	·4694	8·741	5·41
Error	·2685	5	·0537		
Total	1·7970	10	-	-	-

Therefore there is a significant difference between daily

means, but no significant difference between dose rates.

Calculation of the fiducial limits of the means indicates where significant differences occur. (Snedecor, "Statistical Methods", 4th ed., page 266).

The fiducial limits, calculated from the product of the standard error and the value of "t" at the 5% level of probability for 5 degrees of freedom are ± ·486 mms. Application of this to the daily means of length shows a significant difference between the eighth and ninth days only.

The coefficient of variation is much reduced, being 9·8%.

FEMALE WORMS

Mean Lengths in millimetres.

	Dose rate (No. of Larvae)			Mean
	10,000	22,000	20,000	
6 days	2·27	2·30	2·38	2·32
7 "	y= 2·72	2·42	2·45	2·53
8 "	2·59	2·55	2·53	2·56
9 "	4·19	3·55	3·07	3·60

'y' represents a value fitted by the formula described above.

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	5% Point
Dose Rate	·2376	2	·1188	1·300	5·79
Days	3·0052	3	1·0017	10·960	5·41
Error	·4568	5	·0914		
Total	3·6996	10	-	-	-

Again, there is a significant difference between daily means, but no significant difference between dose rates.

The fiducial limits of the means are ± ·635 mms which indicates that the only significant difference is between the means for 8 and 9 days.

The coefficient of variation is again considerably lowered, being 10·99%.

(B) COMPARISON BETWEEN WORM LENGTHS AFTER 22 DAYS, REGARDING DOSE LEVELS AS IDENTICAL.

MALE WORMS

Mean Lengths in millimetres.

Day	Group A	Group B
12	4·60	x = 4·43
14	4·96	5·00
16	5·16	4·85
18	5·20	4·61
20	y = 5·05	4·88
22	4·48	4·65

'x' and 'y' represent values fitted by minimising the

error sum of squares, as described previously.

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	5% Point
Groups	.0884	1	·0844		
Days	·4922	5	·0984	1·66	9·01
Error	·1778	3	·0593		
Total	·7584	9	-	-	-

Therefore there are no significant differencos between daily means. The coefficient of variation is 5·94%.

FEMALE WORMS

Mean Lengths in millimetres.

Day	Group A	Group B
12	5·83	x = 5·77
14	6·26	6·81
16	6·35	6·02
18	6·50	5·43
20	y = 6·19	6·13
22	5·30	5·92

'x' and 'y' are estimated values derived by the formula already described.

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	5% Point
Groups	·0102	1	·0102	
Days	1·0571	5	·2114	·901
Error	·9637	3	·3212	
Total	2·0310	9	-	-

Therefore there are no significant differences betweem daily means. The coefficient of variation is 9·38%.

(C) COMPARISON BETWEEN WORM LENGTHS FOR POPULATIONS 2 TO 18 DAYS OLD.

This analysis is concerned only with sheep killed in the Main Experiment. Dose levels are regarded as equivalent.

MALE WORMS

Mean Lengths in millimetres.

Day	Group A	Group B	Mean
2	·73	·73	·73
3	1·02	·97	1·00
4	1·45	1·35	1·40
5	1·79	1·62	1·71
6	1·99	2·11	2·05
7	x = 2·32	2·19	2·26
8	2·26	2·21	2·24
9	3·40	2·88	3·14
10	3·52	y = 3·39	3·45
11	3·92	4·09	4·01
12	4·60	z = 4·47	4·54
14	4·96	5·00	4·98
16	5·16	4·85	5·01
18	5·20	4·61	4·91

In the foregoing table, 'x', 'y' and 'z' are estimated values, using the formula previously cited. Red lines indicate where significant differences exist (see analysis below).

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	5% Point
Groups	·1222	1	·1222	4·007	4·96
Days	61·1778	13	4·7060	154·295	2·89
Error	·3048	10	·0305		
Total	61·6048	24	-	-	-

Therefore there are significant differences between daily means. Fiducial limits of these means are ·389 mms. Reference to the table of means for males given above shows that significant differences are found between the 3rd and 4th day, 5th and 6th, 8th and 9th, and between 10th and 11th, 11th and 12th, and 12th and 14th days. These are indicated in the table by red lines.

The coefficient of variation is 5·91 %.

FEMALE WORMS

Mean Lengths in millimetres.

Day	Group A	Group B	Mean
2	·73	·73	·73
3	1·02	·97	1·00
4	1·49	1·38	1·44
5	2·00	1·69	1·85
6	2·27	2·30	2·29
7	x = 2·57	2·41	2·49
8	2·59	2·55	2·57
9	4·19	3·55	3·87
10	4·45	y = 4·29	4·37
11	4·76	5·02	4·89
12	5·83	z = 5·67	5·75
14	6·26	6·81	6·54
16	6·35	6·02	6·19
18	6·50	5·43	5·97

'x', 'y' and 'z' are estimated values, using the formula perviously cited.

Red lines indicate significant differences between daily means (see analysis below).

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	5% Point
Groups	·1713	1	·1713	1·822	4·96
Days	109·2754	13	8·4058	89·423	2·89
Error	·9404	10	·0940		
Total	110·3871	24	-	-	-

Therefore there are significant differences between daily

means, and fiducial limits of these means are ·683 mms. Reference to the table of means above shows that significant differences exist between the 2nd and 4th days, 3rd and 5th, 4th and 6th, 5th and 8th, 8th and 9th, 9th and 11th, and between 11th and 12th, and 12th and 14th days. These are indicated in the table by red lines and arrows.

(D) BETWEEN HOST VARIATION.

The experimental design precluded any possibility of analysing this variable. (See the discussion on the Experimental design, page 49 seq.)

(E) COMBINED ANALYSIS FROM 2 TO 18 DAYS FOR BOTH SEXES, DOSE RATES FOR THE MAIN EXPERIMENT BEING REGARDED AS IDENTICAL.

TABLE OF COMBINED LENGTHS (IN MILLIMETRES).

Days	Dose rates		Sex	
	A	B	Male	Female
2	1·46	1·46	1·46	1·46
3	2·04	1·94	1·99	1·99
4	2·94	2·73	2·80	2·87
5	3·79	3·31	3·41	3·69
6	4·26	4·41	4·10	4·57
8	4·85	4·76	4·47	5·14
9	7·59	6·43	6·28	7·74
11	8·68	9·11	8·01	9·78
14	11·22	11·81	9·96	13·07
16	11·51	10·87	10·01	12·37
18	11·70	10·04	9·81	11·93

Derivation of the Table.

Treatments A and B are the sum of the means for males and females for each separate treatment.

Male and female columns are the sum of all the males' mean lengths (irrespective of treatment) and sum of all the females' mean lengths (irrespective of treatment), respectively.

ANALYSIS OF VARIANCE.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	5% Point
Replications	·2284	1	·2284	2·056	4·96
Days	146·8331	10	14·6833	132·160	2·97
Error (1)	1·1114	10	·1111		
Sexes	3·4440	1	3·4440	281·238	4·84
Sex x Day	2·9945	10	·2995	24·549	2·86
Error (2)	·1347	11	·0122		
Total	154·7461	43	-	-	-

Therefore there are significant differences between daily means (over both sexes), between sex means (over all), and be-

tween daily sex means.

The Combined Analysis gives answers to the questions already discussed, as well as additional information on sex differences. However, the validity of a combined analysis, when two sets of data show unequal variability is open to question, and the interpretation of some of the results is to be made with caution.

This interpretation will now be considered.

The means for male and female populations are as set out below:-

Day	Males	Females	Daily Means
2	·730	·730	·730
3	·995	·995	·995
4	1·400	1·44	1·42
5	1·71	1·85	1·78
6	2·05	2·29	2·17
8	2·24	2·57	2·40
9	3·14	3·87	3·51
11	4·01	4·89	4·45
14	4·98	6·54	5·76
16	5·01	6·19	5·60
18	4·91	5·97	5·44

(1) Differences between daily means for both sexes can be derived from the fiducial limits of the means, calculated in the manner already described. Fiducial limits at the 5% value of "c" for 10 degrees of freedom are ± ·53 mms. Significant differences occur between days 2 and 4, 3 and 5, 4 and 6, 5 and 8, 8 and 9, 9 and 11, and 11 and 14.

(2) Sex means are already shown to be significantly different, the F ratio, 132·16, exceeding the 5% level, 2·97.

(3) Sex means within days, i.e. the significance of the differences between males and females of the same age:- fiducial limits for the mean are ± ·24 mms. The differences are barely significant at 6 days, and are significant thereafter.

(4) Significance of differences between daily means within the sexes:- the fiducial limits for significance are ± ·24 mms. The interpretation of this analysis is doubtful. For example, it shows significant differences between day 2 and day 3, which is contrary to the previous analysis.

The significance of these analyses will be discussed in relevant sections of this work.

C. THE LENGTHS OF LARVAE RECOVERED FROM THE FAECES.

Many infective larvae were recovered from the faeces, all of which were active and apparently normal. They were killed with heat and measured with the ocular micrometer.

LENGTH OF LARVAE RECOVERED FROM FAECES.

Days from Infestation	Sheep No.	Nos. of larvae recovered	Mean Length
3	39	21	776
3	44	7	811
4	14	5	765
4	16	31	802
8	19	2	675
12	26	1	760
16	30	1	820

The numbers are not comparable, because the size of the dung sample varied from 30 to 60 gms.

D. THE PRESENCE OF UNDEVELOPED LARVAE.

In order to direct attention to the presence of worms which were retarded in development when compared with others in the population from ehich they were drawn, certain results are collected and presented under this heading.

It is obvious from graphs 1 to 75 in the Appendix that nearly all populations have associated with them small numbers of undeveloped parasites. These are more common in older populations, where in some cases both immature adults and fourth stage larvae are associated with fully developed adult populations.

For convenience, the results are presented in three sections.

(a) Undeveloped Adults. The presence of these forms is discussed, and data acquired in a spedial study of them is presented.

(b) Undeveloped Fourth Stage Larvae. These are examined from two aspects:-

(i) Those present in populations older than eight days.

(ii) Those present in younger populations.

(c) Undeveloped Third Stage (Parasitic) Larvae.

(a) UNDEVELOPED ADULT POPULATIONS.

(i) The female distributions.

Examination of the frequency curves for the lengths of some adult female populations showed marked irregularities. This was especially true of the twenty-two day old population from lamb 18 (graph 73). This distribution is bimodal with the modes approximately 4·0 mms and 5·80 mms. Since it was unlikely that the shorter females were part of a second infestation following close behind the first, it was concluded that certain of the females in the original population had been unable to grow to full size. Since the corresponding distribution for the male population (graph 72) did not show any very marked bimodal effects, it was concluded that the differences in length within the female population were likely to be associated with, if not caused by, some inherent female characteristic. (The obvious falsity of this statement will be discussed shortly.) Being a female characteristic, ovulation was selected as a likely factor.

Measurements were made of the lengths of one hundred and twelve females from the population of lamb 18, and the numbers of eggs present in the uteri of each worm were counted. Distributions were then drawn up of the lengths of worms with no eggs, and the lengths of worms with eggs, as shown in graph 82. C. curticei with no eggs had a mean and standard deviation of 4·62 ·66 mms. Those with eggs had a mean and standard deviation of 5·92 ·16 mms, and the distribution was very close to normal.

The mean of the numbers of eggs in the females was found to be 19 71 the distribution of these ova is shown in fig. 7.

Similar examinations were made on two normal distributions. For this purpose the female populations of lambs 9 and 52 were selected (graphs 67 and 61).

Of fifty females examined in the population from lamb 52, only six were without eggs, and the mean length was 5·48 mms. The mean value for the lengths of the remainder was 6·27 ·43 mms.

In lamb 9 only two out of fifty females examined were without ova; both were very much shorter than the others, the mean being 5·0 mms. The remainder of the population was divided into three arbitrary groups on the basis of the total numbers of

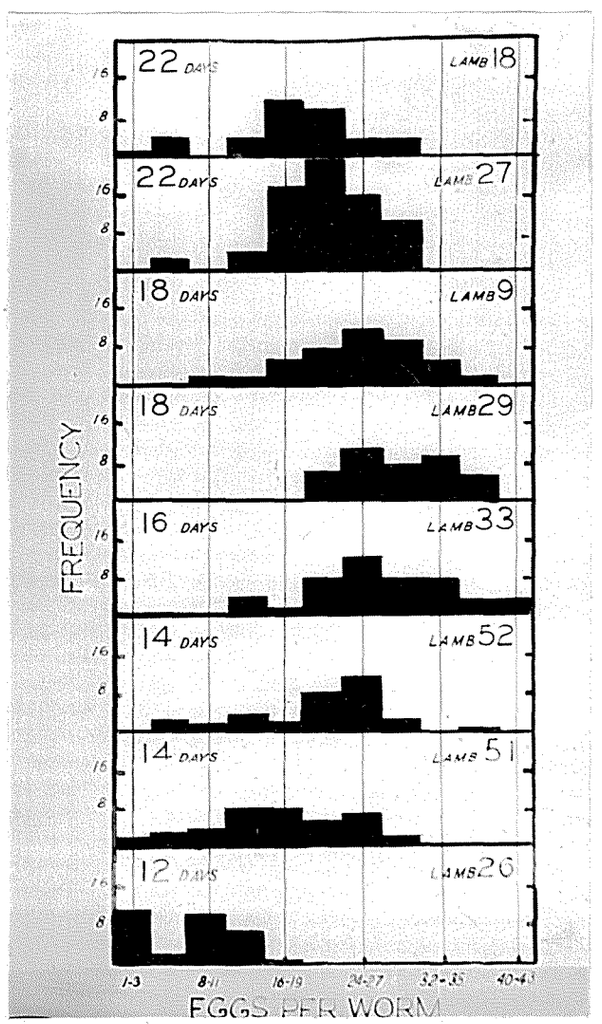


Fig. 7.

Fig. 7. Distributions of the numbers of eggs per female.

eggs in the uteri, as follows:- 1 - 19 eggs, 20 - 29 eggs, and those with 30 or more eggs. The mean lengths and standard deviations for these divisions were 6·19 ± ·10 mms, 6·35 ± ·52 mms, and 6·70 ± ·45 mms respectively. This is shown in graph 80.

The mean of the numbers of eggs found in the females from lamb 9 was 24·75. The distribution of these egg numbers is shown in fig. 7.

As a result of these findings, it was decided to examine irregularities in other female distributions in respect of total length of worms and egg numbers. Only three distributions were not analysed in this manner: those for lambs 6, 30 and 38 (Graphs 71; 63; 55). These frequency distributions were very close to the normal curve, and it was assumed that they would be similar to the distributions for lambs 9 and 52 (already examined) in respect of the numbers of barren females.

Ova were not found in female worms less than twelve days old, with the exception of 2 worms at eleven days.

The frequency curve for females twelve days old is shown in graph 57. It will be noted that this has a very marked negative skew. Measurement of 100 females, and their classification into two distributions, "with ova" and "without ova", resulted in the histogram reproduced in graph 76. About 30% of the females examined contained eggs. Their mean length was 6·80 ± ·33 mms, and as graph 76 shows, the distribution was very close to normal. The lengths of the females with no eggs form a bimodal curve, but by doubling the class intervals this disappears, and the distribution has a flat top (or negative kurtosis). The mean is 5·63 mms and the standard deviation ·86 mms, which is larger than the corresponding figure for the females with eggs.

The distribution of the numbers of eggs within the worms is shown in fig. 7. The mean is 8 eggs.

Of the two female populations fourteen days old, one (lamb 52) is a normal distribution, and its analysis has already been described. The other distribution (lamb 51) is shown in graph 59. Analysis of this distribution is shown in the histogram in graph 77. Of fifty females examined, it was found that those shorter than 6·2 mms had from no eggs up to 10. Of those

longer than 6·2 mms, four had no eggs, and seven fewer than 10, these forming one quarter of the females examined which were longer than 6·2 mms.

The mean length and standard deviation of all females with fewer than 11 eggs was 6·32 mms ± ·70 mms. The females with more than 10 eggs averaged 7·10 ± ·41 mms. It is likely that the majority of females shorter than about 6·6 mms in this population (see graph 59) would have few or no eggs.

Out of fifty females from the population of lamb 33 (graph 79), only four contained no eggs. One of these worms was quite long, being near the middle of the distribution. The remaining three averaged 5·24 mms in length. Females containing ova were divided into two arbitrary groups, those with 11 to 20 and those with 21 or more eggs. The mean lengths of females in these categories were 5·93 ± ·18 mms, and 6·12 ± 1·11 mms.

The analysis of the number of ova per female is set out in fig. 7. The mean number per worm is 27.

The frequency distribution for lamb 29 (graph 69) shows a very pronounced skew effect. Analysis of the number of ova in fifty females showed (graph 81) that no worms shorter than 5·19 mms were fertile. All the remainder, except two, contained more than 20 ova. The mean length of these forms was 5·97 ± ·37 mms, and the mean of seven females with fewer than 11 eggs was 4·79 ± ·31 mms.

The female population from lamb 27 (graph 75) contained a small number of Cooperia shorter than 5·00 mms. Examination of 84 females selected at random from this population showed that the majority of the females below this length had from no eggs up to 20 eggs per female. However, many worms longer than 5·0 mms had less than 20 eggs, as illustrated in graph 83.

Using the divisions of "0 - 10 eggs", "11 - 20 eggs" and "over 21 eggs", the means of the lengths of females in these categories are 4·61 mms (6 worms); 5·45 ± 2·01 mms (21 worms); and 5·76 ± ·45 mms (55 worms).

The distributions of the numbers of ova in the females is shown in fig. 7. The mean of this distribution is 21·73 eggs per worm.

(ii) The male distributions.

The frequency distribution for male Cooperia in lamb 18 (graph 72) shows only a slight deviation from normality.

Previously it was assumed that some "factor" had affected the growth of the females but not the males, and it was suggested that this factor would therefore be associated with a particular female characteristic, such as the production of ova. It has been shown that the degree of sexual development has been related to the length of the females. Though the conclusion was correct, the premise was not sound. It is most unlikely that there would exist a differential "factor", operating on one sex and not on the other. It was decided to examine this male population to determine whether any of the individuals were sexually immature, as had already been done with the females.

Sexual maturity in the male could be determined in two ways, firstly by the presence or absence of spermatozoa in the genital tract, and secondly by the colour of certain parts of the genital organs, especially the spicules.

Weglia (68) working with H. contortus, found that "frequently" he was able to see spermatozoa in the vesicula seminalis. Specimens of C. curticei were examined for the presence of spermatozoa. In some cases they were observed, but in many cases their presence was doubtful. This was largely due to the state of the material, which had been for a year in formalin and glycerine, leaving it very clear and transparent.

The possibility of staining the spermatozoa was examined. Goodey (20) recommends Nile blue sulphate stain for the reproductive organs of living plant parasite nematodes. It has been tried by the writer on Ostertagia spp., but has not been found very effective.

The spicule colour was relatively easy to determine and was unlikely to be changed by the formalin preservative. Monnig (37) working with Trichostrongylus spp. reported that at about eighteen days from infection the spicules darkened to a light brown colour. He regards this as an indication that copulation has taken place. (This conclusion is not necessarily true. As C. curticei matures sexually the genital organs darken, this being an indication of sexual maturity, but it may not nec-

essarily imply that copulation has taken place.)

A sample of males from the population of lamb 18 was examined and the spicules classified as "light" or "dark". Light spicules appeared under the microscope to be grey, while the dark spicules ranged in colour from light yellow to dark orange-brown. As standards for these colours, spicules from lamb 38 (where all the females had been found to be sexually immature) and from lamb 6 (where the females were mature) were referred to. Worm lengths were plotted as two frequency distributions, depending on the colour of the spicules. These are shown in graph 84. The mean length of males with light spicules was 3·94 ± ·46 mms and the corresponding figure for males with the darkened spicules was 4·65 ± ·49 mms.

Examination was made of the males from population of lamb 9. It has already been shown that the female distribution, which was normal, contained only two forms with no ova. When 50 males were examined under the microscope, only two were found with light-coloured spicules. No lengths were recorded.

(b) UNDEVELOPED FOURTH STAGE LARVAE.

Forms which had been arrested in development in the larval stage and had not passed the final ecdysis fall into two divisions:

(i) those associated with populations older than eight days

(ii) those associated with younger populations.

(i) Those associated with older populations.

Reference to the graphs in the Appendix will show that many adult populations (i.e. those older than nine days) had a few fourth stage larvae associated with them. No third stage larvae were found in such populations.

Adult populations which had no fourth stage larvae were found in lambs 6, 9, 30, 32 and 52.

Those which did contain fourth stage forms are summarised in fig. 8 for the males and fig. 9 for the females. In each figure there is a typical nine day old population of larvae which are about to moult, drawn from lamb 25 ("washings"). This acts as a basis of comparison between normal and undeveloped forms.

Examination of fig. 8 for males shows that the distributions have means which are closely similar to one another and to the

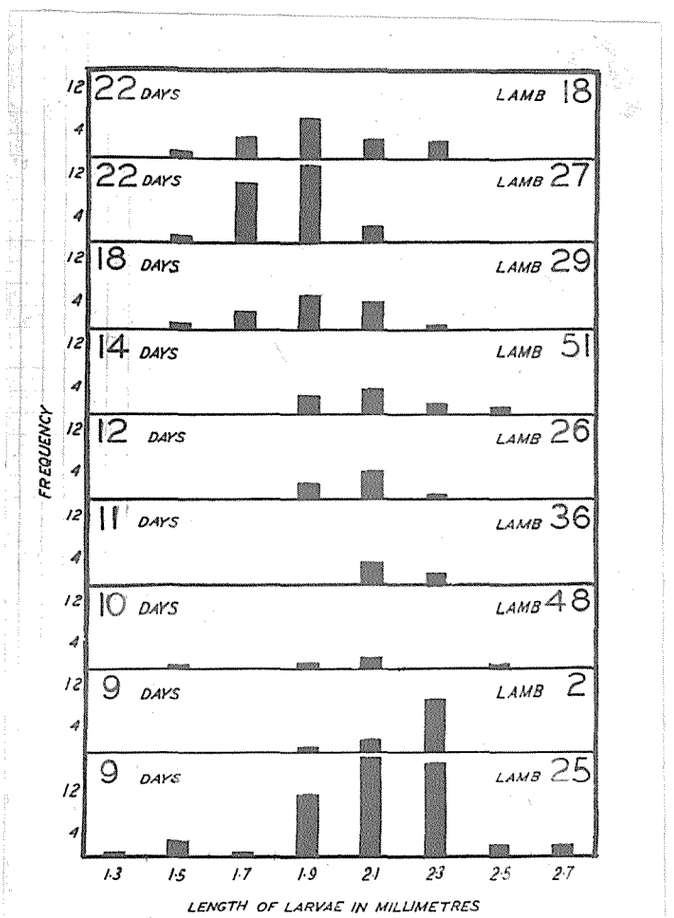


Fig. 8. Distribution of male fourth stage larvae.

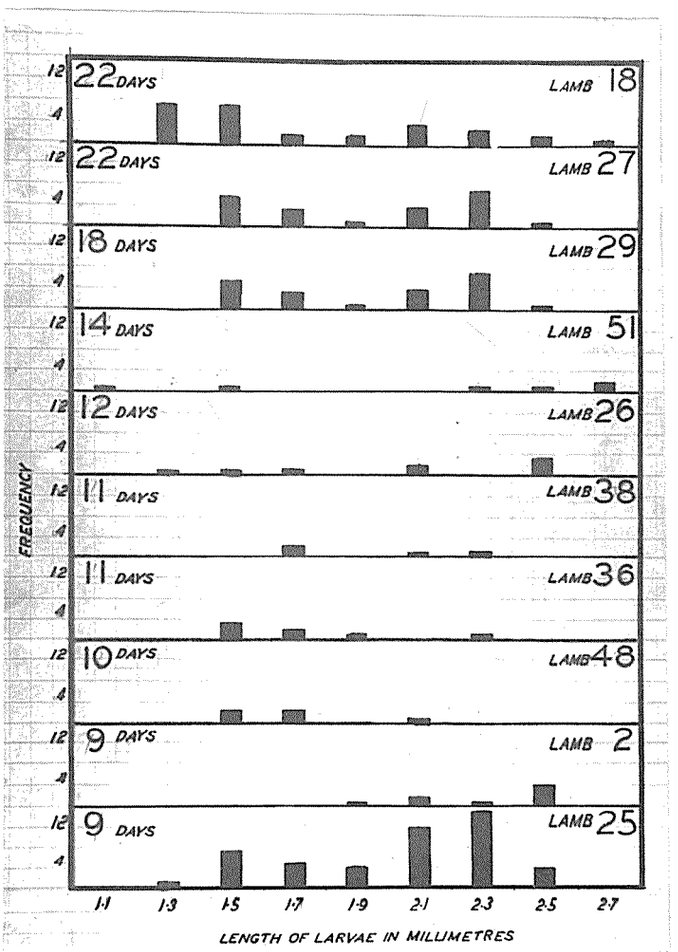


Fig. 9. Distribution of female fourth stage larvae.

normal forms from lamb 25. It was at first thought that the larvae from lambs 18, 27 and 29 were significantly shorter than the remainder. An analysis of variance was made and the F ratio indicated that the chances were against the differences being significant. This analysis is summarised below.

ANALYSIS OF VARIANCE.

Undeveloped fourth stage larvae.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
Total	13·87	149	-
Between Groups	1·66	7	0·2371
Within Groups	12·21	142	0·085
$F = \frac{·2371}{·085} = 2·7$, which is below the 1% level of probability, 2·76 for 7 and 142 df.			

The distributions for the females are shown in fig. 9. Though the numbers of females and males are closely equivalent, the females appear to be fewer than the males because the distributions are more scattered.

Only in lambs 2, 18, 27 and 29 are the worms sufficiently numerous or well enough grouped to give a clear picture. As with the males, these larvae tend to group near to or slightly below the average length at which females moult (as indicated by lamb 25).

There is evidenced in populations from lambs 18 and 27, as well as in lamb 25, a tendency for a second mode to form at 1·5 mms.

(ii) Undeveloped larvae associated with populations less than nine days old.

Most populations have forms present which are less mature than the norm. No males of this description are found in populations from lambs 8, 16, 19, 23, 24, 28, 34, 37 and 40. Other populations have a negative skew frequency, and it is often very difficult to decide whether the shorter larvae should be regarded as being significantly less developed than the others in the same population. Two typical examples of this are to be found in graphs 29 and 31.

So far as the females are concerned, there is a greater tendency towards skew curves than with the males. Odd individ-

uals, obviously undeveloped, can be seen in graphs 28, 38, 40 and 49. It is more difficult with the females to decide whether the lower values in the skewed distributions are significantly less developed. One notable exception to this is shown in graph 32. Here the distribution is definitely bimodal, with a small number of larvae in a distribution having a mean of 1·51 mms and the remainder with a mean value of 2·64 mms.

(c) UNDEVELOPED THIRD STAGE LARVAE.

In graphs 6 and 7 there are a small number of third stage parasite larvae, with a modal length of 740 μ. It is probable that these were undeveloped third stage larvae which had neither been able to grow nor moult.

After three days from infestation, no third stage larvae, either parasitic or infective, were found in the contents of the intestine. This is interesting in view of the fact that in the faeces of five lambs killed up to sixteen days after infestation odd numbers of infective larvae were found. It was not definitely verified however that these were Cooperia larvae; they may have been Ostertagia spp.

: : : :

CHAPTER X.

THE MAIN AND SUBSIDIARY EXPERIMENTS - DISCUSSION OF EXPERIMENTAL PROCEDURE.

A. CRITIQUE OF THE EXPERIMENTAL DESIGN.

Although the experimental design enabled a study of the linear growth of C. curticei to be made, it failed to give any indication of the degree of inter-host variation which might be expected. This is an important aspect of the growth study, because without an estimate of inter-host variation in growth, it is not possible to predict the differences which must be expected between development of worms of the same age, but from different hosts. A second weakness of the plan is that it is not possible to determine the effect of size of infecting dose on development.

Both of these questions could have been answered if several sheep with the same dose rate had been killed each day. In addition larger numbers of sheep would have increased the precision with which the tests of significance could have been applied. An example from the statistical analysis of data will make this clear.

Reference to the analyses of the lengths of the male worms for 6, 7, 8 and 9 days, at infestation rates of 10,000, 22,000 and 20,000 larvae, will show that the difference between means has to be less than .486 mms for significance at the 5% level (pages 70-71). This calculation (52, page 266) is made as follows:-

$$d_{.05} = 2.571 \sqrt{\frac{2(.0537)}{3}} = .486 \text{ mms},$$

where 2.571 is the value for "t" at the 5% level for 5 degrees of freedom (52, page 65), and .0537 is the error mean square from the analysis of variance. The divisor, 3, represents the number of population mean lengths (i.e. sheep). If it is assumed that the variation is the same, it can be seen that increasing the number of sheep will give a larger number of degrees of freedom for the error variance and thus increase the sensitivity of the whole experiment.

It may be argued that the killing of so many sheep after growth had ceased (i.e. from about fourteen days) was unnecessary. These sheep did not provide any data on the development of C. curticei, but they did give valuable information on variations in final

size and on ovulation. This in iteslf justifies the late killings.

B. THE METHODS OF REARING "WORM-FREE" LAMBS.

The degree of care taken to rear "worm-free" lambs varies. In Threlkeld's (67) study of the life history of Ostertagia circumcincta, the lambs were removed from their mothers as soon as possible, fed with pasteurized cow's milk and later with oats and hay which had been steamed in an autoclave. The lambs were housed in raised cages, and water was so placed as to avoid faecal contamination. Though Coccidia appeared at about ten weeks, their numbers were small and eventually they disappeared. The animals did not have any nematodes parasitic in the alimentary tract until the experimental infestation was made. As a check on the presence of parasites, he made faecal egg counts at regular intervals and in addition two lambs were slaughtered in order that their intestinal tracts could be examined for nematode parasites. Blood counts and haemoglobin percentages were also made as an additional check upon the normality of the lambs.

The contrast to this system is found in Andrews' (2) life history studies of C. curticei. Andrews reports that he used seven lambs. (The experiment records ten separate stages of the life history, which presumably would require 10 lambs.) Of these lambs, only three were not "relatively free from parasitism." Two of these had been on pasture during the weeks immediately preceding the experiment, while the third had been on pasture the previous summer. Andrews reports that the presence of parasites in these lambs did not interfere with the experiment and that the developmental stages of C. curticei could be readily distinguished from other species and from older C. curticei.

Threlkeld perhaps went to unnecessary lengths to check on the presence of parasites in the gut when he killed two lambs for examination. The egg counts, especially when they were made at weekly intervals, should have been sufficient.

Indoor feeding is neither so difficult nor so little understood that sheep cannot be kept in a healthy state without the aid of blood tests to check on their well-being. It is questionable as to how the parasites Threlkeld later gave the sheep would be affected by physiological conditions giving rise to a blood

count or haemoglobin percentage outside the limits of normality used by him as standards. Extreme variation would be evidenced in a sick animal which would not normally be used for any experiment of this type. The life-cycle of the parasitic nematode would scarcely be influenced significantly by changes in the blood determinations, if these were not of sufficient magnitude to produce clinical symptoms in the lambs.

Though Andrews apparently did not encounter any difficulties with those lambs which had previously been in the field, it may well happen that such sheep would develop some degree of immunity to parasitism which would interfere with normal development of worms. It is also possible that a number of parasites could be present which were immature and these could cause confusion if the artificially administered parasites were at about the same stage of growth when the sheep was killed.

The methods used for rearing "worm-free" lambs in this experiment, while neither as unnecessarily painstaking as Threlkeld's nor as simple as Andrews', were entirely satisfacroty in their final results, as an examination of pre-experimental parasitism will show.

C. THE LIMITATIONS AND SIGNIFICANCE OF THE FAECAL EGG COUNTS.

The value of the faecal egg counts of the experimental "worm-free" lambs lay in their indication of the presence or absence of nematode parasites in the alimentary tract.

Tetley (65, page 28) states that the method of dilution egg counting which was used in this work is not reliable for small numbers of eggs. This is not important for the present experiment, where all that was required from the data was an indication as to whether the pre-infection was likely to be big enough to confuse the outcome of the investigation.

So far as negative egg counts are concerned, Tetley (ibid.) believes that a negative count can only be accepted as an indication of the absence of parasites if repeated for several weeks. Those lambs which recorded negative egg counts were not necessarily absolutely free of parasites; however, it can be accepted that any parasites which might be present would be very few in number.

One further advantage of the egg counts was that the species

of parasites which were present could be identified, making sure that there would be no uncertainty or confusion in later experiments.

D. PRE-EXPERIMENTAL PARASITISM.

Species present prior to Experimental Infestation.

Egg count data and post mortem analysis showed that the following species of parasites were present in the experimental lambs prior to artificial administration of infective larvae:-

Strongyloides papillosus

Nematodirus spp.

Trichostrongylus spp.

Ostertagia spp.

Cooperia curticei

Trichuris ovis.

Strongyloides papillosus.

It is extremely difficult to rear lambs free from this nematode, because of the ability of the infective form to penetrate the skin of the host. In lambs, it is likely that the soft skin about the hoof and muzzle serves as a means of entry. In this connection, it is to be noted that Monnig (37a) does not regard S. papillosus as being a strong skin penetrator, infection through the mouth being the most important method, in his opinion, of entering the host. Tetley (65) states that S. papillosus has been found in lambs only a few weeks old and still on a milk diet. For this reason he believes that "percutaneous invasion by this species accounts for a greater part of the early parasitism in lambs". Considering these circumstances, it is not unexpected that lambs 39 and 50 had S. papillosus ova in the faeces when examined on 12/11/48. Presumably most of the lambs would be similarly parasitised at that time.

Exposure to infection would occur between birth and housing in the shed, though this period was as short as possible. More significant exposure would be during the feeding times when the ewes came into the shed. Dirt from the hooves and dirty udders would probably be sources of infective larvae.

No records were kept of the actual numbers of S. papillosus recovered from the ingesta, but they were low. There was no

danger of confusing S. papillosus with C. curticei at any stage, the anatomy of the two forms being very different. The long oesophagus of S. papillosus was a valuable differential factor. This species was therefore of no significance in this experiment.

Nematodirus spp.

No attempt was made to differentiate between N. filicollis and N. spathiger. Counts were kept of the numbers of these species present. Some, selected at random, are:-

Lamb	36	33	Nematodirus spp.
"	29	20	"
"	18	2	"
"	48	12	"
"	7	25	"
"	51	25	"

These are insignificant compared with an average maximum number in summer of 1,033 N. filicollis and 433 N. spathiger found in lambs on pasture (Tetley 64).

Adult forms would not be confused with C. curticei. Larval forms are longer and wider than C. curticei of the same age. Boulenger (5) noted that the forked tail and the terminal processes of the parasitic third stage larva are present in the male up to the time of the fourth ecdysis, and even after this in the females.

Nematodirus cannot penetrate the skin like Strongyloides. Work by Boulenger has demonstrated its ability to remain viable with dessication and at high temperatures (60° C) if dry. It is therefore possible that free living forms of Nematodirus were present in the hay which was the only feed in the latter part of the lambs' life. These free living forms can be removed by autoclaving with steam, a practice adopted by Threlkeld (67) in feeding his worm-free lambs. Another possible source of infection would be dirt from the feet of the ewes and from their dung. Though the shed was cleaned out regularly, it may have been possible for some eggs to develop to the infective stage and be absorbed with the hosts' feed.

Trichostrongylus species.

The number of adult Trichostrongylus species present in the intestines of lambs killed in both the Main and Subsidiary experiments was about the same as the number of Strongyloides.

being uniformly very low. No record was kept of the actual number, however.

In measuring immature forms of C. curticei, very occasionally larval forms were found, which were deduced to be Trichostrongylus species. The obvious difference in these forms lay in the anterior region, which tapered in a fashion distinctly different from C. curticei. They also failed to take on the coiled form, characteristic of Cooperia from about the fourth day of parasitism.

The low numbers of Trichostrongylus spp., and the fact that they were recognised in the immature stages, eliminated them as insignificant factors in this study.

Trichuris ovis and Ostertagia species.

With the exception of a rare adult Ostertagia in the mature Cooperia populations, neither of these species are of concern in this work. No immature phases of Ostertagia were observed in the immature Cooperia populations; it is possible that an odd individual was overlooked.

Cooperia curticei.

The presence of odd adult C. curticei in lambs 10 and 40, these being part of the Subsidiary Experiment, was probably due to infective larvae picked up in the lamb shed. During rotation of stock from pen to pen for cleaning purposes, accidental infection could have occurred, as the Stock Culture animals and the infected animals of the Main Experiment were included in such rotation, although housed in pens separate from the other experimental animals.

The larvae could have lived long enough to produce Cooperia ova in the faeces. This was the only case in which C. curticei were not derived directly from an experimental infestation.

To conclude, it can be definitely stated that the few parasites present in the experimental lambs prior to the experimental infestations were of no significance in the growth study.

E. SIGNIFICANCE OF THE SPECIES PRESENT IN THE LAME EWE LAMB.

Cultures of infective larvae used in the Trial Experiment came directly from the Lame ewe lamb. All other cultures were derived from stock culture animals, which were infected in turn

with Ostertagia species or with "Ostertagia" larvae from the Lame ewe lamb. The species present in this lamb which lived in the same habitat as the subject of this experiment are therefore important.

It has already been explained that this study, originally concerned with Ostertagia species, was changed to a study of C. curticei, because the latter was more numerous. C. curticei lives in the small intestine, and the species in the Lame ewe lamb which would be associated with this same habitat were Nematodirus filicollis, N. spathiger, Trichostrongylus vitrinus, T. colubriformis, Strongyloides papillosus and Bunostomum trigonocephalum.

B. trigonocephalum is never present in large numbers in the Manawatu (59) and is not therefore likely to be important. Nematodirus species and Strongyloides have been shown to be easily differentiated from C. curticei in all stages of their life history. Trichostrongyles species alone are likely to present difficulties in identification in immature stages. The post mortem of the Lame ewe lamb was not well conducted, no counts being made of the relative number of Trichostrongylus species present. However, in all experimental populations which had reached maturity, the number of adult Trichostrongylus species were very low, and it is reasonable to assume that similar insignificant numbers would occur amongst the immature populations of C. curticei.

It is concluded that, in spite of the multiplicity of species present in the original source of infective material, there was no possibility of confusion with C. curticei.

F. THE DEVELOPMENT OF A STOCK CULTURE.

(i) The incubation of nematode ova.

The exact reasons for the failure of the ova to develop on the charcoal-sterilized faeces mixture are not known. It is possible that lack of feed for the larvae, or excessive moisture, were contributory causes.

The faeces was sterilized prior to use, in order to kill unwanted spp. of nematodes, and it was at one time thought that this had also killed the bacteria. McCoy (33) has shown that infective larvae of the dog hookworm could not grow unless live bacteria were present; however, it is extremely unlikely that all the bacteria were killed under the conditions of sterilization

employed in this experiment. Other workers (Dickmams and Andrews (14) and Morgan (39)) have used heat-sterilized sheep dung successfully, though in the work of the former it is apparent that the faeces was sterile in respect of nematode ova only; doubtless the same applies to Morgan's work. The only conclusion which can be drawm is that in this experiment it is most unlikely that lack of feed was the cause of the failure of the ova to develop.

The practice adopted in making cultures of nematode ova is to avoid excessive moisture. A damp, crumbly condition is considered desirable. Lapage (30) states that many observations have shown that nematode eggs need oxygen to develop, which is in agreement with the avoidance of excessive water. McCoy (34) has shown that very low tensions of oxygen in water will allow eggs of the dog hookworm to develop, but that amounts of less than 0·4 cc per litre are inhibitory. It is not safe to conclude that the same applies to ova of C. curticei. It is likely, however, that attempts to culture ova under conditions of excessive moisture do fail because of inadequate oxygen tensions. But the subject can be little more than mentioned here, with the conclusion that excessive water was probably a cause of the failure of the culture to develop.

(ii) Infestation with live adults.

The persistent failure of live adult worms to find lodgment in lambs 1, 2, 3 and 22 cannot be definitely explained. The adult stage is a means of reproduction, while the infective stage alone has the ability to locate a habitat. It is not surprising that only a few adults would manage to settle on to the walls of the abomasum, but the number which were successful seems unexpectedly small. It is possible that some did remain in the abomasum however, but ceased to lay eggs, so that their presence would not be revealed in faecal egg counts. It is unlikely that many would remain very long under these conditions.

Most likely the nematodes were too long away from their original host, both in the laboratory and in the rumen of the new host, and they would therefore be weakened by the time they reached the abomasum. It is possible that the healthy state of the new host and the concentrated feed were also significant

factors in this connection.

G. MEASUREMENT OF SIZE OF DOSE.

The mathematical statements of the number of infected larvae given to each sheep cannot be regarded as being highly accurate. Their main value is in the fact that they give a reliable indication of the size difference between populations administered. Thus it is reasonable to say in the case of the Main experiment that the lambs receiving 22,000 larvae had a population approximately double that given to the lambs receiving 10,000. Since the samples for each lamb were prepared with the greatest care, they would be closely similar in the number of larvae they contained.

It can be said therefore that, within any particular population group, the lambs received equal numbers of infective larvae, but that the figures of different populations indicate a relative order of magnitude only.

In both Trial and Subsidiary experiments, many living as well as parasitic forms of S. papillosus were present in the infecting dose, and also, in the latter experiment, numerous first and second stage larvae of unidentified species. These were all included under the heading of "Strongyloides" and a separate count made of them. The individual samples would contain an equal number of these "Strongyloides" larvae.

H. SOME ASPECTS OF SAMPLING.

The location of C. curticei in relation to sampling.

Tetley (60) has shown that C. curticei occur in greatest numbers in the jejunum, five to twenty-five feet from the pyloric sphincter. Infection in any one sheep may be confined to narrow limits, but regions of peak numbers may vary from seven feet to thirty feet from the anterior end of the small intestine.

In this experiment, the small intestine was cut at a point about thirty-nine feet from the pyloric valve, so that the anterior section should include the vast majority of the population. The posterior section of the small intestine was retained. In one case (lamb 49), where it was suspected that the population was being eliminated, this section was examined. This was not done for any other sheep, it being reasoned that since this study required only a sample of the population, any parasites which may

have been in the posterior section would not be of any importance.

The method for the removal of the ingesta.

The method described for collecting ingesta and parasites from the small intestine was the standard technique adopted in this laboratory.

It has already been shown that the Subsidiary experiment was carried out partly with a view to investigating the drop in the total numbers of parasites which Tetley (in concurrent work - unpublished) had observed on the seventh day of parasitism. This aspect was independent of the growth study, which, as mentioned previously, needed a sample of population rather than total numbers for measurement. Consequently it was not investigated exhaustively. The results did show, however, that many parasites were retained in the small intestine after it had been run through the fingers. Complete counts of the populations were not available, but the table below sets out those which are known.

RETENTION OF PARASITES IN THE SMALL INTESTINE.

Age of Population (Days from infection)	Lamb No.	Nos. of C.curticei collected by (i) Standard Method	(ii) Additional Washing.
6.....	(28	-	13760
	(40	-	113
7.....	23	7600	-
8.....	34	-	7846
9.....	(49	5910	1861
	(25	1370	1600

Though the figures are incomplete, they do indicate that the method employed did leave a variable proportion of the population behind in the gut. The numbers thus retained were larger than expected by the writer. The crucial point, in so far as this thesis is concerned, is whether the larvae retained are in any way different in development from those collected by the usual method. If this were so, then the populations recovered from the gut would not be truly representative of the entire population for any given age. To test this, two hundred male and female worms were measured from each of the two populations from lamb 25. The frequency distributions are shown in graphs 45 and 46 (worms collected by usual method) and graphs 47 and 48 (worms retained and subsequently washed out). The means of the populations are

set out below.

SAMPLE MEANS FOR THE POPULATIONS FROM LAMB 25.

Sex	Age	Mean values for populations collected by:- (i) Standard method	(ii) Additional washing
MALES	4th instar	1·99 mms	2·06 mms
	Adult	2·64 mms	2·79 mms
FEMALES	4th instar	1·93 mms	2·24 mms
	Adult	3·17 mms	3·30 mms

The writer does not believe that there is any significant difference between the two populations collected. However, the possibility does exist that the standard technique could on certain occasions have a selective effect in the parasites it removed.

There is no reason to suppose that this would be likely in the adult stages. If Andrews (2) is correct in saying that the early fourth stage larvae are free in the intestine, while those still in the crypts are in the third stage, then a population collected at this stage of development by running the intestine through the fingers would be likely to have too low a proportion of third stage forms.

The results obtained here, incomplete as they are, draw attention to the fact that it is possible to leave large proportions of the nematode populations behind in using the method described. The subject is worthy of further investigation, though Tetley (personal communication) considers that, on the average, the method is a sound one.

The possibility of bias in selection of worms for measurement.

Care was taken to avoid any suggestion of bias in selecting nematodes for measuring. The ingesta was well shaken with a little water and divided into halves. Usually all the C. curticei were picked out of one of these portions. In a few cases some had to be picked from the remaining half, and all worms had to be removed for measuring in one case (on the seventh day (lamb 43)).

When these nematodes were to be measured, they were well shaken up in the 2 oz. bottle in which they were stored, and a portion of the formalin and glycerine solution containing the worms poured into a small dish. Care with the shaking ensured an even distribution of the parasites through the formalin, so that

it was unlikely that there would be any bias to this stage.

In picking Cooperia out of the dish, an area was selected and all the nematodes removed and measured. In some cases the whole dish would be used, in others only a part.

The writer is confident that these precautions in the selection of parasites for measurement gave a representative sample of the entire population.

I. EXPERIMENTAL ERRORS.

Errors in the measurement of C.curticei fall into the following categories:-

(1) Those associated with methods of fixing and preserving.

(2) Those associated with the optical instruments.

(3) Those arising from drawing and measuring.

(4) Those in the table used for converting results to millimetres.

These will now be discussed under their respective headings.

Errors due to fixing and preserving.

The nematodes and gut contents were all placed in 5% formalin as soon as possible after the lamb was killed. They remained in this in some cases up to six months, by which time nearly all worm populations had been transferred into 5% formalin and glycerine. Nematodes were measured after variable periods of from one to nine months in this medium.

The only differences between groups are in the times spent in the formalin and formalin/glycerine mixture. It is not likely that this difference in treatment would make any significant changes in length between age groups.

It is not known whether the formalin solution induced any gradual shrinkage or expansion of the parasites over a long period of time. Measurements should have been made of a number of live C. curticei first in a thin film of water, and then in formalin at successive intervals for several months. This was not done however, and definite knowledge of the effect of preservation is therefore lacking. Indirect negative information can be deduced from the fact that the parasites measured after several months in the preservative had closely similarfrequency distributions to those of the same age measured earlier. This applies especially to lamb 18, where investigations on the bimodal

curve led to a second series of measurements some five months after the first. No obvious differences existed between the two distributions. It is considered unlikely that the period of time spent in the preservative affected the length to any significant degree.

The use of hot fixatives were first considered in this experiment. Hot water and formalin, or hot 70% alcohol could have been employed. Eventually the idea was abandoned in favour of cold formalin, not only because it was much more convenient, but also because work by Scott (50) showed that in order to get complete straightening of Ancylostoma caninum, the worms had to be introduced a few at a time into the hot fixative. This was not possible under the conditions of the experiment, where all the ingesta, along with the parasites, was introduced into the fixative at the one time. Later, when measuring began, it was found that even very tightly coiled C. curticei could be measured just as accurately as straight specimens, so that it was not necessary to straighten the worms with a hot fixative.

Errors associated with the Optical instruments.

(1) The Microprojector.

(a) Pressure of the Cover Slip.

A nematode lying partly in the vertical plane would give a geometrical projection of its length onto the paper, and its image would thus be shorter. To overcome this difficulty, a cover slip was always used. Excess glycerine and formalin mounting media was drawn off with blotting paper so that the cover slip rested on the worms and forced them into the horizontal plane. Undue pressure would probably alter the width, but it is very unlikely that the length would be distorted to a significant degree.

(b) Incorrect Focus.

The projector was always adjusted to give as clear a definition as possible. Very bad focussing, a condition under which worms were not measured, produced small errors. For example, the image of an object was 3·45 mms long when in focus. When out of focus, readings of 3·68 mms, 3·93 mms and 3·62 mms were obtained.

(c) Aberration of the Lens.

When an image in the centre of the field was corr-

ectly in focus, images on the outer edges would be out of focus. In order to minimise errors of this type, nematodes were kept as nearly as possible to the centre of the field by moving the slide as described previously. A better quality lens would have largely overcome this difficulty.

(d) Vertical movement of the Lens.

Any vertical movement of the lens relative to the drawingdrawing board would introduce errors. An object 3·54 mms long was placed on the stage and the lens moved upwards 1·5 mms. The image of the object was then 3·68 mms long. Moving the lens downwards 2 mms and 4 mms below the usual position gave measurements of 3·41 mms and 3·30 mms respectively.

The lens support was clamped lightly to the vertical stand and the position checked occasionally against marks on the stand. At no stage were measurements made with the lens in any other position. This care eliminated any possibility of errors from this source.

(2) The Camera-Lucida.

It has already been explained that when this instrument was used the microscope had to be tilted backwards so that the lower edge or basal face of the prism was directly over and parallel to the paper. A less convenient method was to tilt the drawing paper until it was parallel to the face of the prism. This method was not employed.

If the basal face of the prism was not in the same plane as the paper, but in a plane at an acute angle to either the front or back edge of the paper, distortion occurred. This was particularly obvious when the images of the equidistant lines of the stage micrometer were drawn on the paper. The lines became further and further apart as the distance between the plane of the prism and the plane of the paper increased.

In addition to distortion, changes in the angle at which the microscope was tilted (that is, changes in the angle of the lower face of the prism relative to the paper) produced slight differences in magnification. They were not obvious and could only be detected with a stage micrometer. The microscope was set so that the image of a line 100 microns long when drawn on the paper was the same length as the circumference of the measuring wheel.

Repeated checks were made while working to ensure that the magnification remained constant.

Errors arising from Drawing and Measuring.

In order to determine the accuracy of the methods of measurement, the images of a group of nine adults, nine fourth stage larvae and seven infective larvae were drawn and measured ten consecutive times.

In addition, one nematode from each of these three groups was drawn and a series of measurements made of the one drawing. The object of this was to gain information on the variations resultant on running the measuring wheel along the line representing the worm. In the case of the adult worms, the measurements were made from alternate ends in an attempt to see whether there was any variation in measuring from one end as opposed to the other. The existence of such a variation was suspected because the interaction between the configuration of the line and the handling of the wheel made some worms more awkward to measure from one end than from the other.

The measurements obtained are set out in Appendix . Using the standard technique for the analysis of variance as set out by Snedecor (52), the figures were analysed to determine the standard error, the time error, and the error due to measuring worms from one end as opposed to the other. The analyses are summarised in tables 1 to 8 which accompany the data in the Appendix. XIV

Time Errors. Analysis of data derived from repeated drawings and measurements of the same worms, taking out the "time" variance, showed that there was a significant difference between measurements from time to time in some cases. This was not so with the fourth stage larvae, the analysis of variance (table 1) indicating that differences between measurements of the same worm from time to time were not significant.

With the adult worms (table 3) the F ratio was 2.08, which was significant at the 5% level of probability. Examination of the data shows that at time 1 the length in a number of cases was greater than lengths recorded at other times for the same worms. When time 1 readings were dropped from the analysis, the time means did not differ significantly.

The time error of the microprojector was not likely to be due to fatigue. Neither was it likely to be due to actual alterations in the instruments' magnification, because the setting was unchanged throughout. Apart from a personal error, it is possible that the worms were not in focus when measured. Incorrect focusing has already been shown to be a likely source of errors of the same general order of magnitude as displayed in measurements made at time 1.

The error is small, as can be shown by a study of the standard errors. Reference to table 3 shows that the error mean square is ·0047, giving a standard error of ·069 mms. This is the value which should have been obtained for the standard error if the time means were entirely consistent. This is of course impossible. By including the time factor in the error sum of squares, the error mean square becomes ·0052 and the standard error ·073 mms. The actual effect of the time factor is therefore small, in spite of the fact that one series of measurements showed a significant difference from the others.

Before making an analysis of time errors in measurements with the Camera-Lucida, allowance had to be made for one observation accidentally omitted in the tenth series of measurements. Rather than discard all the other readings for this series, recourse was made to a formula described by Snedecor (52) by means of which a missing item may be supplied in a Randomised Block treatment.

The missing value $$X = \frac{tT + bB - S}{(t-1)(B-1)},$$

where t = number of treatments
b = number of blocks
T = sum of the times with the same treatment as the missing item
B = sum of the times in the same block as the missing item
S = sum of all the observed items.

Substituting, $$X = \frac{10(4314) + 7(7069) - 50584}{(9)(6)}$$

$$= 779 \text{ microns.}$$

The analysis of variance proceeds as before, with the exception that the degrees of freedom for "total" and "errors" are reduced by 1, in accordance with the requirements for the use of this formula.

The analysis is shown in table 5, and it reveals that the differences in length from time to time are barely significant, as shown by the F value of 2·07, which is a little greater than the 5% level of probability, 2·06 for 9 and 53 degrees of freedom. Unlike the data on the adult worms, no set of observations at any particular time caused this, as far as is known. There is some general inconsistency involved which may possibly be explained in two ways:-

(a) The Camera-Lucida with which these measurements were taken was particularly difficult to use. Drawings would tend to become less accurate as time passed.

(b) The magnification could be altered by movement of the draw tube and the angle at which the microscope was tilted. After each set of seven drawings had been made, it was necessary to re-adjust these, using the stage micrometer to restore the correct magnification. All this would contribute to errors. If measurements had been entirely consistent, then the standard error per observation would have been the square root of the error mean square, 36·78 (table 5) which is 6·06μ.

The actual standard error is the square root of 42·50 (table 6) which is 6·52 . The effect of these incosistent measurements is therefore very small.

Position Effects. It was thought possible that the awkward shapes of some worms may have made them more difficult to measure from one end than the other.

In table 7 (Appendix XIV) the analysis of variance in measurements of one drawing from either end shows that there are no significant differences.

The Estimation of Standard Errors. For adult worms, the standard error per observation (redrawing the worm each time for measuring) is $\sqrt{\cdot 005265}$, i.e. the square root of the error mean square, ·073 mms (see table 4, Appendix XIV). Similar values for fourth stage larvae (table 2) and infective larvae (table 6) are shown in the analysis of standard errors set out below:-

ANALYSIS OF STANDARD ERRORS.

Group	Redrawing for each measurement (A)	All measurements from the same drawing (B)	Drawing Errors (A-B) (C)
Adult worms (4mms to 8mms)	0·073 mms	0·028 mms	0·045 mms
Fourth stage larvae (1·6mms to 2·3mms)	0·057 mms	0·010 mms	0·047 mms
Infective larvae (600 to 800)	6·5	2·9	3·6

From data on repeated measurements of the same drawing, Appendix XIV, similar standard errors can be calculated and are shown in column B of the above analysis. These values are partly derived from analyses of variance shown in tables 8, and and other analyses not in appendix. They represent standard errors due to measuring, time factors and fatigue.

The standard errors determined by redrawing (column A of the above analysis) include, in addition to those mentioned above, errors due to the drawing process itself. Therefore the difference between the figures in column A and those in column B represents the standard error due to drawing alone, recorded in column C.

These results may be more readily apprehended in the following form:-

Maximum errors due to different causes for 95% of cases.

Group	Drawing errors	Measuring errors	Total error
Adults	·09mms	·056mms	·146mms
Fourth stage larvae	·094mms	·020mms	·114mms
Infective larvae	7·2	5·8	13·0

For example, if an adult worm 5 mms long is measured, the measurement is within ·146 mms of the correct figure, and within ·056 mms of the correct length of the line drawn. The line drawn is iteslf within ·09 mms of the correct length of the worm. Note:- The above does not refer to the REAL length of the line, but to the real length divided by the magnification.

Rounding errors. Rounding errors should not exceed 1/4 of the standard error per observation. Therefore, in measurements of adult and fourth stage larvae, rounding errors should not exceed ·018 mms and ·014 mms respectively. The second decimal

place can be rounded to even numbers; but it cannot be discarded. In measurements of the infective larvae, rounding errors must not exceed 1/4 of 6.52 or 1.6 . Therefore the unit figure in the micron measurements is needed, but it may be reduced to even numbers.

All data has been taken to the second decimal place in the case of the millimetre measurements, and to the unit figure in micron measurements. No figures have been rounded.

Errors in the Tables.

Three tables were used to express the revolutions and fractions of a revolution of the measuring wheel in terms of millimetres. Two were made for use with the microprojector, the other with the camera-lucida. All three tables are reproduced in Appendix XI. The tables were constructed from a whole series of repeated measurements, as explained in a preceding section. The only significant errors are rounding errors due to correcting certain values to two decimal places.

The other errors in the tables would be insignificant. Possibly the most important is the error which arises in drawing the magnified image of the six millimetre line on the paper. The image was not very easy to see. In the light of the numerous observations made before the tables were constructed, the errors are known to be insignificant. This statement is well illustrated by the differences between the original and corrected millimetre transposing tables. (Appendix XI)

J. ENVIRONMENT AND GROWTH.

In any growth study, it is important that the variations in the environment be known and, where possible, controlled. In some work it is necessary to destroy the environment in order to take measurements. A complete picture of growth cannot be obtained without a number of entirely separate environments, and it is often impossible to regulate these so that they are all equal for the purposes of the experiment.

In this experiment, each sheep represents an entirely separate environment in which the parasites grow. In addition, it must not be forgotten that within each individual nematode there is also an environment which will be variable from worm to worm to a greater or lesser degree.

This section is a study of these environments and will poin(

out, as far as possible, where differences exist which are likely to have a significant influence on growth.

The Internal Environment.

Brody (42) regards the internal environment as being resultant on three factors. He describes these as firstly, the inherent force within a cell to grow indefinitely; secondly, the retarding influence of the finite medium in which the cell exists; and thirdly, hormonal factors.

Comparatively little is known of the internal environment of the Nematoda, and this is not an occasion for discussion of the subject. Hobson (23) has recently reviewed the knowledge of nematode physiology, and more recent work of importance has been done by Rogers (44) on aerobic metabolism, by Bueding (8) on the effects of drugs on parasite metabolism, by Geiman and McKee (18) on protein metabolism, and by von Brand (6) on carbohydrate metabolism.

Within one species, individuals may show varying reactions to the same environment because of differences in their internal environments. Thus in some populations measured in this experiment, there are present one or two isolated individuals very much shorter (and occasionally much longer) than the majority of the population (see graphs 26, 33, 36 and 38 in the Appendix). These may be interpreted as being worms which are in some way abnormal in their internal environment, and as a result unable to develop normally. These isolated variations are few in number and are of little concern in this work. This observation, however, is made with caution, and does not extend to certain small groups of aberrant individuals to which particular reference is made in later discussion.

Sometimes big differences are reported in the reaction of one species to the same or similar environments, and the groups are known as physiological strains. The differences in internal environment, which are exhibited by odd individuals, may be due to chance variations; the differences between groups or strains are the result of some physiological differences in the internal environment between two groups of the same species. Strains of parasites are well known. Scott (49) has shown that differences exist between members of the species Ancylostoma caninum which are

parasitic in the dog and cat. These differences, he says, are exemplified in the differing ability of any one particular strain to infect the same host. Koino (as quoted by Lapage(27)) has shown that the "pig" strain of Ascaris lumbricoides will not develop in man, whereas the morphologically similar "man" strain will do so.

The existence of strains in C. curticei has not been demonstrated, nor have they been definitely shown to exist in the related type H. contortus. Stoll (55) stating that he can find "few if any measurable differences" between several strains used by him.

It is not very likely that the differences in growth rates which exist within the populations examined are due to strain differences. If it be allowed that there were any such differences, it must be remembered that the infective larvae used were well mixed together, and that there would not be any differences between individual doses in respect of relative numbers of each strain present. Therefore, variations within populations would be reasonably constant from host to host. However, while some populations exhibit great irregularity, others show a normal frequency distribution of length, with low standard deviations. It is concluded that the population of infective larvae was homogenous with regard to growth rate of worms, and that strain differences are not apparent.

The External Environment.

The external environment as far as C. curticei is concerned is the jejunum. The suitability of this habitat for the parasite is governed by a number of factors, which may be summarised as (a) the quality and quantity of the host's feed, (b) the host's health (which may be concomitant with its ability to resist parasitic infections), (c) age, and (d) host individuality.

The feeding of the lambs has already been discussed. It was thought that because the lambs were growing well they would not make ideal subjects for a study of development. However, this statement is made with some reservations. There is record of a series of experiments by Fraser (16) culminating in work which showed a well-fed flock to harbour fewer parasites than a poorly fed flock which had been given the same number of infective larvae. Fraser's experiment was not designed chiefly to show

whether the rate of development of the parasites was slower in the well-fed healthy lambs than in the poorly fed lambs. The fact that the average rate of development of C. curticei in the present experiment followed that given by Andrews (2) as the normal life cycle would seem to indicate that the general good health of the experimental flock did not upset the broad outlines of this cycle.

The flock as a whole was fairly even as regards size, weight gains per week, age, and, within limits, the parasitic infestations already in situ. The pre-infestation parasitic populations were so small that it is unlikely that they would produce any acquired immunity.

The question of the development of acquired resistance to the experimental infestations is not strictly a subject for discussion under this heading. In a later section evidence will be presented which has enabled the writer to draw the conclusion that such resistance would not develop over the comparatively short period of the experimental infestation.

In so far as each sheep, as an individual, varies from its fellows in spite of uniformity of age, feeding and general conditions, it would be expected that the intestinal environment would also vary in its general suitability to parasitism. This may be termed a form of natural resistance, and the literature (58) on parasite immunity shows that this is a well known, if little understood, phenomenon.

So far as this experiment is concerned, it was not possible to have any control over this little known variant; it may have been measured, however, by having several host animals for the one age group.

To conclude, it is believed that the only major difference between the environments which the parasites encountered in the different hosts was due to individual variation or 'natural resistance'. The extent to which this was the source of variations observed will be discussed later.

: : : :

CHAPTER XI.

THE MAIN AND SUBSIDIARY EXPERIMENTS - DISCUSSION OF THE RESULTS.

A. UNDEVELOPED PARASITES.

SUMMARY.

The possible origins of immature parasites associated with older populations are considered. The significance of populations of eggless females and the numbers of eggs per female are examined and attention paid to the onset of ovulation. The occurrence and significance of fourth stage larvae, and the association of ecdysis with inhibition of development are studied, and the inhibition of growth is examined.

POSSIBLE ORIGINS OF UNDEVELOPED PARASITES.

Three likely explanations for the presence of undeveloped parasites in the experimental lambs can be suggested. They are (i) re-infection of the host; (ii) delayed passage to the intestine; (iii) arrested development in the intestine.

(i) Re-infection of the Host.

So far as the thirty sheep used in the Main Experiment are concerned, it was not possible for them to become infected with Cooperia curticei larvae other than those administered experimentally.

The eight sheep used in the Subsidiary Experiment ran a risk of exposure to accidental infestation. They were associated with the sheep from the Main Experiment when some of the latter were passing ova of C. curticei in the faeces, and were, until killed, in the same shed as the stock culture animals, though in separate pens. Two of these eight sheep were found to have one or two adult C. curticei in them when slaughtered. These were not derived from the experimental infestation, but were probably acquired accidentally when the sheep were moved from pen to pen in the cleaning operations.

It is not possible therefore to explain the presence of immature forms found in sheep from both Main and Subsidiary Experiments as being the result of accidental infestation with C. curticei.

(ii) Delayed passage through the Rumen.

So far as the fourth stage larvae are concerned,

the shapes of the frequency distributions suggest the simultaneous arrival in the intestine and parallel development of a number of larvae. (See figs. 8 and 9, pages 85 and 86).

It is not likely that any larvae held in the paunch for a long time would remain as a small group and enter the intestine together. Food material is well mixed before entering the intestine, and larvae, if they were left behind, would more likely enter the intestine in a steady but decreasing stream. This would give a skew distribution of length, similar to those found in distribution curves of some adult populations, e.g. graphs 57 and 69. On these grounds it is unlikely that the fourth stage larvae represent worms which have been delayed in their passage to the intestine.

So far as the adult populations in graphs 57 and 69 are concerned, in spite of the negative skew which might be expected to be associated with delay in arrival in the intestine, the writer does not believe that this factor has operated. Work on the passage of larvae through the gut indicates that they are not likely to take more than a few days in reaching the lumen of the intestine.

Monnig (37) has measured the rate at which infective larvae of Oesophagostomum columbianum passed through the paunch and reached the intestine. After five hours from administration, he found 70% of the larvae in the rumen, at eight hours only 8%, and after twelve hours they were "rare", over 90% being in the intestine. Less critical work by Veglia (68) showed "very few" infective larvae of H. contortus in the rumen between 24 and 36 hours after infestation. None were found 48 hours after infestation. It is unlikely in view of this work that larvae would be more than two or three days in the rumen and associated organs before reaching the intestine, and the undeveloped forms cannot be explained in terms of delay in reaching the intestine.

(iii) Arrested development.

This is the most likely explanation for the presence of undeveloped forms with mature populations. Any growing population, plant or animal, can be expected to contain individuals in varying stages of development, and in this instance the undeveloped larvae are believed to be representative of such forms.

UNDEVELOPED ADULTS.

(i) Implications of the Results.

The results indicate that though C. curticei has been from 12 to 22 days in the host, it does not follow that all the population will consist of sexually mature adults. It is possible that a variable proportion of the adult population will not be mature. This will be reflected in the shorter lengths, the absence of eggs in the uteri of the females, and in the light-coloured spicules of the males.

In general, the shape of the frequency distribution of total length of females will show whether or not these forms are present. Some nearly normal distributions may have a few agamic forms associated with them. The same will apply to the male frequency distributions for length, although the differences may not show up so well because of the smaller variation and shorter total length of males compared with females.

(ii) Agamic females and Interpretation of the Egg Count data.

In most populations examined, the number of agamic females has been low. The small error which this would introduce into faecal egg counts would be of no significance. Where the proportion of agamic to fertile individuals is high, the egg counts may lead to an erroneous conception as to the true magnitude of the parasite infection.

The table on page 116 gives the percentage of agamic females. In two cases this is high.

The first case, that of the population from lamb 26, occurs at a time when the females are just commencing egg production. The fact that the mean total number of eggs per female is only about 8, as compared with values of 18 and more for mature populations is indicative of this. Since egg counts made in the early stages of egg laying are necessarily accepted with the reservation that the population has not yet reached its maximum rate of laying, the presence of agamic females at this stage is of little importance.

The second case where a large number of agamic individuals occur is in lamb 18, killed when the parasites had been 22 days in the host. It can be argued that, since the egg count would probably still be rising at this stage, it would not be accepted

as an indication of the degree of parasitism of the animal.

The importance of this example lies in the fact that it shows that an animal can harbour a mature parasitic population, and only show in its faeces eggs equivalent to a population less than half that which is actually present. This could be a serious factor in experimental work, especially that connected with the use of egg count data in resistance studies. The importance of the barren females in this connection has been emphasised by Schwartz, Alicata and Lucker (48). They state that

> "the counts of worm eggs in the faeces, especially in connection with resistance studies, give no direct or positive information in regard to males, immature worms or senile females, which might be present in the host in considerable numbers. This limitation has been pointed out from time to time in the past, but even yet is not always taken into consideration or kept in mind. The significance of the possible presence of agamic parasites in a state of arrested development has been entirely overlooked in the past."

These workers base their observations on work with Nippostrongylus muris in rats. These animals were super-infected after the eggs resulting from a previous infection had entirely disappeared. Killed later when the worms were expected to be in full egg production, the hosts were found to have many undeveloped larvae in the lungs, and most of the worms in the intestines were not fully grown. Few of the females had attained an egg laying stage and they contained relatively few eggs in the uterus. Eggs in the faeces immediately prior to killing were insignificant in number compared with the numbers present at a corresponding stage of development in the first infection.

The cases they cite involve acquired resistance and are more extreme than those which occurred in the sheep used in this experiment, where acquired resistance is not a factor.

It would be interesting to know how many sheep can hold parasitic worms in a reduced state of development similar to sheep 18. Tetley (63) working with Nematodirus concluded that "at any one point of time almost the entire population of worms is

laying eggs". This observation is in line with Stoll's work (53) from which the latter concluded that the average egg count on a daily basis as well as on a per gram of faeces basis can be correlated with the number of worms in the host.

These observations suggest that in sheep, and in other hosts, the presence of many agamic individuals over a long period of time is unusual, and consequently errors in egg counts from this source are likely to be low. However, agamic worms may become more numerous when super infection takes place, and could easily become the source of a serious experimental error in resistance studies following re-infection, and in interpretation of faecal egg counts generally.

(iii) The Significance of the Distribution of the Number of eggs per worm.

Distributions of the numbers of ova per worm are shown in fig. 7, page 80.

The means of these distributions are as follows:-

Lamb No.	Age of population.	Mean No. of eggs.
26	12	7·9
51)		(16·8
52)	14	(21·7
33	16	26·5
29)		(28·6
9)	18	(24·7
27)		(21·7
18)	22	(18·5

The low numbers of eggs per female in lamb 26 may be due to the fact that at 12 days the female is just commencing to produce eggs in the uteri. This particular population has many individuals without eggs which are very nearly the same mean length as those with eggs, and it is suggested that they might have become gravid shortly afterwards.

Populations examined at 11 days were found to have (in the case of lamb 38) two worms containing eggs out of 28 examined. In both cases, these were present in the posterior uterus, two eggs in one, and one in the other.

This is additional evidence for the belief that females do not become gravid until on or after the twelfth day of parasitism.

The figure of 16·8 eggs per female in the case of lamb 52 may be interpreted as indicating that production of ova had only recently commenced.

Of the other values, it is considered that all are probably

comparable, except lamb 18, with 18.5 eggs as the mean number. In this case, the female adult distribution of length has already been shown to give evidence of marked inhibition of growth, and it is probable that this lower figure is a further reflection of the slow development. Taylor (58) says that the numbers of eggs in the female worms present in resistant sheep may be small compared with the numbers carried by females in non-resistant sheep. The population in lamb 18 (graph 73) gives every indication of some degree of natural resistance, so that Taylor's statement is confirmed.

It has been assumed in this work that the total number of eggs in the uteri of each female is an indication of the fecundity of each parasite. Tetley (63) believes that unsegmented eggs, rather than the total number of eggs, is a more reliable basis on which to judge fecundity.

In spite of this, it is probable that the distributions do indicate the general order of relative egg production between populations.

(iv) The onset of Ovulation.

The data obtained in this work shows that eggs first made their appearance in the uteri of females after twelve days of parasitism. Thereafter, the numbers of females without eggs could be expected to fall. This is illustrated in the table:-

Lamb No.	Age of population	% Agamic females.
36	11	100
26	12	66
51	14	18
52	14	12
33	16	6
9	18	4
27	22	8
18	22	64

It must be remembered that this table is from a series of different populations in different hosts, and care must be taken in using comparative figures in this way. This is emphasised by the percentages of eggless females recovered after 22 days of parasitism. The particular case of lamb 18 will be discussed under a separate heading.

Tetley (65, page 160) has shown from faecal egg counts for lambs given a single infection of H. contortus that eggs commence to appear 13 days after infestation and rise to a peak at 35 days

from infestation. Under the controlled conditions of his experiment only two factors could have caused this rise in the numbers of eggs per gram of dung. First, increase in the rate of egg-laying, and second, increase in the numbers of females carrying eggs. It is probable that C. curticei would show a similar curve of egg production. In this case it can be argued that, since in all populations except one older than 14 days 90% of the females are producing eggs, the increase in absolute numbers of eggs in the faeces would be due largely to an increase in the rate of egg production per individual. It is reasonable to argue back to Tetley's experiment, and likewise consider that increase in the numbers of eggs per gram in the case of H. contortus was probably due to increased production per individual.

UNDEVELOPED LARVAE.

Where there are numerous immature fourth stage larvae in populations older than nine days, the frequency distributions of the lengths of the adult females are either very strongly skewed or bimodal. Examples of this are seen in populations from sheep 18, 27 and 29 (graphs 69, 73, 75).

Where there are no undeveloped forms present, the adult females have a normal frequency distribution for length. Examples of this are seen in graphs 61, 63, 67, 71.

This suggests that some lambs provide an environment which is not to the liking of a proportion of the worms at any time while they are developing. Where the life cycle presents difficult phases, these worms which are not in complete harmony with their environment are either unable to grow any further or their development is slowed up. The difficult phases appear to be the final lethargus and attainment of full maturity a day or so afterwards; perhaps at the time when the body commenced to make demands for additional nutrients for reproduction. Possibly the very strong growth impulse which takes hold as soon as the larvae enter the adult stage is sufficient to prevent any worms from falling out for a matter of 24 or 48 hours, because all the graphs of older worms show that there are very few young adults left undeveloped at lengths just past the typical length or the final molt.

It will be noted that it is the female distributions which

are cited as showing the skew effect. As explained elsewhere, undeveloped males are present where females in the same population are undeveloped; this has been shown clearly for lamb 18 (graphs 72 and 73). If a proportion of a population is delayed in development and the remainder are measured a few days later, the females, growing faster than the males, will show a skewed or bimodal distribution. The later group of males will not be very much longer proportionally than those which have failed to develop, and the distribution curve may be only slightly skewed or even appear normal. For this reason, the females are emphasised in this discussion.

There are several exceptions to the statements made at the beginning of this section concerning the association of fourth stage larvae and adults. In three graphs (53, 57, 59) there are small numbers of fourth stage larvae, i.e. 6 or 8 associated with skewed curves in the adult stages. So far as graphs 53 and 59 are concerned, the adult females have not very markedly skewed curves, and there are a number of short adults at odd lengths between the fourth stage larvae and the main adult distribution. Graph 57 has a distribution curve with a very pronounced negative skew and adults of almost all lengths except the shortest are present.

The anomalous feature of these distributions, so far as the preliminary statements made are concerned, is the scarcity of fourth stage larvae. Such skewed distributions would be expected to have a larger number associated with them. It is probable that these distributions represent populations which were not really arrested in development until they reached the adult stage. But it is equally true that fourth stage larvae may have been present, yet eliminated soon after development was arrested.

Another exception is provided by the distributions shown in graphs 51 and 55. In these, two or three larvae and a similar number of undeveloped adults are associated with normal distributions of developed adults. Here it is likely that the environment was quite suitable for the development of the parasites as a whole, as evidenced by their normal distribution curve of length. The few undeveloped individuals, it is suggested, are themselves abnormal and fall behind for this reason.

Only one other case has not been examined in the light of the propositions advanced above. This is in graph 65. Here there were no undeveloped forms in the population measured, but the distribution is slightly skewed. When this distribution was analysed in terms of the number of ova per worm associated with different lengths, it was found that the lower end of the distribution contained worms with reduced numbers of eggs (see graph 73). It appears likely that just prior to the end of the growing period some worms were arrested slightly in development. Consequently, they did not grow quite as much as the rest of the population and produced a skewed effect in the distribution curve. Some were able to produce eggs, but a little later than the longer members of the population (assuming here that the number of eggs in the uteri is an index of relative fecundity) as shown by the fewer eggs they contained. Others, shorter still, had not produced any when the host was killed - though one isolated case is an exception to this.

To conclude, it is suggested that where a population has been able to develop without hindrance to maturity, the distribution curve of length will be normal, and only odd, presumably abnormal, individuals are likely to be found at shorter lengths. Where a population has received some check it will be likely to have a number of immature adults, or fourth stage larvae associated with it, and the distribution for the adult female worms will be negatively skewed.

THE ECDYSES AND ARRESTED DEVELOPMENT.

From the data summarised in fig. 8, it is evident that many male larvae are able to grow to about 2 mms, but are unable to proceed any further. This is close to but slightly below the mean length for all fourth stage larval males about to moult (2·3 mms). Evidently the ecdysis and lethargus are difficult growth phases which some individuals are unable to negotiate.

Many animals have similar critical periods in their growth history which present obstacles to development. Brody (7) quotes work which shows that the curve of specific mortality (or ratio of dead to living individuals at the same time) for the chick embryo has two maxima. One, at eight days, coincides with the maximum concentration of lactic acid prior to the organisation of

a mechanism for its elimination. The other, at sixteen to nineteen days, coincides with cessation of growth consequent on the change in respiratory mechanisms.

If we regard the undeveloped larvae as equivalent to the mortality, this case cited by Brody is analogous to C. curticei, and it is not surprising if the data gives evidence of higher "mortality" at a period of adjustment and change such as the final ecdysis.

So far as fourth stage female larvae are concerned in fig. 9, it is noticeable that lambs 26, 36, 38, 48, and 51 show little or no evidence of increased "mortality" about the time of the fourth ecdysis. This at first glance may be regarded as a consequence of fewer numbers, but is not so because the male and female larvae are present in approximately equal numbers. However, in lambs 2, 18, 27 and 29 the association of inhibition of development with the ecdysis is obvious.

An additional factor enters with the presence of larger numbers of larvae at the lower ends of the frequency distributions for lambs 18, 27, 29, 36 and 25, the modal value being about 1·5mms This is not the only case of this nature.

In graph 32 concerning female populations the frequency distribution has two peaks and the mean for the shorter one of these is 1·51 mms.

This corresponding effect is completely lacking in the male populations; but in graph 31, which shows the distribution of the males from the same population just mentioned, a pronounced negative skew is present. This is interpreted as indicating that the males were likewise inhibited in growth at the same time as the females, but due to their slower growth rate no bimodal effect is shown.

If the hypothesis is adopted that some physiological change, such as an ecdysis, is associated with increased "mortality", i.e. undeveloped forms, then the question may well be asked:- why are so many parasites seemingly arrested in development at 1·5 mms length? Is it risky to reason from effect to cause, and say that this is an indication of some physiological change taking place when the females are about 1·5 mms long. With the small numbers concerned, such an inference is probably unwarranted.

The presence of undeveloped larvae of this length in several sheep is nevertheless an interesting coincidence; larvae are normally about this length on the fourth day of parasitism, and if data collected in this work is accepted as normal, this is one day after the majority have passed the third ecdysis. If Andrews' observation that the third ecdysis took place on the fourth day is more normal, then these larvae may represent forms which have had their development inhibited immediately after the third ecdysis. They have succeeded in passing the third ecdysis, and as such the position is not strictly the same as with fourth stage larvae which have failed to moult into the adult stage.

Apart from this doubtful case, there is no evidence that the third ecdysis is as difficult to pass as the fourth. The complete lack of larvae in the third parasitic stage from all populations older than the second and third day does not, however, necessarily mean that all have succeeded in becoming fourth stage larvae, because it is not known whether any undeveloped forms were eliminated.

It is important to realise that while "mortality" may reach different peaks of intensity, there is at the same time a continual loss of individuals at a low rate from any population. The odd individuals representing arrested development scattered along all lengths of the distribution may be a part of this regular normal loss.

GROWTH OF THE UNDEVELOPED FORMS.

The writer believes that the undeveloped forms were not growing when the host was killed, with the possible exception of some of those in populations twelve days old and less. No very convincing evidence can be advanced in support of this hypothesis. The following two points are worthy of consideration however:-

(1) If fourth stage larvae in association with a young adult population (graphs 56 and 57) are still developing, why is it that fourth stage larvae of about the same length are still present in populations from sheep killed over a week later? (Graphs 72, 73). It might be expected that, if these larvae are developing, they would have passed the final ecdysis by the time they had been twenty-two days in the host.

It seems more logical to believe that the larvae have been arrested at the ecdysis and have made no later progress. A sim-

ilar arguement may be applied to undeveloped adults in older populations, with a similar conclusion.

(2) In the absence of personal experience the writer has accepted the statement made by Schwartz, Alicata and Lucker (48) that "...agamic worms remain in the host and may ultimately come to fertile maturity, apparently a few at a time".

The writer believes that this may be interpreted as meaning that the agamic forms are not developing slowly and steadily, but that over a period of time some may recommence growing and eventually may reach maturity.

THE CAUSE OF INHIBITION OF GROWTH AND REPRODUCTION.

The title of this section opens into a field beyond the scope of the present study. Data already discussed has indicated that the growth of C. curticei in the lambs was not a direct unhindered process, but that in certain sheep some, or even all, of the parasites were definitely unable to reach full maturity.

Inhibition of growth and reproduction is well known in nematode parasitism. The work of Schwartz et al (48) has already been referred to. They claim that their work demonstrates experimentally the development of a growth inhibiting mechanism on the part of the host, which checks propagation and development. Chandler (10) records finding reduced egg production in a single long-standing infection of Nippostrongylus muris.

Taliafferro (56) has suggested that nematodes (and N. muris in particular) are subjedt to an infection of reproduction associated ultimately with a general impairment of the metabolic activities of the host.

This work directs attention to the fact that the environment in which the parasite finds itself is not always the optimum for full development. The actual degree to which it departs from the optimum is called by Tetley (63) "the pitch of the host-parasite relationship". According to him it may be vaguely indicated by, inter alia, the size to which the parasites have grown and the fecundity of the parasite.

The undeveloped parasites found with developed populations are evidence of some incompatibility. When odd aberrant individuals are found, the incompatibility is probably a function of the individual, which may be physiologically abnormal. Where

many are found the environment is more likely to be the source of the incompatibility. This incompatibility in the environment may be due to the development of some immune mechanism. The work of Schwartz, Chandler and Taliafferro cited above is more concerned with this type of reaction. It is most unlikely however that the experimental lambs used had been able to develop an immunity mechanism in even the oldest populations studied. Tetley (65, page 159) has data which he believes indicates that resistance to C. curticei is slow to develop, and would not be likely to occur in three weeks of parasitism. It is also known (53) that egg production does not reach a maximum value in H. contortus until about 14 or 15 weeks after infection, which would indicate the development of resistance about that time at the earliest. Sarles (47) has reported a simllar period for the development of immunity to Trichostrongylus calcaratus in rabbits.

It is believed that the undeveloped worms are evidence of individual inherent differences between sheep as regards their suitability for parasitism; this may be a type of natural resistance. Just what form these differences take it is not really possible to say. Possibly the pH of the intestinal secretions may be involved.

Veglia (68, page 425) found that H. contortus reached maturity in a shorter period in a hot season and a longer period in a cold season. If this observation can be accepted, it still does not affect the data obtained in this work. Reference to meteorological observations (40) for the time the sheep were infected with growing worms shows that no extremes of temperature were encountered. The lamb-shed would reduce temperature variation to an even smaller degree.

B. THE SIGNIFICANCE OF THE DEGREE OF SYMMETRY OF THE DISTRIBUTION CURVES.

SUMMARY.

A study is made of the degree of normality of the frequency distributions of length, and the possible relationship between evidence of natural resistance and the shape of the frequency distributions.

TYPES OF DISTRIBUTIONS.

The following forms of frequency distributions are evident

in graphs 1 - 75 illustratedin the Appendix.

I. Normal distributions.

II. Negatively skewed distributions.

III. Bimodal distributions.

IV. Positively skewed distributions.

V. A distribution showing pronounced negative kurtosis.

MEASUREMENTS OF SYMMETRY.

No tests were made to determine the degree of normality of the data. It was considered that, for the purposes of this discussion, departures from normality which were not immediately obvious to the eye were of no likely significance.

VALIDITY OF THE DATA.

The writer is aware that the shapes of distribution curves can be profoundly altered by the numbers of observations, and that in general the greater the number the more symmetrical the curve tends to become.

The distributions are from samples of approximately 1/20th to 1/40th of the populations; in some cases, they represent an even higher proportion of the total, and in one lamb (lamb 43) all available worms were measured. There are only four exceptions to this; the distributions for lambs 23, 28, 32 and 49 represent smaller fractions of the populations, possibly as low as 1/75th or 1/100th. In view of the relative sizes of sample and population, it is believed that the symmetry of the distributions is indicative of the relative order of normality of the whole populations.

ANALYSIS OF THE DISTRIBUTIONS.

(1) Normal distributions. Infective larvae used in this experiment exhibit a normal distribution of length. As populations mature, they show greater variation and the distributions in some cases lose their symmetry.

It is believed that a normal curve in an adult population is indicative of unhindered development at that stage. This is supported by the fact that normal adult distribution curves (e.g. lamb 6, graphs 70 and 71) have either none or else only isolated individuals with retarded development at the shorter lengths, and within the distribution curve itself, there are found very few or no females without ova.

The same inference about the normality of distribution curves of fourth stage larvae has less validity (see graph 49 of undeveloped larvae). It is possible that inhibition of development is less likely to alter the shape of the frequency distribution.

Lamb 49 (graph 49) has a population of fourth stage larvae with a frequency curve which does not conform to the hypothesis, being normal. The population of this lamb exhibited a greater degree of undevelopment than any other studied. The possibility does exist that the population did actually develop normally but was late in commencing development. It is more likely, however, that development commenced at the usual time and proceeded normally. Sudden cessation of development would then "freeze" the curve in its normal shape.

(2) Negatively skewed distributions. Frequency curves exhibiting a negative skew are common, especially in older age groups. Some however are found in young age groups, as shown in graphs 8, 18, 24, 29, 30 and 31. In the case of graph 8, the negative skew is due to the presence of both third stage parasitic larvae and fourth stage larvae.

With exceptions of this nature, it is probable that negative skew distributions indicate that the population encountered some form of incompatibility in the intestinal environment. As a result, some individuals would be delayed in their development to varying degrees, and the distribution assumes an asymmetrical form.

This hypothesis can be supported by the fact that distributions of this type are commonly associated (in the adult stage) with numbers of immature fourth stage larvae which have failed to reach maturity. In addition, many adult females are without ova (lamb 29, graphs 68, 69 and 81). Additional evidence is apparent in the population from lamb 43 (graph 31). The distribution, which has a pronounced negative skew, represents the entire male population (eighty-four worms).

Other lambs receiving a comparable number of infective larvae contained between two and five thousand Cooperia*, of which about

* Data supplied by J.H. Tetley.

half would be males. It is believed that the small population indicates an environment which was not entirely optimal for parasitism by C. curticei; and it is highly probable that the negative skew of the distribution is a further expression of this incompatibility.

(3) Bimodal Distributions. Two forms of bimodal distribution are to be found:-

(i) Those due to the presence of adults and fourth stage larvae in the one population, e.g. lamb 7 in graphs 43 and 44.

(ii) The second type is seen in graphs 73 and 32. It has been shown elsewhere that the two modes in graph 72 are a result of different degrees of development - see graph 82 for an analysis of this. No criterion of relative development was studied in the population represented by graph 32.

It is believed that bimodal curves of this second type are indicative of some degree of incompatibility in the environment, similar to and possibly stronger than that which has been suggested as giving rise to a negative skew. It should be noted that the corresponding male distributions (graphs 72 and 31) do not show evidence of bimodal curves. This point will be discussed later.

(4) Positively skewed distributions. Only a few examples of this type of asymmetry are known; see graphs 19, 27 and 28, drawn from populations of lamb 28 and (for the two latter graphs) lamb 23.

It has already been stated that the distributions of these lambs are representative of samples smaller than 1/40th part of the total population. In spite of this, it is highly probable that the whole population likewise exhibits positive skew of the same general order of magnitude.

It is believed that the positive skew is an indication of a fairly high degree of incompatibility in the environment, greater than that responsible for negative skew or bimodal distribution. It is suggested that the result of this incompatibility has been to allow a few individuals to grow normally while a majority experienced a high degree of delayed development.

It is relevant to mention that both these populations were administered in numbers far above any others with the exception of the comparable levels given to lambs 34 and 49. The possible

influence of size of infestation on development is discussed in a later section.

(5) Negative Kurtosis. A pronounced degree of negative kurtosis is exhibited by the female distributions from lamb 28 (graph 20). While the exact mechanism of the derivation of such a curve is not easy to suggest, it is worthy of note that the population administered to this lamb was of the same order of magnitude as that administered to lambs 23, 34 and 49. In all of these lambs, the populations exhibited various degrees of abnormal development, reflected in the lengths of the larvae and in the symmetry of the frequency distributions of length. In view of this, it is believed that the flat-topped distribution shown in graph 20 is indicative of some derangement in normal development; possibly this is associated with the level of population originally administered.

It is concluded that a frequency distribution with a negative skew is evidence of some degree of incompatibility in the environment of the parasite and of inhibition of growth and development. A similar conclusion can be drawn for bimodal distributions within one population. The validity of interpreting distributions with a positive skew, or with negative kurtosis, as indicative of resistance is questionable. If it be allowed that a normal distribution of length represents unhindered development, then any departure from normality may represent some degree of abnormal growth.

C. THE GROWTH OF THIRD STAGE LARVAE.

SUMMARY.

Differences between the mean lengths of several populations of infective larvae are analysed. The possibility of an increase in length of larvae while still in the third parasitic stage is studied.

(1) INFECTIVE LARVAE.

In the table below are given the mean lengths of the Cooperia-Ostertagia infective larvae. The data is derived from three sources: (i) the larvae used for infestation on 13/4/49; (ii) larvae from the rumen of the lamb infected on 13/4/49 and killed on 14/4/49; and (iii) Larvae from the faeces of several lambs, the numbers of which are given in the table on page 78.

MEAN LENGTHS OF INFECTIVE LARVAE FROM THREE SOURCES.

Source	Mean Length and Standard Deviation	Method of Measurement
Infecting dose	746 ± 34 μ 771 ± 30 μ	Microprojector Camera lucida
Rumen of lamb 46 killed one day from infestation	759 ± 41 μ	Ocular Micrometer
Taken alive from faeces of infected lambs	799 ± 44 μ	Ocular Micrometer

The means show great variation, some of which is due to the different techniques employed in measuring the larvae. The camera lucida was the most reliable instrument for the measurement of very small forms, and the mean value of 771 μ for the infective larvae can be accepted as being fairly accurate. Less reliance can be placed in the Microprojector for measuring larvae. The fact that the larvae in lamb 46 were shorter on the average than those taken from the infecting dose as measured by the camera lucida is assumed to be due to the ocular micrometer method of measurement, which was also unreliable. It is believed that the larvae in the rumen of lamb 46 were probably unchanged in length and that (allowing for sampling variations) a mean length nearer 770 μ would be more accurate than 759 μ.

It is considered that the difference between the means of the population from the rumen of lamb 46 and the larvae recovered from the faeces of the infected lambs is a real one, and not caused by any variation in the technique of measuring with the ocular micrometer, which was used for both populations. It is likely that the lengths obtained by the ocular micrometer are consistently lower than the real values; the mean of the larvae recovered from the faeces may be higher.

Taking into account errors in measurement, it appears that the infective larvae found alive in the faeces of the lambs were longer than the mean length of larvae in the population from which they were originally obtained. Two alternative conclusions may be drawn. Either the larvae have increased in length or else there has been a selection in favour of shorter larvae remaining in the intestine.

It is appropriate to mention here that Hung (quoted by Scott

(25)) found that there was an apparent increase in the length of infective larvae of Ancylostoma caninum and Nector americanus when recovered alive in the faeces of rats three days after skin infection. This is in agreement with the results described above.

Scott (51) investigated the problem and found "great variation" from case to case. He concluded that there was a decrease rather than an increase in length, and advanced the idea that longer larvae had been selected to stay in the gut. It was from this suggestion that the writer derived the opposite conception of selection of the shorter larvae to remain in the gut. This would explain the results presented above. No reason can be advanced as to why larvae of any particular length should be selected to stay in the gut. The hypothesis of selection on the basis of length is not valid without evidence.

Scott has attempted to prove his case by measuring the larvae remaining in the rats, from the faeces of which he recovered the short larvae; he found (51) that "an increase in size may have occurred", but he could not show that it was significant. Fulleborn (quoted by Scott (17)) working with Uncinaria stenocephala obtained similar results.

From this review, it is apparent that the situation is confused; and there is no definite proof for either shorter or longer larvae in the faeces than in the infecting dose, and no logical explanation has been advanced by these workers in support of their claims.

Though it cannot be accepted in the light of published evidence, neither as a result of this present work, that there was a selection in favour of one particular extreme length to inhabit the intestine rather than another, the alternative conclusion that the infective larvae increased in length is also difficult to accept.

The mean length of the larvae recovered from the faeces represents an increase of 5% over the mean value for the lengths of the infective larvae from the rumen of lamb 46, as measured by the ocular micrometer. Such a small increase may be the result of the fixing and preserving methods employed. The larvae recovered from the rumen of lamb 46 on the first day of parasitism were fixed in approximately 5% formalin and glycerine, and mounted in glycer-

ine for measuring. Larvae recovered from the faeces were killed by heating in a drop of water on a slide. Care was taken to keep the temperature low, and the slide was not allowed to become hot enough to sting the back of the hand.

It is believed that the increase in length is due to the heat treatment of these larvae.

It is most unlikely that infective larvae would have any ability to increase in length. Their function is to find a habitat in the host and their energy reserves must necessarily be limited because they are not able to feed; what food reserves they have are probably used in the process of finding a habitat. It is also doubtful whether the external cuticle would be capable of extension, since it is really "dead" in the sense that another cuticle lies beneath it, separating it from the true larva. Lapage (29) has shown that increased pressure, resulting from the permeability of the sheath when placed in mixtures of NaOH and HCl, ruptures the inner layer of the sheath first and then the outer layer, in a line just below the head. If the infective larva could grow, it would probably rupture at least the outer sheath, just as does the increased pressure.

(2) THIRD STAGE PARASITIC LARVAE.

The mean lengths for third stage parasitic larvae and young fourth stage larvae are set out below.

LENGTH OF THREE DAY LARVAE.

Sheep No.	Graph No.	Stage	Mean and Standard deviation
39	6 & 7	3rd parasitic stage	877 ± ·14 μ
		4th stage	996 ± ·09 μ
44	8	3rd parasitic stage	754 ± 161 μ
		4th stage	1050 μ

In an experiment on the differentiation and measurement of the infective larvae of C. curticei and Ostertagia spp. described later, p166 it was concluded that the mean length and standard deviation of C. curticei was 761 ± 26 μ.

It is reasonable to assume that the larvae recovered from lambs 39 and 44, coming originally from the same population of infective larvae as those measured, had initially a mean length of about 760 μ. It is also reasonable to assume that the loss of

the second stage sheath reduced the average length to 730μ approximately, which is in agreement with the length of third stage parasitic C. curticei larvae on the second day of parasitism in this experiment. (See graphs 4 and 5 (in the Appendix)). The mean lengths of the third stage infective larvae recorded above are much greater than the mean lengths which characterise larvae just entering into the third parasitic stage. This is especially obvious in lamb 39 where some third stage parasitic larvae are over one millimetre long (see graph 7 of the Appendix).

Apparently the third stage parasitic larvae measured have increased in length in varying degrees up to 300μ, or even more in some cases. Many must make very small increases in length, because both graphs 7 and 8 show some fourth stage larvae which are about the same length or a little longer than the mean minimum size of third stage parasitic larvae immediately after the second ecdysis.

Andrews' (2) account of the life history of C. curticei is vague on this point. Apparently he noticed some larvae which had grown a little, because he says that the larvae "appeared to increase very little in length". On the other hand, he records observing some which were shorter than the infective larvae. These were probably third stage parasitic larvae which had just passed the second ecdysis. Others which were evidently not shorter than the infective larvae, but the same length (or even larger?) may have been larvae which had increased in length slightly, after passing the second ecdysis. There is an alternative possibility. They may represent the longer infective larvae, which on losing their sheaths would be about the same length as the average.

It is not possible to decide from Andrews' data whether all third stage parasitic larvae increased in length before they changed into the fourth stage; nor is it possible to decide whether third stage parasitic larvae which have increased in length are not forms which have been unable to leave the cuticle at the third ecdysis, but rather have received the strong stimulus to grow just the same.

Work by Monnig (37) on Trichostrongylus spp. shows that the parasitic third stage larvae grew on the average from 683μ at 48 hours, to (at 72 hours) 865μ for males and 942μ for females.

Threlkeld (67) makes no mention of any increase in length of third stage parasitic larvae of O. circumcincta and Veglia (68) working with H. contortus states that "it appears that in the parasitic part of the third stage the larvae do not increase in length when compared with mature larvae of the free living stage".

It is concluded that there is a possibility that third stage parasitic larvae of C. curticei increased in length. The subject warrants further investigation, however.

D. THE ECDYSES OF C. CURTICEI.

SUMMARY.

This section is a discussion of the second, third and fourth ecdyses in relation to time, place, length of worm, constancy from host to host and of the effect of the final lethargus and ecdysis on the growth curve.

THE SECOND ECDYSIS.

All larvae recovered from the lamb killed after one day of parasitism had retained the second stage sheath (graph 3). In the two lambs killed two days after infestation all larvae recovered were in the third parasitic stage (graphs 4 and 5). Therefore the second ecdysis occurred between the 24th and 48th hours after the larvae were administered.

Since the larvae recovered from the rumen of the lamb killed on the first day of parasitism had not ex-sheathed, it seems probable that the second ecdysis took place in some part of the alimentary tract posterior to the rumen, i.e. either the abomasum or else the small intestine. It is possible that the acid in the abomasum has a similar effect on infective larvae of both C. curticei and Trichostrongylus species. The latter, according to Monnig (37), will readily ex-sheath in dilute hydrochloric acid, and he suggests that the acidity of the abomasum has a similar effect.

It is not known whether the second ecdysis of C. curticei will take place in a more anterior portion of the alimentary tract if the larvae are administered in solid food instead of in a liquid suspension. Veglia (68) found that H. contortus larvae passed the second ecdysis in the abomasum if administered in water, but when introduced in solid food they commenced to lose the sheath

within the mouth of the sheep.

Andrews (2) found, in agreement with the results recorded in this experiment, that C. curticei larvae were in the small intestine forty-eight hours after administration, and were without the second stage sheath, i.e. in the third parasitic stage. He made no observations for the first day of parasitism. In contrast to this, Monnig (37) found Trichostrongylus instabilis and T. rugatus larvae in the third parasitic stage in the intestines of lambs only twenty-four hours after administration.

Whether these differences in times taken by the larvae of Cooperia and Trichostrongylus spp. to reach the intestine are a function of the species themselves, or of the environment within the gut, is not possible to decide with the data available.

THE THIRD ECDYSIS.

All larvae found in the intestines on the second day of parasitism were in the third parasitic stage. In populations one day older only a few were still in this stage. The remainder, (comprising 87% of the population examined from lamb 44, and 78% of the population examined from lamb 39) were in the fourth stage (see graphs 6, 7 and 8). It is concluded that the third ecdysis for the majority of these larvae took place between the 48th and 72nd hours from infestation.

This is contrary to Andrews (2) who found that the larvae of C. curticei did not pass the third ecdysis until the fourth day from infestation, one day later than recorded in this experiment.

Possibly the time at which the third ecdysis takes place will vary with different hosts, feeds or even strains of worms (assuming such latter exist; there is no evidence of them in C. curticei). It should be noted however that there is close agreement between the two lambs 44 and 39 as to the time of this ecdysis. This is suggestive of a major difference between these sheep and those used by Andrews. Previously it has been noted that a few of the sheep he used had already acquired infestations of C. curticei in the field. It is possible that some of them were the hosts used in the early stages of Andrews' experiment. If it is assumed that they had some degree of acquired immunity, they may have delayed the ecdysis. This is one way to account for the differences in results, but it is purely conjectural.

It is interesting to compare C. curticei with other related species in respect of the third ecdysis. Threlkeld (67) working with Ostertagia circumcincta is confusing; his work may be interpreted to mean that the ex-sheathed larvae found in the walls of the abomasum at 90 hours were in the fourth stage. Veglia (68) was able to distinguish between third stage parasitic larvae of H. contortus when in the active period and in the lethargus. He believes that the latter commences about 30 to 36 hours after the larvae enter the host, and that it lasts 12 hours, after which the skin is shed.

Andrews' observations on C. curticei are more in line with Monnig's on Trichostrongylus, which is not unexpected when it is considered that the worms both live in the same habitat and are closely related genera. The observations recorded in the present experiment would make C. curticei more like H. contortus in the time of the third ecdysis; on a priori grounds this seems less reasonable.

THE FOURTH ECDYSIS.

The fourth ecdysis is the final change or moult through which the larval form must pass to reach maturity. Whether it is any more perilous from the point of view of the nematode than the other ecdyses is not known, though in data discussed earlier it was shown that there were many larvae which were unable to pass the fourth ecdysis. In contradistinction to this, few or no forms appeared to be held up by the third ecdysis. This does not necessarily indicate that it is less difficult for the larvae to pass this phase, because it is not known whether any would be eliminated if they failed to develop. It may be that the change from a free living to a parasitic form, even though it is accomplished gradually with two moults (the delayed second, and the third moult), is more perilous than the fourth ecdysis.

The fourth ecdysis (and possibly the third) may be likened to a dam across a river. The larvae build up behind this "dam" and growth ceases, corresponding to the formation of a pool of water. When the ecdysis takes place there is a sudden and vigorous spurt of growth, as if the dam had burst and water was released.

This sudden spurt of growth can be seen in any graphs of populations killed on the 8th and 9th days of parasitism (e.g. graphs

35, 36, 37, 38). The fourth stage larvae have a mean of about 2·3 mms for males and 2·5 mms for females. The average length for adults, even if very young, is greater than the mean for the fourth stage larvae in any particular population. This explains the bimodal frequency distributions of some populations in hosts killed at about 8 or 9 days (graphs 33 - 48). These are really separate frequency distributions partly or entirely superimposed. In the latter (graph 33), the young adults have not increased in length enough to show the bimodal effect, and in outline the two curves appear as one normal curve.

THE CONSTANCY OF THE THIRD AND FOURTH ECDYSES.

Scott (49) was not able to measure the early growth of Ancylostoma caninum in its host largely because the time of passing through the different moults varied so much that "even average figures were unreliable". In C. curticei no such difficulty was experienced because both third and fourth moults were fairly regular. Graphs 6, 7 and 8, prepared from populations three days old show approximately equivalent numbers of third stage forms in each host.

The fourth ecdysis was taking place on the 8th and 9th days of parasitism in all cases except one - lamb 49 (graph 49). This animal proved to contain an exceptional population which is discussed later (page 138). From the other sheep, only one adult was recorded on the seventh day of parasitism - a male from lamb 10 (graph 25). By the ninth day the great majority of worms examined had passed the ecdysis. With the exception of lamb 49, it is believed that the sheep contained larvae which were passing through the final ecdysis in approximately the normal time. It is possible that some hosts may have had some adults by the seventh day of parasitism, as with lamb 10, but it is not likely that the number would be high. But, while development is not likely to be much faster than evidenced here, it could easily be slower, as the population of lamb 49 shows. Whether C. curticei in lambs in the field develop and pass the final ecdysis within the lengths and times found here is not known, but it is highly probable that they do. Where a sheep has been able to develop some degree of resistance however, it is likely that development would be slower.

LENGTH AT THE FOURTH ECDYSIS.

The average fourth stage male larva at eight to nine days is

2·3 mms long, and the average female 2·5 mms. More extreme lengths are common. The longest were found in lamb 34 (graphs 37 and 38) where males were 3·60 - 3·79 mms and females 4·60 - 4·79 mms, both in the fourth stage. The shortest adults found were males at 1·40 - 1·59 mms in lamb 25 (graph 47). The shortest adult females came from lamb 42 (graph 40). They were 1·60 - 1·79 mms long.

The ecdysis is likely to take place between 1·50 mms and 3·70 mms in males with the mean at about 2·3 mms, and in females between 1·70 mms and 4·70 mms with the mean at about 2·5 mms. Indirectly, length, in so far as it is correlated with development, would be the deciding factor in determining the time of the ecdysis. A certain stage of development must be reached before the worm can moult, and there would be a lower limiting size below which the worm would be insufficiently developed to do this. It is probable that there is an upper limiting size beyond which the worm cannot grow without moulting. These extremes doubtless approximate the limits set out above.

Normally, the worm is developed sufficiently to moult at about eight days. Time in itself does not determine the moult; as this thesis has shown, there are undeveloped forms which may persist in the fourth stage for up to three weeks at least.

An attempt was made to relate Prizbam's rule (quoted by Wigglesworth (70)) to the moulting. This empirical law (which incidentally is by no means universal) states that the weight of insects is doubled during each moult, and at each moult the linear dimensions are increased by 1·26. The rule is based upon the hypothesis that every cell in the body divides once and grows to its original size and moults. This was found to have no relationship to the length of C. curticei at the different moults. This is to be expected because quite apart from the empirical nature of this rule, C. curticei increases in length between moults, and not at each moult.

EFFECT OF THE LETHARGUS AND ECDYSIS ON THE GROWTH CURVE.

The effect of the fourth lethargus on the growth curve is to be seen in the discontinuity or "step" effect about the 6 - 7 day period. (See figure 10, page 143)

Scott (49) did not construct a growth curve for the dog hookworm for forms younger than the fourth ecdysis. He suggests that growth prior to this moult takes place at the same rate as after it (a point to be elaborated later) and he also suggests that the discontinuity at moulting is probably slight. The data examined here does not bear this out for C. curticei, though it may be true for Ancylostoma caninum. The fourth lethargus extends over nearly two of the twelve days which C. curticei takes to reach maturity, or nearly one sixth of its growth period, and the growth is profoundly affected.

Such discontinuities are the rule rather than the exception in growth curves of all but the more simple organisms (66, page 159). An annual plant like the lupin will show the simple growth cycle. Expressed as a summation curve it will show a characteristic sigmoid form. Expressed as its differential it will show a simple rise from a minimal to a maximal value, and back to a minimal like a normal distribution curve.

In most organisms however it is only a section of the whole growth cycle which shows this simple form. The integral curve of growth rises in a series of steps and stairs, and is really a series of sigmoid curves end to end. The differential curve shows a series of peaks instead of one simple peak, indicating several periods of maximum growth velocity, interspaced with periods of minimal velocity.

Some workers go further than others in describing growth curves in terms of a series of short discontinuous curves. Brody (7) for example has divided the simple S-shaped sigmoid curve into two phases, which he has designated the self-accelerating phase and the self-inhibiting phase. The former is that portion of the differential curve which shows increasing velocity, the latter the portion showing declining activity. In the summation curve of growth the division between these two curves is marked by the point of inflection, which coincides with puberty in man, or flowering in plants. The self-accelerating phase Brody further subdivides into a series of cycles. This is shown in the differential growth curve of the rat from shortly after conception, where four cycles appear.

Within each of these segments or cycles of the curve, the

percentage growth rate is constant and less than the preceding segment, and each segment can be represented by a comparatively simple formula.

It should be mentioned that Brody's division of the sigmoid curve into a number of independent exponential phases are by no means universally accepted. Weymouth et al (69) has criticised the emphasis Brody places on the point of inflection in the sigmoid curve and Gray (22) points out that any curve can be expressed as a series of straight lines or exponential curves within suitable limits. Unless there is good evidence for the division of a growth curve into a "finite number of successive and different processes, the process sub-division of the growth curve is merely arbitrary".

In the growth curve of C. curticei, there is "good experimental evidence" for the sub-division of the growth curve; in a later section, an attempt will be made to express these segments of the curve in terms of growth equations.

Both the summation curve and its differential, the velocity curve, are shown in figs. 10 and 11 on page 143 and 144

The velocity curve, fig. 11, especially shows the effect of the fourth ecdysis in reducing the growth rate to a zero value at 7 days. The drop at two days is due to the second ecdysis, and the length of the larva from that point has been taken as the base. This loss of sheath is not "growth" in the real sense, and the declining line from 1 to 2 days is not really analogous to the decline from 6 to 7 days.

E. EFFECT OF SIZE OF INFESTATION ON GROWTH OF THE PARASITES.

SUMMARY.

The relationship between size of infestation and its effect on the growth rate is discussed, and a hypothetical "latent reaction" is suggested to explain the observed results.

One of the objects of this experiment was to investigate the possibility of the size of infestation affecting development. Statistical analysis (see page 70) did not reveal any significant differences in development between populations originating from infestations of 10,000, 20,000 and 22,000 infective larvae. Statistically, the populations originating from infestation of

nearly 400,000 larvae were not significantly different from those from the smallest infestations.

Four lambs, nos. 23, 28, 34 and 49, received nearly 400,000 larvae each and were killed at 7, 6, 8 and 9 days respectively. When the parasitic populations were examined, it was found that three of them exhibited some differences in development when compared with smaller populations of the same ages from other sheep.

The population from lamb 28 (graphs 19 and 20), killed at 6 days, is not really significantly different from the others of the same time.

Populations of lambs 23 (graphs 27 and 28) and 49 (graph 49), killed at 7 and 9 days respectively, are both shorter than the other populations for the same days, while the worms from lamb 34 (graph 37 and 38) are far longer than any others at 8 days.

In some cases, the frequency distributions of length were different from the usual form. That from lamb 49 (graph 49) is normal, and the distributions for lamb 34 (graphs 37 and 38) are likewise normal. These two populations are the most abnormal of the four in terms of length in spite of the normality of their distributions. The frequency curves for lamb 23 (graphs 27 and 28) have a slight positive skew. This is very unusual, only one or two other populations out of all those measured showing this effect. One of these is graph 19, the distribution of the males from lamb 28; the female curve is not skewed but gives evidence of a strong negative kurtosis (graph 20).

Thus none of these populations were really "normal". Even where the means corresponded closely to the mean for "normal" populations, as with lamb 28, the distributions presented features not found elsewhere.

Unfortunately the experiment was very poorly designed in respect of the subject of this section, and in the statistical analysis it was found that there were no significant differences between the mean lengths of the populations receiving 400,000 larvae and those receiving numbers of the order of 10,000 and 20,000. This is largely due to the fact that the variability within the 400,000 larvae group was high and also the numbers of sheep killed were too small - one only - for each of the "dose-level - day" cells. Consideration of all relevant data leads t

the conclusion that the effect of the large dose level is a real one, but that the experiment is not sufficiently refined to detect it.

The observed effect of a large dose of larvae on the development of its members supports Mayhew's (32) suggestion that large numbers of developing larvae seem to interfere with the rate of development. It is in contradiction to Scott's (49) finding that different sized infestations of A. caninum made no difference to the lengths of the worms in the host. He used doses of all sizes up to one thousand; it may be that more than this would be required to produce an effect.

It is not easy to give a satisfactory explanation of these variations in growth. Taylor (58) discussing the acquisition of heavy and rapidly acquired infestations of some of the Trichostrongylidae suggested that their development was inhibited because some substance stimulating the growth of the larvae is used up quickly.

This theory may be a possible explanation for the undeveloped populations of lambs 23 and 49. Lamb 28 has a normal population so far as length is concerned, which may be explained if it is suggested that extra large amounts of the growth stimulating substance were available. Taylor's theory does not explain the population of lamb 34. In effect this is a "giant" population. Not only are the adults longer for their age than any others, but some of the fourth stage larvae have reached amazing lengths; a few fourth stage males are nearly double the length at which most normal larvae molt. In searching for an explanation we must take account of this last population.

One possible theory is as follows:-

Assume that lamb 34 is a lamb which is very susceptible to parasitism, i.e. its intestinal environment is very suitable for parasites.

Suppose lamb 28, while it is not unduly susceptible, is not very resistant, i.e. it has an intestinal environment which may be termed as "average".

Assume lambs 23 and 49 have a natural inherent resistance, the latter more than the former.

If we place an equal number of infective larvae into these

it may be that their potential degree of infestation is emphasised. That is, susceptible lambs weaken in the face of overwhelming numbers and allow the parasites to develop to an above-average size. Potentially resistant lambs act in the opposite way; instead of being overwhelmed they have their powers of resistance enhanced by the stimulus of large numbers. It is as though the potential level of the natural susceptibility or resistance is already decided by the responsible mechanism in the lamb, but it is latent; a big population can make it react in its true manner, while a small one may not evoke any difference between the hosts. A lamb with a hypothetical "average" susceptibility like no.28 would produce worms of a"normal" length.

The problem of size of infestation and development of the resultant population is an important one and it needs further specially planned investigation.

F. THE INFLUENCE OF SEX ON GROWTH.

SUMMARY.

The consequences of the faster growth rate of the females over the males on the shape of the frequency distributions are discussed. An analysis is made of the commencement of the sexual differentiation of length.

The data shows that females have a greater length than males of the same age over the latter portion of their growth. Since they commenced growth from the same length it follows that the females must grow faster than the males. Measurements of this growth rate have been attempted using the velocity constants of a logistic equation. For reasons explained in a later section, page , such a measurement cannot be regarded as strictly correct. No other of the many possible ways of measuring growth rates has been attempted.

Consequences of the differential growth rate.

As the populations mature, their "spread" or "scatter" increases. Comparison of frequency distributions of young and old populations reveals this. This spreading effect is greater in females than in males, because of the faster growth rate. It follows from this that distributions which may be hidden in the male frequency curves are revealed in female curves. This is

well shown in a comparison between graphs 72 and 73 and between 31 and 32. It has been emphasized elsewhere that, because the females will grow more than males in any given time, the increas in length over any undeveloped portion of the population will be greater for females than for males. Therefore a "trough" will appear in a female distribution which will be bimodal, but the corresponding males may show a skew distribution only (see page

The Differentiation of sex in terms of length.

Males and females could be distinguished at the fourth da merely on the shape of the tail. After the fourth day, the ma are shorter than the females, but the statistical analysis show this is barely significant at 6 days of parasitism and definite significant at 8 days. See the Combined Analysis (page 76).

Andrews (2) has observed that, on about the fourth day of parasitism, i.e. immediately after the third ecdysis, the genita primordium of the females was nearer to the posterior end of the larva than in the male. Since in this experiment the third ecdysis took place on the third day, Andrews' findings should apply to three-day old worms.

An attempt was made to measure the distance of the genital primordium from the posterior end for each three day old larva at the same time as the total length was measured. It was hoped to be able to draw a frequency distribution of these distances (from the genital primordium to the posterior end); this would probably be bimodal. It would then be possible to relate this distribution for each worm back to the length distribution, which would be a normal curve, and resolve it into potentially male and female distributions.

Attention to more important sections prevented this from being carried out, beyond a few preliminary measurements.

G. THE GROWTH CURVE.

SUMMARY.

A freehand curve of growth has been drawn through points representing the mean lengths of populations of known ages. The validity of this curve as a representation of the individual growth curve is examined. The points have been fitted to a curve using a logistic equation, and certain growth constants

derived. The empirical nature of this procedure is discussed, and the application of a logistic equation to the growth curve of C. curticei is compared with similar work by Scott on Ancylostoma caninum.

FREEHAND GROWTH CURVE.

As a final study the means for the different age populations were plotted and a curve drawn through these points to give an outline of the course of growth of C. curticei. (See fig. 10). This curve is not mathematically fitted but a freehand curve drawn through the greatest possible number of points. It illustrates more exactly than any previous analysis the growth of the worm.

The length of the worm does not change till the second day of parasitism, when it decreases due to the second ecdysis. From the third to sixth days the curves rise steeply and the male and female curves diverge. At any given age after about four days, the females have a greater length than the males and must therefore grow faster. The effects, if any, of the third ecdysis on the shape of the curve are not shown because the time intervals were too large to bring it out. Had shorter intervals been used it is possible that the curve would show a "step" effect (similar to that for 6, 7 and 8 days) due to the third lethargus. It must be remembered however that the evidence for growth in the third parasitic stage put forward in this thesis is scanty. It is quite definite that whatever growth there is, is very small - say from 730 to 800 or 900 on the average. In order to produce a decided break in the curve like the fourth ecdysis, or like the second ecdysis as shown by McCoy (fig. 3, page 6), there must be steady growth prior to the lethargus. The third stage parasitic larvae do not show evidence of steady growth, and if there is any break in the growth curve at the third ecdysis it must be small and would be very hard to show, even with smaller time intervals.

At 6, 7 and 8 days the curve flattens out; as already mentioned this represents the final lethargus. From 9 days onwards, the curve rises steeply, this rise being associated with the onset of the growth impulse immediately after the fourth ecdysis. The curve flattens out at about 6 mms for females and just below 5 mms for males.

At the end of the growth curve the means of 20 and 22 day

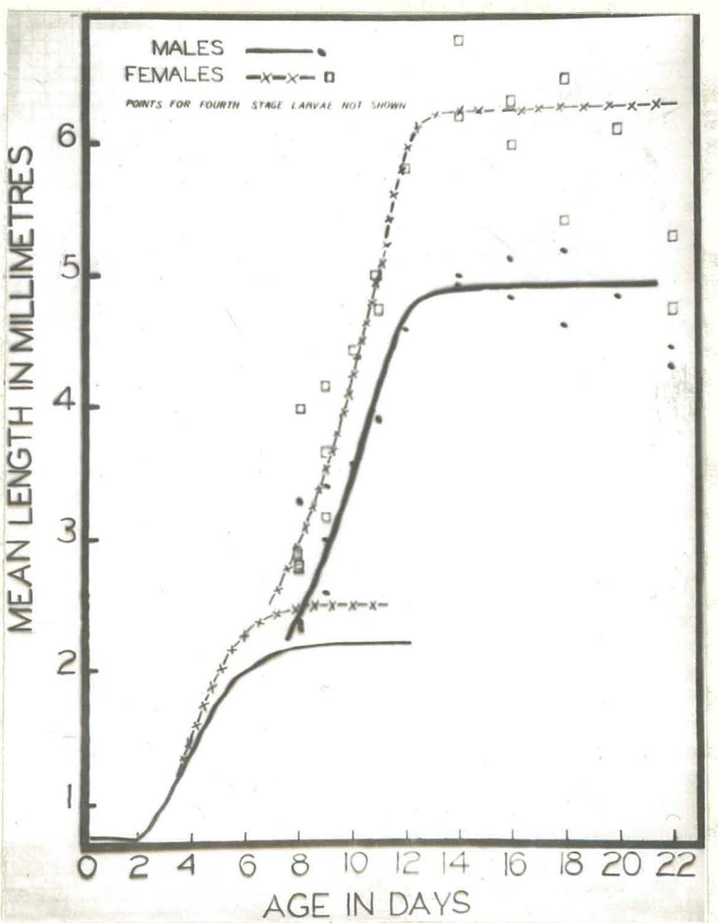


Fig. 10. Summation curve of growth.

populations are lower than the means for the other adult populations. This of course must not be interpreted to indicate that the curve should drop. By chance the average lengths in the oldest populations recovered were shorter than the majority of the adults for other populations. Statistical analysis has already shown that there are no significant differences between the mean values for any of the populations after twelve days.

As an extension of this cumulative or summation curve, its differential or velocity curve was drawn by plotting the increments of length for successive ages. This curve is shown in fig. 11.

Two complete cycles are involved for both males and females. From 0 days to 2 days, the curve drops in concession to the lowered length consequent on the loss of the larval tail. From a zero velocity at 2 days the curve rises to a maximum for 4 days to 6 days, and drops again to zero, or nearly so, at 7 days. This represents the first phase of growth up to the fourth ecdysis. As already explained it is not likely that this curve is resolvable into two curves by the third ecdysis.

The second curve rises steeply to a series of peak values and falls again to zero after about twelve days.

The irregular nature of the velocity curves is due both to the variability of the material and to the smallness of the number of observations. In theory such curves should be smooth, with growth velocity rising from minimal to maximal values and pulling back to minimal values again. The high peak reached by a population at twelve days does not imply that the greatest velocity of growth is reached at this time. Indeed fastest growth in the adult stage is reached at about 8 days, immediately after the fourth ecdysis, and the velocity of growth is declining at 12 days.

THE SIGNIFICANCE OF THE FREEHAND CURVES OF GROWTH.

In considering the curves presented in figs. 10 and 11 the question arises as to what these curves really represent. Do they faithfully portray the course of growth which an individual worm would follow, or are they little more than a line drawn through a series of average points.

It must be remembered that individuals do not grow exactly alike and that individual curves (both integral, fig. 10, and differential, fig. 11) will differ from organism to organism and will

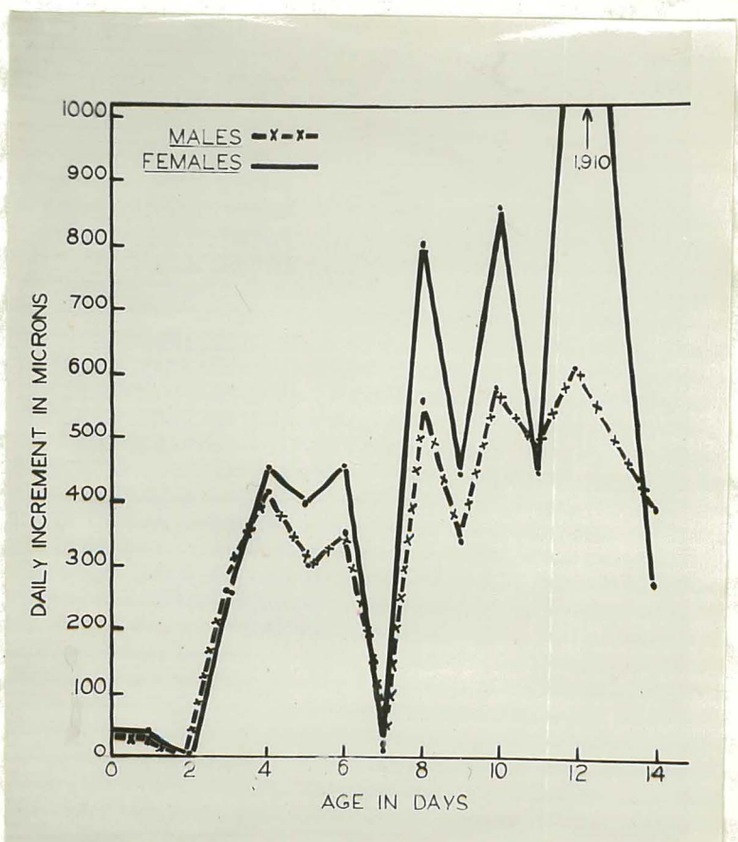


Fig. 11. Velocity curve of growth.

also be different from a curve compounded from mean values of large populations of known ages.

The interpretation of average curves was the subject of much discussion two decades ago when some workers, following the footsteps of P.W. Robertson (43), interpreted cycles or fluctuations in average growth curves as being representative of epochs in the life of the individual. Attempts were then made to associate these with physiological changes in the individual. These interpretations became the subject of severe criticism by other writers of whom M. Merrell (36) and C.B. Davenport (13) are the best known.

Merrell analysed mathematically a series of growth curves of the rabbit. She found that an average curve was not a reflection of an individual curve, but that, especially where the material exhibited great variability, certain differences were introduced. These are the result of the averaging process and may take the form of cycles and of varying degrees of skew.

She showed the average of a group of logistics to contain more waves than a simple logistic, and that these variations could be entirely lacking in the individual growth curve. In biological material of what Merrell calls "ordinary variability", such fluctuations do not show up to a significant extent. The skew effect is however more sensative, and in material with a comparatively small amount of variability it can produce significant differences in the degree of symmetry of the average curve when compared with individual curves. A rather unexpected effect is introduced if the individual curves are themselves skew. Merrell found here that the average curve was more symmetrical than the individual. Normal individual curves however produce skewed average curves.

From this work of Merrell's it follows that curves of the types in figs. 10 and 11 are not a true picture of individual growth. Individuals' curves can be expected to vary considerably from these. However, the variability of the material has obviously not been such as to cause these growth curves to give an entirely erroneous picture, because they do conform to our knowledge of the growth of C. curticei, at least in outline.

THE GROWTH EQUATIONS.

In the review of literature it has already been mentioned that Scott (49) fitted logistic curves to the mean lengths of

different aged adult populations of Ancylostoma caninum. McCoy (34) extended this to the free-living larvae of the same species.

The equation used by both workers was of a logistic type:-

$$L = \frac{b}{1 + e^{c - dT}}$$

where L = Length in mms.

T = time in days from infestation

b = final size of the worm

and c and d are constants, d being used by Scott and McCoy as a measure of the intrinsic growth rate.

In fitting curves of this type to the data on the fourth stage larvae, account had to be taken of the fact that they did not start growth from a zero value. The formula was slightly modified by the insertion of another value on the left hand side to allow for this. The final curves fitted were as follows:-

Males.

1 - 8 days $L - .650 = \frac{1.513}{1 + e^{4.9125 - 1.2048T}}$

8 - 16 days $L = \frac{5.200}{1 + e^{3.8158 - .4654T}}$

Females.

1 - 8 days $L - .650 = \frac{1.850}{1 + e^{5.2654 - 1.2056T}}$

8 - 16 days $L = \frac{6.600}{1 + e^{3.2928 - .4294T}}$

The value 0.650 has been selected by a process of trial to be the best allowance which can be made for the fact that the larvae commenced growth at a finite length. The values for "b", the final size, are "educated guesses" only. The constants c and d were selected by trial and arror as giving a curve which fitted the given points more closely than any other values.

Using the above formulae, curves have been plotted and are shown in fig. 12. The data from which these curves were derived is reproduced in Appendix XIII.

Certain information can be obtained from these curves, though it must be accepted with reserve. It is all based upon the calculated theoretical curves, and these err in two major ways.

(1) They are very empirical. Not only could other values

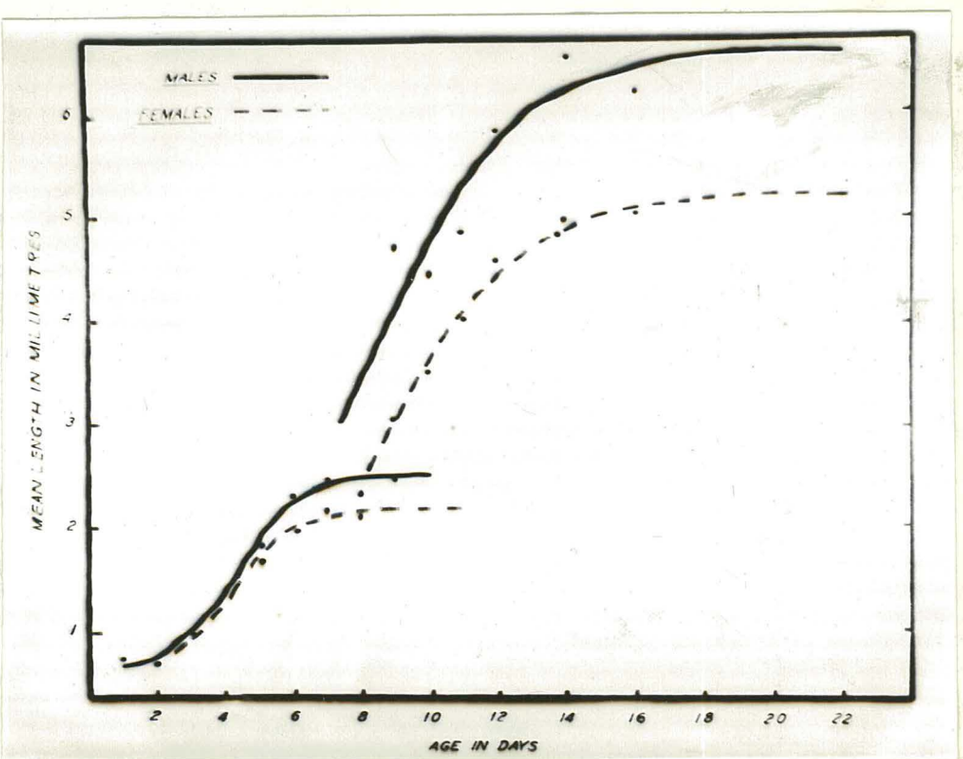


Fig. 12. The growth curves calculated from the logistic equations.

for the equation give nearly as good a fit to the points (if not just as good) but probably other types of equations such as the Gompertz or algebraic forms* would fit the points also. These would all give different values for the constants. The values for "b", the maximum size, are an estimate only, as is the value 0·650 to allow for the initial length of the fourth stage larvae.

(2) It has already been emphasised that curves of growth drawn from averages are likely to give erroneous results. Consequently, any information derived from them cannot be applied to individual cases, but is merely an approximate to some true parameter.

The following relevant information can be derived from these growth equations:-

(1) Maximum length — For adults, "b"
For fourth stage larvae (a + b)

(2) Length at which maximum rate of growth occurs
For adults, $\frac{1}{2}b$
For fourth stage larvae $\frac{b + 2a}{2}$

(3) Time at which maximum rate of growth occurs
For all forms $\frac{c}{d}$

(4) Maximum rate of growth
For all forms $\frac{1}{4}bd$.

These values are set out on the next page.

---

* Gompertz equation $y = ke^{-e^{-b-cx}}$

Algebraic " $y = k\left(\frac{t}{T}\right)^3\left(2 - \frac{t}{T}\right)^3$

See Wilson (71).

A. FOURTH STAGE LARVAE.

		Males	Females
(1)	Maximum lengths	2.163mms	2.50 mms
(2)	Length for maximum growth rate	1.407 mms	1.575 mms
(3)	Time of maximum growth rate	4.08 days	4.29 days
(4)	Maximum growth rates	0.456 mms/day	0.558 mms/day.

B. ADULTS.

		Males	Females
(1)	Maximum lengths	5.2 mms	6.6 mms
(2)	Length for maximum growth rate	2.6 mms	3.3 mms
(3)	Time of maximum growth rate	8.2 days	7.67 days
(4)	Maximum growth rates	0.605 mms/day	0.709 mms/day.

Maximum lengths, as already explained, are purely estimates and as such will be only approximately correct.

It will be noted that the time given for the maximum growth rate is about the time of the ecdysis; this applies to both adult and fourth stage forms. This is to be expected. The frequency distributions for populations eight and nine days old show that both male and female adults are longer on the average than the fourth stage larvae, and in some cases the difference is quite considerable (graphs 36, 37, 38 and others of the same time). The comparative absence of adults (in older populations) just slightly longer than the length at which the ecdysis takes place may also be significant. Possibly the strong growth impulse after the ecdysis does not allow many young adults to fall by and remain undeveloped.

If this is true it is to be expected that likewise there would be very few if any larvae at lengths corresponding to the lengths of young fourth stage larvae, i.e. immediately after the third ecdysis. Evidence has already been presented (see figs 8 and 9, pages 85 and 86) which indicates that in some cases there is an accumulation of larvae at this length, i.e. 1.5 mms or thereabouts. In the absence of larger numbers, it is hard to say whether the presence of these larvae is significant.

In the data above, maximum rates of growth for females occur earlier than for males so far as adults are concerned. This would seem to imply that the females pass through the fourth ecdysis before the males, but counts of relative numbers of male and female adults in populations passing the ecdysis have not shown any real

differences to exist in this connection (see fig 6a, page 67). It is possible that the figures given for the time of maximum growth rate are not significantly different. For fourth stage larvae, the maximum growth rate according to the formula is reached first by the male. Since sexes can only just be identified by the tail shape and length at this time, it seems unlikely that there would be any real difference between these values.

GROWTH RATES.

Males and females commence growth at the same length, but by the time they have reached maturity, there are big differences between them in length. It follows that females must have grown faster than males, and this is in agreement with the growth rates for males and females calculated from the growth equation.

In view of the known errors inherent in this mathematical analysis, it may be argued that the differences between the sexes in respect of growth rate only coincide with what actually happens by chance. Perhaps a slightly different set of values for the constants b and d would have altered the relationship of the figures purporting to give the rate of growth of the sexes.

To arrive at the true growth rate would probably mean an analysis along the lines of Brody's true instantaneous growth rates (7).

In spite of their disadvantages, it is within all probability reasonable to accept the fact that the maximum growth rate ($\frac{1}{4}bd$) is faster in millimetres per day in adults than in fourth stage larvae.

THE VALIDITY OF THE LOGISTIC CURVE AS A DESCRIPTION OF GROWTH.

The logistic growth curve of Robertson's type is not satisfactory as a picture of growth and has been criticised amongst others by Brody (7).

He believes that Robertson's (43) fundamental idea of the logistic equation as a description of growth is incorrect. Growth is not necessarily an autocatalytic process as Robertson inferred. The equation he advanced represents a true logistic curve, i.e. a symmetrical curve, and Brody attacks this belief. Growth curves according to him are not usually symmetrical because the inflection does not take place in the centre of the curve.

The growth curve of *O. curticai* has presented just this very difficulty. It is not truly symmetrical because the fastest growth rate, i.e. the steepest slope, is not in the centre of the curve. As a result it has not been possible to make an exact fit of a logistic curve to the data. Fig. 12 shows this clearly; it is the best fitting logistic curve and yet the curves do not reach their upper asymptote till about 18 or 20 days for the females and 16 to 18 days for the males. This implies that growth is still taking place at this time, whereas the velocity curve of growth, crude though it is, pictures correctly the cessation of growth at about 12 days.

To overcome difficulties of this type, Brody found that he had to subdivide the curve of growth into a number of component parts and fit different equations to each. The single growth curve is too complicated for representation by a single simple mathematical expression.

It is only fair to note that Brody has been criticised (22), it being pointed out that his subdivisions of the sigmoid curve into a number of distinct exponential curves or straight lines is purely arbitrary in many cases, and can be done with any curve of this type.

Whatever form of growth equation various workers support, many of them believe that in our present stage of knowledge we are not on safe ground if we venture beyond using growth equations as an empirical classification. Gray (22) believes that in such an empirical formula only two symbols have any real meaning, those which denote time and size. All others are meaningless, and can have values assigned to them which give the best representation of the data available.

To conclude this section, it is obvious that little use can be made of the facts concerning rate of growth derived from the logistic formula. Not only are the equations largely empirical, and the average curves distorted, as already described, but also, in making use of symbols (velocity constants) other than those denoting realities which we can measure directly, i.e. the *Metrical* constants (to use Medawar's (35) terminology) we are on dangerous ground. These *velocity* constants (i.e. c + d) are as already emphasised too readily manipulated in an arbitrary

fashion to give the best fit to the curve. They have, as used here, no real significance.

SCOTT'S USE OF THE LOGISTIC CURVE.

It is not proposed to attempt comparisons between values of the "d" constant of the logistic used in this thesis with its equivalent as used by Scott (49). In view of the numerous errors involved in their application such a comparison is scarcely valid. It is interesting to note however that Scott makes no mention of having any difficulty in fitting his logistic curves to the points on the graph, in contra-distinction to what was found here. He did not find any differences between the intrinsic growth rates of male and female hookworms, as measured by the "d" constant (referred to by him as the "b" constant). This is in accordance with the implications of the equations made here.

He did however find differences in the intrinsic growth rates of hookworms from the dog when they managed to grow in the cat, so that the formula used was able to pick growth rates of that order at least.

H. AGE ESTIMATION IN C.CURTICEI.

SUMMARY.

The use of the data obtained in this experiment to determine age in a population of C. curticei is studied and the difficulties are noted. An analysis is made of the possible ways in which field acquired infestations would be expected to differ from the experimental data, and a number of suggestions are advanced for the practical use of the method in the age determination studies.

INTERPRETATION OF THE DATA IN TERMS OF AGE.

Data obtained in this experiment is to be applied to the determination of the age of infestations of C. curticei acquired by a worm-free lamb when it is placed on contaminated pasture. Two questions arise. First, supposing all worm populations grew in much the same manner as those examined in this experiment, with what degree of reliability could the data on lengths be interpreted in terms of age? Secondly, what variations in the growth of C. curticei are to be expected, and to what extent will they negate the application of the knowledge already gained?

To answer the first question, it will be presumed that a

series of populations are available corresponding to those found in the experiment. These will be examined to find out the difficulties presented by the data on an estimation.

(To aid in this discussion, the means of populations have been marked in figs. 13 (males) and 14 (females) and numbered according to the sheep from which the population was recovered. Graph numbers refer as usual to the Appendix.)

"Infective Larvae in the Rumen" Graph 3.

ONE DAY

In so far as this data is concerned, the combination of rumen and infective larvae can probably be regarded as indicating parasitism of up to twenty-four hours duration. At no other stage were infective larvae found in the lambs, including, as well as the small intestines of all lambs, the reticulum of the lamb killed on the first day and reticula of the two lambs killed on the second day. In view of the rate of passage of larvae through the rumen (see page) it is not likely that any significant numbers would be present after the first twenty-four hours. However, no observations were made on the rumens of lambs killed on the second day, to confirm this statement.

(The fact that no infective larvae were recovered from the small intestines of any of the lambs is interesting because live ones were found in the faeces many days after infestation (see page 78). This would seem to indicate that they were overlooked in searching the ingesta of the small intestine. This is surprising because the examination of the ingesta was most thorough).

"Third stage parasitic larvae, with a mean length of 730 , located in the small intestine and without fourth stage larvae present".

TWO DAYS Graphs 4 and 5.

"Immature fourth stage larvae with a mean length close to 1 mm, associated with smaller numbers of third stage parasitic larvae showing some signs of being near the third ecdysis".

THREE DAYS Graphs 6 to 8.

The actual proportions of third to fourth stage larvae

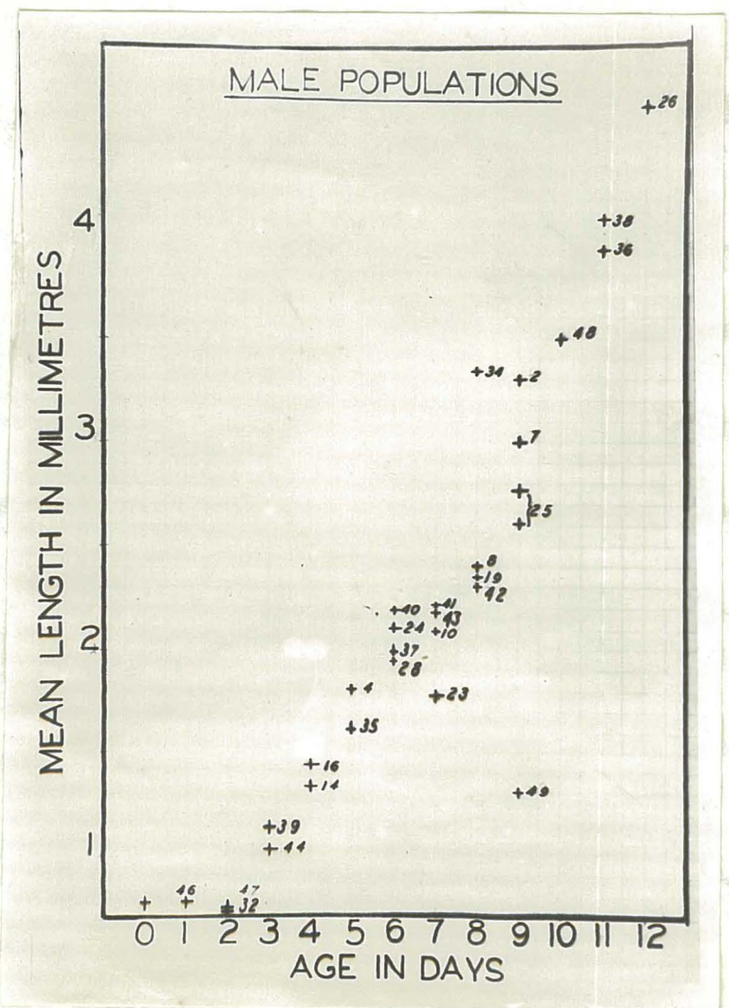


Fig. 13. Means of male populations (numbers are those of individual hosts).

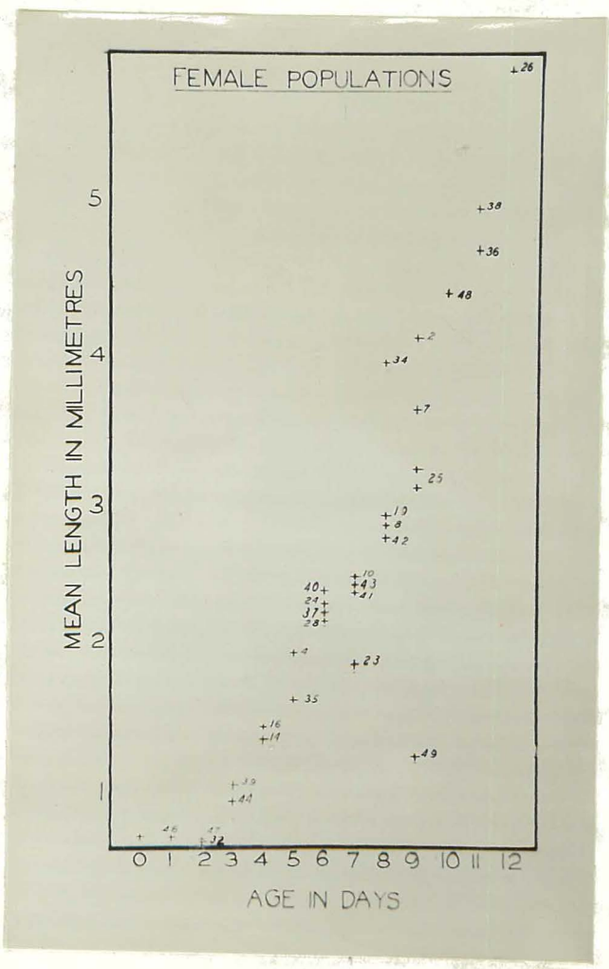


Fig. 14. Means of female populations (numbers are those of individual hosts).

will possibly vary considerably, and may not be a very useful criterion. Length is a more valuable feature, larvae about one millimetre long being likely to have just passed the third ecdysis.

"Fourth stage larvae, with a mean length of 1·3 to 1·5 mms and with sex differences becoming evident in the shape of the tail".

FOUR DAYS Graphs 9 to 12.

In lamb 44, containing a three day old population, some larvae did show differences in the shape of the tail tip. Very few of them could be classified with certainty as male or female, and no such differences were found in the population from lamb 39, also killed on the third day. Sex differentiation along with the distinctive mean lengths (see figs. 13 and 14) are sufficient to distinguish between three and four day populations obtained in this experiment.

"Fourth stage larvae, with a mean length for males of 1·6 to 1·8 mms and for females of 1·7 to 2·0 mms".

FIVE DAYS Graphs 13 to 16.

With increase of length, the possible range of lengths also increased. Standard deviations are likely to be greater in five-day than in four-day populations, especially with faster growing female worms, as shown below:-

SEX	POPULATIONS 4 DAYS OLD	POPULATIONS 5 DAYS OLD
Male	0·16 and 0·11	0·15 and 0·17
Female	0·13 and 0·16	0·25 and 0·22

The data above also shows that it is likely that on the fifth day female populations will show a greater standard deviation than males. This does not occur on the fourth day.

"Males about 2·1 mms long and females about 2·3 mms long".

SIX OR SEVEN DAYS Graphs 17 to 32.

It is not possible to distinguish between populations six and seven days old in terms of length. If a population with a mean corresponding to those given for this age contained a few adults, it would almost certainly be seven days old. The difficulty is that there are very few adults found at seven

days; only one was recovered in this experiment, a male from lamb 10.

Reference to figs. 13 and 14 shows that the mean lengths for the worms from lamb 23 are sufficiently short to place them in a five day old population. The significance of this population will be discussed later.

(N.B. Where the frequency distribution is bimodal as with females of lamb 43 (graph 32), the mean for the higher distribution is taken as the population mean. The faster growing larvae are regarded as standard.)

"Immature adults and fourth stage larvae present".

EIGHT OR NINE DAYS Graphs 33 to 49.

The mean length for all adult males at eight days is 2·63 mms and for females 3·13 mms. Corresponding figures for nine days are 2·96 mms and 3·68 mms respectively.

In order to differentiate between 8 and 9 day populations, it was first decided to base the lengths of the populations on the mean value for both 4th stage and adults. It was found, however, that especially where there were many larvae still in the fourth lethargus, as happens at eight days, the mean length for the whole population became almost the same as the mean for the larvae in the fourth lethargus and the mean for 8 days then differed very little from the next lower group for six and seven days. On this account it was decided to use the mean of the adult worms in 8 and 9 day populations. This is only reasonable when it is considered that these adults are growing, whereas the fourth stage larvae are not. This principle is a natural extension of that adopted with bimodal curves (lamb 43, graph 32) where the shorter mode was regarded as being a measure of worms with slower growth rates, and was therefore not included in the population mean.

In addition to the length, the proportions of fourth stage larvae present may be useful in deciding whether a population is eight or nine days old. Where less than half the population is adult, the chances are that it is eight days.

The shape of the frequency distribution may also be of

value. Eight-day-old worms will probably form a normal distribution. In nine day populations, the adults will have been growing for a longer time, and the distribution will tend to be bimodal.

Two anomalous values occur; lamb 34 at eight days, and lamb 49 at nine days. This will be discussed later.

The presence or absence of fourth stage larvae should make the distinction between seven and eight day populations quite clear.

"Adult males 3·5 mms long and adult females 4·4 mms long, and a few fourth stage larvae present".

TEN DAYS Graphs 50 and 51.

Because only one population was examined here, it is not possible to make any estimate of the probable variability. In figs. 13 and 14 the mean values for the population from lamb 48 are quite distinct from both 9 and 11 day old worms.

"Adult males about 4 mms long and females 4·8 to 5 mms long. A few may have 1 or 2 eggs in the uteri, but the majority are devoid of ova".

ELEVEN DAYS Graphs 52 to 55.

The presence of odd eggs is likely to be a useful identifying feature. It is most unlikely that any females at 10 days would have many eggs. On the other hand, it is just as likely that there would be no females with eggs in populations of this age.

"Males 4·6 mms long, females 5·8 mms. A number of females containing ova, averaging 8 per worm. These will be mainly the longer worms. The shorter females show darkening of the ovaries in some cases".

TWELVE DAYS Graphs 56 and 57.

Here again data is confined to ome population. The number of ova, and the proportion of larvae containing ova, as well as the length of the worms, makes this an easy age group to identify. The population on which this data is based shows a pronounced skew, which may even be interpreted as being bimod-

al. It was not possible to divide the distribution into two curves however. By eliminating all forms shorter than 5·4 mms. the mean becomes 6·4 mms.

It is not possible to determine the age of a population older than twelve days with certainty because growth ceases about this time.

PROBLEMS IN INTERPRETATION.

The foregoing illustrates how the data could be used to determine age. It now remains to study the obvious defects which appear in this scheme. They are:-

(1) The reliability of the onset of ovulation as an index of age.

(2) The interpretation of Bimodal and skewed distributions of length.

(3) The differentiation between 5 and 6-7 days.

(4) The ecdysis as an index of age.

(5) Abnormal rates of development.

(1) Onset of Ovulation. The presence of a few ova in odd worms was cited as one of the identifying features of a population eleven days old. At the same time, it was mentioned that this may not be a very reliable index. Similarly, the use of the presence of ova in a prtoportion of twelve day old worms as an indication of age should likewise be accepted with caution. Data obtained in this work (see page 79) has shown that there is a big possibility of variation in the number of fertile females amongst growing mature female populations. It is likely that a twelve day old population could be found without any eggs, and it is also possible, but much less likely, that a large proportion of females eleven days old could have eggs.

It is therefore hard to accept the presence or absence of ova as an absolute indication of age to within a day.

(2) Bimodal and skewed curves. Bimodal and skewed distributions of length are not very numerous in younger populations, but their incidence and importance, especially in female frequency distributions, increases with age. They are likely to cause some confusion and doubt in age estimation.

Obvious and easily explained bimodal curves are common in

populations eight and nine days old. These are caused by the fast growing adults associated with fourth stage larvae in the lethargus. It has already been explained why, in these curves, the mean value of the adult frequency distribution is accepted as an age index.

However, in certain cases where the true explanation of these bimodal curves is not obvious, there is a real danger that they will be interpreted as representing two populations. For example, the females from lamb 43 (graph 32) show a bimodal frequency distribution with mean values 1·51 and 2·64 mms respectively. This could be described as being a mixture of a 4 day and a 6 - 7 day population. Similarly, lamb 26 (graph 57) shows a very skewed curve, tending almost to a bimodal distribution. Though it is only one population it could easily be regarded as being a mixture of eleven and twelve day populations.

Certain other populations show only slight degrees of skew which should not cause any confusion. The mean for these distributions differs very little from the mode however; elimination of the extreme values makes little difference to the mean.

With mature populations, there are very big departures from normal. The classic example of these is the female distribution from lamb 18 (graph 73). Had this been submitted for age estimation it would have been classed as a mixture of populations ten and twelve days old.

Where two distribution curves occur, the mean for one being fourth stage larvae about the length at which larvae pass the final ecdysis, and the mean for the other a much greater length, special difficulties in interpretation occur. There are two possible alternatives for this type of distribution:-

(i) It may represent a single comparatively old population with a few individuals which were unable to pass the ecdysis.

(ii) It may represent two populations, one 6 or 7 days old, and the other much older.

If the fourth stage larvae are few in numbers in comparison with the adults, and are not combined in a distribution

with adults of the same or slightly greater lengths, it is likely that they represent immature forms of the same age as the adult distribution, as suggested in the first alternative above. Examples of this are common, e.g. lamb 2 (graphs 41 and 42).

If the older population had associated with it in the second distribution an approximately equal number of worms in a frequency curve typical of those found at the eighth or ninth days (i.e. adults and larvae in close association) then it could be fairly regarded as a double age population, as in the second alternative.

There is no obvious way round this interpretation of certain bimodal or skew curves. In practice, probably the best scheme would be a study of populations from several lambs killed on the same day. If a bimodal curve in any one population was due to worms of different age groups, then it would be repeated in each lamb, assuming they were all equally exposed to the factor which initiated infestations.

(3) Differentiation between five and six - seven days. Prior to five days, population characteristics are very definite for each day and it is not likely that they would present any difficulty in interpretation.

There may however be some doubt as to whether a population was in the five day group, or in the six - seven day group. An additional test would be to examine live specimens. Those which moved actively could be placed in the fifth day group, or perhaps sixth day. Any which were more or less mobile would be seven days old, or perhaps six. Veglia (68) was able to use this method to pick larvae of H. contortus in the final ecdysis. No attempt was made to apply it to C. curticei.

(4) The Importance of the Ecdysis. The third ecdysis has been suggested as an aid in differentiating between populations three and four days old. In view of the fact that Andrews found this to take place on the fourth day, while in this experiment it was found to be nearly concluded at sventy-two hours, it cannot be regarded as a very stable index of age.

A population of third stage parasitic larvae under the

data obtained in this experiment would be classed as a two day population. It must not be overlooked however that it may be a three day old population, which will pass the third ecdysis on the fourth day of parasitism.

The fourth ecdysis has also been regarded as a relatively constant factor for age identification. This assumption has been confirmed by all populations recovered when eight or nine days old with the exception of lamb 49.

(5) Abnormal rates of Development. Mention has already been made of one outstanding example of abnormal development, lamb 49, (graph 49). At nine days, the mean length of this population was only equivalent to a four day old population.

Similarly, at seven days, the population from lamb 23 shows signs of being much shorter than other seven day populations. The mean is more compatible with a five day population. Lamb 34 (graphs 37 and 38) contained a population which at eight days was much longer than other eight day populations, not only in young adults, but also in fourth stage larvae, which were the longest of this stage found.

These three populations, from lambs 49, 23 and 34, all had one feature in common - the infective dose was many times larger than any of the others. One other population had a similar dose (lamb 28), but it is no different in mean length from others of its age group (figs. 13 and 14).

This aspect has been discussed in the section on the effect of the size of infestation on growth. It is sufficient to indicate here that such variation can exist and is likely to be a serious source of error in the interpretation of lengths into ages. The only obvious solution is to use a larger number of lambs as suggested in the discussion on bimodal or skewed curves.

FIELD ACQUIRED INFESTATIONS.

So far, the value of the data in estimating the age of C. curticei has been discussed. This data was obtained from artificial infestations. It remains to consider how naturally acquired infestations may be expected to differ in terms of development.

In interpreting measurements of worms obtained from nat-

ural infestations in hitherto worm-free lambs, the significance of the following factors must be kept in mind:-

(1) Individuality of the sheep.
(2) The effect of the change of nutrition.
(3) Acquired resistance.
(4) Elimination of infestations.
(5) Size of dose and rate of dosing.

(1) Individuality of the sheep. Attention has been drawn in a study of ovulation to the fact that all lambs do not produce environments which are exactly alike in so far as the growth of O. curticei is concerned. The data just discussed also provides examples of this and it is reasonable to presume that these differences in environments will be significant features in naturally acquired infestations.

Of the thirty-eight sheep used in this experiment, nine produced populations which were partly or wholly undeveloped in comparison with others of the same age. In all except two cases this could only be due to differences inherent in the environments within the intestines of the lambs. The two possible exceptions are lambs 49 and 23 (discussed elsewhere) and the other lambs are nos. 18, 24, 26, 29, 40, 41 and 43.

If we can accept these situations as typical, it is possible that approximately one out of every five lambs used may show signs of abnormal development in its parasitic fauna, and this will render interpretation of length distributions difficult.

In the field, the best safe-guard against this difficulty would be to kill several sheep at the same time - at least four. This would assist in determining undeveloped and otherwise aberrant populations.

(2) Nutrition. It is not likely that the sudden change in diet from a hay - concentrate feed to a green pasture would hinder infestations. It is even possible that the digestive upsets caused by the change would weaken toe host and aid the parasites in establishing themselves.

(3) Acquired resistance. Elsewhere it has been shown that this is likely to require several weeks to develop. It will therefore be of no significance in age estimation. Since growth of O. curticei ceases at about twelve days, it is not possible to determine age after this time, at least on the data

obtained here. It is thus little use keeping lambs out for more than about twelve days before killing.

(4) Elimination of infestations. Mayhew (32) observed that the faecal egg count of a calf infected with H. contortus dropped sharply almost to zero when a further dose of infective larvae was given to the animal.

If this same phenomenon can take place with C. curticei in lambs, then there is a danger of incorrect conclusions being drawn. It would, for example, be possible for a lamb to acquire an infestation when environmental conditions were favourable for the infective larvae. If in a matter of a few days a further infestation was acquired, which promptly caused the loss of the previous larvae, then there would be no record of the original infestation, and consequently no record of the association with it of the particular environmental condition which proved favourable for parasitic infection.

It would be advisable to repeat Mayhew's work, using lambs and C. curticei. A special attempt should be made to find out whether or not immature forms would be eliminated as a result of the introduction of the infective larvae, and at what dosage level the elimination would take place. It is not likely that there is a similar answer for all sheep to this latter query.

(5) Size of Infecting dose and Rate of acquisition. It has already been shown that the size of dose affects the development of the population. Rate of dosing is also important, according to Leiper (quoted by Lapage (30)), in that a series of small infestations constantly picked up may lead to the development of a resistance somewhat different from that developed by animals receiving isolated or periodic doses. This is not likely to be important in experimental age determination of the type envisaged, because the animals would be out for too short a time for any resistance to develop.

Possibly a low constant intake would result in a frequency distribution with no particularly marked peak and with a very large standard deviation.

Size of dose is thus likely to be an important factor in field-acquired parasitism. Large intakes of larvae may influence the development of the population. The use of several

sheep will not necessarily overcome this because they will not all be affected in the same way, as observed in this experiment. Where anomalous curves of distribution of length are found in several lambs killed at the same time, it may be advisable to count the whole population. If the numbers are very high, this may be an indication that excessive numbers of parasites have interfered with the growth process.

SUGGESTED EXPERIMENTAL PROCEDURE.

It is suggested that the technique for studying factors which predispose a lamb to parasitism by an estimation of the age of its nematode fauna be defined in the light of the following points based upon the data obtained in this experiment.

The numbers of worm-free lambs required.

Because all lambs do not present the same environment for the growth of parasites, it would be wise to use several, preferably not less than four lambs at any one killing.

The length of the free grazing period.

If lambs are killed after a period of more than twelve days in the field it will become difficult to estimate the age of any C. curticei they may acquire. For example, the age of a mature population from a lamb which had been left out for sixteen days could only be estimated as being between twelve and sixteen days.

The sex to measure.

In C. curticei male worms have the advantage of being shorter and less variable within a population. They therefore take less time to measure and a smaller sample can be used. The frequency curve of distribution is more regular than with the females, and irregular or delayed growth is less likely to show up as an abnormal distribution. A further advantage is that in mixed infestations the males would be easier to identify from other species than females.

To females goes the advantage of faster growth, which means that differences between mean values of populations from day to day are greater than in males. Many of the variations concealed in a male frequency distribution may be of value in age estimation. These will frequently become evident in the female distribution. A comparison of graphs 72 and 73 will

illustrate this point.

In spite of these advantages, it would probably be only necessary to measure male worms where several sheep are killed. If numbers are limited, it would be advisable to measure female worms as well. For the smaller forms, five days old or less, there would be no harm in combining males and females in the same distribution, because the differences in length between the sexes are not great.

The Species to measure.

In some cases it may be advantageous to study the ages of two species conjointly, for example Cooperia and Ostertagia, assuming that they are affected in a similar manner by those variations in the environment which influence the rate of infestation in sheep. This would help in the analysis of some abnormal populations, especially where only a few sheep could be killed at the one time.

It would also be an advantage to study a species which took longer to reach maturity than C. curticei, while at the same time retaining the simple life cycle. H. contortus may prove a wise choice, provided it could be obtained in adequate numbers.

Size of sample.

In collecting this data two hundred worms of each sex were measured from populations varying in size (with some exceptions) from one thousand to five thousand individuals.

It is important to have an estimate of the size of sample which should be measured. A method is furnished by Snedecor (52). On page 46 of his work he quotes a formula for the estimation of sample size when the mean and standard deviation of a sample are known.

The sample size, $n = \frac{t^2 V}{L^2}$

when V = variance

t = ratio of mean to standard error

L = fiducial limits of the mean.

Since the variation in length within a population increases with age, three populations of different ages were selected in this experiment. They were those from lamb 35 (5 days old), lamb

48 (10 days old) and lamb 18 (22 days old).

In all cases, the variance was already known. Values of t for the 1% and the 5% level were obtained from a table given by Snedecor (52, page 65). Two values were selected for L, ·5 and ·3 mms. Results of these calculations are:-

AGE		5 days (lamb 35)				10 days (lamb 48)				22 days (lamb 18)			
SEX		Male		Female		Male		Female		Male		Female	
Fiducial limits of the mean (mms)		0·5	0·3	0·5	0·3	0·5	0·3	0·5	0·3	0·5	0·3	0·5	0·3
Size of population 'n' for values of 't'.	t @ 5%	41	115	45	127	196	544	313	870	294	817	406	1130
	t @ 1%	72	200	79	220	341	947	545	1515	494	1382	707	1966

It is obvious that whatever limits are set for the values of t and L, the sample must be bigger for older populations than for younger populations, and that more females must be measured than males for the same values of $\frac{L}{\sigma}$ and t.

Snedecor emphasises that this formula is only a rough guide to sample size. Rigid adherence to these estimates is not necessary in this method of determining age. Where a curve of distribution is made from young worms, and especially where only a simple curve is involved, very small numbers are probably sufficient to determine the age to within a day or so, and measuring the large numbers as determined by the formula is not likely to make the estimate of age more accurate.

The population from lamb 18 was selected because it is probably the nearest approach to the slightly complicated distribution drawn from lambs in the field. Large samples are needed to make fairly certain of placing a mean within the fiducial limits selected. It is likely however that the fiducial limits of even 0·5 mms are needlessly small for a mature population. Given a knowledge of the growth of C. curticei, an investigator should not need limits as small as those suggested, and he could accordingly measure smaller numbers. In addition, if several sheep are killed at the same time, and the populations they have acquired together have all grown normally, then by superimposing the distributions of a few worms for several sheep,

an accurate picture of the ages of the populations could be obtained. Therefore, in general terms, the more lambs which can be used the lower need be the number of worms measured.

So far as other species which grow similarly to *C. curticei* are concerned, it is likely that their growth and variation will be similar to *Cooperia*. Therefore the remarks concerning sample size in *C. curticei* may be applied to them also.

; : ; :

CHAPTER XII.

THE DIFFERENTIATION AND MEASUREMENT OF INFECTIVE LARVAE.

SUMMARY.

Concluding that the culture was mainly Cooperia curticei and Ostertagia spp., a study was made of the literature to determine the most suitable way in which to differentiate between the two species. Total length of the larva, and the shape of the tail tip were selected as being together the most suitable characteristics, and an estimation of the mean length of both C. curticei and Ostertagia spp. was made.

INTRODUCTION.

In order to complete the study of the parasitic growth of C. curticei, it was decided to measure the lengths of a sample of the infective larvae used in the Main Experiment.

Two problems were involved. Firstly, there were several species present in the culture. Secondly, the material was found to have deteriorated with storage, and finer details of the anatomy were not readily discernible.

It was known from examinations of the populations originating from this culture that the majority of the larvae were Ostertagia and Cooperia species. Only a few Trichostrongylus species were found. Nematodirus spp. and Strongyloides papillosus were also present, but as they were present in the experimental lambs prior to infestation, their presence is not necessarily an indication that they were in the culture. It is unlikely however that they were entirely absent from it. Though no search was made in the abomasum or the large intestine of any of the experimental lambs for other species, a study of the source of the cultures shows that others were likely to be present (pages 39 and 52). These were Oesophagostomum venulosum and Haemonchus contortus.

REVIEW OF LITERATURE.

Dickmans and Andrews (14) have examined the infective larvae of C. curticei and Ostertagia spp. They say "the larvae of C. oncophora and C. curticei may be distinguished from the others by the presence of prominent oval cuticularized struct-

ures at the anterior end of the oesophagus and by the very blunt and rounded posterior end of the larva proper. The tail sheath is slightly longer than that of O. circumcincta".

In describing O. circumcincta they note the presence of cuticularized structures around the base of the buccal capsule. In their own words:- "The buccal cavity is ovoid and is connected to the oral opening by a fine canal. The walls just posterior to the buccal capsule are slightly more conspicuous, being more highly cuticularized but still very small". The tail they describe as "bluntly pointed".

The difference between the "very blunt and rounded tail" of C. curticei and the "bluntly pointed" tail of O. circumcincta is not much in words. However, drawings from Dickmans and Andrews' work (14, plate V, figs. 3 and 4) show that the tail tip of C. curticei is distinctly more blunt than that of O. circumcincta.

Monnig (38) found the larvae of Cooperia spp. and Ostertagia spp. the most difficult to differentiate. His drawing (38, page 265/266) shows that he recognised the "bluntly pointed" tail of O. circumcincta and O. trifurcata. However, he was not working with C. curticei but with a mixed collection of C. fuelleborni, C. serrata and C. antidorca, all parasites of wild antelopes in the Union of South Africa. These larvae, all very much alike, have a more pointed tail than O. circumcincta, and are therefore not the same as C. curticei in this respect.

Length has been used by many workers as a means of differentiating between species of infective larvae. The literature contains only one measurement of the length of the infective larvae of C. curticei. This is given by Dickmans and Andrews as being from 711μ to 850μ with the mean 752μ.

Many measurements have been made of O. circumcincta, and there is a wide variation between these. The values given are as follows:-

Source	Species	Range (Microns)	Mean (Microns)
Morgan (39)	O. circumcincta O. trifurcata	not given	830
Monnig	" "	888-907	907
Dickmans & Andrews	O. circumcincta	797-866	819
Gordon (21)	O. circumcincta O. trifurcata	656-880	779

Monnig says "the measurement shows the most frequent (not the average) followed by the shortest and longest obtained". It is strange that the most frequent measurement (or mode) should have no values higher than it whatsoever. Nematodes measured in the course of this investigation have not shown such a pronounced skew frequency.

Gordan's measurements are taken from 1000 larvae, and give a lower limit than the others. Commenting on the variation between the results of different workers, he says that it is a "matter for speculation".

It would appear that the two most important variables are likely to be (1) variations in the environments in which the larvae were cultured and (2) methods of fixation employed.

The following systems were used by various workers in preparing cultures:-

Dickmans and Andrews poured sterile water containing ova and detritus of female worms on to a mixture of sand and the contents of the caeca of a sheep. This mixture had previously been heated at a low temperature to kill any nematode eggs or larvae already there. Both Monnig and Gordon used dung from animals which were hosts of a pure infection of the nematode studied. This dung was kept in a glass jar and (with Monnig) this was left at a temperature of 26°C and where possible in the dark. Morgan's procedure was similar to Dickmans and Andrews. Female worms were teased out, releasing the ova, which were cultured at 25°C in sterilized sheep's faeces mixed with animal charcoal or sterilized soil.

Glasser and Stoll (19) studying the growth to the infective stage of Haemonchus contortus on sterile nutrient media, compared the lengths of these larvae with larvae cultured on unsterilized sheep dung. The former were found to be on the average shorter than the latter, the figures being 569± 19μ as opposed to 679 ± 4μ and 664 ± 7μ for samples of the larvae reared on sheep dung. This work shows the variation which can be expected from different cultures. It is not unreasonable to suggest that some of the variation in larval lengths of Ostertagia species may be due to the different conditions under which the larvae were reared, involving perhaps variations in

quantity and quality of available nutrients.

The second source of variation suggested is that of the method of fixing employed. Dickmans and Andrews made all their measurements on larvae killed in warm Fleming solution, while both Gordon and Morgan used gentle heat to fix their larvae prior to measurement. Monnig does not state which method he employed. These different techniques may have contributed to the variation between different workers.

Where the differences in length between species are so small with many nematode infective larvae, and where methods of handling the material may cause variations greater than the variation within a species, an accurate technique in both rearing and measuring the larvae seems very necessary.

For this study, the work of Dickmans and Andrews was used as a guide, because it is the most complete account available. The differences between infective larvae of C. curticei and Ostertagia spp. as enumerated by these workers are set out below, all the measurements being in microns:-

	C. curticei	Ostertagia spp.
Total length	711-850	787-866
Dimensions of intestinal cells	50-80 by 10	60 by 7
Length of Oesophagus	130-160	150-190
Distance from anterior end of genital primordium	340-448	280-430
Tail of larvae proper	52-99	30-40
Anus to tip of sheath	97-122	94-110

METHODS EMPLOYED IN DIFFERENTIATION.

A preliminary attempt was made to separate the two species present by measuring the length of two hundred larvae, using the microprojector and the measuring wheel. Later the Camera lucida was employed to measure larval length.

In addition to length, it was decided to study other characteristics. The measurement in the table above which shows the greatest difference between the two species is the length of the larval tail. This is the measurement from the anus to the tip of the larval tail. The state of the material was such that it was extremely difficult to identify the anus, even when the larva was rolled over under the cover slip. This also pre-

cluded possibilities of using the measurement from the anus to the tip of the sheath. There is little difference between the species in this respect however. Another measurement which is not greatly different between the species is that from the anterior end to the genital primordium. The variation within each species is very large, being, in the case of the Ostertagia, over twice the variation in length. In nearly all cases, the intestinal cells could not be distinguished as separate entities, so that measurements were impossible. Disintegration of the larvae also made it extremely difficult to pick out any details of the buccal capsule, even when using the oil immersion lens.

It was finally decided to measure the length of the oesophagus, mainly because it was well defined. In a few cases, however, the junction of the oesophagus with the intestine was obscure due to disintegration of the intestinal cells.

In addition to total length and the length of the oesophagus the shape of the larval tail tip was used as a means of identification. This in practice was not as easy as expected. Great care had to be taken with the depth of focus, or else a pointed tail could be misjudged as being round.

RESULTS.

All the measurements obtained are reproduced in Appendix X.

A. Measurements with the Micro-projector.

A frequency polygon was constructed from the data obtained by measuring two hundred larvae with the micro-projector and the measuring wheel. This revealed defects in this system of measurement when very small objects were involved. The data, produced to two decimal points of a millimetre, was arranged with the following class intervals:-

·69 - ·70
·71 - ·72
·73 - ·74
·75 - ·76

In some class intervals no values occurred, for example:-

·69 - ·70	28 larvae
·71 - ·72	nil
·73 - ·74	44 larvae
·75 - ·76	49 "

From the nature of the material, it was realised that this nil result for a class interval could only arise through defective measuring. Examination of the chart used in transposing

revolutions of the measuring wheel into millimetres showed that there were no revolutions or fractions of a revolution equivalent to the following dimensions:- ·49 - ·50 mms; ·60 - ·61 mms; ·71 - ·72 mms; ·82 - ·83 mms. From the class intervals employed it will be seen that the values ·49 - ·50 mms and ·71 - ·72 mms form complete class intervals. However, the values ·60 - ·61 mms and ·82 - ·83 mms fall each within two class intervals, and so no obvious gap occurs in the frequency polygon; thus ·60 - ·61 mms lies partly in the class interval ·59 - ·60 mms and partly within the interval ·61 - ·62 mms.

Re-arrangement of the class intervals produced a frequency polygon which overcame this difficulty (graph 1). Two distinct curves are seen to be involved. The mean for the entire distribution is 746·5μ, and the standard deviation 34·9μ.

B. Using the Camera Lucida.

From measurements of the length of fifty larvae taken with the camera lucida, a frequency polygon was constructed. Each larva was classed as "Cooperia" if it had a blunt tail, and "Ostertagia" if the tail was pointed. This enabled the distribution to be divided into two types of larvae. The forms with the rounded larval tail are centered on a mean of 761μ ± 25·7μ; those with a pointed tail on a mean of 781 ± 30·6μ (graph 2).

The mean length of the oesophagi in the distribution centered on 761μ was 147μ. That of the oesophagi in the distribution centered on 781μ was 155μ.

With total length of the larva as abscissae, and length of the oesophagus as ordinate, a scatter chart was prepared. Since length of the oesophagus and total length are shorter on the average in Cooperia than in Ostertagia, it was expected that two overlapping but distinct groups would be formed.

DISCUSSION.

A. The shape of the Larval tail as a specific character.

In order to test whether the shape of the tip of the larval tail had been used correctly as a differential character in deciding if a larval form was C. curticei or Ostertagia species, an analysis of variance was made (52) on the data, and is set out on the following page.

ANALYSIS OF VARIANCE ON "BLUNT" VERSUS "POINTED" TAIL DATA.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Between Groups	1	4200·9	4200·9
Within Groups	48	37955·5	790·7
Total	49	42156·4	-

$F = \frac{4200 \cdot 9}{790 \cdot 7} = 5 \cdot 31$, which is greater than the 5% level of probability, 4·04, for 1 and 48 degrees of freedom.

If the population of infective larvae is very largely a Cooperia - Ostertagia population, then the significance of the difference between the two groups is an indication that the selection of the species on the basis of tail shape did in fact show a real difference. The likelihood of it being due to chance is only 1 in 20.

Additional evidence can be seen in the mean values for the groups. The shorter mean, 761 microns, lies close to the value for the length of C. curticei infective larvae which is given by Dickmans and Andrews as being 752 microns. The higher mean length, 781 microns, is shown to be in close agreement with the 779 microns given by Gordon as the mean value for the length of Ostertagia larvae.

The predominance of the Cooperia - Ostertagia type in the population used for infection has already been discussed. Had other species been present in more than small numbers, the shape of the tail alone would not be a practicable method by which to isolate the Cooperia and Ostertagia larvae, as some species have rounded tails like Cooperia, e.g. Oesophagostomum columbianum, while others are more like Ostertagia, e.g. Trichostrongylus vitrinus (14).

B. Specific differentiation using the Scatter chart.

The scatter chart made from the combination of total length and oesophageal length is not reproduced and the grouping was not analysed statistically. Since the total lengths of the two groups differed significantly, this method of identification was considered to be superfluous. It may be of use in some cases where lengths alone will not reveal the presence of separate groupings. This remains to be proved however.

C. Comparison between the Camera Lucida and the Micro-projector as measuring instruments for larval forms

Larvae measured with the micro-projector had a mean length of 774 μ, not including forms shorter than 650 μ. The mean length of larvae measured with the camera lucida was 772 μ. (See graphs 1 and 2 of the Appendix).

It is believed that the camera lucida is the more accurate instrument. This is to be expected, because its magnification was very much higher than the micro-projector, being somewhere in the vicinity of 400 diameters. In addition, it avoids the rounding errors which are unavoidable in the table used for transposing the revolutions of the measuring wheel into absolute terms. These errors become very large when lengths under 1000 μ are being considered.

: : : :

CHAPTER XIII.

SUMMARY AND CONCLUSIONS.

1. Literature on the relationship between growth and age, and growth in the Nematoda is reviewed. After a study of the anatomy of C. curticei and related types, length is selected as the most suitable index of growth, and methods for its measurement are investigated. The problem of rearing worm-free lambs and preparing a pure infestation of C. curticei is discussed.

2. A Trial experiment is donducted to investigate technical methods. It is concluded that at least 10,000 infective larvae are needed to infest the sheep for the conditions of this experiment.

3. The infestation of 31 lambs for the Main Experiment, and 8 lambs for the Subsidiary Experiment is described. Results are presented in terms of frequency distributions of the lengths of populations of C. curticei of various ages from one to twenty-two days.

4. The experimental design is not entirely satisfactory.

5. The methods for rearing worm-free lambs compare more than favourably with those of other workers.

6. In spite of the multiplicity of species present in the source culture, the experimental dose is almost pure C. curticei-Ostertagia spp.

7. Delayed passage to the intestine does not cause the presence of undeveloped parasites.

8. Undeveloped parasites are the result of a partial incompatibility between host and parasite. In a partially incompatible host, the females, or a portion of them, are likely to have no eggs, or else fewer eggs, than those in normal hosts will return.

9. Agamic females could introduce erroneous factors into the interpretation of egg count data.

10. Older females have more eggs in their uteri than those which have only just matured.

11. All the females of *O. curticei* in a non-resistant sheep will produce eggs in the uteri at about 12 or 13 days after infestation. Subsequent increase in numbers in faecal egg counts is due to increased egg production in the individual.

12. Skew or bimodal adult distributions of length are usually associated with distributions of undeveloped fourth stage larvae of the same age. Such distributions are evidence of a degree of partial incompatibility.

13. The male distributions of length are likely to approximate more closely to a normal curve than the female curves from the same population.

14. The fourth ecdysis is a phase which a proportion of larvae fail to pass.

15. Undeveloped worms may come to maturity a few at a time.

16. There is some evidence for an increase in length of third stage parasitic larvae.

17. The second ecdysis of *O. curticei* took place in some portion of the intestinal tract posterior to the rumen, between 24 and 48 hours after infestation.

18. The third ecdysis took place in the small intestine between 48 and 72 hours after infestation. This is not in agreement with previous work (Andrews (2)).

19. The fourth ecdysis took place on the 8th and 9th days from infestation. The lethargus and ecdysis cause a break in the growth curve at this time.

20. The growth impulse which breaks the fourth ecdysis is particularly strong. Constants from the growth curve formulae also suggest that the fastest growth rate occurs after the fourth ecdysis.

21. An infestation of the order of 400,000 *O. curticei* larvae is likely to interfere with the normal growth rate in a positive or a negative direction. A theory is advanced to account for this phenomenon.

22. As the populations mature, the frequency distributions of length show a greater "spread". This is more prominent in female distributions, since females grow faster than males.

23. Under the conditions of this experiment there is no significant difference between the lengths of male and female C. curticei until the sixth day of parasitism.

24. The composite growth curves (figs. 10, 11 and 12) are not truly representative of individual growth curves. They do however give a reasonable idea of the individual curve because the data is not highly variable.

25. Constants derived from the growth formulae must be accepted with reserve.

26. The growth of C. curticei is not strictly logistic in the sense that it cannot be represented by a logistic formula with a high degree of accuracy.

27. Using the data and technique described in the text, it should be possible to determine the age of infestations of C. curticei to within 1 day of the correct figure, with worms up to about 12 days of age.

28. A technique to determine the length of C. curticei infective larvae in a mixed Cooperia curticei - Ostertagia spp. population is described.

: : : :

ACKNOWLEDGEMENTS.

Grateful recognition is made for help received from the following:-

Mr J.H. Tetley, to whom the original idea of the thesis is due, and who provided much needed encouragement during difficult times.

Mr A.C. Glenday, of the Applied Mathematics Laboratory of the Department of Scientific and Industrial Research, who gave advice on the application of statistical treatments and on curve fitting.

The College Librarian and staff for considerable help.

Miss V. Bailey and Miss L. Procter for photographic assistance.

Miss Sommerville who typed Parts I and II of this work.

: : : :

BIBLIOGRAPHY.

1. Ackert, J.F. 1931. The morphology and life history of the fowl nematode Ascaridia lineata (Schneider). Parasit. 23:360-379.

2. Andrews, J.S. 1939. Life history of the nematode Cooperia curticei, and development of resistance in sheep. Journ. Ag. Res. 58:771-786.

3. Baker, D.W. 1939. Survival of worm parasite infection on New York State pastures. Cornell Vet. 29:45-48.

4. Baylis, H.A. 1929. A manual of helminthology. London, Bailliere, Tindall and Cox.

5. Boulenger, Ch. L. 1915. The life history of Nematodirus filicollis Rud, a nematode parasite in the sheep's intestine. Parasit. 8:133-154.

6. Brand, T. von. 1949. The carbohydrate metabolism of parasites. Journ. Parasit. 34 (2), Abstract 27.

7. Brody, S. 1945. Bioenergetics and growth. New York, Reinhold Publishing Corporation.

8. Bueding, E. 1949. The effect of drugs on the metabolism and enzyme systems of parasites. Journ. Parasit. 34 (2), Abstract 26.

9. Chandler, A.C. 1929. Hookworm disease. New York, MacMillan Company.

10. Chandler, A.C. 1932. Experiments on resistance of rats to superinfection with the nematode Nippostrongylus muris. Am. Journ. Hyg. 16:750-782.

11. Chitwood, B.G. and Chitwood, M.B. 1937. An introduction to Nematology. Washington, published by the authors.

12. Curtice, C. 1890. The animal parasites of sheep. U.S. Bur. An. Ind. Bull. 57.

13. Davenport, C.B. 1934. Critique of curves of growth and relative growth. Cold Spring Harbour Symposium. 2:203 seq.

14. Dickmans and Andrews, J.S. 1933. A comparative morphological study of the infective larvae of the common nematodes parasitic in the alimentary tract of sheep. Trans. Am. Micr. Soc. 52:1-25.

15. Fenwick, D.W. and Franklin, M.T. 1942. Identification of Heterodera species by larval length. Technique for estimating the constants determining the length variations within a given species. Journ. Helm. 20:67-114.

16. Fraser, A.H.H. 1938. The influence of the nutritional state of lambs on their susceptibility to an artificial infestation with parasitic nematodes. Emp. Journ. Expt. Ag. 6:316-322.

17. Fülleborn, F. 1927. Über das Verhalten der Larven von Strongyloides stercoralis, Hakenwürmern, und Ascaris lumbricoides im Körper des Wirtes (und ein Versuch, es biologisch zu deuten). Arch. f. Schiffs. u. Trop. Hyg. 31, Beihefte 2, 1-56.
- as quoted by Scott (51).

18. Geiman, Q.M. and McKee, R.W. 1949. The protein metabolism of parasites. Journ Parasit. 34 (2), Abstract 28.

19. Glasser, R.W. and Stoll, N.R. 1938. Sterile culture of the free living stages of the sheep stomach worm Haemonchus contortus. Parasit. 30:324-332.

20. Goodey, T. 1933. Plant parasitic nematodes. London, Methuen.

21. Gordon, H. McL. 1933. Differential diagnosis of the larvae of Ostertagia spp. and Trichostrongylus spp. of sheep. Aust. Vet. Journ. 9:223-227.

22. Gray, J. 1929. The kinetics of growth. Br. Journ. Expt. Biology 6:248-274.

23. Hobson, A.D. 1948. The physiology and cultivation on artificial media of nematodes parasitic in the alimentary tract of animals. Parasit. 38:183 seq.

24. Hubbs, C.L. and Perlmutter, A. 1942. Biometric comparison of several samples, with particular reference to racial investigations. Am. Nat. 76:582-592.

25. Hung, S.L. 1925. Report to Helminthological Society. Journ. Parasit. 12:113 seq.
- as quoted by Scott (51).

26. Kates, K.C. 1943. The overwinter survival on pasture of pre-parasitic stages of some nematodes parasitic in sheep. Proc. Helm. Soc. Washington. 10:23-25.

27. Koino, S. 1922. Experimental infection on the human body with ascarids. Japan Med. World 12:317-320.
- as quoted by Lapage (30).

28. Lane, C. 1928. The mass diagnosis of hookworm infection.
Am. Journ. Hyg. 8, May supplement, H48.

29. Lapage, G. 1935. The second ecdysis of nematode infective larvae.
Parasit. 27:186-206.

30. Lapage, G. 1937. Nematodes parasitic in animals.
London, Methuen.

31. Lipson, M. and Gordon, H. McL. 1940. The passage of phenothiazine through the alimentary canal of sheep.
Aust. C.S.I.R. Journ. 13:240-244.

32. Mayhew, R.L. 1941. Studies in bovine Gastro-Intestinal parasites. V. Immunity to the stomach worm with a note on the pre-patent period.
Am. Journ. Hyg. 33, Sect. D, p.103-111.

33. McCoy, O.R. 1929. The suitability of various bacteria as food for hookworm larvae.
Am. Journ. Hyg. 10:140-156.

34. McCoy, O.R. 1930. The ingluence of temperature, hydrogen-ion concentration and oxygen tension on the development of the eggs and larvae of the dog hookworm.
Am. Journ. Hyg. 11:413-448.

35. Medawar, P.B. 1940. Growth, growth energy and ageing of the chicken's heart.
Proc. Roy. Soc. (B) 129:332 seq.

36. Merrell, M. 1931. Relationship of individual to average growth.
Human Biology 3:37 seq.

37. Monnig, H.O. 1926. The life histories of Trichostrongylus instabilis and T. rugatus of sheep in South Africa.
Union Sth Afr. Dept. Ag., Dir. Vet. Ed. and Res. Rept. 11-12:231-251.

37A. Monnig, H.O. 1930. Studies on the Bionomics of the free living stages of Trichostrongylus spp. and other parasitic nematodes.
Union Sth Afr. Dept. Ag. Dir. Vet. Services and An. Ind. Rept. 16:175-198.

38. Monnig, H.O. 1931. The specific diagnosis of nematode infestation in sheep.
Union Sth Afr. Dept. Ag., Dir. Vet. Services and An. Ind. Rept. 17:255-364.

39. Morgan, O.O. 1930. On the differential diagnosis of the larvae of some helminth parasites of sheep and goats.
Journ. Helm. 8:223-228.

40. New Zealand. Air Dept. 1949. Meteorological

Bureau. Meteorological observations. Station 19, April 1949.

41. Ransom, B.H. 1911. Nematodes parasitic in the alimentary tract of cattle, sheep and other ruminants. U.S. Bur. An. Ind. Bull. 127.

42. Robbins, W.J. et al. 1928. Growth. Yale University press.

43. Robertson, P.W. 1923. The chemical basis of growth and senescence. Philadelphia, J.B. Lippincott.

44. Rogers, W.P. 1949. The relative importance of aerobic metabolism in small nematode parasites of the alimentary tract. I. Oxygen tensions at the normal environment of the parasite. II. The utilisation of oxygen at low partial pressures by small nematode parasites of the alimentary tract. Aust. Journ. Sc. Res. (B) 2:157-174.

45. Sadun, E.H., Totter, J.R. and Keith, C.K. 1949. Effect of purified diets on the host-parasite relationship of chickens to Ascaridia galli. Journ. Parasit. 35 (6), Sect. 2, Abstract 10.

46. Sadun, E.H. 1949. The antibody basis of immunity in chickens to the nematode Ascaridia galli. Am. Journ Hyg. 49:101-106.

47. Sarles, M.P. 1932. Development of acquired resistance in rabbits by repeated infection with an intestinal nematode, Trichostrongylus calcaratus, Ransom 1911. Journ. Parasit. 19:61-82.

48. Schwartz, B., Alicata, J.E. and Lucker, J.T. 1931. Resistance of rats to superinfections with a nematode, Nippostrongylus muris, and an apparently similar resistance of horses to superinfection with nematodes. Wash. Acad. Sc. Journ. 21:259-261.

49. Scott, J.A. 1929. Host induced variation in the growth curve of the dog hookworm, Ancylostoma caninum. Am. Journ. Hyg. 10:125-139.

50. Scott, J.A. 1929. The length of specimens of the dog hookworm after various methods of fixation. Journ. Parasit. 16:54-55.

51. Scott, J.A. 1930. The biology of hookworms in their hosts Quart. Rev. Biol. 5:79-97.

52. Snedecor, G.W. 1946. Statistical methods. 4th edition. Iowa State College press.

52A. Sproston, N.G. 1944. The genus Kuhnia n.g. (trematoda: Monogenea). An examination of the value of some specific characters, including factors of relative growth. Parasit. 36:176 seq.

53. Stoll, N.R. 1929. Studies with the Strongyloid nematode Haemonchus contortus. I. Acquired resistance of hosts under natural re-infection conditions out-of-doors.
Am. Journ. Hyg. 10:384-418.

54. Stoll, N.R. 1930. On methods of counting nematode ova in sheep dung.
Parasit. 22:116-136.

55. Stoll, N.R. 1936. Certain nett effects in Helminth parasitism with special reference to the sheep host.
Cornell Vet. 26:171 seq.

56. Taliafferro, W.H. 1948. The inhibition of reproduction of parasites by immune factors.
Bact. Rev. 12:1 seq.

57. Taylor, E.L. 1928. Syngamus trachea from the starling transferred to the chicken, and some physiological variation observed.
Ann. Trop. Med. Parasit. 22:307-318.

58. Taylor, E.L. 1934. Field experiments on the immunity of lambs to parasitic gastritis caused by a mixed infection of Trichostrongylid nematodes.
Journ. Helm. 12:143-164.

58A. Taylor, E.L. 1934. The epidemiology of parasitic gastritis of sheep. Observations on the relative importance of factors concerned in the disease.
Journ. Ag. Sci. 24:192-208.

59. Tetley, J.H. 1934. The nematodes of sheep in Manawatu district, New Zealand.
Journ. Helm. 12:183-196.

60. Tetley, J.H. 1937. The distribution of nematodes in the small intestine of the sheep.
N.Z. Journ. Sc. Tech. 18:805-817.

61. Tetley, J.H. Spicule length in Cooperia curticei as a measure of favourable intestinal environment for intestinal nematodes of sheep.
Journ. Parasit. 27:449 seq.

62. Tetley, J.H. 1941. The differentiation of the eggs of the Trichostrongylid species Nematodirus filicollis and N. spathiger.
Journ. Parasit. 27:473 seq.

63. Tetley, J.H. 1941. Egg laying function of a nematode as shown by a study of Nematodirus eggs in utero.
Journ. Parasit. 27:481-491.

64. Tetley, J.H. 1941. The epidemiology of low-plane nematode infestation in sheep in the Manawatu district, N.Z
Cornell Vet. 31:243-265.

65. Tetley, J.H. 1949. Rhythms in nematode parasitism of sheep.
N.Z. Dept. Sc. Ind. Res. Bull.96.

66. Thompson, D'Arcy W. 1942. On growth and form. Cambridge, University press.

67. Threlkeld, W.L. 1934. The life history of Ostertagia circumcincta. Virg. Ag. Expt. Sta. Tech.Bull.52.

68. Veglia, F. 1915. The anatomy and life history of the Haemonchus contortus (Rud). Union Sth Afr. Dept. Ag., Dir. Vet. Ed. and Res. Rept. 3 and 4:349-500.

69. Weymouth, F.W., McMillan, H.C., and Rich, H.W. 1931. Latitude and relative growth in the razor clam Siliqua patula. Br. Journ. Expt. Biol. 8:228-249.

70. Wigglesworth, V.B. 1939. The principles of insect physiology. London, Methuen.

71. Wilson, E.B. 1934. The mathematics of growth. Cold Spring Harbour Symposium 2:199 seq.

72. Yorke, W. and Maplestone, P.A. 1926. The nematode parasites of vertebrates. London, J.and A. Churchill.

: : : :