STUDIES ON COOPERIA CURTICEI

(RANSOM 1907) A NEMATODE

PARASITE OF SHEEP

A thesis

presented in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

at Massey University

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1970

ABSTRACT

This thesis records in part I studies on the ecology of the free living stages of Cooperia curticei, both under controlled and natural conditions.

At constant temperatures free living stages developed throughout the temperature range of $10-37^{\circ}C$. At all temperatures each larval stage occupied the same proportion of the total developmental time to reach the infective stage. The relationship between the rate of development in log days and temperature was found to be linear. Under natural conditions the rate of development was most strongly correlated with mean maximum air temperature and was not significantly different to that observed under controlled conditions. When faecal cultures were kept at $10^{\circ}C$, $27^{\circ}C$ and $37^{\circ}C$ a higher proportion of eggs completed development to the infective stage at $27^{\circ}C$ than at the other temperatures. Under natural conditions the percentage recovery was influenced by weather conditions particularly rainfall.

Submergence of the free living stages in water inhibited their further development. First and second stage larvae survived longest at temperatures between $5^{\circ}C$ and $15^{\circ}C$ but for a much shorter time than infective larvae. Between the extremes of -6 and $52^{\circ}C$, the longest survival of infective larvae was 312 days at $10^{\circ}C$.

Techniques are described for the recovery of Cooperia curticei larvae from sample units of pasture, soil and faecal pellets. Under natural conditions the maximum survival of larvae from monthly experiments ranged from 9 - 26 weeks. Maximum survival was particularly influenced by temperature. Infective larvae survived through the winter. There was an exponential relationship between the percentage survival and percentage of larvae recovered from the herbage. Vertical migration of larvae appeared to be primarily affected by rainfall and evaporation.

It is concluded that infective larvae of Cooperia curticei are available to grazing sheep throughout the year. Theoretically the nematode can complete from 9 - 11 generations in each year.

Part II of this thesis records experiments on the relationship between <u>Cooperia</u> <u>curticei</u> and the host sheep.

Experiments carried out <u>in</u> <u>vivo</u> and <u>in</u> <u>vitro</u> demonstrated that infective larvae of <u>C.curticei</u> exsheath under conditions provided by the rumen. The process of exsheathment was similar to that described for <u>H.contortus</u>.

A series of experimental observations were made on the effect of <u>Cooperia</u> <u>curticei</u> infection in sheep using animals of differing ages, on different diets and with various sizes of infection. The prepatent period of infection was 14-16 days. Peak egg counts were recorded 5-7 days after infection became patent. Thereafter they declined gradually in sheep given 10,000 larvae but in sheep given 50,000 to 100,000 larvae the decline was more abrupt. The egg output per female worm was found to range up to 1,958 eggs per day.

No clinical sign of infection was observed from any experimental animal. Body weights, wool growth and blood analyses showed no significant changes and no gross lesions or significant histopathological changes were observed. The results indicate a well balanced relationship between <u>C.curticei</u> and the sheep.

The distribution of the <u>C.curticei</u> in the small intestine was skewed, and most of the worms were recovered from 5-10 feet from the gastric pylorus. A predominance of female worms was observed at all levels of the small intestine. Maximum percentage recovery of <u>C.curticei</u> was observed in sheep given 10,000 larvae. Experimental animals with higher doses besides giving a lower rate of recovery showed inhibition of development and stunted growth of worms.

Serum and intestinal mucus samples from infected animals were tested for precipitating antibodies by gel diffusion against five antigens. Antigens were prepared from first stage, second stage, ensheathed third stage, exsheathed third stage larvae and exsheathing fluid. Variable numbers of precipitin lines were obtained with serum and mucus from infected sheep more than 6-7 months old. Sheep 2-3 months old showed no such response but did show evidence of an acquired resistance to infection.

ACKNOWLEDGEMENT

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INTRODUCTION

About 50 species of nematode parasites have been recorded from
sheep and considering the relatively wide range of ecological conditions
under which domestic sheep occur it is ·surprising to find that so many
of the species infecting them have an equally wide distribution. Some
of the most pathogenic species are found in the suborder Strongylata.

Strongylate nematodes are present in sheep throughout New Zea-
land. Among them are the species of the genus Cooperia and of particu-
lar interest is Cooperia curticei.

This nematode is normally found in the small intestine and has
frequently been dismissed as being relatively non-pathogenic. However,
it is present in a high proportion of sheep and often in larger numbers
than has generally been reported elsewhere in the world.

Strongylate nematodes have many common features but there are
specific differences which frequently have important implications in the
epidemiology and pathogenesis of infections.

Research work has been directed mainly to the study of those
strongylate nematodes which are believed to be of major economic impor-
tance. As a result the study of species usually considered to have little
or no pathogenic significance has tended to be comparatively neglected.
Among these, Cooperia curticei is an outstanding example. Since Cooperia
curticei is present in large numbers in New Zealand sheep, (Charleston,
pers.comm.), it was considered important to investigate the signifi-
cance of this parasite to the host. The results of experimental
infections of sheep with this parasite are recorded in Part II of this
thesis.

At the same time it was felt desirable to investigate the epid-
emiological factors that might give rise to the relatively high burdens
of Cooperia curticei encountered in sheep in this country.

There is an extensive literature on the ecology of the free
living stages of strongylate nematode but the reported observations are
frequently contradictory and difficult to interpret. Often the con-

ditions under which they have been made and the techniques used have
been inadequately described and sometimes inappropriate. Attempts to
correlate extensive and detailed laboratory observations with detailed
field observations using a single isolate are lacking. Obviously some
attempt at synthesis is necessary to avoid confusion in the application
of the results of various investigators. From the literature, it is also
obvious that various strains of parasite from different geographical
areas of the world are distinctly different in biology. Studies on these
isolated strains under laboratory and field conditions would be especial-
ly useful in tracing adaptations to specific environments (ecotypes).

The first part of the thesis presents a planned study of Cooperia
curticei under laboratory and simulated field conditions. It was intend-
ed to correlate the laboratory and field observations with the hope that
this might provide a model which could be applied to other species of
strongylate nematode.

ECOLOGY

OF

COOPERIA CURTICEI

PART ONE

Chapter I   <u>REVIEW OF LITERATURE</u>

1. I.   <u>CLASSIFICATION AND GENERAL DESCRIPTION OF THE GENUS COOPERIA</u>:-

The majority of nematologists believe that Strongylate nematodes
originated from free living ancestors of todays rhabditiforms.   Even today
strongylate nematodes preserve numerous features common to their putative
ancestors including characteristics of both a morphological and physiological
nature.   There is a great number of strongylate species distributed
exceptionally widely throughout all areas of the globe and many of these cause
serious diseases of domesticated animals.   In ruminants nematodes of the
superfamily <u>Trichostrongyloidea</u> are particularly important.   Amongst these
nematodes are species of the genus <u>Cooperia</u>.

The superfamily <u>Trichostrongyloidea</u> (Cram, 1927) includes the family
<u>Trichostrongylidae</u> (Leiper, 1912) and within this is the subfamily <u>Cooperiinae</u>
(Skrjabin <u>et al</u>, 1952) which includes the tribe <u>Cooperiea</u> (Skrjabin and Schulz,
1937).   This tribe contains three genera, namely <u>Cooperia</u> (Ransom, 1907a),
<u>Cooperioides</u> (Daubney, 1933) and <u>Paracooperia</u> (Travassos, 1935).   The
speciation within the genus <u>Cooperia</u> has been revised by a number of workers
(Skrjabin <u>et al</u>., 1954;   Yamaguti, 1961).

In 1890 Curtice first described a nematode from sheep which he named
<u>Strongylus ventricosus</u> (Rudolphi, 1809).   Giles in 1892 established that the
species described by Curtice was an independent form and called it <u>Strongylus</u>
<u>curticii</u>, while Ransom (1907a) placed the species in the genus <u>Cooperia</u> as
<u>Cooperia curticei</u>.

Soulsby (1965) listed four species occurring in sheep and goats,
namely <u>C.curticei</u>, <u>C.oncophora</u>, <u>C.mcmasteri</u> and <u>C.serrata</u> (syn. <u>Paracooperia</u>
<u>serrata</u>), but the main sheep parasite of this genus is <u>Cooperia curticei</u>.
<u>Cooperia punctata</u> and <u>Cooperia pectinata</u> may be found in sheep but their
principal host is cattle.   <u>Cooperia oncophora</u> and <u>C.mcmasteri</u> are primarily
parasites of cattle.

Tetley (1934, 1937) studied the nematodes of sheep in the Manawatu
district of New Zealand and reported the occurrence of <u>Cooperia curticei</u>,
<u>Cooperia punctata</u>, <u>Cooperia oncophora</u> and <u>C.mcmasteri</u>.   <u>Cooperia curticei</u>

is now known to be widespread in distribution throughout the world though said to be generally present in small numbers in individual animals (Gordon, 1950; Parnell et al., 1954; Soulsby, 1965).   However in New Zealand, _Cooperia curticei_ are often found in sheep in large numbers,(Charleston, pers.comm.).

The known hosts of _Cooperia curticei_ are sheep (_Ovis aries_), goat (_Capra hircus_), cattle (_Bos taurus_), red deer (_Cervus elephus_) and fallow deer (_Dama dama_) (Skrjabin et al., 1954).

I. 2.   MORPHOLOGY AND DEVELOPMENT OF THE PRE-PARASITIC STAGES AT DIFFERENT TEMPERATURES :-

i)   The egg:-   The egg of _Cooperia curticei_ has a typical strongylate morphology (Fig. I).   The size of the eggs of _Cooperia curticei_ has been recorded by various authors and their findings are summarised in Table I. Tetley (1941a) compared the sizes of _Haemonchus contortus_ eggs from the nematode uterus with those recovered from faeces of sheep and noted that swelling of the eggs took place during the interval between laying and their appearance in the faeces.   Tetley also suggested the sizes of nematode eggs also vary with the strain of nematode, the host species and the technique used to recover the eggs, although he presented no evidence in support of this.   These factors may account for the variations in size recorded by different authors.

Experimental work on embryonic development of gastro-intestinal nematodes of sheep has been carried out mostly utilising _Haemonchus contortus_ and _Trichostrongylus spp._   (Ransom, 1906; Veglia, 1915; Ross and Gordon, 1936; Shorb, 1944; Dinaburg, 1944; Silverman and Campbell, 1959; Ciordia and Bizzall, 1960; Wang, 1964; Crofton and Whitlock, 1964, 1965; Crofton, 1965).

Crofton and Whitlock (1964) examined the rates of hatching of eggs of different species of trichostrongyle and showed that under controlled and standardised conditions of incubation, different phenotypes could be detected whose response to temperature differed.   These phenotypes were derived from different geographical locations and reflected the selection of phenotypes under different environmental conditions.   This may explain the variation in results obtained by different workers using the same nematode species derived from different geographical locations.   Crofton and Whitlock (1965) also

Table I

The dimensions of _Cooperia curticei_ eggs
according to various authors:-

Length μ	Breadth μ	Mean μ	Reference
63 - 70	30 - 32	-	Hall (1924)
73 - 82	34 - 42	-	Wood (1931)
60 - 80	30 - 35	-	Ross & Gordon (1936)
70 - 82	35 - 41	76.8 x 37.7	Shorb (1939, 1940)
			&
			Shorb & Kates (1943)
77 - 94	34 - 39	-	Tetley (1941a)
67.5 - 87.5	30 - 40	77.5 x 35.6	Cunliffe & Crofton (1953)
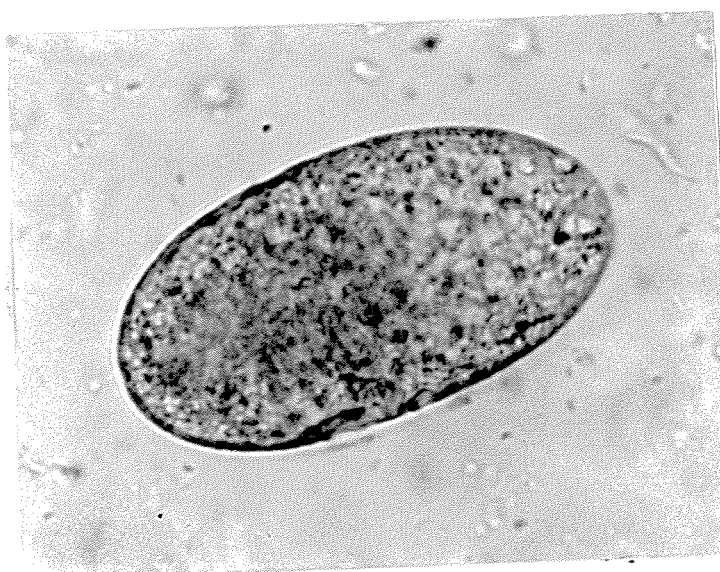
Fig. I.   Egg of _Cooperia curticei_
magnification x 1000

produced evidence that the volume of the egg is correlated with the time required for development to the hatching stage.

The only work on the egg of Cooperia curticei is that of Crofton (1965). He examined the time taken for eggs of Cooperia curticei to hatch under constant temperatures in aerated water and found that no hatching occurred at temperatures below 16°C or above 38°C; at 17-18°C hatching occurred after 7 days incubation, at 20°C after 48 hours incubation, at 25°C after 24 hours, and at 38°C after 15 hours. Crofton concluded that the eggs of Cooperia curticei require a relatively high temperature to hatch compared with other tricho-strongyles. The culture of C.curticei used was derived from South West England.

There is no literature on the hatching or embryonic development of other species of Cooperia. Silverman and Campbell (1959) while studying the embryonic development of H.contortus under controlled conditions stated that for hatching a temperature above 9°C was necessary. They also found that eggs placed in well aerated water developed rapidly, whereas eggs kept in water that was not aerated or in faecal pellets saturated with water became inhibited. It was considered that this was due to an interference with aeration, presumably resulting in a decreased availability of oxygen.

ii)   First and second stage larvae:-   There is no literature on development of the pre-parasitic larvae of Cooperia curticei and other species of Cooperia, under controlled conditions but Andrews (1939) studied the morphology and development of preparasitic stages of Cooperia curticei cultured at "room temperature". He noted that the newly hatched rhabditiform larvae were approximately 300 μ long and that they grew to a length of approximately 500 μ within about 12 hours after which they entered a period of lethargus. Later they became active again and cast their sheaths approximately 30 hours after hatching. The second stage larvae resembled those of the first stage, except that they were larger and the intestinal cells contained more granules. The second stage larvae fed actively for approximately 48 hours before entering the second lethargus, which lasted about 12 hours. About 90 hours after hatching the larvae became active again and underwent an incomplete moult and reached the third ensheathed larval stage, which is the infective stage.

Silverman and Campbell (1959) studied the larval development of H.contortus under constant temperatures and their results are shown in Table 2.

Table 2

The relationship between various constant temperatures and the rate of
embryonation, survival and hatching of Haemonchus contortus
(From Silverman and Campbell, 1959)

Temperature						
°C	0.0	7.2	11.0	14.4	21.7	37.0
°F	32.0	45.0	51.0	58.0	71.0	98.0
Time (days) required for development from blastomere stage						
EGGS						
Morula						
Minimum	-	3	1	0.5	0.5	0.25
Median	-	8	2	1	0.5	0.6
Gastrula						
Minimum	-	6	2	1	1	0.25
Median	-	18	3	2	1	0.5
Tadpole						
Minimum	-	14	3	1	1	0.5
Median	-	20	5	4	2	0.67
Pre-hatch						
Minimum	-	16	4	2	1	0.5
Median	-	24	8	5	3	1
LARVAE						
First stage						
Minimum	-	-	6	3	2	0.5
Median	-	-	10	6	4	1.5
Second stage						
Minimum	-	-	7	6	3	1
Median	-	-	13	10	5	2
Third stage						
Minimum	-	-	15	9	5	3
Median	-	-	20	12	8	4
Mean survival %						
Minimum	-	1	60	50	50	10
Maximum	-	10	90	80	70	30

iii)   Third stage infective larvae:-   Monnig (1931) while comparing the
infective larvae of several different species of the Trichostrongylidae
described the infective larvae of Cooperia spp.   Dikmans and Andrews (1933a)
studied the third stage infective larvae of Cooperia curticei and stated that
larvae measured from 711 to 850μ in length including the sheath.   The buccal
cavity was globular and the posterior end of this structure was cuticularised
giving the appearance of two oval bodies.   The genital primordium was situated
340 to 448 μ from the anterior end.   The tail of the larvae was broadly rounded
and 58 to 70μ in length.   The tail of the sheath projected 39 to 52 μ beyond
the end of the larva proper and terminated in a sharp point.   The distance
from the anus to the tip of the sheath ranged from 97 to 122 μ.   Later, Andrews
(1935) published a figure showing that the third stage larvae is characterised
by having a band of fibres surrounding the buccal capsule.   Andrews (1939)
later criticised his own observation and said that the band shown in figure
of his 1935 paper were actually the transverse striations of the cuticle.

Much of the data on the development of Trichostrongylus and Haemonchus
larvae to the infective stage relates the rate of development to different
temperatures, an aspect with obvious epidemiological significance.   The
temperatures at which the infective larvae of sheep nematodes show optimal
development, in terms of both rapid development and low mortality appear to fall
between 20 to 30°C (Crofton, 1963).   At lower temperatures, down to 9 - 10°C,
some development may take place but the rate is much slower.   At temperatures
above 30°C, to about 36-37°C, development is more rapid than at the optimum
levels, but mortality is higher, and larvae die rapidly at temperatures
above 40°C.

There appears to be no published information relating to the effect of
temperature on the development of larvae of Cooperia curticei to the infective
stage.   Rose (1963) while studying the development of Cooperia oncophora,
found that at 22-23°C the times for development to the infective stage ranged
from 3 to 9 days, at 14-16°C, 7 to 21 days and at 10-11°C, 21 to 56 days.

Larval development of Haemonchus and Trichostrongylus at different
temperatures was studied by Ransom, 1906; Veglia, 1915; Ross and Gordon, 1936;
Shorb, 1944; Berberian and Mizzelle, 1957; Silverman and Campbell, 1959;
Ciordia and Bizzall, 1960; Gupta, 1961 and Conway, 1964.   Except for Silverman
and Campbell (1959) these investigations have been only concerned with the time

taken by larvae of species of these two genera to reach the infective stage at specified temperatures. In all cases the observation has been that within certain limits the rate of development increases with temperature. Observations of the minimum, optimum and maximum temperatures at which development of a single species will occur show variations which are probably accounted for by phenotypic variations within nematode species and variations in technique used. It does not seem appropriate to cite the findings of these workers in detail.

The experiments on Haemonchus contortus carried out by Silverman and Campbell (1959) however, were far more detailed than any of the others. They examined the rates of development of all the larval stages, up to the third stage, under constant conditions using one isolate of H.contortus. Their work, the results of which are summarised in Table 2 , is a model of careful experimentation. They obtained development of the infective larvae at temperatures of from $11^{o}C$ to $37^{o}C$ and a maximum rate of survival at $11^{o}C$. The rate of development was expressed as minimum and median times.

The meaning of "median" as used by these authors is not clear. They state, "The term median is however qualified here to have reference not to the total population, but to that part of it which completed development." (Silverman and Campbell, 1959). "Median" may refer to the time when the majority of the developing larval population reach a particular stage of development. It is not defined in terms of the percentage of the population that has developed to the stage in question. Silverman and Campbell (1959) observed that some individuals in the larval population may take much longer to develop than the majority of the population so that the maximum development time can be "several weeks" longer than the median time.

I. 3.   SURVIVAL OF PRE-PARASITIC STAGES UNDER LABORATORY CONDITIONS :-

The development of the pre-parasitic stages of strongylate and other nematodes is only possible within certain defined temperature limits. The temperatures to which these developing stages are subjected under natural conditions frequently fall outside this range. It is of some interest from an ecological and epidemiological standpoint to examine the ability of the different pre-parasitic stages to survive at temperatures outside the range that allows development so that they may resume development when the temperature returns to within that range. In general it has been found that in the

presence of adequate moisture the infective third stage larvae of strongylate nematodes are better able to survive extremes of temperature than the earlier pre-parasitic stages.

i)   The egg:-   Nothing has been reported on the survival of eggs of any species of Cooperia under controlled laboratory conditions.   Survival of eggs of Trichostrongylus spp. has been studied by Ross and Gordon (1936), Poole (1954), Wang (1964), and Anderson et al. (1966).   Their findings were that eggs survived better at lower than at higher temperatures and embryonated eggs were more resistant than unembryonated eggs.   Storage at 40°C and above for periods longer than 4 days was said to be lethal.

Silverman and Campbell (1959) reported that saturation of faecal cultures with water inhibited development of eggs of H.contortus although the eggs survived 21 to 68 days at 7.2°C, 45 to 81 days at 11°C, 40 to 56 days at 14.4°C and 26 to 33 days at 21.7°C.

ii)   First and second stage larvae:-   There is little information on the survival of the pre-infective larvae of strongylate nematodes under controlled conditions.   This is probably due to the difficulty in securing preinfective larvae at a particular stage of development free from other stages.   This would necessitate a detailed knowledge of development times of each larval stage at different temperatures.

Prasad (1959) and Anderson et al (1966) stated that preinfective larvae of Trichostrongylus spp. resisted freezing for up to a month and at temperatures of 0 to 4°C, they survived up to 4 months.   Maintenance at 40°C was lethal in 24 hours.

iii)   Third stage infective larvae:-   The survival of the infective larvae of Cooperia curticei appears not to have been studied under controlled conditions.   Cooperia oncophora has been studied by Rose (1963) and he stated that infective larvae can survive for more than 2 years at temperatures of 6 to 11°C, whereas longevity was 15 months at 15-16°C and 13 months at 24-25°C. Infective larvae survived up to 24 weeks when kept frozen in water at -4 to -6°C.

The survival of the infective larvae of <u>Trichostrongylus</u> <u>spp.</u> has been studied by Monnig (1930), Nnochiri (1950), Poole (1954), Prasad (1959), Gupta (1961), Anderson <u>et</u> <u>al</u>. (1966) and Herlich (1966).   The findings vary from one author to another which can presumably be accounted for by the different species, strains and techniques used.   In general infective larvae survive extremely well at lower temperatures.   The most detailed data is provided by Anderson <u>et</u> <u>al</u> (1966).   They found that at 4°C, 75% of infective larvae were alive after 425 days, at 20 to 30°C maximum survival ranged from 128 to 312 days. Storage at 45 to 50°C was said to be lethal.   High proportions of larvae survived 4 day periods at -10°C.   Gupta (1961) concluded that larvae of <u>T.retortaeformis</u> which had developed at various temperatures have different survival rates under controlled conditions.   He found in his experiment that the most favourable temperature for survival was 10°C when the infective larvae had developed at about 20°C.

I. 4.   <u>ECOLOGY OF FREE-LIVING STAGES</u> :-

i)   <u>General considerations</u>:-   Strongylate eggs are passed in the faeces, and under natural conditions are subject to complex ecological influences: Climate and weather influence the microclimate of the eggs and infective larvae until they are ingested by the final host.   In nature there are a number of factors which moderate or otherwise influence the effects of weather and climate on nematodes in soil and on herbage.   For instance, soil type, particularly in relation to particle size is thought to influence the structure of moisture films in the soil and the ability of the soil to retain moisture.   Most of the work on this subject has been related to phyto-parasitic nematodes and has been reviewed by Wallace (1961, 1963).   Information on the relationship of soil type and survival or migration of larvae of zoo-parasitic nematodes is scanty. Observations on <u>Ascaris</u> eggs indicate that the eggs remain viable longer in heavy clay soils than in other soil types (Beaver, 1952).   There is evidence to suggest that strongylate infective larvae migrate most readily in sandy soils (Payne, 1923; Bruns, 1937, cited by Wallace, 1961; Lucker, 1938).

The type of herbage and the presence or absence of a herbage mat moderates the effects of changes in air temperature and humidity (Crofton, 1963).   Thus, it has been found that a cover of clover (<u>Trifolium</u> <u>repens</u> & <u>T. subterraneum</u>) favour survival of infective larvae of <u>H.contortus</u> more than a cover of ryegrass (<u>Lolium perenne</u>) and velvet grass (<u>Holcus lanatus</u>).   The

clover sward had the greatest abundance of foliage and heavier mat (Knapp, 1964). The type of herbage also affects the formation of moisture films which are necessary for larval migration (Crofton, 1954).

The size and persistance of the faecal mass are also factors to be considered and there are marked differences in effect according to the host species involved. The faecal mass deposited by cattle has a much greater moderating influence on the micro-environment than the faecal pellets produced by sheep (Christie, 1963). The influence of the faecal mass is, in turn affected by the microbial and invertebrate fauna of the soil which, together with rain, lead to the destruction of the faecal mass. It seems likely too, that these organisms may exert a direct effect on the survival of nematode eggs and larvae through what may loosely be termed 'predation' (Wallace, 1963). It is not known how quantitatively important this direct effect is.

The environment influences both the development of infective larvae and their survival and the conditions which favour one process may not favour the other. For example the optimum temperature for development is generally higher than the optimum temperature for survival (Levine, 1963). Within limits, survival time decreases with increasing temperatures. Unfortunately, most reports of critical laboratory studies on temperature effects on development of strongylate nematodes have not been correlated with field events. Many workers have also failed to differentiate between development and survival, so that it is impossible to analyze their findings adequately.

ii)   Ecology and development of free living stages:- Nematode larval development is strongly influenced by temperature, though moisture and aeration also have appreciable affects on the rate of growth (Silverman and Campbell, 1959). Although the warm period of summer is the one when egg and larval development is most rapid, it is not to be expected that hot weather will inevitably result in a more rapid expansion of population. Silverman and Campbell (1959) stated that an increase in temperature has a two-fold effect, the first is a direct acceleration of developmental rate, the second is an indirect inhibition, through desication. The net result clearly depends upon the time at which the drying process overtakes the development process. The changes in the physical conditions of faecal pellets take place more rapidly in the superficial than in the deeper parts so that there is a differential effect on the developmental rates of contained nematode eggs according to

their situation.   These conditions lead to an extension of the time limits
of appearance of third stage larvae.   However, faecal pellets are not permanent
structures and their rate of disintegration has an important influence on the
duration of this effect on larval development.

Christie (1963) suggested that disintegration of faecal pellets is due
to rain, insects, bacteria, fungi and earthworms.   In Scotland he observed the
complete disintegration of faecal pellets in as short a time as 6 days and few
pellets persisted as long as 3 months.   When faecal pellets were thoroughly
wetted by rain immediately after being dropped on the pasture, they very soon
became mushy, and rain on subsequent days produced rapid disintegration, but
when there was no rain in first 24 hours, the pellets were much more resistant
to subsequent wetting.   Faecal pellets persisted longest when dried rapidly
immediately after deposition.

There is no literature on the development of the free living stages of
Cooperia curticei under natural conditions.   Rose (1963) reported the outdoor
observations on the rate of development of the free living stages of Cooperia
oncophora at different times of the year.   During the winter the majority of
the eggs in cattle faeces deposited on pastures at Weybridge, England failed to
develop into infective larvae.   During the remainder of the year mortality was
lower and the rate of development was more rapid as the temperatures increased.
During the months of April to September the time taken for larvae to reach the
infective stage ranged from less than a week up to 3 weeks.   The greatest
numbers of infective larvae were recovered from faecal pats during the summer
despite the dry weather prevailing.   Dissemination of faeces over pastures
during summer months influences development and the precise effect is determined
principally by the weather.   During a dry spell it may limit the development,
whereas under moist conditions it may facilitate development.

Monnig (1930) suggested that the eggs of Trichostrongylus spp. can
develop to infective third stage larvae on pastures in the cold dry winter of
South Africa provided the eggs can reach the complete embryo stage.   Crofton
(1948b) reported on the rate of hatching of eggs of T.retortaeformis in Britain.
When maximum temperatures were below $50^{o}F$, there was no hatching; when the
maximum temperature was 50 to $55^{o}F$ eggs hatched after between 8 to 15 days and
when the maximum temperature was 60 to $80^{o}F$ eggs hatched in 8 days or less.
Gibson and Everett (1967) studied the development of the free living stages of

<u>T.colubriformis</u> at Weybridge, England. The observations were carried out for 3 years and in general similar results were obtained in each year. Eggs placed on the ground from November to February failed to develop (temperature range was below 10°C). In March a large proportion of the eggs disintegrated and those that survived took 8 to 12 weeks to develop to infective third stage larvae. Later in the season development was more rapid, taking 8 weeks in April, 6 weeks in May, and 1 week in June, July and August.

Dinaburg (1944) studied the effect of climate on the development of <u>Haemonchus contortus</u> eggs from sheep of Beltsville, Maryland, U.S.A. Infective larvae did not develop when monthly mean maximum temperature was below 65°F (18.3°C), regardless of rainfall but when mean maximum temperature was between 66 to 84°F (18.9 to 28.9°C), the numbers of larvae recovered under a cover of ryegrass in flower pots placed outside varied with the amount of rainfall. Dinnik and Dinnik (1958) stated that in Kenya the temperature is high enough (range 53 to 75°F) throughout the year to permit the development of <u>H.contortus</u>. However, the development of larvae was largely confined to those seasons of the year when there was adequate rainfall. Silverman and Campbell (1959) reported that under British field conditions the development of infective larvae of <u>H.contortus</u> require upwards of 2 weeks in summer and considerably more at other times of the year. They further stated that from preliminary studies on other strongyloid species, the evidence indicates that their cycle of development is even more prolonged and that their ability to endure adverse conditions is better developed.

iii)   <u>Ecology and survival of infective larvae</u>:- Infective larvae differ from the other pre-parasitic stages in that they do not feed but subsist on food reserves stored in the intestinal cells. The ability of infective larvae of strongylate nematodes to survive under various climatic conditions has been studied by a number of workers in various countries, with conflicting conclusions. Crofton (1949, 1952) demonstrated that the microclimate of the field was more important than the macroclimate and difference in management and stocking of the fields effectively altered the rate of increase of larval population.

Some of the earliest work on survial of infective larvae of <u>Cooperia curticei</u> was that of Baker (1939). He reported that infective larvae can survive for 21 months on New York State pastures. Baker gave no weather data.

Shorb (1942) stated that infective larvae of Cooperia curticei disappeared in summer after 3½, 2½ and 2 months exposure on pastures in Maryland, U.S.A. He supported the view of Leiper (1937) and Taylor (1938) that larvae of sheep nematodes die rapidly in the open, but his weather data was empirical. Dinaburg (1945) reported that no infective larvae were obtained from eggs of Cooperia spp. in Maryland when the mean maximum temperature during about 14 days fell below 42°F and mean minimum below 29°F. He concluded that low temperatures probably killed the infective larvae in a fortnight on pasture during winter. Kates (1943, 1950, 1965) studied survival of infective larvae of sheep parasites in Maryland, using parasite-free lambs to graze contaminated pasture at different times of the year. In 1943 he stated that Cooperia larvae survived less than 2½ months in summer. He later reported (1950, 1965) an optimum survival for Cooperia spp. of 2 months or more in warm moist weather; intermediate survival of 1 to 2 months in cool moist and in warm dry weather and little or no survival in cool dry weather or over winter.

Using test animals to graze pasture contaminated in summer, Goldberg and Rubin (1956) in the U.S.A. found that larvae of Cooperia punctata and Cooperia curticei remained available for 122 days and those of Cooperia oncophora for 256 days. In similar work Goldberg and Lucker (1959) reported that Cooperia punctata remained available for 63 days and Cooperia oncophora for 329 days. In these papers insufficient information is given on temperature and precipitation. Drudge et al. (1958) and Anderson et al. (1964) also reported the survival of Cooperia oncophora and C.punctata for several months over the winter period in Kentucky and Illinois, U.S.A.

Rose (1963) reported that the infective larvae of Cooperia oncophora survived on the herbage of the experimental plots in Weybridge, England, for almost 2 years and in the faeces until such time as the pats had disintegrated, which varied from 6 to 16 months. He further stated that the ability of the infective larvae to over-winter suggests a good degree of tolerance to low temperatures.

In England survival of the infective larvae of Trichostrongylus spp. on pasture was studied by Taylor (1938), Crofton (1948b) Gibson (1966) and Gibson and Everett (1967). The maximum survival varied from 39 to 50 weeks for T.colubriformis and 20 weeks for T.retortaeformis.

In Canada the survival of the infective larvae of _Trichostrongylus_ spp. was studied by Griffiths (1937), Swales (1940) and Smith and Archibald (1965); in U.S.A. by Dinaburg (1945), Kates (1943), Hawkins et al. (1944, 1947), Shorb (1942), Seghetti (1948), Goldsby and Eveleth (1947), Levine (1963) and Anderson et al. (1965); and in Australia by Durie (1961), and Donald (1967c). In general their findings were that few, if any infective larvae of _Trichostrongylu_ spp. survive the winter.

Anderson et al. (1965) are reported to have carried out detailed observations on _T.colubriformis_. Their results however, have only been published in abstract form and are difficult to interpret. Each week from April to August, Anderson and his co-workers contaminated grass plots with faeces containing eggs and recovered infective larvae each week for 10 week periods after the faeces were put out. Development was slowest in April (mean soil surface temperature $51.4^{\circ}F$) and fastest in July and August (mean soil surface temperature of $86.5^{\circ}F$ and $85.6^{\circ}F$ respectively). However, it is difficult to reconcile their relative recovery rates with the meteorological data provided because the presentation of the data appears to be erroneous in that the soil temperatures quoted are consistently higher than the mean air temperature. The authors comment that only in "early spring" (presumably April) did larvae survive 10 weeks and then only in small numbers. In general, they could not be recovered for more than 5 to 6 weeks during the summer.

Levine (1959, 1963) studied the "potential transmission periods" for _Haemonchus_ and _Trichostrongylus_ spp. in sheep, in Illinois, U.S.A. The "potential transmission period" was based on monthly mean temperature and monthly precipitation. For _Haemonchus_, the temperature range was 15 to $37^{\circ}C$ with a soil-water deficiency of not more than 2 cm and for _Trichostrongylus_ 6 to $20^{\circ}C$ with a soil-water deficiency of not more than 2 cm.

Survival of _Haemonchus contortus_ infective larvae on pasture in the U.S.A. was studied by Dickmans and Andrews (1933b) Doll and Hull (1946) Goldsby and Eveleth (1947) Seghetti (1948), Dinaburg (1944) Kates (1943, 1950), and Knapp (1964). In general all authors reported little or no survival over winter. Kates (1950) stated that a small number of infective larvae can survive the winter depending on the time of pasture contamination. When pasture was contaminated in June and July there was no winter survival, but when pasture was contaminated in August and September a few larvae survived

the winter.   Becklund and Gilmore (1955) reported that infective larvae of
H.contortus can survive 27-112 days in New Mexico depending on when pasture was
contaminated.   Swales (1940) reported no survival of H.contortus larvae in
winter in Canada.   Kauzal (1933, 1941) stated that under 20% of H.contortus
infective larvae survived $7\frac{1}{2}$ weeks in Australia when minimum temperature was
40-50°F, maximum temperature 60-65°F and rainfall 2-3 inches.

iv)   Migration of infective larvae:-   Several workers have investigated the
effect of environmental conditions on the movement and behaviour of infective
larvae on herbage.   The main factors affecting the migration of infective
larvae are moisture, temperature and light.

Effect of moisture:-   Larval stages are dependant upon a moist
environment for normal activity and survival.   Adequate rainfall and high
atmospheric humidity maintain adequate moisture surrounding nematode larvae in
soil.   Consequently, in order for free larvae to become available to grazing
animals, they must migrate to stems and leaves of pasture vegetations in thin
films of water when suitable temperature and moisture conditions prevail.
Crofton (1954b) demonstrated that larvae followed existing water film pathways
in soil and that vertical migration of larvae on to the pasture plants was due
to random movements in all directions independent of gravity.   He stated that
vertical migration may be described without reference to geotropism or
undescribed special receptors in the larvae.   Rogers (1940) stated that
moisture in the grass was found to favour larval migration.   More than 0.12 ml
of water per square cm of soil surface, however, retarded larval movements.
Increasing quantities of moisture in the soil up to 85% saturation assisted
larvae to move on the grass.   Above this value larval migration was retarded.
It was found that presence of water on the soil surface tended to lessen the
ability of larvae to move on the grass.   Rees (1950) stated that high humidity
(continuous precipitation) favours migration and low humidity (absence of rain)
inhibits vertical migration on the grass.

Effect of temperature:-   The effect of temperature on larval migration
has been studied by Rogers (1940), Kauzal (1941) and Crofton (1948a).   Rogers
(1940) reported that infective larvae of Trichostrongylus, Haemonchus,
Ostertagia and Chabertia spp. were found to move up the grass in largest
numbers at 5.°C and 45°C.   Kauzal (1941) stated that larvae survive for
longer periods in soil than on herbage and contaminate the herbage only when

there is suitable temperature and moisture.   Crofton (1948a) stated that
during periods when the maximum temperature never exceeded 55°F, little or no
migration of larvae occurred and that at temperatures above 55°F migration
increased.   Rise in temperature was however associated with increase in the
rate of evaporation and high rates of evaporation reduced the number of larvae
migrating on the grass blades.

Effect of light:-   Infective larvae are said to be phototropic and are
attracted by light of moderate intensity, but repelled by strong light.
During the day highest numbers of larvae have been recovered from the grass
early in the morning with a second but smaller peak of recovery occuring in the
evening (Rogers, 1940; Rees, 1950).   Rees (1950) stated that provided
temperature and humidity are favourable, light intensity is of great importance
in determining the time of the day at which the maximum numbers of larvae are
on grass blades.   She also stated that the evening peak was not as well
defined as that in the morning, even though the light intensity, temperature
and humidity was similar at both times.   The time of the morning maximum
became progressively earlier passing from winter to summer and the time of the
evening maximum progressively later.   The reverse was true in the second half
of the year.   She further stated that infective larvae respond more readily
to light of comparatively low intensity, a condition obtaining shortly after
sunrise and before sunset.   The light intensities measured at the time of
maximum recovery were low, varying from 15 to 560 foot candles, the majority
being below 200 foot candles.

Crofton (1948b) stated that larvae normally occur on the lower portion
of the herbage, near the base of the blades, where there is least climatic
change.   The type and density of the herbage modifies the effect of climatic
factors which influence the extent of their movement.

The work of Silangwa and Todd (1964) has shown that fewer larvae may
migrate onto the herbage than was widely believed.   They demonstrated that
only 2 to 3% of sheep trichostrongyle larvae present at the base of the grass
migrated up the blades and of these about $\frac{2}{3}$ reached the first inch above the
soil and a mere 0.8% (of 2 to 3%) reached more than 5 inches.

There is no literature on the migration of the infective larvae of
Cooperia curticei.   Rose (1963) studied the rate of migration of Cooperia

oncophora larvae from the cattle faecal pats on to the herbage at Weybridge,
England.   In the first series of observations, started in June, larvae were
recovered from the herbage until August and the numbers recovered reached a
peak in November.   In the second series, started in July of the following year,
larvae were recovered from the herbage within a few days of becoming infective
and recovery of increasing numbers was made throughout August to reach a peak
in September.   He stated that the differences in two series of experiments
were due to changed weather conditions.   In the first series of experiments,
the summer was dry and sunny with little rain and whole pat dried out completely
in a few days;   during the second series of experiments the summer was quite
different, rain fell frequently and faecal pats remained soft and moist for most
of the time.   He further stated that the rate of migration of larvae from
cattle faeces on to the herbage depends on the condition of the faecal pats.
The infective larvae were virtually unable to migrate from the hard dry pats
but migrated readily from those which were soft and moist.   Rose found that at
all times of the year most of the larvae were in the lower levels of the herbage
i.e. less than 2 inches above soil level but that at times large numbers could
be recovered more than 2 inches above the soil.

I. 5.   SEASONAL VARIATIONS OF WORM BURDENS OF COOPERIA CURTICEI IN SHEEP :-

The factors which influence the rate and extent of infection of sheep
with gastro-intestinal nematodes are complex.   Almost every animal of the flock
harbours worms and the environment is contaminated continuously.   The factors
which determine the numbers of helminths in individuals of a group of animals at
any point in time, are complex.   They include the micro-climate for the
development, survival and migration of larvae, the physiological condition and
immune status of the host and the rate of uptake of infective larvae.   This
last is in turn affected by grazing patterns and intensity.   There are seasonal
variations in worm burdens and consequent disease patterns in various parts of
the world which depend principally on climate.   Within similar geographic and
climatic regions the seasonal patterns of fluctuations of parasite populations
may be predicted.

Tetley (1941b, 1949, 1959a, b) described the epidemiology of low plane
nematode infection in sheep in Manawatu district, New Zealand.   He noted
the seasonal incidence (temperate, Southern hemisphere) of Cooperia spp. by
slaughtering the animals at two monthly intervals and by faecal egg counts.

He stated that infection of <u>Cooperia</u> <u>curticei</u> was acquired throughout the year and was present in all age groups. The nematode appeared to accumulate over a long period in the animal, so that it was not until the second year of the sheep's life that worm numbers were at a maximum. Following the elimination of the greater part of the early infection, low numbers of worms persisted. It was found that in spring large numbers of <u>C.curticei</u> existed in 1 year-old sheep while this species was starting to accumulate in lambs. In this situation the year-old sheep probably influenced the intensity of infection in the lambs.

A flock of spring-born lambs, were reared free of parasites. One group of these lambs was exposed to infection in the field in late summer, one group in autumn and one in winter. Larger populations of <u>C.curticei</u> were found in the lambs exposed in late summer than in the other two groups. The build up of the larval population in the summer, & the subsequent high rate of infection was correlated with rainfall. However, there was evidence that the phenomenon of host resistance came into operation to curtail rapidly the intensity of parasitism. Small populations found in sheep grazed in the late autumn and winter were associated with dry autumn conditions and with the onset of frosts. Brunsdon (1963b) also reported that infective larvae of <u>Cooperia</u> <u>curticei</u> are most numerous on pasture in autumn and early winter in the area close to Wallaceville, New Zealand.

Morgan <u>et al</u>. (1951) described the seasonal variation in the worm burden of Scottish hill sheep and noted that <u>Cooperia</u> <u>curticei</u> increased in numbers in the spring and tended to persist throughout the summer. This increase was due to larvae which had overwintered, being ingested at that time, for there was no evidence that any were lying dormant throughout the winter in the mucous membrane of the small intestine, and being involved in the spring rise of the ewes.

The incidence of helminths in sheep in winter rainfall region of Australia were studied by Gordon (1953, 1958a) and Forsyth (1953). They noted that worm burdens tended to show an increase in the late winter and early spring and a decline in late spring and early summer. Heaviest infections were generally recorded in March to April before the autumn seasonal rains bring fresh growth to pastures. Durie (1961) reported the seasonal fluctuations in population of nematode larvae in cattle in Australia and he recovered the larvae of <u>Cooperia</u> <u>spp</u>. at all seasons.

Crofton (1954a, c, 1955) described the seasonal incidence of helminth parasites of sheep in Great Britain and noted that Cooperia spp. were present in small numbers throughout the year but their number increased greatly in late summer (June and July) and autumn.

Later Crofton (1957) suggested that the pattern of seasonal incidence is not necessarily one that can be attributed to seasonal changes of climate but rather was a consequence of the life history pattern of individual species, being directly connected with the rate of egg production and the time required for the development of each generation.   He stated that the minimum time required for the development of the infective stage of Cooperia curticei under the most favourable field conditions is five days, and 15 days of prepatent period, make the generation interval 20 days.   A weeks delay in development of Cooperia spp. increases the generation time by less than 35%, similarly 14 and 28 days delay in development increases the generation time to 70% and 140% respectively.   He postulates that delays in generation time will have greatest effect on the rate of increase of these species which are the most prolific egg layers and have the shortest generation time and least effect on the less prolific species with the longer generation time.   If the egg laying capacity of H.contortus is represented by 100, the corresponding figures for other species are Trichostrongylus spp. 20;   Cooperia curticei 20, and Ostertagia spp. 55.   As Trichostrongylus and Cooperia spp. have short generation times with relatively low fecundities, their rate of increase is much slower than that of H.contortus and Ostertagia spp.   This explains the occurrence of peak numbers of C.curticei larvae on pasture late in the year.

As can be seen there is little factual information on the biology of Cooperia curticei.   It could be expected that observations on other strongylate nematodes could be extrapolated, if only in general terms, to Cooperia curticei. However, there is even controversy and doubt about the basic facts of the biology of these nematodes, particularly in terms of larval ecology.   This is attributable to some extent to the inadequately controlled experiments carried out and the qualitative nature of many of the observations.   Differences between species of strongylate nematode and the existence of different strains within species make it impossible to extrapolate from detailed data on one nematode isolate to another with any degree of certainty.   This situation is aggravated by the diversity of technique used by different workers.   It is surprising to find that, apparently, no-one has attempted a detailed study of

the biology of the free-living stages of a single isolate under laboratory and field conditions. It would be difficult for any one person to encompass every aspect of the larval ecology but enough is known to indicate the most important lines of investigation. Without a complete set of observations on one isolate it is surely difficult to establish realistic generalisations and hypotheses which may apply to other similar organisms.

Chapter II. GENERAL MATERIALS AND METHODS

II. I. GEOGRAPHICAL DEFINITION OF MANAWATU:-

Observations on the development and survival of C.curticei have been made outdoors under natural conditions, so it is pertinent to describe briefly the geographical definition of the area.

Massey University is situated in the district of Manawatu which is regarded as that portion of the basin of the Manawatu river which is contained by Southern Oroua, Kairanga and Manawatu Counties. It has an area of six hundred and fifty one square miles most of which is alluvial less than 100 feet above sea level. The soils of the district range from clayey silts to sandy loams and is devoted mainly to sheep farming and dairying. The climate is temperate and although the seasons are well defined, it is not characterised by severe extremes. Rainfall is moderate and evenly distributed throughout the year. In the summer and autumn it is insufficient to prevent parching of grassland. Summer temperatures are not excessive and in the shade, they seldom rise above $80^{O}F$ ($26.7^{O}C$). Frost may be recorded over eight to ten months of the year, and winter minimum temperature on grass seldom goes below $20^{O}F$ ($-6.7^{O}C$).

II. 2. THE ESTABLISHMENT AND MAINTENANCE OF A PURE CULTURE OF COOPERIA CURTICEI

Specimens of Cooperia curticei were collected from the small intestine of sheep slaughtered in the Post mortem room of Animal Health Department, Massey University and from the Abattoir (Freezing Works), Longburn, Palmerston North. The female nematodes were carefully washed in physiological saline solution until they were entirely free from the debris and were then cut up in a petri-dish with a fine scalpel. The eggs thus liberated were cultured in a mixture of sterile sheep faeces and vermiculite. The mixture was placed in small wide mouthed bottles with lids on and was left in the incubator at $27^{O}C$ temperature for 8 days. The infective larvae that developed from the eggs were then removed from the culture using a Baermann technique. The infective larvae suspended in water were administered per os by means of a plastic pipette to a parasite-free sheep. This process of giving infection was repeated three times in the same sheep until a patent infection was developed in the host. After infection this donor sheep was kept as a source of material

for the infection of other sheep.   At least two donors were always kept and
faecal pellets were collected by means of faecal bags.

II. 3.   TECHNIQUE USED FOR EGG COUNTS:-

Two gram faecal pellets were weighed on 'Harvard trip' balance and were
transferred to a wire sieve in a porcelain cup 9 cm in diameter and 3 cm in
depth.   28 ml of saturated salt solution was added to the faecal pellets.   The
faecal pellets while immersed in the salt solution were broken on the sieve with
a plastic spoon and the coarse fibres collected in the sieve were removed.   The
suspension was thoroughly mixed with a Pasteur pipette, McMaster slides were
filled.   Eggs on both sides of the McMaster slide were counted microscopically
and total numbers of egg per gram (e.p.g.) were estimated by multiplying the
numbers of eggs counted by 50.   The technique will be referred to as the
modified McMaster method.

II. 4.   ROUTINE CULTURE OF FAECES:-

The faecal pellets were broken up in a plastic bag and were transferred
to a large tray (45 x 30 x 3 cm).   Then vermiculite and water was added.   The
material was mixed thoroughly with the hands until the moisture content was
judged satisfactory and was transferred to 1 or 2 pint screw cap glass jars.
Lids were loosely screwed down on the jars to control water evaporation.   The
material was incubated for 8 days at $27^{o}C$ in a 'Qualtex' water jacketed incubator
The infective larvae were recovered by means of a Baermann technique and were
stored in tap water at $10^{o}C$ in a 'Gallenkamp' cooled incubator with a
temperature control of ± 1-$2^{o}C$.

II. 5.   TECHNIQUE USED FOR LARVAL COUNTS

The following technique was primarily used in the estimation of larval
numbers for administration to experimental animals.   The larval suspension
was shaken thoroughly in a wide mouthed glass bottle until there were no clumps
of larvae.   With a one ml graduated pipette 1 ml of the larval suspension was
removed and diluted in 9 ml of tap water and 0.1 ml of larval suspension was
similarly diluted in 9.9 ml of tap water.   Five samples of 0.1 ml and 1 ml
respectively were taken from each dilution and the larvae present in these
samples counted using a dissecting microscope.   The mean of the ten counts was

then calculated and total numbers of larvae per ml of original suspension estimated.

II. 6. PARASITE-FREE SHEEP

The sheep used in all the experiments described in this thesis were Romney x Perendale or Romney x Romney. Most were removed from their mothers when 24 hours old and reared artificially on cows milk. They were gradually weaned onto a mixture of lucerne chaff and a concentrate feed. They were subsequently maintained on this concentrate feed. Some of the lambs were born of ewes brought indoors and treated with pyrantel tartrate as an anthelmintic. The ewes were removed when the lambs were approximately 8 weeks old and the lambs were then maintained on the same concentrate food as the other sheep.

Precautions were taken throughout to avoid the introduction or transmission of helminths in the sheep. Initially, the sheep were kept on concrete which was cleaned daily and later on a slatted floor. At no time was any helminth infection found in any of the sheep in the house. Sheep under experiment were transferred to another building and kept in individual cages with slatted floors.

Composition of Concentrate feed.

Lucerne meal	60%
Barley meal	20%
Wheat husk	20%

Vitamin A and D_3, a standard mineral mixture and a special supplement of anhydrous molybdenum sulphate (1.8 gm/1000 lb) and sodium sulphate (2 kg/1000 lb) were added. The molybdenum and sodium sulphates were necessary to avoid the copper poisoning which otherwise occurs with this diet.

Chapter III   STUDY ON DEVELOPMENT AND SURVIVAL OF THE FREE LIVING
                STAGES OF COOPERIA CURTICEI UNDER LABORATORY CONDITIONS

Introduction: Adult Cooperia curticei occur in the small intestine of sheep
and related ruminants, and eggs are passed in the faeces.   Under suitable
conditions of temperature and moisture, they develop to first, second and third
stage larvae, which may migrate onto the vegetation and subsequently be ingested
by grazing animals.   Two main processes are thus involved, development from the
egg to the infective larva, and survival thereafter.   Within limits, the rate
of development increases with increasing temperature, whereas survival time
decreases with increasing temperatures.   But nematode species differ in their
response to environmental conditions.   It is desirable, therefore, to determine
the factors affecting development and survival of the free living stages of
Cooperia curticei under laboratory conditions.   Amongst other things this is
necessary for the understanding of the performance of the nematode under field,
i.e. natural conditions.   From the time the eggs are deposited on the ground
and the infective larva is ingested by a host animal the free-living stages are
exposed to complex and variable environmental conditions.   The microenviron-
ment of the egg or larva is affected by changes, chiefly of a meteorological
nature, in the macroenvironment.   Under natural conditions those environmental
factors such as temperature, humidity and precipitation are subject to long and
short term fluctuations and are related to one another in a complex fashion.
In order to examine the effect of single factors in the environment, laboratory
studies are necessary where environmental variations are minimised, except in
respect of the factor being studied.

Experiments were carried out to investigate the effect of temperature and
of moisture on the development and survival of the preparasitic stages.

III. I. DEVELOPMENTAL STUDY OF COOPERIA CURTICEI AT DIFFERENT TEMPERATURES:-

i)   Materials and methods:-   Pelleted faeces were collected from sheep with
a pure infection of Cooperia curticei (refer II.2).   For embryonic studies
faecal pellets were obtained from the rectum of the donor animals and for larval
studies faecal pellets were allowed to accumulate in a faecal bag for 16 to 18
hours (from about 4.00 p.m. in the evening to 8.00 a.m. in the morning).   Egg
counts estimated by a modified McMaster method ranged from 700 to 1800 eggs per
gram.   Faecal pellets were broken in a plastic bag and were transferred to a
tray.   To each 100 grams of broken faecal pellets, 60 to 65 grams of

vermiculite and 170 to 180 ml of water were added and mixed thoroughly.
Cultures prepared in this way will be referred to as having 'normal' moisture
content.   Culture was carried out in a pint glass jar, $\frac{1}{2}$ to $\frac{2}{3}$ filled with
loosely packed faeces.   The jars were covered with lids to control loss of
water through evaporation.   Each collection of faeces was divided into equal
parts for comparison of development at different temperatures.   Observations
were made on cultures kept at constant temperatures of 6°C (42.8°F), 10°C (50°F),
15°C (59°F), 20°C (68°F), 27°C (80.6°F), 32°C (89.6°F), 37°C (98.6°F) and 42°C
(107.6°F).   Culture samples were taken as frequently as conditions warranted.
Three to 5 replicate samples were cultured at each temperature.

For the embryonic study eggs were recovered by a concentration technique.
The faecal sample was suspended in water and centrifuged for 3 minutes at 1000
rpm. and the supernatant was removed.   The material was then mixed with satur-
ated sugar solution and again centrifuged for 3 minutes.   The upper layer of
the sugar solution containing the eggs was collected on a coverslip and was
examined microscopically.   For the recovery of larval stages faeces were placed
on a wire screen in a glass funnel filled with water at 37°C and closed with a
clipped rubber tube.   This was left for 2 to 3 hours and the larvae which had
gravitated to the bottom of the funnel were run off.   Microscopic observations
were made on larvae killed and stained with aqueous iodine solution.   For each
set of observations 100 individuals were classified according to developmental
stages.   The classification categories comprised three egg and three larval
stages; eggs in morula and gastrula (E_1), tadpole or early vermiform (E_2), late
vermiform to prehatch larva (E_3), first stage larva (L_1), second stage larva
(L_2), and third stage larva (L_3).

Observations on the percentage recovery of infective larvae were made at
10, 27 and 37°C.   Faecal pellets (100 grams) were cultured with vermiculite and
'normal' moisture, as described above.   Egg counts were repeated 5 times by the
modified McMaster method and taking the mean count the total number of eggs per
culture was estimated.   The material was then incubated for 40 days at 10°C, 8
days at 27°C and 5 days at 37°C.   The time of cultivation chosen were based on
observations of the time taken for 90% or more of the larval population to reach
the infective stage at these temperatures.   To examine the effect of leaving
the faecal pellets intact on the percentage larval recovery, 100 gram samples of
faeces were cultured.   The pellets were sprayed with distilled water before the
jars were closed to ensure continual saturation of the atmosphere in the jars
with water.   Culturing was only carried out at 27°C for eight days.   Larvae

were obtained by the Baermann technique and total numbers of infective larvae
from each culture were estimated by counting the larvae from 0.5 to 1.0 ml of the
larval suspension under a dissecting microscope.   Volumes of suspension examined
were measured in a one ml graduated pipette.   Five replicates were set up at
each temperature.

ii)   Results:-   It was observed that eggs of Cooperia curticei in the same
culture may develop at different rates and may fail to develop or die at any
stage from unknown causes.   Unembryonated eggs cultured at 6°C (42.8°F) failed
to develop beyond the gastrula stage.   All eggs were found to be disintegrated
at 55 days.   Hatching did not occur unless the eggs were maintained at a temp-
erature of 10°C (50°F) or above.   At a temperature of 42°C (107.6°F) unembryo-
nated eggs died very rapidly and failed to develop.

Newly formed first stage larvae were observed to emerge from the egg
through a hole that had been produced by their sharp pointed tail and not by
prodding through with their heads as described by Veglia (1915).   Hatching was
thus similar to that described by Silverman and Campbell (1959) for H.contortus.

Because of variation in the rate of embryonation, the data on develop-
ment rates have been presented as minimum and T90 times.   The term 'minimum'
refers only to the time when a very small proportion of any given population
(less than 5%) and 'T90' refers to the time when 90% or more of the total
population had completed development of that stage.

The relation between temperature and the rate of development of
Cooperia curticei from egg to the third stage infective larvae is summarized in
Table 3.   At a constant temperature of 10°C (50°F), 15°C (59°F), 20°C (68°F),
27°C (80.6°F), 32°C (89.6°F) and 37°C (98.6°F) with 'normal' moisture, freshly
passed Cooperia curticei eggs require about 22, 12, 6, 5, 4, 3, and 2.5 days
respectively, before the first of them reach the third larval stage.   For 90%
or more to become third stage infective larvae, it requires 40, 23, 13, 8, 6,
and 5 days respectively (see fig. 2).   These data are mean figures derived
from at least three replicates at each temperature.   In the studies of
embryonic development on the egg there were no detectable differences between
replicates.   During larvae development after hatching differences between
replicates ranged from up to 6 hours at 20°C and above, to up-to 12 hours at
temperatures below 20°C.

Table 3

The relation between various constant temperatures and
the rate of development of _Cooperia curticei_.

Temperature:	10°C 50°F	15°C 59°F	20°C 68°F	27°C 80.6°F	32°C 89.6°F	37°C 98.6°F

Time (days) required for development from
blastomere stage

	10°C	15°C	20°C	27°C	32°C	37°C
E_1, Gastrula						
Minimum	1	0.25	0.25	0.25	0.25	< 0.25
T90	2	0.75	0.50	0.50	< 0.50	< 0.50
E_2, Tadpole						
Minimum	2	0.75	0.50	0.25	0.25	0.25
T90	4	2	1	0.75	0.50	< 0.50
E_3, Prehatch						
Minimum	4	2	1	0.50	0.50	< 0.50
T90	7	3	1.75	1	< 1	0.75
L_1, First stage larvae						
Minimum	7	3	1.50	1	0.75	0.75
T90	10	5.5	3	1.75	1.50	1.25
L_2, Second stage larvae						
Minimum	10	5	2.75	1.5	1.25	1
T90	22	12	6.75	4.25	3.25	2.75
L_3, Third stage larvae						
Minimum	22	12	6.5	4	3	2.50
T90	40	23	13	8	6	5

Fig. 2
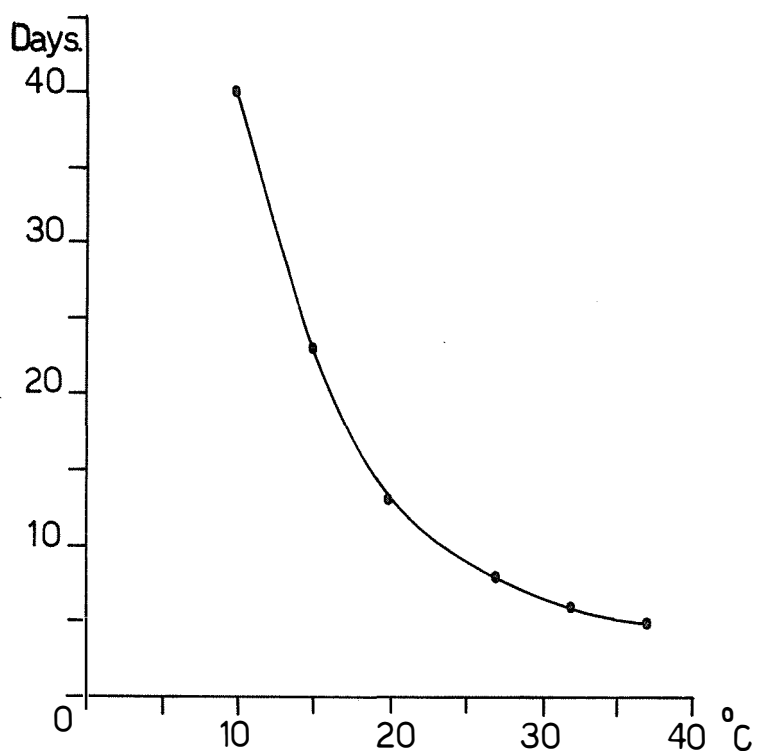
Fig. 3
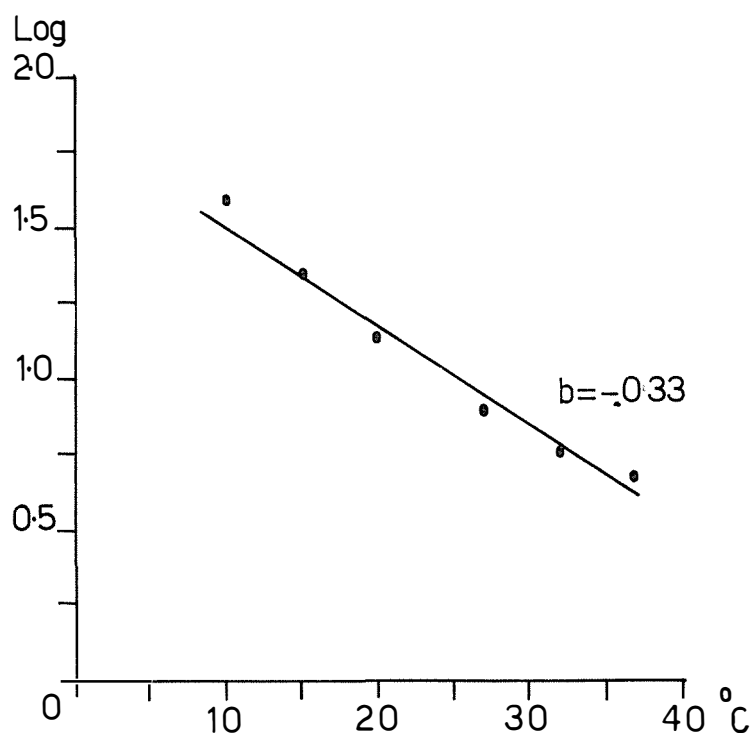
Fig. 2: Days development to infective stage (T90) at controlled temperatures.

Fig. 3: Regression of development time (log days) on temperature.

To examine further the relationship between temperature and rate of development the log time of development was plotted against the temperature. A straight line was obtained (Fig. 3), the slope of which is -0.033 (Appendix I) and is constant throughout the temperature range of 10 to 37°C. The slope indicates that the development time to the infective stage changes by 0.033 log units of time for each degree centigrade change in temperature. The observed development time at 10°C appeared slightly longer than would be expected from the calculated regression line but this difference is not statistically significant.

Table 4 show the total percentage time taken for development of first, second and third infective larval stage at T90 at different temperatures. An analysis of variance was carried out on the data given in Table 4 after arcsin transformation of the percentage to stabilize the variance. The analysis is shown in Appendix 2. The variance "Between temperatures" is not significantly greater than the error variance. The result suggest that each of the larval stages occupies the same proportion of the development time throughout the temperature range of 10 to 37°C. This also indicates that the slope of a regression line of development time on temperature will be the same for all the larval stages.

Table 5 shows the 95% confidence limits for development of the first stage larvae which occupies 22.50 to 25.35% of the time, second stage 28.40 to 30.70% and third stage infective larvae occupies 45.00 to 48.00% of the time at T90 stage.

Percentage recovery of infective larvae at different temperatures is given in Table 6. Maximum recovery of 87.6 to 95.1% (mean 92.16%)of infective larvae was observed at 27°C and minimum 5.1 to 12.0% (mean 7.14%) at 37°C. Percentage recovery at 10°C ranged from 31.9 to 47.5% (mean 40.22%). Percentage recovery of infective larvae from intact faecal pellets cultured at 27°C was markedly lower than after routine culture of faeces with vermiculite and 'normal' moisture at 27°C, and ranged from 28.0 to 47.2 per cent (mean 35.24%).

Table 4

Total percentage time for development of first,
second and third stage infective larvae to T90
at different temperatures.

Temperature	10°C	15°C	20°C	27°C	32°C	37°C
	50°F	59°F	68°F	80.6°F	89.6°F	98.6°F

Percentage of the total time for development

	10°C	15°C	20°C	27°C	32°C	37°C
L_1, First stage larvae	25.0	23.9	23.0	21.8	25.0	25.0
L_2, Second stage larvae	30.0	28.2	28.9	31.3	29.1	30.0
L_3, Third stage larvae	45.0	47.9	48.1	46.9	45.9	45.0

Table 5

Confidence limit of 95% on the *percentage of
the total time taken in each larval stage of
development at T90

Stage of Development	Total of all temperature	Mean percentage	Variance	Standard Deviation	Confidence Limit Lower and Upper	True Limits Lower and Upper
First stage larvae	175.76	29.29	0.80	0.9	28.34 to 30.24	22.50% to 25.35%
Second stage larvae	197.69	32.94	0.46	0.7	32.20 to 33.64	28.40% to 30.70%
Third stage larvae	257.84	42.97	0.63	0.8	42.12 to 43.82	45.00% to 48.00%

*   After arcsin transformation.

Table 6

Percentage recovery of infective larvae of Cooperia
curticei at different temperatures.

Temperatures:	10°C	27°C	37°C
	50°F	80.6°F	98.6°F

Days of incubation	40	8	5

Sample number	Percentage recovery of infective larvae		
1	47.5	94.0	6.1
2	42.6	95.1	7.3
3	34.0	93.4	5.1
4	31.9	90.7	12.0
5	45.1	87.6	5.2
Mean	40.22	92.16	7.14

III. 2. <u>EFFECT OF EXCESS MOISTURE ON DEVELOPMENT OF THE EGGS</u>
<u>AND PREINFECTIVE LARVAE OF COOPERIA CURTICEI AT 27°C</u>:

When eggs and preinfective larvae of strongylate nematodes are cultured
in the presence of excess water interference in aeration occurs. This experi-
ment was designed to examine the development of the eggs and preinfective larvae
of <u>Cooperia curticei</u>, when cultured in excess water.

i)   <u>Materials and methods</u>:-

<u>Eggs</u>:- Freshly obtained faecal pellets containing eggs of <u>Cooperia</u>
<u>curticei</u> were broken up in water. The suspension was sieved (60 meshes per
inch) and centrifuged to deposit the eggs. The supernatant was removed and the
deposit was then resuspended in water and centrifuged. This procedure was
repeated four times. The eggs collected as the final sediment were suspended
in water and this comprised the stock suspension. The number of eggs in the
stock suspension was estimated by a modified McMaster egg count substituting
2ml of suspension for 2 grams faeces. An estimated 250 eggs per ml were
present.

From the stock suspension three different types of culture were set up.

1.   Stock suspension containing eggs was placed in pint glass jars in 1 cm
        depth of water.
2.   The stock suspension of eggs was mixed with vermiculite to give a creamy
        (semi-liquid) consistency and placed in pint jars.
3.   Stock suspension was passed through a filter paper to retain the eggs
        and the wet filter paper and eggs placed in a closed petri dish.

Faecal pellets (700 e.p.g.) were placed in water in jars so that they
were just submerged. Other pellets were just lightly sprayed with water and
similarly placed in glass jars to provide 'normal' conditions for larval
development.

All cultures were incubated at 27°C and material was examined as
frequently as conditions warranted. Eggs were recovered by a flotation
technique using saturated sugar solution. Larvae were obtained by the
Baermann technique (see page 27).

Preinfective larvae:-   First and second stage larvae were obtained from 1 and 3 day old normal cultures at 27°C.   The larvae were free from other stages of development and were kept in 1 cm depth of water in wide mouth glass bottles.

Control cultures were set up by mixing vermiculite with suspensions of first or second stage larvae until the moisture content was judged the same as that used in 'normal' routine cultures.   Duplicate cultures were set up for both larval stages and development was examined at 27°C.   Larvae were examined microscopically and daily observations were carried out until all larvae were dead.

ii)   Results:-   The effect of excess water on the development of the egg is summarised in Table 7.   It can be seen that embryonation was considerably inhibited.   Few eggs reached the tadpole stage and none developed further. Normal faecal pellets or eggs on filter papers developed to the third infective larval stage in 8 days.   Eggs with vermiculite in semi-liquid cultures developed to the third larval stage in 6 to 15 days but only in small numbers.

No further development of the first stage larvae was observed when kept in water at 27°C.   All larvae were dead after 7 days incubation.   Controls kept in vermiculite with 'normal' moisture developed to the second larval stage in 3 to 5 days and to the third larval stage after 6 to 9 days.

The development of the second stage larvae when kept in water at 27°C is shown in Table 8.   For the first 2 to 3 days most of these larvae were active, later they became lethargic.   After 7 to 11 days of incubation only 2 to 5 per cent had reached the third larval stage and the others had died.   In controls, cultured with vermiculite and 'normal' moisture content, 90 to 95% developed to the third larval stage in 5 days.

Table 7

Effect of excess moisture on the rate of embyonation of Cooperia curticei eggs at $27^{O}C$ ($80.6^{O}F$).

T i m e   (d a y s)   r e q u i r e d   f o r   d e v e l o p m e n t

	1 Eggs Suspended in water	2 Eggs in faecal pellets submerged in water	3 Eggs with vermiculite (semi-liquid consistency)	4 Eggs in faecal pellets (normal culture)	5 Eggs on filter papers (normal moisture)
Eggs morula stage	0.5	0.5	0.5	0.25	0.25
Eggs gastrula stage	1 to 9	1 to 9	1 to 9	0.25 to 0.50	0.25 to 0.50
Eggs tadpole stage	1 to 15	1 to 15	1 to 9	0.50 to 1	0.50 to 1
Eggs prehatch stage	-	-	1 to 6	1 to 2	1 to 2
First stage larvae	-	-	6 to 7	1 to 3	1 to 3
Second stage larvae	-	-	6 to 9	3 to 5	3 to 5
Third stage larvae	-	-	6 to 15	4 to 8	4 to 8

Table 8

Effect of excess water on the development
of the Second stage larvae of Cooperia
curticei at 27°C (80.6°F).

Period (days) of incubation at 27°C (80.6°F)	Larvae in excess water	Controls
1	All L_2 active	All L_2 active
3 to 5	Most L_2 lethargic and no L_3 stage	90 to 95% developed to L_3
7	2% developed to L_3	Do.
11	5% developed to L_3. All others dead.	Do.

L_2 =   Second stage larvae.

L_3 =   Third stage larvae.

III. 3. EFFECT OF MOISTURE ON SURVIVAL OF THE EGGS OF COOPERIA CURTICEI AT DIFFERENT TEMPERATURES:

When faecal pellets were saturated with water, the contained eggs became inhibited and this appears to be due to interference with aeration (refer Chapter III.2). The present experiment was designed to determine the ability of the eggs of Cooperia curticei to survive when kept in water at $5^{O}C$ and $27^{O}C$.

i) Materials and methods:- Faeces were obtained from the donor sheep. Faecal pellets were broken up in water, sieved (60 meshes per inch) and the material was resuspended and centrifuged 6 times with clean water. Eggs were then suspended in tap water and were kept in wide mouth pint glass jars in a depth of 5 cm of water at $5^{O}C$ ($41^{O}F$) and $27^{O}C$ ($80.6^{O}F$). One ml of material contained approximately 100 eggs as estimated by the modified McMaster method, (2 ml of material was substituted for 2 grams of faecal pellets). Faecal pellets (700 e.p.g.) were also kept at $5^{O}C$ ($41^{O}F$). Eggs were in the morula stage at the start of the experiment.

The survival of the eggs was judged on their ability to resume development when transferred to a more favourable environment. Samples of eggs in water were taken at 5 day intervals and 2 ml of the suspension was placed on a filter paper and incubated at $27^{O}C$ in a closed petri dish. At the same time 2 gram of the faecal pellets stored at $5^{O}C$ were mixed with vermiculite and water to give a 'normal' mixture and cultured in wide mouthed glass bottles at $27^{O}C$.

Culture was continued for 4 to 5 days and then attempts were made to recover larvae using the Baermann technique. Larvae were examined microscopically.

At the same time as eggs in water and faeces were taken for continuing culture, eggs were also recovered by sugar flotation and examined microscopically.

At the commencement of the whole experiment a sample of faeces was cultured under 'normal' conditions as a check on the viability of the eggs used.

ii)   Results:-   Culture under 'normal' conditions of a sample of faecal pellets used in the experiment showed that eggs were viable and the majority of them reached the infective stage after 8 days.

Eggs stored in water died and disintegrated with time.   As the eggs degenerated the cells of the embryo became indistinct leaving an amorphous mass which became progressively shrunken;   egg shells became thinner and finally disappeared.   At $27^{O}C$ over 90% of the eggs were affected and contained shrunken embryonic remains after 25 days incubation.   At $5^{O}C$ approximately 20% were affected after 25 days incubation, 35% after 35 days, 50% after 45 days, 75% after 55 days and more than 90% after 65 days.   Similar changes in the eggs were seen in faecal pellets maintained at $5^{O}C$.   In these approximately 50% were distorted after 25 days incubation, 80% after 45 days and over 90% after 50 days.

The results of the observations on the ability of eggs to resume development after storage are summarized in Table 9.   Even after 5 days storage a comparatively small proportion of eggs were capable of resuming development. This and the subsequent recoveries parallel the disintegration of the eggs with storage.   There was a noticeably longer survival of a small number of eggs in water at $5^{O}C$ than at $27^{O}C$.   Even in faecal pellets the survival of eggs was distinctly limited and no larvae could be recovered after the pellets had been stored for 50 days at $5^{O}C$.

Table 9

Percentage survival of Cooperia curticei eggs in
water and in faecal pellets at
different temperatures.

Period in days	Survival in water		Survival in faecal pellets
	27°C (80.6°F) %	5°C (41°F) %	5°C (41°F) %
5	13	27	16
10	10	20	14
15	5	15	12
20	1	8	8.7
25	0.3	7	2.7
30	0	4	1.2
35	—	2.5	0.6
40	—	2	0.4
45	—	1	0.01
50	—	0.6	0
55	—	0.4	—
60	—	0.2	—
65	—	0.1	—
70	—	0	—

III. 4. <u>SURVIVAL OF THE FREE LIVING STAGES OF COOPERIA CURTICEI</u>
<u>AT DIFFERENT TEMPERATURES</u>:

The survival of the free living stages of gastro-intestinal nematodes in
nature, depends on complex ecological phenomena but temperature and moisture are
important. This experiment was designed to test the effect of various constant
temperatures on the survival time of the free living stages of <u>Cooperia curticei</u>
in water.

i) <u>Materials and methods</u>:- Three different free living stages were
studied, first, second, and third stage infective larvae. These were obtained
by incubating the faecal material from the donor sheep with a monospecific
infestation of <u>Cooperia curticei</u> for predetermined periods of time at $27^{\circ}C$.
First stage larvae were recovered after one day, second stage larvae after 3
days and infective larvae after 8 days of incubation. At these times larvae
could be secured free of the other stages and overlapping in development time
was avoided. Observations were recorded separately for each group of free
living stages.

The effect of storage temperatures on first, second and third stage
infective larvae was determined by placing 200 freshly harvested larvae each in
3 ml tap water in screw-cap 'Universal' glass bottles (capacity 28 ml). The
depth of the water was one cm which allowed observations at each time as larval
development is inhibited in the water (refer chapter III.2). Five replicate
samples were kept at temperatures of $5^{\circ}C$ $(41^{\circ}F)$; $8^{\circ}C$ $(46.4^{\circ}F)$; $10^{\circ}C$ $(50^{\circ}F)$; $15^{\circ}C$
$(59^{\circ}F)$; $20^{\circ}C$ $(68^{\circ}F)$; $27^{\circ}C$ $(80.6^{\circ}F)$; $32^{\circ}C$ $(89.6^{\circ}F)$; $37^{\circ}C$ $(98.6^{\circ}F)$; $40^{\circ}C$ $(104^{\circ}F)$;
$45^{\circ}C$ $(113^{\circ}F)$; and $52^{\circ}C$ $(125.6^{\circ}F)$. The samples were examined regularly for
activity under a dissecting microscope when the temperature of the larval
suspensions had been allowed to equilibrate with room temperature.

To determine the effect of freezing temperatures (-3 to $-6^{\circ}C$) samples
of infective larvae were kept in 'Universal' glass bottles and were first kept
at $8^{\circ}C$ for half an hour and then at $5^{\circ}C$ for 2 hours to avoid sudden shock of
falling temperature. After each time interval, one sample was removed from
the storage chamber and activity was observed after allowing the larvae to
equilibrate at room temperature for 1, 2, 3 and 24 hours. Controls were kept
at $10^{\circ}C$ temperature.

'Survival' was judged on the basis of the numbers of larvae showing mobility.

ii)   Results:-   The average percentage survival of first and second stage larvae at the temperatures and time intervals indicated are given in Tables 10 and 11.   Maximum survival times are shown in Fig. 5.   In general, a higher percentage of second stage than the first stage remained alive at most temperatures and times tested.   Maximum survival of 21 to 23 days for first stage larvae was observed at 5° to $10^{\circ}C$ (41° to $50^{\circ}F$) and for second stage larvae maximum survival of 35 days was seen at $15^{\circ}C$ ($59^{\circ}F$) (Fig.5).   At extremely high or low temperatures neither stage survived well.   First and second stage larvae survived up to 1 day and 2 days respectively at $40^{\circ}C$ ($104^{\circ}F$) and up to 1.5 hours and 2.5 hours only at $45^{\circ}C$ ($113^{\circ}F$).   Survival at freezing temperatures of -3 to $-6^{\circ}C$ (26.6 to $21.2^{\circ}F$) for first and second stage was only up to 24 hours.

The infective third stage larvae survived longer than preinfective larvae.   Table 12 gives the mean per cent survival of third stage infective larvae at the temperatures and time intervals indicated.   Fig. 4 shows essentially the same information graphically.   Fig. 5 shows the maximum survival times plotted against temperature.   While the infective larvae died quite rapidly at the temperature extremes, they survived extremely well (312 days) at $10^{\circ}C$ ($50^{\circ}F$) and moderately so (187 to 312 days) from 8 to $20^{\circ}C$ (46.4° to $68^{\circ}F$).   After 118 days storage at $10^{\circ}C$, 75% were alive and active when brought to room temperature, 50% were still alive after 176 days, and 25% were still alive after 225 days.   At temperatures above $10^{\circ}C$ the percentage survival was inversely proportional to the temperatures as can be seen most clearly in Figs. 4 and 5.   Survival at $8^{\circ}C$ ($46.4^{\circ}F$) and $5^{\circ}C$ ($41^{\circ}F$) was up to 187 and 74 days respectively, survival at $40^{\circ}C$ ($104^{\circ}F$) was up to 13 days, but storage at $45^{\circ}C$ ($113^{\circ}F$) and $52^{\circ}C$ ($125.6^{\circ}F$) was rapidly fatal to the infective larvae and survival was 4 hours and three-quarters of an hour respectively.

Survival of the infective larvae at freezing temperatures is given in Table 13.   At freezing temperatures of -3° to $-6^{\circ}C$, 5% of the infective larvae survived as long as 6 days.   It was observed that larvae which had been stored at freezing temperature were not active for 1 to 3 hours after return to room temperature and most of them took a longer time to regain activity.

Table 10

Survival of first stage larvae of
Cooperia curticei in water at
various constant temperatures.

Temperatures		Survival in days			
°C	(°F)	75%	50%	25%	0%
5	(41)	10	13	15	21
8	(46.4)	11	14	16	22
10	(50)	15	19	21	23
15	(59)	9	12	14	16
20	(68)	5	6	7	10
27	(80.6)	3	5	6	7
32	(89.6)	1	2	3	5
37	(98.6)	1	1.5	1.75	2
40	(104)	up to 1 day			
45	(113)	up to 1.5 hours			

Table 11

Survival of Second stage larvae of
Cooperia curticei in water at
various constant temperatures.

Temperatures		Survival in days			
°C	(°F)	75%	50%	25%	0%
5	(41)	3	5	8	16
8	(46.4)	5	8	13	21
10	(50)	10	14	19	25
15	(59)	18	23	28	35
20	(68)	14	16	20	23
27	(80.6)	5	7	10	15
32	(89.6)	3	5	9	11
37	(98.6)	1.5	2	3	5
40	(104)	up to 2 days			
45	(113)	up to 2.5 hours			

Table 12

Survival of third stage infective larvae
of Cooperia curticei in water at
various constant temperatures.

Temperatures		Survival in days			
°C	(°F)	75%	50%	25%	0%
5	(41)	49	55	62	74
8	(46.4)	71	117	152	187
10	(50)	118	176	225	312
15	(59)	106	162	214	299
20	(68)	80	122	164	206
27	(80.6)	24	46	60	86
32	(89.6)	16	24	37	52
37	(98.6)	5	8	14	19
40	(104)	4	7	10	13
45	(113)	up to 4 hours			
52	(125.6)	up to 45 minutes			

Fig. 4: Percentage survival of infective larvae of Cooperia curticei in water at controlled temperatures. Temperatures (^{O}C) are shown adjacent to the corresponding survival line.

Fig. 5: Maximum survival of first, second and third stage larvae of _Cooperia_ _curticei_ in water at controlled temperatures.
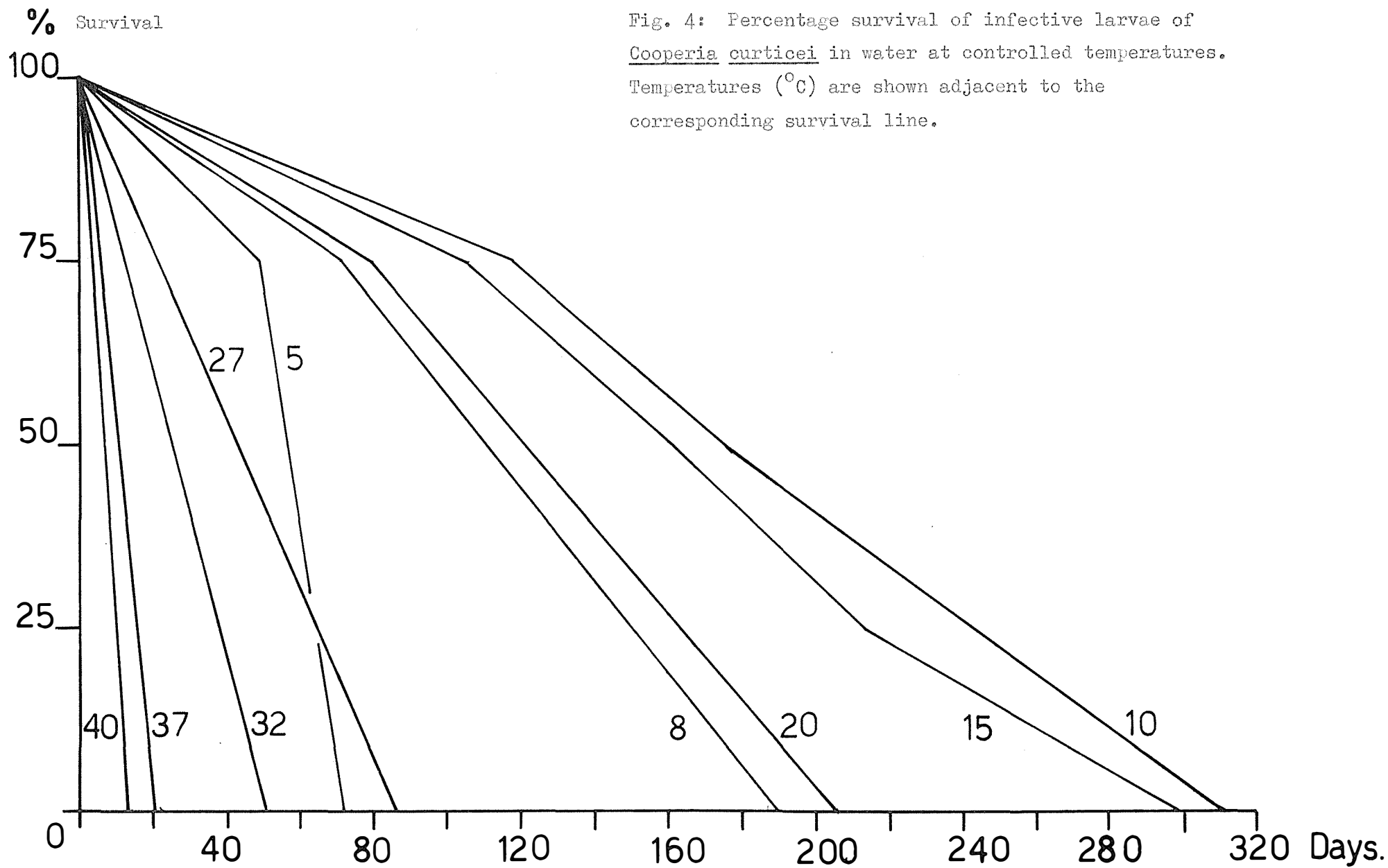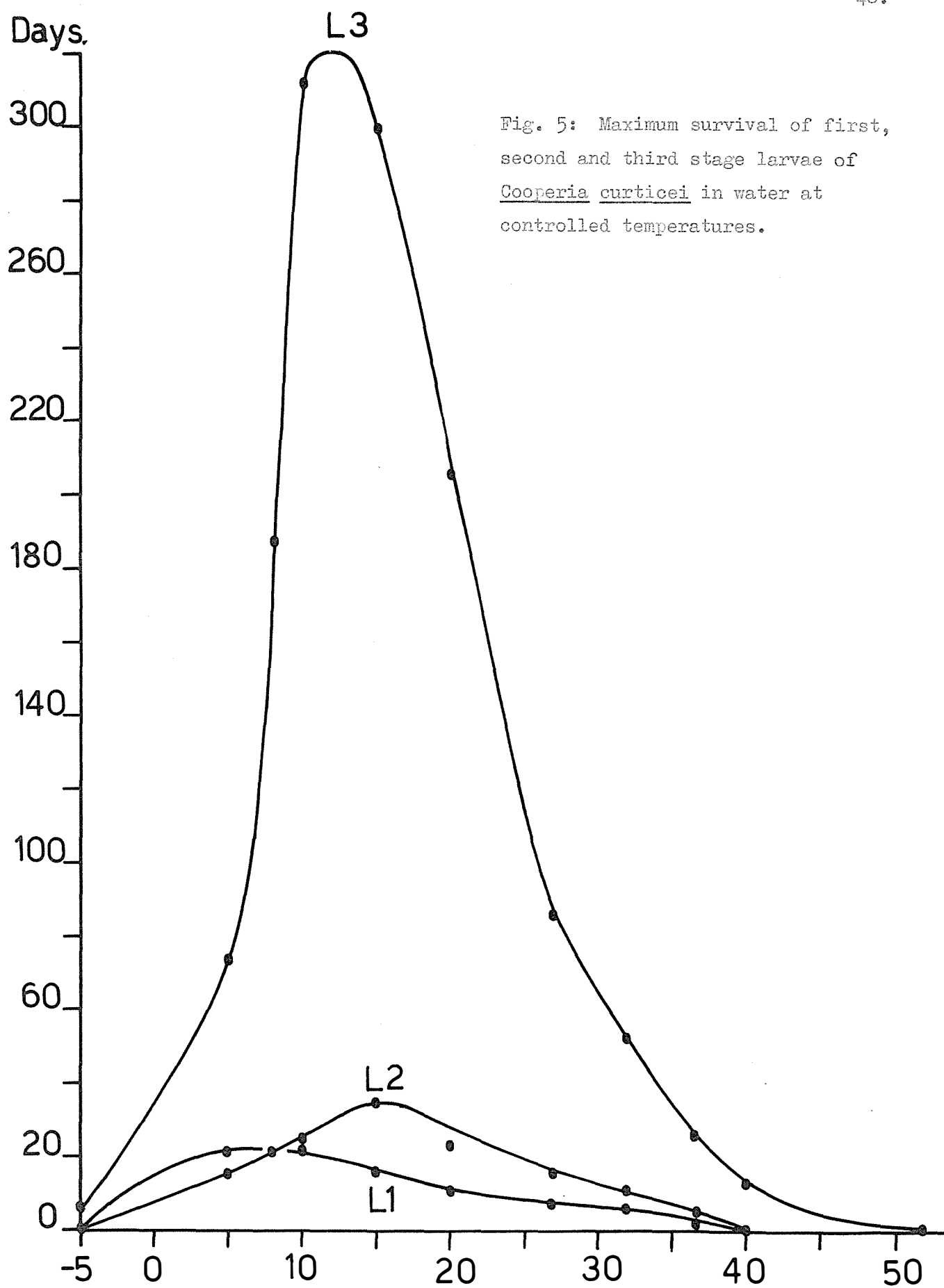
Table 13

Survival of infective larvae of Cooperia curticei at temperatures below freezing point.

Period frozen in days	Percentage activity at room temperature after				Daily temperatures	
	1 hour	2 hours	3 hours	24 hours	Min. °C (°F)	Max. °C (°F)
0.25	53	70	85	99	-6.1 (21)	-4.4 (24)
0.5	32	46	69	95	-6.1 (21)	-5.0 (23)
0.75	19	37	61	92	-5.6 (22)	-4.4 (24)
1	15	17	21	84	-6.1 (21)	-4.4 (24)
2	11	18	22	78	-5.6 (22)	-3.3 (26)
3	10	16	25	64	-6.1 (21)	-3.3 (26)
4	5	7	19	38	-6.7 (20)	-5.6 (22)
5	3	5	10	20	-6.1 (21)	-5.6 (22)
6	0	2	3	5	-6.7 (20)	-5.6 (22)
7	0	0	0	0	-6.1 (21)	-6.1 (21)
8	0	0	0	0	-6.1 (21)	-5.6 (22)

III. 5. SUMMARY OF EXPERIMENTAL FINDINGS:-

Studies on the larval development and survival of the free living stages of <u>Cooperia</u> <u>curticei</u> were carried out under constant temperatures and the following observations were recorded.

1. Individual eggs of <u>Cooperia</u> <u>curticei</u> were found to develop at different rates and some failed to develop or died at any stage from unknown causes.

2. Hatching did not occur unless the eggs were maintained at a temperature of $10^{\circ}C$ and above. At a temperature of $42^{\circ}C$ eggs died very rapidly and failed to develop.

3. At constant temperature of $10^{\circ}C(50^{\circ}F)$; $15^{\circ}C(59^{\circ}F)$; $20^{\circ}C(68^{\circ}F)$; $27^{\circ}C(80.6^{\circ}F)$; $32^{\circ}C(89.6^{\circ}F)$; and $37^{\circ}C(98.6^{\circ}F)$ with adequate moisture, freshly passed <u>C.curticei</u> eggs required about 22,12,6,5,4,3, and 2.5 days respectively before the first of them reach the infective third larval stage. For 90% or more to become third stage infective larvae, 40,23,13,8,6, and 5 days respectively were required.

4. Throughout the temperature range of 10 to $37^{\circ}C$, the rate of development of infective larvae was altered by 0.033 log units of time for each degree centigrade temperature change.

5. All larval stages occupied the same proportion of the development time throughout the temperature range of 10 to $37^{\circ}C$. For 90% or more to become first stage larvae 22.5 to 25.3% of the total time was required whereas second and third stage infective larvae occupied 28.4 to 30.7% and 45.0 to 48.0% of the time respectively (95% confidence limits).

6. The percentage of eggs which completed development to the infective larvae varied with temperature. In 'routine' culture the mean percentage recovery of the infective larvae at $10^{\circ}C(50^{\circ}F)$, $27^{\circ}C(80.6^{\circ}F)$ and $37^{\circ}C(98.6^{\circ}F)$ was 44.22 (range 31.9 to 47.5%), 92.16 (range 87.6 to 96.1%), and 7.14 (range 5.1 to 12.0%) respectively. When intact faecal pellets were cultured at $27^{\circ}C(80.6^{\circ}F)$ the recovery was markedly lower than in routine culture and ranged from 28.0 to 47.2%(mean 35.24%) only.

7.   Development of the eggs, first, and second stage larvae was inhibited in excess water at 27°C, probably due to interference with aeration.

8.   Over 90% of the eggs stored in water at 5°C and 27°C were disintegrated after 65 and 25 days respectively. All eggs in faecal pellets stored at 5°C were disintegrated after 50 days. The period of survival of the eggs as judged by their ability to resume development when removed to a favourable environment paralleled the observed degeneration of the eggs held in water at different temperatures.

9.   At various constant temperatures the maximum survival time of the pre-infective larvae in water was much less than that of the infective larvae. The maximum survival of the first stage larvae was 21 to 23 days at 5 to 10°C. For second stage larvae, maximum survival of 21 to 35 days was observed at 8 to 15°C.

10   The maximum survival of the infective larvae in water at 10°C was 312 days. At temperatures above 10°C survival decreased and was inversely related to temperature. Survival at 5°C and 8°C was also poorer than at 10°C. The maximum survival time at different temperatures are as follows, 5°C - 74 days; 8°C - 187 days; 10°C - 312 days; 15°C - 299 days; 20°C - 206 days; 27°C - 86 days; 32°C - 52 days; 37°C - 19 days and 40°C - 13 days. Larvae died after 4 hours at 45°C and after 45 minutes at 52°C.

11.   At freezing temperature (-3 to -6°C) the maximum survival of the infective larvae was 6 days. Larvae which had been stored at these temperatures took up to 24 hours to regain activity.

III. 6. <u>DISCUSSION</u>:

Laboratory experiments on the development of the embryonic and larval stages of <u>Cooperia</u> <u>curticei</u> under constant temperatures demonstrated that this nematode confirms in general terms to the pattern which might be expected from studies made by several workers on other strongylate nematodes (Ransom, 1907a; Veglia, 1915; Ross and Gordon, 1936; Shorb, 1944; Berberian and Mizzelle, 1957; Silverman and Campbell, 1959; Gupta, 1961; Ciordia and **Biszall**, 1960; Crofton, 1963; Rose, 1963). However, only few have been studied in the detail described in this thesis and with the known occurrence of different strains of species of nematode adapted to different temperature conditions (Crofton and Whitlock, 1964, 1965) make the detailed comparison of the present results with those of other workers difficult.

It is apparent that the temperatures at which larval development occurred fall into the same general range (10° to 37°C) as that compatible with development of other strongylate nematodes (Crofton, 1963). Although the minimum temperature at which hatching would occur was not accurately determined because of the lack of suitable apparatus it was found that with the strain of <u>C.curticei</u> being used that this minimum temperature fell between 7°C and 9°C. It is noteworthy that Crofton (1965) in his experiment with a strain of <u>C.curticei</u> from S.W. England found that 16°C was the minimum temperature at which hatching would occur. Although Crofton kept his eggs in aerated water and in the present experiment eggs were in vermiculite-faeces mixtures, it is unlikely that this would account for the differences in the two sets of observations. It is most likely that this may be due to strain differences which indicates that the "New Zealand" strain is adapted to develop at lower temperatures than the one examined by Crofton. This result is surprising. The temperature range in Bristol, England, where Crofton was working, is lower than that occurring in Palmerston North, New Zealand. It would be expected therefore, that the strain of nematode from Bristol would be adapted to lower temperatures than the local strain.

It was observed by Silverman and Campbell (1959) in their work on <u>H.contortus</u> that the rate of development of individual eggs varied considerably at constant temperatures. This may be linked as suggested by Crofton and Whitlock (1965) to variations in the volume of individual eggs. Whilst it is comparatively easy to determine "minimum" times of development, it is

impracticable to consider maximum development times i.e. when 100% of the
larval population have reached a particular stage of development. It was
necessary, therefore, to adopt some other criterion to mark the end point
of a particular phase of development. For this purpose the time taken for
90% or more of a given population to reach the stage in question was used.
The term "median" as used by Silverman and Campbell (1959) but not clearly
defined by them, was not considered appropriate as this term has specific
mathematical connotations which do not apply to the situation being des-
cribed. The use of the "T90" proved highly satisfactory in the present
experiments.

The relationship between "T90" times for development of the infect-
ive larvae and temperature was found to be logarithmic (fig. 2) and the
data were fitted almost perfectly to a linear regression of log days on
temperature (fig. 3). This linear relationship permitted the quantification
of the effect of temperature on development rate. On examining the data on
larval development of H.contortus and T.retortaeformis described by Silver-
man and Campbell (1959), Berberian and Mizzelle (1957), and Gupta (1961), a
linear relationship between log rate of development and temperature was ap-
parent. Using the "median" times of development of the infective larvae for
H.contortus at different temperatures (Silverman and Campbell, 1959) a
regression slope of -.024 is observed. This shows that a change of one de-
gree centigrade of temperature will alter the rate of development of H.
contortus by 0.024 log units of time. This compares with a change of 0.033
log units for Cooperia curticei (Appendix I). It seems probable that a
linear regression of log development time on temperature could be similarly
calculated for other nematodes.

The results on the relationship between development and temperature
limits within which the metabolism of the developing larvae can function are
not surprising. A constant relationship of temperature and development would
be expected since these animals are poikilothermic. The fact that the pro-
portion of the time taken for intervening stages up to the infective stage
was approximately constant at all the temperatures at which development pro-
ceeded, indicates that the effect of temperature on development rates is
virtually the same at all stages. If this were not the case one would have
to propose the existence of different metabolic mechanisms at different
stages of development. The logarithmic relationship of temperature and rate
of development can simply be explained on the basis of the effect of in-
creasing temperature on the speed of chemical reactions which also increases
exponentially.

The linear relationship of temperature and development demonstrated, if it is assumed to apply to other strongylate nematodes, has useful implications. In order to arrive at a standard line from which the developmental time of the infective stage at different temperatures may be calculated, it is only necessary to establish, as accurately as possible, the "T9o" times at two or three constant temperatures. Obviously the larger the number of temperatures tested, the more accurate the calculated regression slope will be. But for practical purposes it would appear that a sufficient close approximation to reality could be obtained with comparatively little work. The value this may have in predicting rates of development under natural conditions will be discussed later.

Percentage development of the infective larvae also varied with temperature; in faecal cultures when the pellets were broken and mixed with vermiculite, the mean percentage recovery of the infective larvae was 40.2% at $10^{o}C$; 92.2% at $27^{o}C$ and 7.1% at $37^{o}C$ (Table 6). Intact faecal pellets however, only yielded a mean percentage recovery of 35.2% at $27^{o}C$. It is suggested that the lower recovery from faecal pellets was due to an interference with migration of larvae which reduced their chances of recovery and probably to variations in the micro-environment of the developing eggs and larvae situated in various positions in the faecal pellet.

It could be argued that the use of intact faecal pellets for the experimental observations as was done by Silverman and Campbell (1959) would more closely simulate field conditions. However, as Silverman and Campbell pointed out, sheep faecal pellets vary considerably in size and consistency, and their use only adds a further variable to the experimental observations. In any case, under field conditions faecal pellets are not permanent structures and when wetted by rain may disintegrate in a few days (Christie, 1963).

Little work has been carried out to measure precisely the oxygen requirements of developing nematode eggs or larvae (Schwabe, 1957; Desowitz, 1962). In the present experiments where the atmosphere was excluded by the presence of water, development of the eggs, and first and second larval stages was inhibited. It is assumed that this was due to a reduction in the amount of oxygen available. The majority of first and second stage larvae died after a few days in water. Embryonation of the eggs became inhibited at the

tadpole stage of development and only a small proportion of these eggs were capable of resuming development (Table 9).

The survival of the eggs in water was better at 5°C than at 27°C but it would appear from the survival rate of eggs in faecal pellets that a temperature of 5°C itself caused a comparatively rapid mortality of the eggs. It might have been expected that the eggs in the faecal pellets would survive longer than those in water, but their maximum survival was less than eggs kept in water. This suggests that there was some advantage in survival to the eggs in water and that in some way or other the oxygen deficiency protected the eggs against the effects of low temperature. The interaction of temperature, hypoxia and larval development is complex and warrants some careful investigation.

Inhibition of the development of nematode eggs saturated with water has also been observed by Veglia (1915); Shorb (1944); Silverman and Campbell (1959) and Anderson et al. (1966). These latter two groups of workers also observed that survival of eggs saturated with water was generally better at lower than at higher temperatures. It is notable that eggs of H.contortus survived less well at 7.2°C than at 11°C although this was not commented on by the authors (Silverman and Campbell, 1959).

The observations that the development of the pre-infective larvae could be retarded by keeping them in water, allowed comparison of their rates of survival at different temperatures. At the same time the survival of the infective larvae was also studied.

It was found that the maximum survival time was closely related to temperature but not in a linear fashion (figure 5). The maximum survival of first stage larvae (23 days - Table 10) was obtained at 10°C and above and below this temperature survival times were shorter. Similarly second stage larvae showed a maximum survival (35 days - Table 11) at 15°C and on either side of this temperature survival rates decreased. Maximum survival of third stage larvae was obtained at 10°C (312 days - Table 12) and again survival rates decreased both above and below this temperature. These results suggest that, as with eggs saturated with water, the effect of decreasing temperature is not simply to induce a reversible state of hibernation, the depth of which is directly related to temperature. In this connection it is interesting to note that the optimum temperatures for survival approach that below which no

hatching of eggs occur. So it may be hypothesised that temperatures lower than those giving optimal survival cause irreversible damage to the metabolism of the nematode. At temperatures above that compatible with maximum survival the situation is complicated by the possible effects of increased metabolism, exhausting supplies of stored metabolites, though one would expect this to apply particularly to the third stage larvae which does not feed. In these experiments, obviously, dessication was not involved in the larval mortality.

The maximum survival of third stage larvae at the optimum temperature of $10^{O}C$ was considerably greater than that of first and second stage larvae at their optima. It is generally assumed that the sheath of the infective larvae protects them against dessication and adverse conditions (Rose, 1955). But in a limited study Marquardt and Anderson (cited by Anderson et al. 1966) found that cutting off the ends of the sheath of the infective larvae of trichostrongylids did not seem to affect larval survival. So it is not clearly understood just why infective larvae survive better than pre-infective ones. In spite of the fact that infective larvae could not feed they survived longer and it appears that something more subtle than ensheathment must be offered as an explanation for their longer survival. It might be suggested that this may be due to a difference in the requirement of oxygen for metabolism between the infective larva, which neither feeds nor grows, and the first and second larvae, which do both.

In the present experiment, in relation to temperatures, eggs and infective larvae were more resistant than pre-infective larvae. The maximum survival times at $5^{O}C$ were for eggs 65 days; first stage larvae - 21 days; second stage larvae - 16 days; and for third stage larvae 74 days. Similarly at $27^{O}C$ the maximum survival were for eggs - 30 days; first stage larvae 7 days; second stage larvae - 15 days and third stage larvae 86 days. This observation further confirms the widely held view that of all the preparasitic stages of strongylate nematodes, the eggs and the infective larvae are most resistant to environmental variations (Crofton, 1963).

The observations on the ability of the infective larvae to survive freezing temperatures for short periods of time are in general agreement with those of other workers (Monnig, 1930; Stewart and Douglas, 1938; Poole, 1954; Prasad, 1959; Rose, 1963; Anderson et al, 1966). The maximum survival of the

infective larvae at temperatures ranging from -3.3°C to -6.7°C was 6 days.   A
notable feature of this experiment was the long period necessary at room temp-
erature for maximum numbers of larvae to regain their motility.

Chapter IV   THE ECOLOGY OF THE FREE LIVING STAGES OF
                              COOPERIA CURTICEI:-

          As infection with gastro-intestinal nematodes is acquired by sheep
following the ingestion of infective larvae whilst grazing, a study of develop-
ment and survival of the infective larvae of the nematodes concerned is relevant
to the control of parasitic gastro-enteritis in sheep.   This work is a part of
a larger study devoted to the ecology of the free living stages of Cooperia
curticei, since relatively little attention has been paid to this species.

IV. I. MATERIALS AND METHODS:

          Observations were made on the rate of development of the free-living
stages in faecal pellets and on the migratory activity and longevity of larvae
in faeces, on herbage and in soil each month of the year.   All observations
were carried out using herbage grown in flower pots 9 cm high and 5 cm in
diameter.   To avoid contamination with other nematodes the soil was sterilised
(400°F for 3 hours) and it was placed approximately 6 cm deep in the flower pots;
the soil was watered with tapwater and perennial rye grass was sown.   The pots
were not watered thereafter.   When the herbage had grown for 2 months it was
clipped to a height of 4 cm before the start of each experiment.   The total
faecal output of two donor sheep, with monospecific infestations of Cooperia
curticei was collected during the preceeding day by means of a faecal collecting
bag.   The faecal pellets were first thoroughly mixed to ensure, as far as
possible, an even distribution of Cooperia curticei eggs.   The faecal egg count
was estimated by modified McMaster method and an average of ten counts on two
samples was recorded.   Then 10 gm of faecal pellets was spread evenly over the
herbage in each of 65 to 75 flower pots and these were placed outdoors so that
they were exposed to the local climatic conditions.   To check the eggs' ability
to develop to third stage infective larvae, faecal pellets from each experiment
were cultured with vermiculite and 'normal' moisture at 27°C.

          Examination of the faecal material was carried out daily until first
stage larvae appeared, then on alternate days until third stage infective larvae
appeared in the faecal pellets.   Eggs were recovered by a flotation technique
using saturated sugar solution, the stages of development were determined and
the percentage which had reached each stage of development was recorded.
Further faecal pellets were placed on alternate days on a small Baermann

apparatus to recover any first, second or third stage larvae.   Microscopic
observations were made on iodine stained preparations.

During the winter months when larval development was poor larvae were
obtained from faecal pellets by Baermann technique and then the same faecal
pellets were examined by sugar flotation technique to determine the stages of
development of eggs, if any.   Even when no larvae were recovered by Baermann
apparatus, faecal examinations were continued until either eggs could no longer
be found in faeces or until the faecal pellets finally disintegrated.   In each
observation 100 individuals were classified according to developmental stages.
The classification categories comprised three egg and three larval stages, eggs
in morula and gastrula (E_1), tadpole or early vermiforms (E_2), late vermiform
to prehatch larva (E_3), first stage larvae (L_1), second stage larva (L_2) and
third stage larva (L_3).

To determine the migratory activity and longevity of larvae in faeces,
on herbage and in soil, 4 samples were taken from each flower pot which consisted
of whole faeces, herbage clipped from 2 cm above in height, 0 - 2 cm in height
from soil level and 1 cm top soil.   This procedure yielded quantities of
herbage per sample unit of up to 5 grams wet weight.   Samples were examined at
weekly intervals in duplicate.

Separation of the infective larvae from faecal pellets, herbage and
soil is discussed in detail in the Chapter, IV 2, with a test of the technique.

All samples were collected in the morning and observations were carried
out until two consecutive collections were free from Cooperia curticei larvae.

In each monthly experiment, when infective larvae developed outdoors
from the faecal pellets, five replicate samples of 200 infective larvae not more
than a week old were obtained from faeces cultured at $27^{O}C$ and placed in water
outdoors adjacent to the pots.   The larvae were kept in water, 1 cm deep in
screw cap 'Universal' glass bottles.   Their survival (percentage motile) was
observed at weekly intervals using a dissecting microscope (refer III, 4 (i)),

A complete series of the experiment described above was set up in the
first week of the months of March, April and May, 1968, and then on the first
day of each month throughout the year from June 1968, to February, 1969.

Meteorological records were obtained during the whole of the period of observation from Grasslands Division, D.S.I.R., Palmerston North, which is about one quarter of a mile from the site of this experiment.

IV. 2. TEST OF A TECHNIQUE FOR THE RECOVERY OF INFECTIVE LARVAE
        OF COOPERIA CURTICEI FROM SMALL SAMPLE UNITS:-

A number of methods for the recovery and counting of infective larvae in samples of pasture has been described (Taylor, 1939; Crofton, 1954b; Parfitt, 1955; Durie, 1959; Donald, 1967a). Most of these were designed to deal with bulked samples of the order of 8oz. or more. Taylor (1939) described the separation of larvae from pasture which involved grass washing and sedimentation followed by concentration on filter paper and subsequent separation of the larvae from the sediment in a Baermann funnel. The inefficiency of the Baermann funnel separation and the loss of larvae during sedimentation was recognized by Taylor. Crofton (1954b) described the separation of larvae from pasture by a flotation method using a saturated solution of zinc sulphate. He also stated that zinc sulphate solution dehydrates the infective larvae which form a curl in a tight spiral, thereby reducing the number of larvae which can be examined carefully. Parfitt (1955) reported that when known numbers of larvae were placed on grass, his technique, based on flotation, yielded an average of 43% recovery, and the rest were lost during the processes of flotation, sedimentation and washing. Durie (1959) tested his technique which relies on the difference in density of larvae and debris under the influence of a stream of water flowing upwards at a gradually decreasing flow rate. The soil and pasture sediment was treated with a saturated solution of iodine in potassium iodide, to kill the larvae. By adding known numbers of larvae to sediment obtained by washing samples of pasture which were free of infective larvae, recovery from pasture ranged from 61 to 94%, with an average of 74% and from soil ranged from 55 to 94% with an average of 78%. Donald (1967a) described a technique for the recovery of strongylate infective larvae from small sample units (25 gm herbage) of pasture by flotation of the sediment with saturated potassium iodide solution; his technique yielded approximately 90% recovery. But in all these techniques mentioned above, it was difficult to establish whether the larvae were alive or not at the time the pasture was sampled.

In order to estimate efficiently the frequency distribution and survival of infective larvae in pasture, soil and faecal pellet samples, a simple technique is required which will recover a high proportion of larvae from each sample unit and also isolate the larvae sufficiently to allow them to be counted individually in a living condition, as motility appears to be the only simple criterion of life in infective larvae.

Hence the following technique was devised which relies on the active migration of the larvae from the medium, under the stimuli provided by temperature, moisture, light and gravity.

i)   Description of the technique:-   For studies on the distribution and survival of infective larvae of Cooperia curticei in random samples perennial rye grass grown in flower pots (9 x 5 cm in diameter) on sterilised soil were used.   The sample units consisted of herbage clipped at 2 cm and above in height, and 0 - 2 cm in height from the soil surface.   This procedure yielded quantities of herbage, up to 5 grams per sample unit wet weight.   Top soil to a depth of 1 cm and faecal pellets (10 grams) were chosen as separate sample units.

a)   Separation of infective larvae from herbage: -   Each sample of the herbage is soaked in glass beakers for 24 hours in 500 ml of warm water (initially at a temperature of $37^{\circ}C$) for 5 grams of herbage and 250 ml of warm water for 1 gram of herbage, and is stirred at intervals.   The bulk of the herbage is then lifted out with a spatula and transferred to other beakers containing the same quantity of warm water, and is soaked for another 24 hours. Each lot of herbage washing is passed through a sieve of 60 meshes to the linear inch, in order to remove remaining blades of grass and is allowed to stand for 6 hours.   The supernatant water is syphoned off to leave the sediment in about 100 ml of water which is transferred to a 100 ml glass beaker and is again allowed to settle overnight.   The supernatant fluid is then again syphoned off to leave the sediment in about 25 ml of water which is transferred to a "Universal glass bottle (capacity 28 ml).   The bottles are centrifuged at 500 rpm for 10 minutes or sediment is allowed to settle for 6 hours.   The supernatant fluid is then carefully drawn off to leave the sediment in about 4 ml of water.   At this point 3-4 drops of dilute aqueous Lugol's iodine solution is added to the sediment collected from each sample and living infective larvae present in the sediment are counted microscopically at a magnification of 100 diameters. This dilution of aqueous Lugol's iodine solution kills the free living larvae but not the infective larvae of Cooperia curticei.

b)   Separation of infective larvae from top soil surface of flower pots:- The separation of the larvae from top soil is carried out in a modified Baermann apparatus containing a glass funnel and a sieve strainer (in place of screen) which also holds the flower pot.   Between the soil surface and the sieve is

placed one layer of 'Snowtex' tissue. The flower pot containing soil is
kept upside down on the sieve in the Baermann apparatus and 1 cm of top
soil is allowed to remain immersed in water for 2 days. At intervals of
24 and 48 hours, 25 ml of liquid are drawn off in "Universal" glass bottles
and larvae are counted after settling, as described for herbage samples.
With this method, recovery of living infective larvae free from soil debris
from top soil can be obtained.

c)   Separation of infective larvae from faecal pellets:- Faecal pellets
containing infective larvae are placed on a small sieve and kept in a Baer-
mann apparatus. Faecal pellets are allowed to remain immersed in water for
2 days. At 24 and 48 hour intervals 25 ml of the liquid is drawn off in
"Universal" glass bottles and larvae are counted after settling as describ-
ed for herbage samples.

ii)   Tests of the technique:- In testing the technique for the recovery
of infective larvae, from herbage, soil and faecal pellets samples, known
numbers of Cooperia curticei infective larvae were added to parasite-free
samples. Perennial rye grass (herbage) grown in the laboratory in flower pots
with sterilised soil and parasite-free faecal pellets were used as samples.

Eighty lots of living Cooperia curticei infective larvae were
counted individually. The 80 lots consisted of 5 replicates within each of
the 4 sample groups covering the range 0-50,100-200,300-500 and 800-1200
larvae respectively. Each lot of larvae was placed either on herbage, soil,
or faecal pellet samples. In order to test all stages of the technique in-
cluding to some extent the separation of the larvae from the samples, the
larvae were allowed to stay for half an hour in the sample, so that water
containing larvae could be absorbed and the infective larvae could adhere
to soil, faecal pellets and herbage samples.

Each sample contaminated with known numbers of larvae was processed
as described above and the numbers of larvae recovered are shown in Table 14 and 17.

The percentage recovery in individual trials on 1 and 5 grams of her-
bage ranged from 79 to 92.4 with a mean of 85.6 and 60.0 to 75.1 with a mean
of 64.5 respectively. The percentage recovery from soil and faecal pellets
ranged from 57.3 to 75.2 with a mean of 64.6 and 50.0 to 66.3 with a mean
of 58.3 respectively. The overall recovery rate from all the samples

Table 14

The recovery of infective larvae of <u>Cooperia</u> <u>curticei</u> after
adding varying numbers to one gram weight of herbage.

Range of larvae in each group	Actual no. larvae added	No. of larvae recovered				
		Counting slides I	II	Rinsing of tubes & pipettes	Total recovered	Percentage recovery
0- 50 1)	22	16	2	0	18	81.8%
2)	31	24	2	1	27	87.1%
3)	40	35	2	1	38	86.4%
4)	50	39	6	0	45	90%
5)	50	38	4	0	42	84%
100- 200 1)	101	69	10	3	82	81.1%
2)	124	103	3	1	107	86.3%
3)	154	129	6	1	136	88.3%
4)	172	143	13	3	159	92.4%
5)	196	169	6	1	176	89.8%
300- 500 1)	308	268	10	1	279	90.5%
2)	353	294	21	1	316	89.5%
3)	405	298	22	5	325	80.2%
4)	453	350	15	6	371	81.9%
5)	496	356	34	9	399	80.4%
800-1200 1)	828	578	67	12	657	79.3%
2)	937	647	125	2	774	82.6%
3)	1009	823	56	8	887	87.9%
4)	1119	972	35	2	1009	90%
5)	1197	913	79	5	997	83.3%

Mean recovery:-   85.6%

The recovery of infective larvae of <u>Cooperia</u> <u>curticei</u> after
adding varying numbers to five grams weight of herbage.

No. of larvae recovered.

Range of larvae in each group	Actual no. larvae added	Counting I	glides II	Rinsing of tubes & pipettes	Total recovered	Percentage recovery
0- 50 1)	21	13	1	0	14	67%
2)	30	18	1	0	19	63.3%
3)	40	20	4	0	24	60%
4)	46	27	3	0	30	65.2%
5)	50	29	5	1	35	75%
100- 200 1)	102	64	5	3	72	70.6%
2)	124	55	15	4	74	60%
3)	154	96	4	2	102	66.2%
4)	175	102	6	6	114	65.1%
5)	198	122	6	1	129	65.1%
300- 500 1)	305	161	16	8	185	60.6%
2)	344	197	13	11	221	64.2%
3)	404	213	24	10	247	61.1%
4)	449	212	42	9	263	60%
5)	499	344	26	5	375	75.1%
800-1200 1)	808	467	24	10	501	62%
2)	910	494	53	11	552	60.6%
3)	1015	543	59	7	609	60%
4)	1106	618	71	9	698	64.5%
5)	1196	649	107	27	783	65.4%

Mean recovery:-   64.5%

Table 16

The recovery of infective larvae of _Cooperia_ _curticei_
after adding varying numbers to soil surface.

No. of larvae recovered.

Range of No. larvae in each group	Actual no. larvae added	Counting I	Slides II	Rinsing of tubes & pipettes	Total recovered	Percentage recovery
0- 50 1)	20	12	2	0	14	70%
2)	31	17	6	0	23	74.2%
3)	40	21	8	0	29	72.5%
4)	45	21	5	1	27	60%
5)	49	25	5	0	30	61.2%
100- 200 1)	101	44	30	2	76	75.2%
2)	126	58	21	3	81	64.3%
3)	154	71	16	5	92	60.3%
4)	175	85	13	9	107	61.1%
5)	198	112	25	8	145	73.2%
300- 500 1)	303	153	40	4	197	65%
2)	346	182	25	8	215	62.1%
3)	410	195	48	12	255	62.2%
4)	447	237	33	5	275	61.5%
5)	497	278	17	3	298	60%
800-1200 1)	811	465	55	6	526	64.8%
2)	899	516	40	8	564	62.7%
3)	986	529	124	5	659	66.8%
4)	1101	569	66	11	647	58.7%
5)	1196	520	156	9	686	57.3%

Mean recovery:- 64.65%

Table 17

The recovery of infective larvae of _Cooperia_ _curticei_ after adding
varying numbers to ten grams weight of faecal pellets.

No. of larvae recovered.

Range of No. larvae in each group	Actual no. larvae added	Counting I	slides II	Rinsing of pipettes & tubes	Total recovered	Percentage recovered
0- 50 1)	22	10	1	0	11	50%
2)	33	18	2	0	20	60.6%
3)	41	27	0	0	27	65.8%
4)	45	23	0	0	23	51.1%
5)	50	29	2	1	32	64%
100- 200 1)	104	50	7	2	59	56.7%
2)	123	70	3	1	74	60.1%
3)	152	76	10	2	88	57.9%
4)	174	91	12	5	108	62.0%
5)	195	101	14	2	117	60%
300- 500 1)	302	146	17	4	167	55.3%
2)	355	183	8	7	198	55.7%
3)	403	198	30	3	231	57.3%
4)	452	235	10	5	250	55.3%
5)	494	285	14	6	305	61.7%
800-1200 1)	828	367	71	9	447	54%
2)	912	479	47	7	533	58.4%
3)	1006	574	83	10	667	66.3%
4)	1103	533	81	8	612	55.4%
5)	1196	607	82	9	698	58.3%

Mean recovery:- 58.3%

was 68.25%

An analysis of variance was carried out on the percentage recovery data
in Table 14 to 17, after arcsin transformation of the percentage to stabilize
the variance.   The analyses were carried out to see if there was any significant
difference in recovery rate due to the presence of differing numbers of larvae
in the samples.

These analyses are shown in Appendices 3 to 6.   In all cases the mean
squares for "Between groups" is not significantly greater than the errors mean
square.   These results suggest that the recovery efficiency of the techniques
is independent of the numbers of infective larvae present within the range
tested.

Analysis of variance was also carried out between 4 treatment groups
(data in tables 14, 15, 16 and 17) and the percentage variation between the
groups was significant, due to a higher percentage recovery from one gram
herbage samples (Appendix 7).   But when analysis of variance was carried out
on the percentage recovery of infective larvae from three groups, herbage 5 gram,
faecal pellets and soil samples (data in tables 15, 16, and 17) after arcsin
transformation, the variation was not significant (Appendix 8).

This analysis indicates that average percentage of the recovery for
each treatment group (5 grams herbage, faecal pellets and soil samples) is
uniform.   However, from one gram herbage samples the mean percentage recovery
rate (85.6%) was significantly higher (P$<$ 0.01).   It would appear that the
lowered efficiency of the recovery from the larger quantites of herbage is
related to the comparative difficulty with which larvae can be extricated from
a larger and more complex matrix of grass blades.

IV. 3. RESULTS:

i)   Development of the free living stages:-   The development of the egg of Cooperia curticei to third stage infective larvae, in faecal pellets, under natural conditions was investigated throughout the year from March 1968 to February 1969.   Observations on the rate of development showed that free living stages developed at different rates and many eggs failed to develop and died due to weather conditions.   Because of these variable reactions the data on development rates have been presented as minimum and maximum times.   The term 'minimum' refers to the period taken for only a small proportion ($<5\%$) of the population to reach a given stage.   For pre-infective larvae 'maximum' refers to the time when 70% or more of the total population completed development; this endpoint was selected because of the observed wide range of development rates within the population.   For infective larval stages 'maximum' refers to the time when 90% or more of the total population had completed development on the day of examination.

At the time faecal pellets were placed in Experiment I conditions for development were good (mean air temperature $69.3^{\circ}F$, $20.8^{\circ}C$).   Faecal pellets were deposited on 6th March, 1968, and maximum development of the infective larvae was recorded on 14th March, 1968.   The rate of development of the free living stages is shown in Table 18.   Minimum and maximum time for development of infective larvae was 6 and 9 days with 10% and 17% recovery from two samples. The daily fluctuations in meteorological data for the period up to maximum development of infective larvae are given in Appendix 9.   Air temperature ranged from 57.0 to $81.2^{\circ}F$ (13.9 to $27.3^{\circ}C$) with a mean minimum and mean maximum of $62.0^{\circ}F$ ($16.7^{\circ}C$) and $76.6^{\circ}F$ ($24.8^{\circ}C$) respectively.   Minimum temperature on grass ranged from 49.9 to $65.3^{\circ}F$ (10.0 to $18.4^{\circ}C$) and the relative humidity range, total rainfall and total evaporation were 61 to 91%, 0.65 inches and 0.86 inches respectively.   A replicate experiment was set up on 8th March and infective larvae were recovered after 10 days, with similar results to those shown in Table 18.

In Experiment 2, faecal pellets were deposited on 7th April, 1968. Development was slow in later stages probably due to decreasing temperatures but percentage recovery of infective larvae was higher than in Experiment 1. Minimum and maximum time for development of infective larvae was 10 and 21 days, with 12.7% and 23.4% recovery from duplicate samples.   The rate of development

Table 18

Rate of development of the free living stages of Cooperia
Curticei in faeces under natural conditions
during the month of March 1968.

Stages of development	Time (days) required for Development		
	Date	Days	Percentage
E_1, Gastrula			
Minimum =	—	.	
Maximum =	6. 3. 68	1 =	E_1 = 91%
			E_2 = 9%
E_2, Tadpole	'		:
Minimum =	6. 3. 68		
Maximum =	7. 3. 68	2 =	E_1 = 2%
			E_2 = 90%
			E_3 = 8%
E_3, Prehatch			
Minimum =	7. 3. 68		
Maximum =	8. 3. 68	3 =	E_3 = 93%
			L_1 = 7%
L_1, First stage larvae			
Minimum =	8. 3. 68		
Maximum =	9. 3. 68	4 =	E_3 = 1%
			L_1 = 72%
			L_2 = 27%
L_2, Second stage larvae			
Minimum =	9. 3. 68		
Maximum =	11. 3. 68	6 =	L_1 = 1%
			L_2 = 84%
			L_3 = 15%
L_3, Third stage larvae			
Minimum =	11. 3. 68		
Maximum =	14. 3. 68	9 =	L_2 = 4%
			L_3 = 96%
Percentage recovery of infective			10 & 17%
larvae from duplicate samples			(mean 13.5%)

is shown in Table 19.   Daily fluctuations in weather records for the period up to maximum development of infective larvae are given in Appendix 10.   Air temperature ranged from 40.6 to 77.6°F (4.8 to 25.4°C) with a mean minimum and mean maximum of 49.5°F (9.7°C) and 63.2°F (17.3°C) respectively.   Minimum temperature on grass ranged from 35.4 to 59.4°F (1.8 to 15.3°C) and the relative humidity range, total rainfall and total evaporation were 66 to 97%, 4.09 inches and 1.41 inches respectively.

By the time faecal pellets were placed on 3rd May, 1968, in Experiment 3, temperatures were decreasing with a few frosts at night (mean air temperature 53.0°F, 11.7°C).   The rate of development was very slow (Table 20) and maximum development of infective larvae was recorded on 30th May, 1968.   Minimum and maximum time for development to infective larvae was 12 and 28 days with 6.82 and 13.6% recovery from duplicate samples.   Daily fluctuations in meteorological records for the period of development are given in Appendix 11.   Air temperature ranged from 33.8 to 66.2°F (1.1 to 19.0°C) with a minimum and maximum mean of 45.6°F (7.6°C) and 65.0°F (18.3°C) respectively.   Minimum temperature on grass ranged from 26.2 to 53.1°F (-3.2 to 11.7°C) and the relative humidity range, total rainfall and total evaporation were 76 to 99%, 4.66 inches and 1.14 inches respectively.

At the time when faecal pellets were deposited in Experiment 4 on 1st June, 1968, the weather conditions for development were unfavourable with frosts at night (mean air temperature 49.2°F, 9.8°C).   Only a few first stage larvae were recorded after 24 days of exposure and no further development was observed (Table 21).   Eggs were disintegrated after 73 days of exposure and most of the faecal pellets were broken down into the soil due to 9 successive wet days with a total of 2.49 inches of rain.   Meteorological records for the month are shown in Appendix 12.   Air temperature ranged from 29.8 to 59.7°F (-1.1 to 15.4°C) with minimum and maximum means of 43.2°F (6.2°C) and 56.2°F (13.3°C) respectively.   Minimum temperature on grass ranged from 21.2 to 49.9°F (-6.0 to 10.0°C) and the relative humidity range, total rainfall and total evaporation were 55 to 96%, 6.02 inches and 0.90 inches respectively.

In Experiment 5 faecal pellets were deposited on 1st July, 1968, and the conditions for development were still unfavourable (mean air temperature 46.1°F, 7.8°C).   Few second stage larvae were recorded on 5th August, 1968.

Table 19

Rate of development of the free living stages of _Cooperia_ _curticei_ in faeces under natural conditions during the month of April 1968

Stages of development		Time (days) required for Development		
		Date	Days	Percentage
E_1,	Gastrula			
	Minimum =	7. 4. 68 (morning)	1	unembryonated 44% E_1 = 56%
	Maximum =	7. 4. 68 (evening)	1.5 =	E_1 = 92% E_2 = 8%
E_2,	Tadpole			
	Minimum =	7. 4. 68		
	Maximum =	8. 4. 68	2 =	E_1 = 2% E_2 = 90% E_3 = 8%
E_3,	Prehatch			
	Minimum =	8. 4. 68		
	Maximum =	9. 4. 68	3 =	E_2 = 2% E_3 = 78% L_1 = 20%
L_1,	First stage larvae			
	Minimum =	9. 4. 68		
	Maximum =	10. 4. 68	4 =	E_3 = 8% L_1 = 76% L_2 = 16%
L_2,	Second stage larvae			
	Minimum =	10. 4. 68		
	Maximum =	16. 4. 68	10 =	L_1 = 2% L_2 = 91% L_3 = 7%
L_3,	Third stage larvae			
	Minimum =	16. 4. 68		
	Maximum =	27. 4. 68	21 =	L_2 = 8% L_3 = 92%

Percentage recovery of infective larvae from duplicate samples   12.7 & 23.4% (mean = 18.0%)

Table 20

Rate of development of the free living stages of <u>Cooperia curticei</u> in faeces under natural conditions during the month of May 1968

Stages of development	Time (days) required for Development		
	Date	Days	Percentage
E_1, Gastrula			
Minimum =	3. 5. 68 (morning)	1 =	mostly unembryonated
Maximum =	3. 5. 68 (evening)	1.5 =	$E_1 = 93\%$ $E_2 = 7\%$
E_2, Tadpole			
Minimum =	3. 5. 68		
Maximum =	4. 5. 68	2 =	$E_1 = 3\%$ $E_2 = 84\%$ $E_3 = 13\%$
E_3, Prehatch			
Minimum =	4. 5. 68		
Maximum =	5. 5. 68	3 =	$E_1 = 2\%$ $E_2 = 13\%$ $E_3 = 84\%$ $L_1 = 1\%$
L_1, First stage larvae			
Minimum =	5. 5. 68		
Maximum =	8. 5. 68	6 =	$E_3 = 2\%$ $L_1 = 96\%$ $L_2 = 2\%$
L_2, Second stage larvae			
Minimum =	8. 5. 68		
Maximum =	14. 5. 68	12 =	$L_1 = 8\%$ $L_2 = 87\%$ $L_3 = 5\%$
L_3, Third stage larvae			
Minimum =	14. 5. 68		
Maximum =	30. 5. 68	28 =	$L_2 = 8\%$ $L_3 = 92\%$

Percentage recovery of infective larvae from duplicate samples

6.8 & 13.6%
(mean 10.2%)

Table 21

Rate of development of the free living stages of <u>Cooperia</u> <u>curticei</u> in
faeces under natural conditions during the month of June 1968

Stages of development		Time (days) required for Development		
		Date	Days	Percentage
E_1, Gastrula				
	Minimum =	1. 6. 68	1	= unembryonated
				= more than 90%
	Maximum =	3. 6. 68		
E_2, Tadpole				
	Minimum =	3. 6. 68	3	= unembryo = 5%
				E_1 = 83%
				E_2 = 12%
	Maximum =	12. 6. 68		
E_3, Prehatch				unembryo = 5%
	Minimum =	12. 6. 68	12	=
				E_1 = 20%
				E_2 = 72%
				E_3 = 3%
	Maximum =	-		
L_1, First stage larvae				unembryo = o%
	Minimum =	24. 6. 68	24	=
				E_1 = 21%
	Maximum =	-		E_2 = 58%
				E_3 = 16%
				L_1 = 5%
L_2, Second stage larvae				
	Minimum =	-		
	Maximum =	-		
L_3, Third stage larvae				
	Minimum =	-		
	Maximum =	-		

All eggs disintegrated in 73 days time.

after 36 days of exposure but no further development was observed.   (Table 22).
All eggs were disintegrated after 64 days and faecal pellets in most of the
samples were broken down into the soil after 44 days, when total rainfall
reached 4.04 inches.   Weather records for the month are given in Appendix 13.
Air temperature ranged from 30.8 to 61.1°F (-0.6 to 16.1°C) with minimum and
maximum means of 39.0°F (3.9°C) and 53.1°F (11.7°C) respectively.   Minimum
temperatures on grass ranged from 23.5 to 48.5°F (-4.7 to 9.2°C) and the
relative humidity range, total rainfall and total evaporation were 69 to 98%,
2.93 inches and 0.78 inches respectively.

When faecal pellets were deposited on 1st August, 1968, in Experiment 6,
weather conditions for development were still relatively unfavourable.
Development was recorded up to second stage larvae but with a high rate of
mortality probably due to frequent frost at nights, only a few infective larvae
were recorded on 2nd September, 1968, after 33 days of exposure (Table 23).
After 36 days eggs were not present in samples.   Meteorological records for
the month are given in Appendix 14.   Air temperature ranged from 31.6 to 65.0°F
(-0.4 to 18.3°C) with minimum and maximum means of 41.4°F (5.2°C) and 56.1°F
(13.3°C) respectively.   Minimum on grass ranged from 25.2 to 47.8°F (-3.9 to
8.8°C) and the relative humidity range, total rainfall and total evaporation
were 74 to 96%, 2.51 inches and 1.36 inches respectively.

By the time faecal pellets were deposited on the first day of September,
October and November, 1968, in Experiment 7, 8 and 9, the weather conditions
were just favourable for development (mean air temperature ranged from 50.7 to
52.1°F, 10.5 to 11.2°C) and there was little difference in fluctuation in
temperatures in these three months.   Maximum time required for development to
infective larvae ranged from 23 to 25 days.   The rates of development for the
month of September, October and November, 1968, are shown in Tables 24, 25 and
26, and the percentage recovery of infective larvae from duplicate samples was
4.5 and 18.3, 10.76 and 14.88, and 6.46 and 12.65 respectively.   Daily weather
data for the period up to maximum development of infective larvae for
experiment 7 (September), 8 (October) and 9 (November) are given in Appendices
15, 16 and 17 respectively.   Meteorological data for the period of develop-
ment for these experiments are summarised as follows.

For experiment 7 (September) air temperature ranged from 35.1 to 65.5°F

Table 22

Rate of development of the free living stages of _Cooperia curticei_ in
faeces under natural conditions during the month of July 1968

Stages of development		Time (days) required for Development		
		Date	Days	Percentage
E_1,	Gastrula			
	Minimum =	1, 7, 68	1	= unembryo = 89%
				E_1 = 11%
	Maximum =	5, 7. 68		
E_2,	Tadpole			unembryo = 5%
	Minimum =	5, 7. 68	5 =	E_1 = 93%
				E_2 = 2%
	Maximum =	14, 7. 68		
E_3,	Prehatch			unembryo = 2%
	Minimum =	14. 7, 68	14 =	E_1 = 12%
				E_2 = 84%
				E_3 = 2%
	Maximum =	–		
L_1,	First stage larvae			Eggs damaged = 3%
	Minimum =	26. 7. 68	26 =	E_1 = 11%
	Maximum =	–		E_2 = 57%
				E_3 = 23%
				L_1 = 6%
L_2,	Second stage larvae			Eggs damaged = 19%
	Minimum =	5. 8. 68	36 =	E_1 = 10%
	Maximum =	–		E_2 = 64%
				E_3 = 7%
				L_1 = 2%
				L_2 = 4%
L_3,	Third stage larvae			
	Minimum =	–		
	Maximum =	–		

All eggs disintegrated in 64 days time.

Table 23

Rate of development of the free living stages of _Cooperia curticei_ in faeces under natural conditions during the month of August 1968

Stages of development		Time (days) required for development		
		Date	Days	Percentage
E_1,	Gastrula			
	Minimum =	1. 8. 68	1	= unembryo = 90%
				E_1 = 10%
	Maximum =	2. 8. 68		
E_2,	Tadpole			unembryo = 8%
	Minimum =	2. 8. 68	2 =	E_1 = 82%
				E_2 = 10%
	Maximum =	5. 8. 68		
E_3,	Prehatch			E_1 = 3%
	Minimum =	5. 8. 68	5 =	E_2 = 81%
				E_3 = 16%
	Maximum =	10. 8. 68		
L_1,	First stage larvae			E_1 = 1%
	Minimum =	10. 8. 68	10 =	E_2 = 11%
				E_3 = 84%
				L_1 = 4%
	Maximum =	15. 8. 68		
L_2,	Second stage larvae			E_3 = 6%
	Minimum =	15. 8. 68	15 =	L_1 = 90%
	Maximum =	—		L_2 = 4%
L_3	Third stage larvae			Eggs = damaged
	Minimum =	2. 9. 68	33 =	L_2 = few (14)
	Maximum =	—		L_3 = few (5)
				Total larvae
				recovered =
				19 only

Table 24

Rate of development of the free living stages of <u>Cooperia</u> <u>curticei</u> in faeces under natural conditions during the month of September 1968

Stages of development		Time (days) required for development		
		Date	Days	Percentage
E_1,	Gastrula			unembryo = 85%
	Minimum =	1. 9. 68	1 =	E_1 = 15%
	Maximum =	2. 9. 68	2 =	unembryo = 3%
				E_1 = 80%
				E_2 = 17%
E_2,	Tadpole			
	Minimum =	2. 9. 68		unembryo = 2%
	Maximum =	4. 9. 68	4 =	E_1 = 3%
				E_2 = 86%
				E_3 = 9%
E_3,	Prehatch			
	Minimum =	4. 9. 68		E_1 = 3%
	Maximum =	6. 9. 68	6 =	E_2 = 16%
				E_3 = 79%
				L_1 = 2%
L_1,	First stage larvae			
	Minimum =	6. 9. 68		E_3 = Few
	Maximum =	9. 9. 68	9 =	L_1 = 96%
				L_2 = 4%
L_2,	Second stage larvae			
	Minimum =	9. 9. 68		L_1 = 2%
	Maximum =	15. 9. 68	15 =	L_2 = 91%
				L_3 = 7%
L_3,	Third stage larvae			
	Minimum =	15. 9. 68		L_2 = 6%
	Maximum =	25. 9. 68	25 =	L_3 = 94%

Percentage recovery of infective larvae from duplicate samples   4.5 & 18.3% (mean 11.4%)

Table 25

Rate of development of the free living stages of _Cooperia_ _curticei_ in faeces under natural conditions during the month of October 1968

Stages of development	Time (days) required for development		
	Date	Days	Percentage
E_1, Gastrula			unembryo = 61%
Minimum =	1. 10. 68	1 =	E_1 = 39%
Maximum =	2. 10. 68	2 =	unembryo = 4%
			E_1 = 87%
E_2, Tadpole			E_2 = 9%
Minimum =	2. 10. 68		E_1 = 2%
Maximum =	3. 10. 68	3 =	E_2 = 94%
			E_3 = 4%
E_3, Prehatch			
Minimum =	3. 10. 68		E_2 = 12%
Maximum =	4. 10. 68	4 =	E_3 = 71%
			L_1 = 17%
L_1, First stage larvae			
Minimum =	4. 10. 68		Eggs = Few
Maximum =	7. 10. 68	7 =	L_1 = 88%
			L_2 = 12%
L_2, Second stage larvae			
Minimum =	7. 10. 68		L_1 = 1%
Maximum =	12. 10. 68	12 =	L_2 = 87%
			L_3 = 12%
L_3, Third stage larvae			
Minimum =	12. 10. 68		L_2 = 4%
Maximum =	23. 10. 68	23 =	L_3 = 96%

Percentage recovery of infective
larvae from duplicate samples

10.76 & 14.88%
(mean 12.8%)

Table 26

Rate of development of the free living stages of <u>Cooperia</u> <u>curticei</u> in faeces under natural conditions during the month of November 1968

Stages of development		Time (days) required for development		
		Date	Days	Percentage
E_1,	Gastrula			
	Minimum =	1. 11. 68 (morning)	1 =	unembryo = 69% E_1 = 31%
	Maximum =	1. 11. 68 (evening)	1.5 =	unembryo = 4% E_1 = 87% E_2 = 9%
E_2,	Tadpole			
	Minimum =	1. 11. 68		E_1 = 14%
	Maximum =	2. 11. 68	2 =	E_2 = 82% E_3 = 4%
E_3,	Prehatch			
	Minimum =	2. 11. 68		E_1 = 0%
	Maximum =	3. 11. 68	3 =	E_2 = 16% E_3 = 82% L_1 = 2%
L_1,	First stage larvae			
	Minimum =	3. 11. 68		E_3 = Few
	Maximum =	6. 11. 68	6 =	L_1 = 88% L_2 = 12%
L_2,	Second stage larvae			
	Minimum =	6. 11. 68		L_1 = 4%
	Maximum =	12. 11. 68	12 =	L_2 = 89% L_3 = 7%
L_3,	Third stage larvae			
	Minimum =	12. 11. 68		L_2 = 2%
	Maximum =	23. 11. 68	23 =	L_3 = 98%

Percentage recovery of infective larvae from duplicate samples

6.46 & 12.65% (mean 8.5%)

(1.7 to 18.5°C), with a minimum and maximum mean of 44.2°F (6.8°C) and 57.1°F (13.9°C) respectively.   Minimum temperature on grass ranged from 28.2 to 49.5°F (-2.1 to 9.7°C), and the relative humidity range, total rainfall and total evaporation were 68 to 95%, 2.06 inches and 1.67 inches respectively.

For experiment 8 (October) air temperature ranged from 34.9 to 64.6°F (1.7 to 18.1°C), with a minimum and maximum mean of 46.1°F (7.8°C) and 58.1°F (14.4°C) respectively.   Minimum temperature ranged from 30.9 to 52.8°F (-0.6 to 11.6°C) and the relative humidity range, total rainfall and total evaporation were 60 to 97%, 3.51 inches and 2.15 inches respectively.

For experiment 9 (November) air temperature ranged from 37.0 to 69.8°F (2.8 to 21.0°C) with a minimum and maximum mean of 47.3°F (8.5°C) and 62.0°F (16.7°C) respectively.   Minimum temperature on grass ranged from 33.8 to 51.4°F (1.0 to 10.8°C) and the relative humidity range, total rainfall and total evaporation were 54 to 83%, 1.37 inches and 3.10 inches respectively.

In experiment 10 faecal pellets were deposited on 1st December, 1968. Data on the rate of development are shown in Table 27.   Minimum and maximum development of infective larvae was in 9 and 15 days time with 3.0% and 8.56% recovery from duplicate samples.   The daily weather chart for the period of development is given in Appendix 18.   Air temperature ranged from 37.1 to 71.1°F (2.8 to 21.1°C) with a minimum and maximum mean of 50.7°F (10.4°C) and 65.7°F (18.7°C) respectively.   Minimum temperature on grass ranged from 31.6 to 54.5°F (-0.9 to 12.5°C) and the relative humidity range, total rainfall and total evaporation were 58 to 98%, 3.37 inches and 2.83 inches respectively.

At the time when faecal pellets were placed outside on the first days of January and of February, 1969, for experiments 11 and 12, conditions for development were good (mean air temperature 62.1 to 62.7°F, 16.8 to 17.1°C) and rate of development was rapid as shown in Tables 28 and 29.   Minimum and maximum development of infective larvae were 6 to 8 and 10 days and percentage recovery from duplicate samples for experiment 11 and 12 was 0.23 and 1.34, and 11.9 and 36.2 respectively.   Poor recovery in experiment 11 (January) was probably due to dry weather during the first week, through which the faecal pellets became very hard and dry.   A replicate experiment was set up on 9th January, 1969 and results were similar to those shown in Table 28.   The infective larvae (maximum > 90) were developed in 11 days with a poor percentage

Table 27

Rate of development of the free living stages of Cooperia curticei in
faeces under natural conditions during the month of December 1968

Stages of development		Time (days) required for development		
		Date	Days	Percentage
E_1, Gastrula				
	Minimum =	1. 12. 68 (morning)	1 =	unembryo = 55% E_1 = 45%
	Maximum =	1. 12. 68 (evening)	1.5 =	unembryo = 5% E_1 = 88% E_2 = 7%
E_2, Tadpole				
	Minimum =	1. 12. 68		unembryo = 2%
	Maximum =	2. 12. 68	2 =	E_1 = 20% E_2 = 76% E_3 = 2%
E_3, Prehatch				
	Minimum =	2. 12. 68		E_1 = 2%
	Maximum =	3. 12. 68	3 =	E_2 = 15% E_3 = 70% L_1 = 13%
L_1, First stage larvae				
	Minimum =	3. 12. 68		Eggs = few
	Maximum =	5. 12. 68	5 =	L_1 = 82% L_2 = 18%
L_2, Second stage larvae				
	Minimum =	5. 12. 68		L_1 = 2%
	Maximum =	9. 12. 68	9 =	L_2 = 92% L_3 = 6%
L_3, Third stage larvae				
	Minimum =	9. 12. 68		L_2 = 4%
	Maximum =	15. 12. 68	15 =	L_3 = 96%

Percentage recovery of infective
larvae from duplicate samples

3.0 & 8.56%
(mean 5.7%)

Table 28

Rate of development of the free living stages of Cooperia curticei in
faeces under natural conditions during the month of January 1969

Stages of development	Time (days) required for development		
	Date	Days	Percentage
E_1, Gastrula			
Minimum =	—		unembryo = 9%
Maximum =	1. 1. 69	1 =	E_1 = 89%
			E_2 = 2%
E_2, Tadpole			
Minimum =	1. 1. 69		unembryo = 1%
Maximum =	2. 1. 69	2 =	E_1 = 15%
			E_2 = 81%
			E_3 = 3%
E_3, Prehatch			
Minimum =	2. 1. 69		E_2 = 3%
Maximum =	3. 1. 69	3 =	E_3 = 62%
			L_1 = 35%
L_1, First stage larvae			
Minimum =	3. 1. 69		L_1 = 70%
Maximum =	5. 1. 69	5 =	L_2 = 30%
L_2, Second stage larvae			
Minimum =	5. 1. 69		L_1 = 2%
Maximum =	8. 1. 69	8 =	L_2 = 84%
			L_3 = 14%
L_3, Third stage larvae			
Minimum =	8. 1. 69		L_2 = 1%
Maximum =	10. 1. 69	10 =	L_3 = 99%
Percentage recovery of infective larvae from duplicate samples			0.23 & 1.34% (mean 0.7%)

Table 29

Rate of development of the free living stages of <u>Cooperia</u> <u>curticei</u> in
faeces under natural conditions during the month of February 1969

Stages of development	Time (days) required for development		
	Date	Days	Percentage
E_1, Gastrula			
Minimum =	-		unembryo = 2%
Maximum =	1. 2. 69	1 =	E_1 = 83%
			E_2 = 15%
E_2, Tadpole			
Minimum =	1. 2. 69		unembryo = 1%
Maximum =	2. 2. 69	2 =	E_1 = 8%
			E_2 = 85%
			E_3 = 6%
E_3, Prehatch			
Minimum =	2. 2. 69		E_1 = 2%
Maximum =	3. 2. 69	3 =	E_2 = 19%
			E_3 = 69%
			L_1 = 10%
L_1, First stage larvae			
Minimum =	3. 2. 69		Eggs = few
Maximum =	4. 2. 69	4 =	L_1 = 96%
			L_2 = 4%
L_2, Second stage larvae			
Minimum =	4. 2. 69		L_1 = 2%
Maximum =	8. 2. 69	6 =	L_2 = 81%
			L_3 = 17%
L_3, Third stage larvae			
Minimum =	8. 2. 69		L_2 = 2%
Maximum =	10. 2. 69	10 =	L_3 = 98%

Percentage recovery of infective		11.9 & 36.2%
larvae from duplicate samples		(mean 24.0%)

recovery.   Meteorological records for the period of maximum development of infective larvae for experiments 11 and 12 are given in Appendices 19 and 20 respectively.   Weather data for the period of development of experiments 11 and 12 are summarised as follows;

For experiment 11 (January) air temperature ranged from 51.0 to 79.5°F (10.6 to 26.4°C) with a minimum and maximum mean of 54.6°F (12.5°C) and 70.7°F (21.5°C) respectively.   Minimum temperature on grass ranged from 45.9 to 60.2°F (10.0 to 15.7°C) and the relative humidity range, total rainfall and total evaporation were 57 to 88%, 1.76 inches and 1.43 inches respectively.

For experiment 12 (February) air temperature ranged from 43.0 to 75.7°F (6.1 to 24.3°C) with a minimum and maximum mean of 54.5°F (12.5°C) and 69.7°F (20.9°C) respectively.   Minimum temperature on grass ranged from 43.0 to 58.2°F (6.1 to 14.5°C) and the relative humidity range, total rainfall and total evaporation were 60 to 90%, 1.83 inches and 1.36 inches respectively.

The results on development of the infective larvae throughout the year from March, 1968 to February, 1969, and their corresponding meteorological records are given in Table 30.   From this table it appears that the rate of development was strongly influenced by temperature.   Relative humidity and rainfall may have produced effects on the rate of survival and percentage recovery of the infective larvae but they did not appear to influence the rate of development.   Development of the infective larvae was not recorded when mean air temperature was below 50°F (10°C) and mean maximum air temperature was between 53 to 56°F (11.7 to 13.3°C) but a few eggs developed to first and second larval stages.   When mean maximum air temperature was between 57 to 65°F (14 to 18°C) infective larvae were developed in 15 to 28 days and when mean maximum air temperature was between 65 to 77°F (18 to 25°C) the development was rapid and infective larvae developed in 9 to 15 days.

To test the rate of development of the infective larvae (maximum $>$ 90%) under natural conditions against the mean maximum air temperature during the period of development these data were compared (Table 31), with the predicted days of development after log transformation from the regression line equation prepared under constant temperatures (refer Appendix 1).   Analysis of variance was carried out and is shown in Appendix 21.   The variance "between developments" under natural and laboratory conditions was not significantly greater

Table 30

Rate of development of the free living stages of <u>Cooperia</u> <u>curticei</u> infaecal pellets deposited out-of-doors at different times of the year, with Meteorological records.

Experiment No.	Infected faeces deposited out-of-doors in 1968.	Time in development to third stage larvae (days)		Air temp. (range) ^{O}F	Mean Max. air Temp. ^{O}F	Mean Minimum air Temp. ^{O}F	Mean Air Temp. ^{O}F	Minimum temp. on grass ^{O}F (range)	Relative humidity (range)	Total rainfall (inches)	Total evaporation (inches)
		Minimum	Maximum > 90%								
1	March	6	9	57.0 to 81.2	76.6	62.0	69.3	49.9 to 65.3	61-91	0.65	0.86
2	April	10	21	40.6 to 77.6	63.2	49.5	56.3	35.4 to 59.4	66-97	4.09	1.41
3	May	12	28	33.8 to 66.2	65.1	45.6	53.0	26.2 to 53.1	76-99	4.66	1.14
4	June	-	-	29.8 to 59.7	56.2	43.2	49.2	21.2 to 49.9	55-96	6.02	0.90
5	July	-	-	30.8 to 61.1	53.1	39.0	46.1	23.5 to 48.6	69-98	2.93	0.78
6	August	-	-	31.6 to 65.0	56.1	41.4	48.7	25.2 to 47.8	74-96	2.51	1.36

Table 30 continued

Experiment No.	Infected faeces deposited out-of-doors in 1968	Time in development to third stage larvae (days)		Air temp. (range) °F	Mean Max. air Temp. °F	Mean Minimum air Temp. °F	Mean air Temp. °F	Minimum temp. on grass °F (range)	Relative humidity (range)	Total rainfall (inches)	Total evaporation (inches)
		Minimum	Maximum > 90%								
7	September	15	25	35.1 to 65.5	57.1	44.2	50.7	28.2 to 49.5	68-95	2.06	1.67
8	October	12	23	34.9 to 64.6	58.1	46.1	52.1	30.9 to 52.8	60-97	3.51	2.15
9	November	12	23	37.0 to 69.8	62.0	47.3	54.7	33.8 to 51.4	54-83	1.37	3.10
10	December	9	15	37.1 to 71.1	65.7	50.7	58.2	31.6 to 54.5	58-98	3.37	2.83
11	1969 January	8	10	51.0 to 79.5	70.7	54.6	62.7	45.9 to 60.2	57-88	1.76	1.43
12	February	8	10	43.0 to 75.7	69.7	54.5	62.1	43.0 to 58.2	60-90	1.83	1.36

Table 31

Month	Time for maximum (> 90%) development of infective larvae		Mean Maximum air temp.	Predicted log days of development of infective larvae (T90) from regression slope −0.033 (Appendix 1)
	Actual days	Log days		
March 1968	9	0.9542	24.7°C (76.6°F)	0.9699
April	21	1.3222	17.3°C (63.2°F)	1.1222
May	28	1.4472	18.3°C (65.1°F)	1.1818
June, July & August	No development	-	-	-
September	25	1.3979	13.9°C (57.1°F)	1.3118
October	23	1.3617	14.4°C (58.1°F)	1.3096
November	23	1.3617	16.7°C (62.0°F)	1.2330
December	15	1.1761	18.7°C (65.7°F)	1.1673
January 1969	10	1.0000	21.4°C (70.7°F)	1.0755
February	10	1.0000	21.0°C (69.7°F)	1.0934
Total:-		11.0210		10.4645

than error variance within months. These results suggest that under
natural conditions rate of development is strongly correlated with mean
maximum air temperatures over the period during which development to the
infective stage occurs.

The maximum development time in days for each month is plotted in
Fig. 6. Conversion of the days to logarithms converted this curve to a
straight line (figure 7) and the slope of this line was calculated
(Appendix 22). The slope of the regression line was -0.049. This slope
indicates that the rate of development of the infective larvae (> 90%)
changes per degree centigrade by 0.049 log units of time. This is little
different from the slope of the regression line (-0.033) obtained under
constant temperatures (refer Appendix 1). The difference in the regres-
sion line under natural conditions may be accounted for by diurnal
fluctuations in temperature during changing seasons (figure 6). Chi
square test was applied to find out the goodness of fit of this regres-
sion line prepared under natural conditions. The calculations are given
in Appendix 23. The calculated line is not significantly different from
the observed data (p <0.05). The two regression lines were compared.
The "T90" times for temperatures of $15^{o}C$, $20^{o}C$ and $25^{o}C$ were obtained
from the regression lines from laboratory and field data (Appendix 24).
The difference in intercept is shown in Appendix 25. The results indi-
cate that there was no statistically significant difference between the
two slopes.

Days.

Fig. 6

Log

Fig. 7

b=-0.49
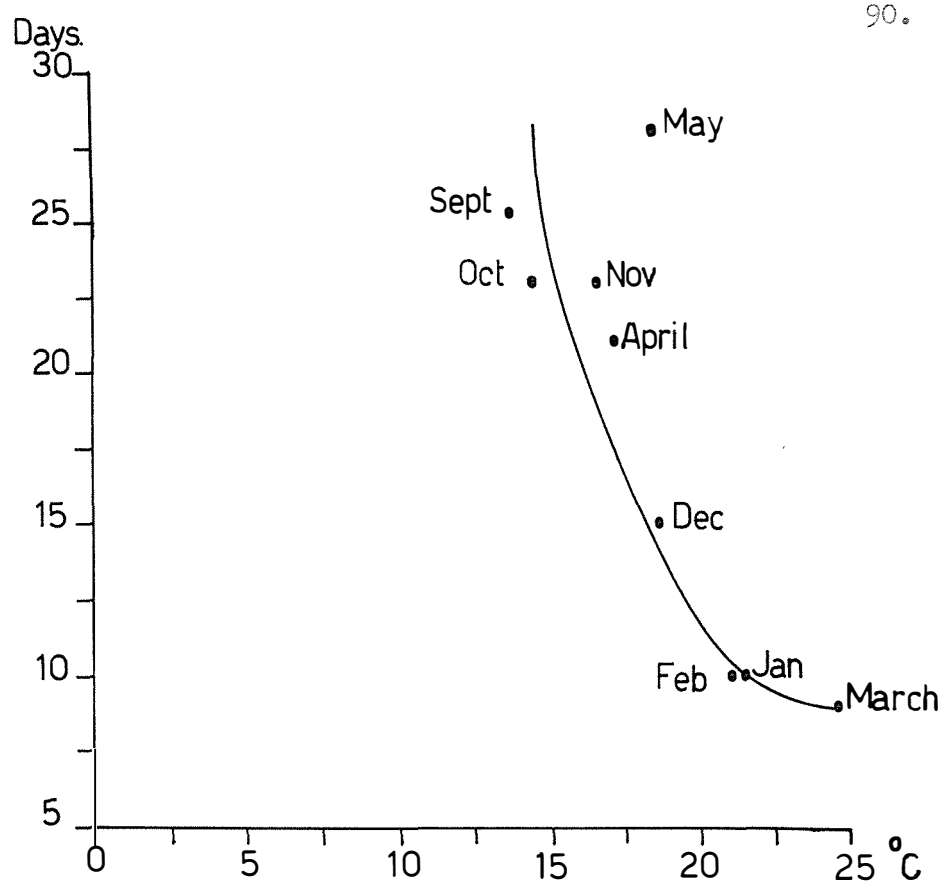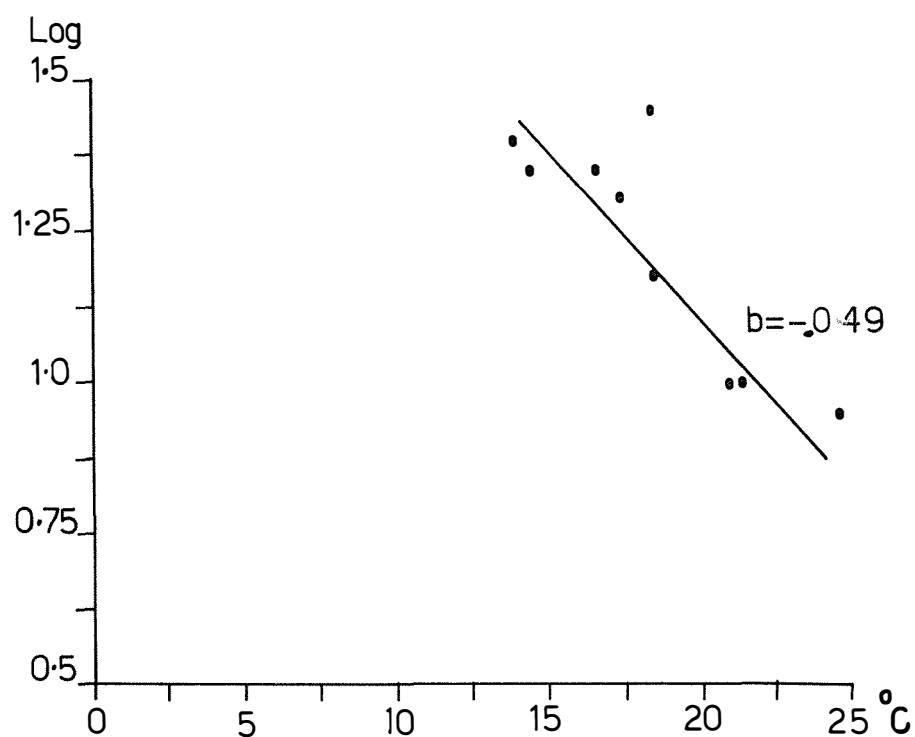
Fig. 6: Days development to infective stage (> 90%) and mean max. air temp. during period of development.

Fig. 7: Regression of development time (log days) on mean max. air temp.

ii)   <u>Survival of the infective larvae</u>:- After more than 90% of the larval stages recovered had reached the infective stage, the survival of these infective larvae was recorded. Duplicate samples were examined each week throughout the year from March, 1968 to February, 1969. Examination of the samples from each month (experiment 1-3, and 7-12) was continued until no infective larvae were recovered in two successive weeks. No observations were made on eggs deposited in June, July and August since no infective larvae were recovered at any time from these samples (see chapter IV 3 (i)).

The numbers of infective larvae recovered from herbage to 2 cm and>2 cm in height, from the faecal pellets and the soil surface are given in Appendix tables 26 to 34, together with the percentages of larvae that survived and larvae that migrated onto the herbage.

The percentage survival and the total percentage of larvae which had migrated onto the grass for each month in which observations were made are summarised in tables 32 to 40. The maximum survival, maximum percentage recovery and maximum percentage migrated are given for each month together with meteorological data in table 41. Maximum survival in ascending order with meteorological data is given in table 42. Weekly larval recoveries and migration with meteorological data are shown in figures 8 to 16. The survival of the infective larvae kept in 1 cm depth of water with each monthly experiment is summarised in table 43, and the survival maxima from table 42 are provided for comparison.

To examine the influence of meteorological factors on maximum survival, correlation coefficients were calculated on data given in table 42. Correlation coefficients are shown in Appendix 35. The calculations indicate that there was significant negative correlation between maximum survival and mean maximum air temperature. There was no correlation between survival and total evaporation. A significant positive correlation can be demonstrated between maximum survival and relative humidity and total rainfall. However, the maximum survival of the infective larvae kept in one cm depth of water was almost precisely the same as that occurring on herbage and soil throughout the year (Table 43). This suggests that in these experiments only fluctuations in the air temper-

Table 32

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 1 (March, 1968).

Period in weeks	First replicate		Second replicate		Mean	Mean
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival
1	1.00	17.00	0.62	10.00	0.81	13.50
2	0.70	7.30	1.5	4.20	1.10	5.75
3	0.46	6.24	0	0.50	0.23	3.14
4	0.50	6.40	0.18	1.00	0.34	3.70
5	1.24	5.50	1.68	4.30	1.46	4.90
6	1.40	3.00	1.28	2.00	1.34	2.50
7	1.00	2.52	0.60	1.80	0.80	2.16
8	1.46	1.82	0.66	0.74	1.06	1.28
9	1.06	1.24	0	0.60	0.53	0.92
10	0.40	0.92	0.30	0.70	0.35	0.81
11	0.66	0.66	0.42	0.48	0.54	0.57
12	0.32	0.32	0.20	0.22	0.26	0.27
13	0.05	0.10	0.07	0.35	0.06	0.22
14	0.02	0.12	0.01	0.12	0.02	0.12
15	0.05	0.05	0.06	0.06	0.05	0.06
16	0	0.12	0	0.08	0.03	0.10
17	0	0.005	0	0	0	0.002
18	0	0.08	0	0	0	0.04
19	0	0	0	0	Nil	Nil
20	0	0	0	0	Nil	Nil

Table 33

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 2 (April, 1968).

Period in weeks	First replicate		Second replicate		Mean	Mean
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival
1	0.6	23.4	2.3	12.7	1.45	18.05
2	15.58	20.85	15.70	19.90	15.64	20.37
3	12.37	18.70	13.25	18.0	12.81	18.35
4	17.16	21.13	17.96	19.5	17.56	20.32
5	13.3	17.35	4.47	7.04	8.89	12.20
6	8.26	11.11	6.58	9.0	7.41	10.56
7	5.70	6.66	2.85	4.0	4.28	5.33
8	5.08	6.70	4.43	5.10	4.76	5.90
9	6.34	7.64	4.59	6.5	5.47	7.07
10	2.61	3.02	2.23	2.83	2.42	2.98
11	0.63	0.75	0.60	0.68	0.62	0.72
12	0.26	0.35	0.41	0.46	0.34	0.42
13	0.38	0.47	0.29	0.32	0.34	0.40
14	0.48	0.52	0.34	0.38	0.41	0.45
15	0.70	0.74	0.23	0.26	0.47	0.50
16	0.23	0.27	0.13	0.18	0.18	0.23
17	0.19	0.21	0.11	0.12	0.15	0.17
18	0.14	0.15	0.06	0.11	0.10	0.13
19	0.07	0.08	0.06	0.06	0.07	0.07
20	0.04	0.05	0.03	0.04	0.04	0.05
21	0.04	0.04	0.02	0.02	0.03	0.03
22	0.06	0.06	0.01	0.01	0.04	0.04
23	0.04	0.04	-	-	0.04	0.04
24	0.03	0.03	-	-	0.03	0.03
25	0.01	0.01	-	-	0.01	0.01
26	0	0	-	-	Nil	Nil
27	0	0	-	-	Nil	Nil

Table 34

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 3 (May, 1968).

Period in weeks	First replicate		Second replicate		Mean	
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Mean Percentage migrated	Mean Percentage survival
1	5.20	13.6	1.63	6.82	3.42	10.21
2	7.05	14.0	2.12	8.20	4.59	11.10
3	4.0	12.0	2.10	8.0	3.05	10.0
4	7.32	12.25	5.15	8.05	6.24	10.15
5	8.0	10.65	4.17	5.37	6.09	8.01
6	2.10	7.93	2.28	4.73	2.19	6.33
7	3.52	9.0	3.59	8.1	3.55	8.55
8	2.55	6.52	2.70	5.72	2.63	6.12
9	3.57	6.4	2.50	4.40	3.04	5.40
10	1.75	3.0	1.93	3.0	1.84	3.00
11	4.42	5.20	2.15	3.65	3.29	4.43
12	2.62	3.72	2.85	3.55	2.74	3.64
13	1.56	2.20	1.55	2.15	1.56	2.20
14	1.30	1.80	0.70	1.32	1.00	1.56
15	1.25	1.95	1.32	1.65	1.29	1.80
16	2.15	2.80	1.40	2.02	1.78	2.41
17	1.65	2.15	1.60	2.07	1.63	2.11
18	1.37	2.40	1.24	1.90	1.32	2.15
19	0.57	1.50	-	-	0.57	1.50
20	0.80	1.05	-	-	0.80	1.05
21	0.77	1.00	-	-	0.77	1.00
22	0.67	0.80	-	-	0.67	0.80
23	0.50	0.62	-	-	0.50	0.62
24	0.35	0.35	-	-	0.35	0.35
25	0.17	0.17	-	-	0.17	0.17
26	0.02	0.02	-	-	0.02	0.02
27	0	0	-	-	Nil	Nil
28	0	0	-	-	Nil	Nil

Table 35

Percentage survival and vertical migration of the
infective larvae of _Cooperia curticei_ under natural
conditions from Experiment 7 (September,1968).

Period in weeks	First replicate		Second replicate		Mean	
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Mean Percentage migrated	Mean Percentage survival
1	1.0	18.3	0	4.5	0.5	11.50
2	0.6	7.30	0.4	2.52	0.5	4.91
3	8.6	14.12	5.88	8.44	7.14	11.28
4	8.28	10.24	3.56	5.40	5.92	7.87
5	10.4	16.08	8.0	11.72	9.20	13.90
6	8.48	13.28	5.0	9.08	6.74	11.15
7	5.16	7.04	2.6	4.04	3.88	5.54
8	4.0	4.64	2.84	3.73	3.42	4.19
9	1.9	3.1	1.8	3.0	1.90	3.10
10	1.76	2.84	1.4	1.92	1.58	2.38
11	2.10	3.40	1.4	1.84	1.75	2.62
12	1.64	3.32	1.0	1.44	1.32	2.38
13	1.4	1.60	0.4	0.4	0.9	1.00
14	1.36	1.56	0.6	0.7	0.98	1.13
15	1.12	2.6	0.6	0.7	0.86	1.65
16	2.52	2.62	1.12	1.12	1.82	1.87
17	0.3	0.3	0	0	0.3	0.3
18	0	0	0	0	Nil	Nil
19	0	0	0	0	Nil	Nil

Table 36

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 8 (October, 1968).

Period in weeks	First replicate		Second replicate		Mean	Mean
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival
1	1.0	11.40	1.8	10.76	1.40	11.08
2	10.88	14.88	8.46	10.52	9.67	12.70
3	24.30	29.44	22.16	29.38	20.23	29.41
4	9.8	10.6	8.52	5.80	7.80	9.56
5	1.32	2.32	1.30	1.86	1.31	2.09
6	1.14	2.20	0.88	1.7	1.01	1.45
7	7.2	8.0	5.4	7.42	6.3	7.71
8	1.74	2.12	0.92	1.22	1.33	1.67
9	1.0	1.22	0.58	0.92	0.79	1.07
10	0.56	0.88	0.20	0.50	0.38	0.69
11	0.36	0.48	0.90	1.04	0.63	0.76
12	1.24	1.24	0.20	0.28	0.72	0.76
13	0.24	0.30	0.18	0.18	0.21	0.24
14	0	0	0	0	Nil	Nil
15	0	0	0	0	Nil	Nil

Table 37

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 9 (November, 1968).

Period of weeks	First replicate		Second replicate		Mean	Mean
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival
1	0.2	8.95	0.3	6.46	0.25	7.71
'2	1.80	9.87	3.40	8.85	2.60	9.36
3	7.87	11.57	2.10	7.01	4.98	9.29
4	9.20	12.65	9.40	11.59	9.30	12.12
5	11.20	12.20	5.90	6.70	8.50	9.45
6	3.11	4.50	1.43	2.30	2.27	3.40
7	1.85	3.00	2.12	2.70	1.99	2.85
8	1.16	1.70	1.00	1.40	1.08	1.55
9	0.50	0.77	0.40	0.57	0.45	0.67
10	0.37	0.40	0.22	0.26	0.30	0.33
11	0.07	0.07	0.01	0.01	0.1	0.1
12	0.01	0.01	0	0	0.005	0.005
13	0	0	0	0	Nil	Nil
14	0	0	0	0	Nil	Nil

Table 38

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 10 (December,1968).

| Period in weeks | First replicate | | Second replicate | | Mean | Mean |
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival
1	0	25.24	0	18.65	0	22.03
2	0	8.56	0	3.00	0	5.78
3	3.22	7.63	1.40	6.73	2.31	7.18
4	2.26	4.84	1.37	2.68	1.82	3.76
5	1.70	2.35	1.14	1.61	1.42	1.98
6	0.13	0.45	0.17	0.43	0.15	0.44
7	0.28	0.45	0.20	0.25	0.21	0.33
8	0.31	0.41	0.13	0.22	0.22	0.32
9	0.17	0.25	0.13	0.14	0.15	0.20
10	0.05	0.10	0.04	0.05	0.05	0.08
11	0	0	0	0	Nil	Nil
12	0	0	0	0	Nil	Nil

Table 39

Percentage survival and vertical migration of the
infective larvae of Cooperia curticei under natural
conditions from Experiment 11 (January,1969).

Period in weeks	First replicate		Second replicate		Mean	Mean
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Mean Percentage migrated	Mean Percentage survival
1	0	1.19	0	0.64	0	0.92
2	0.92	1.34	0.10	0.23	0.51	0.79
3	1.30	1.59	0.28	0.44	0.79	1.02
4	0.21	0.25	0.09	0.14	0.15	0.20
5	0.31	0.40	0.14	0.20	0.23	0.30
6	0.06	0.09	0.03	0.08	0.05	0.09
7	0.04	0.06	0	0	0.02	0.03
8	0.02	0.05	0.02	0.02	0.02	0.04
9	0.02	0.04	0	0	0.01	0.02
10	0	0	0	0	Nil	Nil
11	0	0	0	0	Nil	Nil

Table 40

Percentage survival and vertical migration of the
infective larvae of _Cooperia curticei_ under natural
conditions from Experiment 12 (February,1969).

Period in weeks	First replicate		Second replicate		Mean	Mean
	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival	Percentage migrated	Percentage survival
1	0	36.2	0	11.9	0	24.50
2	9.06	29.34	5.97	25.95	7.52	27.65
3	14.82	19.94	12.30	17.98	13.56	18.96
4	11.54	20.90	6.30	8.55	8.92	14.73
5	4.80	8.28	4.21	6.02	4.51	7.15
6	2.41	4.15	1.10	2.08	1.76	3.12
7	4.65	5.95	3.83	4.52	4.25	5.24
8	1.37	3.76	1.76	2.66	1.56	3.21
9	0.82	1.87	0.68	1.47	0.76	1.67
10	1.44	5.48	0.43	0.97	0.94	3.23
11	0.27	1.33	0.18	0.42	0.23	0.88
12	0.38	0.42	0.09	0.22	0.24	0.32
13	0.72	0.90	0.12	0.20	0.42	0.55
14	0.46	0.48	0.03	0.05	0.25	0.27
15	0.37	0.46	0.03	0.06	0.20	0.26
16	0.12	0.12	0.05	0.05	0.08	0.08
17	0.01	0.01	0	0.02	0.005	0.02
18	0	0	0	0	Nil	Nil
19	0	0	0	0	Nil	Nil

Table 41

Maximum survival of the infective larvae of Cooperia curticei under natural conditions at different time of the year with meteorological records

Experiment No.	Infected faeces deposited outdoors	Maximum survival in weeks	Maximum percentage recovery	Maximum percentage migrated from total recovery	Means for the maximum period of survival				Total rainfall	Total evaporation
					Mean minimum air temp.	Mean maximum air temp.	Mean minimum temp. on grass	Mean relative humidity		
					°F	°F	°F	%	inches	inches
	1968									
1	March	18	13.50	1.46	47.3	61.4	42.5	85	17.40	6.872
2	April	25	20.37	17.56	42.9	56.5	38.1	86	21.45	7.943
3	May	26	11.10	6.24	43.4	56.9	39.4	83	21.54	11.960
4,5 & 6	June; July & August	Infective larvae could not develop refer table 21,22,23, & 30.			–	–	–	–	–	–
7	September	17	13.90	9.20	49.1	63.6	45.7	75	15.80	17.025
8	October	13	29.41	20.23	51.1	66.0	47.5	74	12.07	14.640
9	November	12	12.12	9.30	53.4	68.9	49.1	75	11.48	14.500
10	December	10	22.03	2.31	54.7	70.1	50.5	74	8.49	11.430
	1969									
11	January	9	1.02	0.79	54.7	70.1	49.7	74	4.39	9.661
12	February	17	27.65	13.56	49.5	63.6	43.9	79	8.70	11.843

Table 42

Survival in ascending order of the infective larvae of
Cooperia curticei throughout the year with meteorological records

Starting Months & Exp. No.	Maximum survival (weeks)	Mean maximum air temp.	Mean minimum air temp.	Mean minimum on grass	Relative humidity (mean)	Total rainfall (inches)	Total evaporation (inches)
11 January 1969	9	70.1°F (21.1°C)	54.7°F (12.6°C)	49.7°F ((9.8°C)	74	4.39	9.661
10 December 1968	10	70.1°F (21.1°C)	54.7°F (12.6°C)	50.0°F (10.3°C)	74	8.49	11.430
9 November 1968	12	68.9°F (20.5°C)	53.4°F (11.9°C)	49.1°F (9.4°C)	75	11.48	14.500
8 October 1968	13	66.0°F (18.9°C)	51.1°F (10.6°C)	47.5°F (8.6°C)	74	12.07	14.640
7 September 1968	17	63.6°F (17.6°C)	49.1°F (9.4°C)	45.7°F (7.6°C)	75	15.80	17.025
12 February 1969	17	63.6°F (17.5°C)	49.5°F (9.7°C)	43.9°F (6.7°C)	79	8.70	11.843
1 March 1968	18	61.4°F (16.3°C)	47.3°F (8.4°C)	42.5°F (5.9°C)	85	17.40	6.872
2 April 1968	25	56.6°F (13.6°C)	42.9°F (6.1°C)	38.1°F (3.3°C)	86	21.45	7.943
3 May 1968	26	56.9°F (13.8°C)	43.4°F (6.3°C)	39.1°F (4.1°C)	83	21.54	11.960

Table 43

Maximum survival of the infective larvae of _Cooperia curticei_ in water (depth 1 cm) under natural conditions at different times of the year

Experiment No.	1	2	3	4, 5 & 6	7	8	9	10	11	12
Months	March 1968	April	May	June July & August	Sept.	Oct.	Nov.	Dec.	Jan. 1969	Feb.
Period in weeks				**Percentage survival**						
1	100	100	100		100	100	100	100	100	100
2	92	100	98		100	100	100	97	93	99
3	87	98	98		100	98	98	97	78	99
4	65	97	98		99	96	95	93	71	95
5	44	97	96		94	96	92	90	59	87
6	32	97	95		91	87	90	81	50	79
7	20	96	95		90	85	85	62	35	77
8	18	96	91		87	79	64	46	19	75
9	15	96	90		72	56	34	21	4	68
10	12	95	90		64	36	19	3	1	60
11	10	95	86		56	22	3	0	0	51
12	4	90	81		36	9	0	—	—	36
13	2	84	75		21	3	—	(10)	(9)	23
14	1	78	72		10	1	(12)			11
15	0.5	65	60		3	0				6
16	0.5	50	42		1	—				2
17	0	35	31		0	(13)				0
18	—	23	25		—					—
19	(18)	15	23		(17)					(17)
20		5	20							
21		2	12							
22		2	10							
23		1	8							
24		0	4							
25		—	0							
		(25)	(26)							

(Column 4, 5 & 6 — June, July & August): No infective larvae developed in these months & comparisons not made.

The figures in brackets are the maximum survival in weeks of larvae in pot experiments.

Fig. 8: Larval survival & migration with meteorological data: Exp. 1 (March 1968)
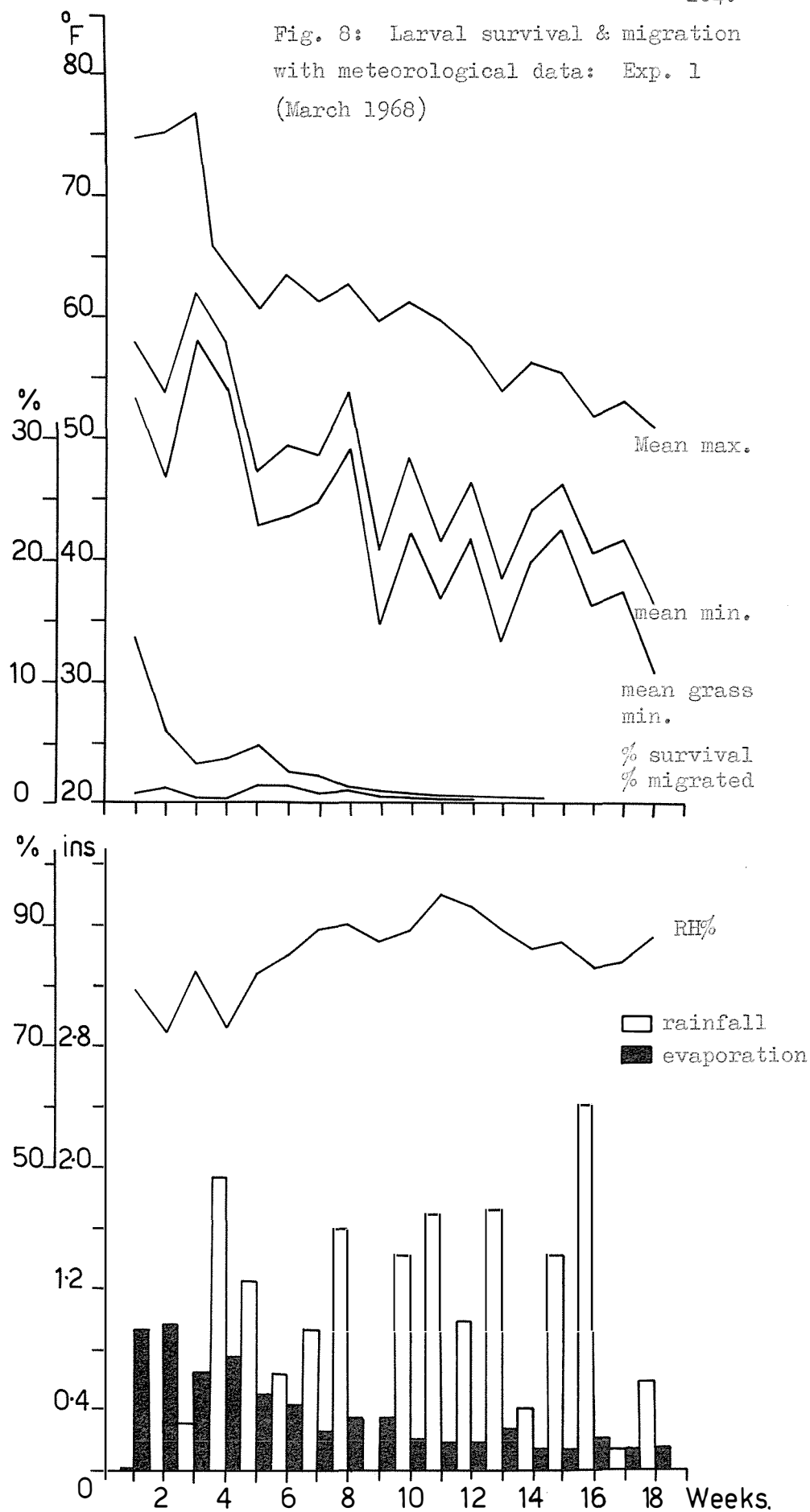
Fig. 9: Larval survival & migration with meteorological data: Exp. 2 (April 1968)
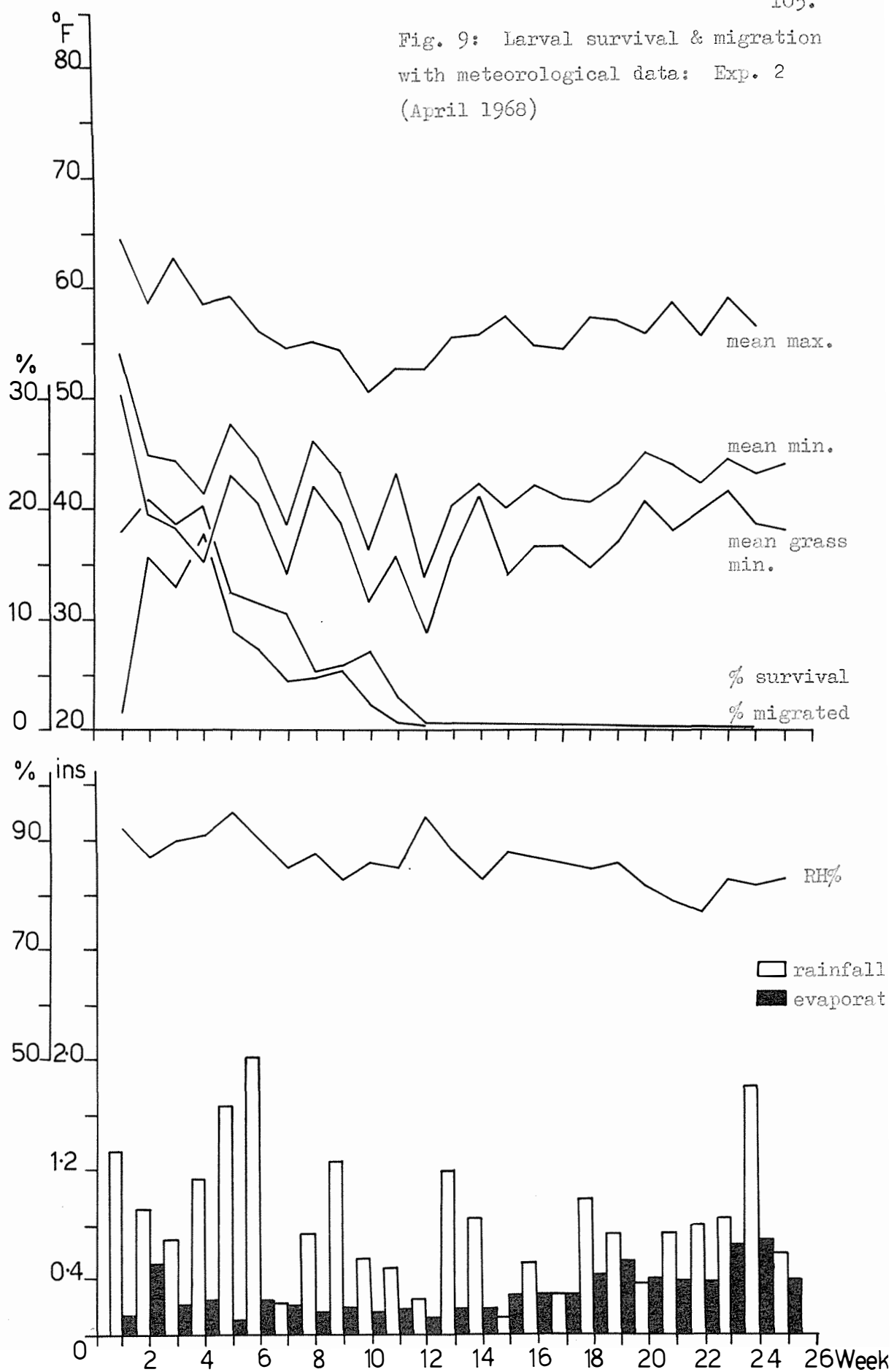
Fig. 10: Larval survival & migration with meteorological data: Exp. 3 (May 1968)
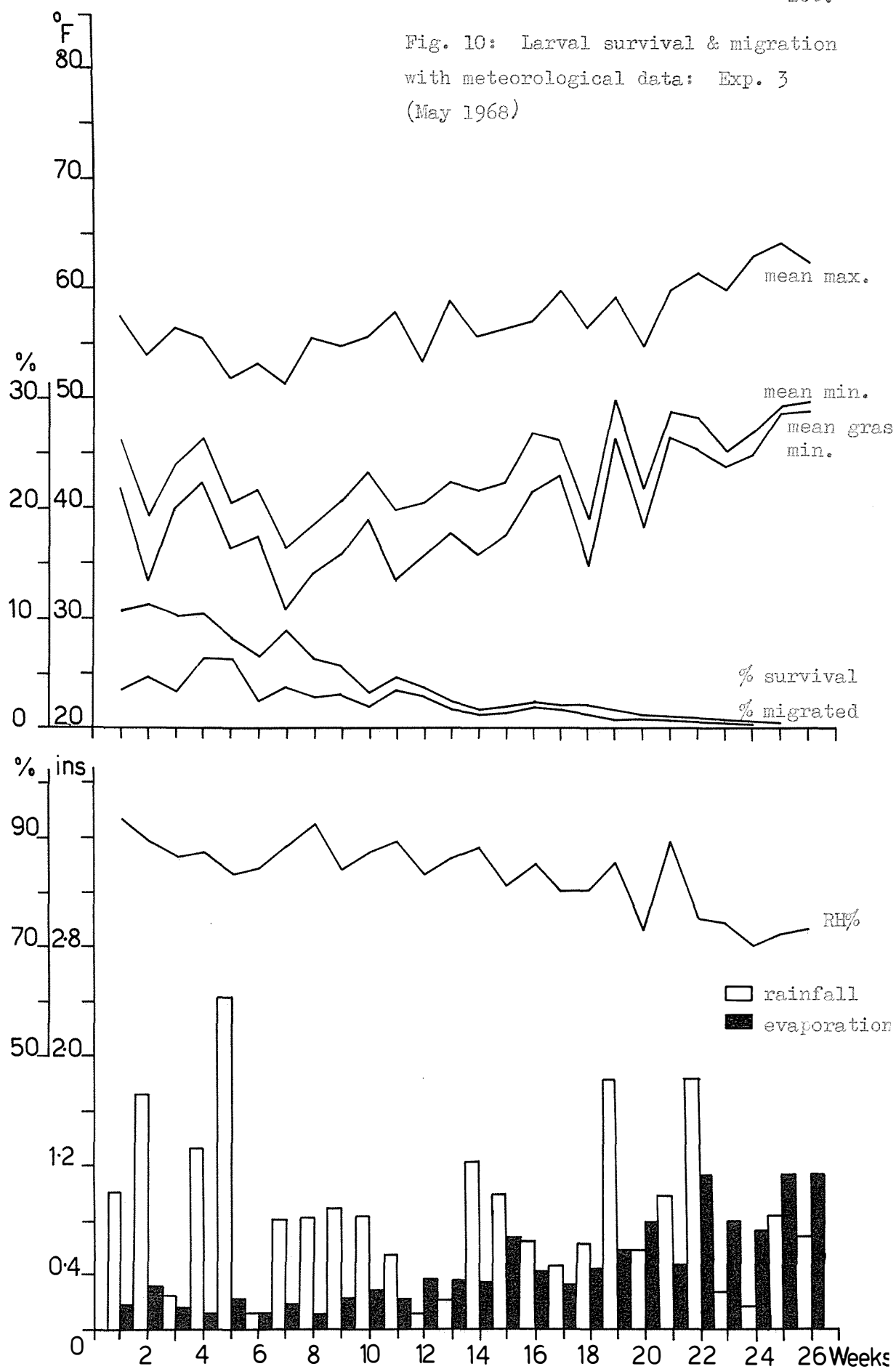
Fig. 11: Larval survival & migration with meteorological data: Exp. 7 (September 1968)
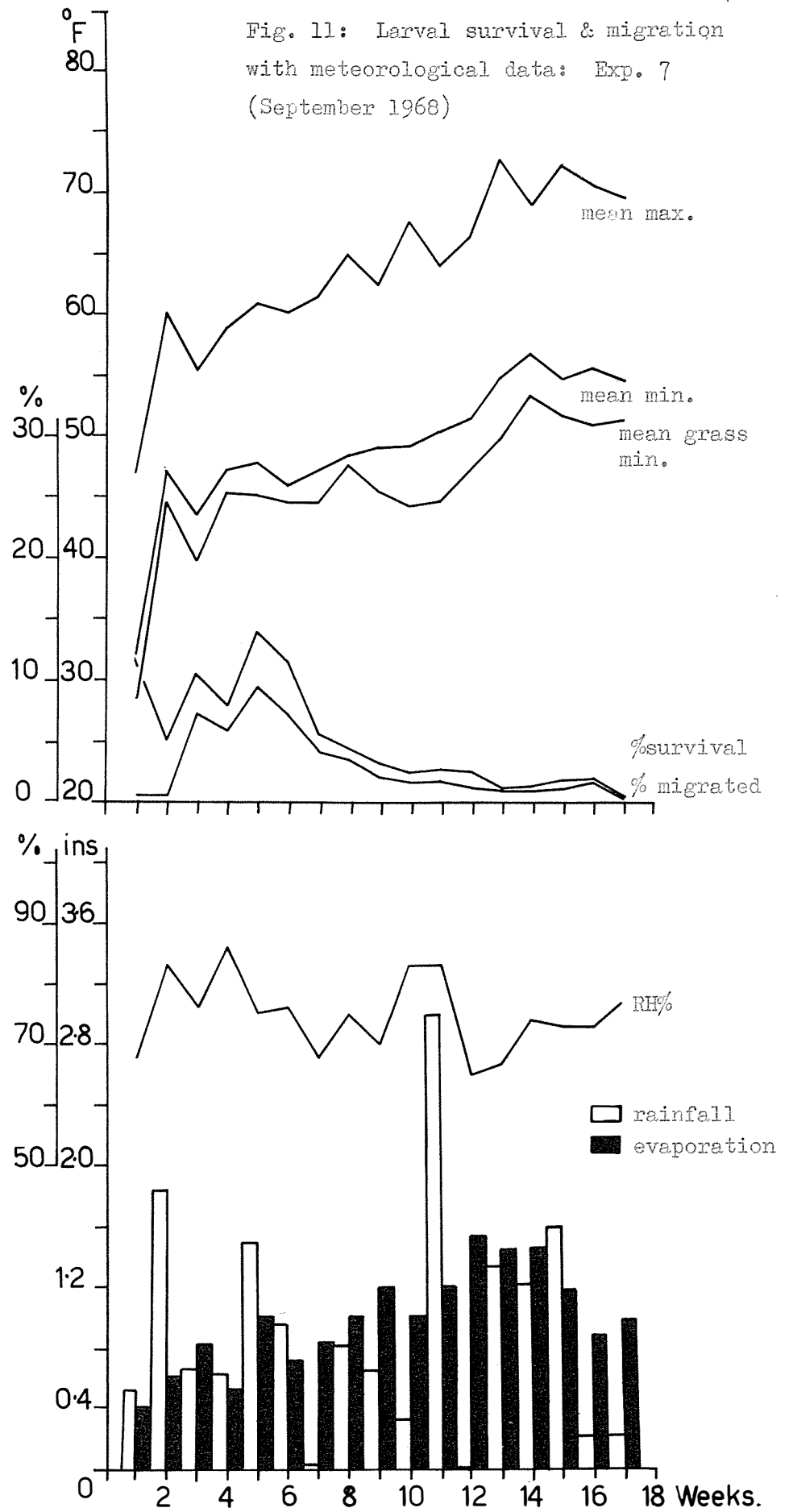
Fig. 12: Larval survival & migration with meteorological data: Exp. 8 (October 1968)

°F

80
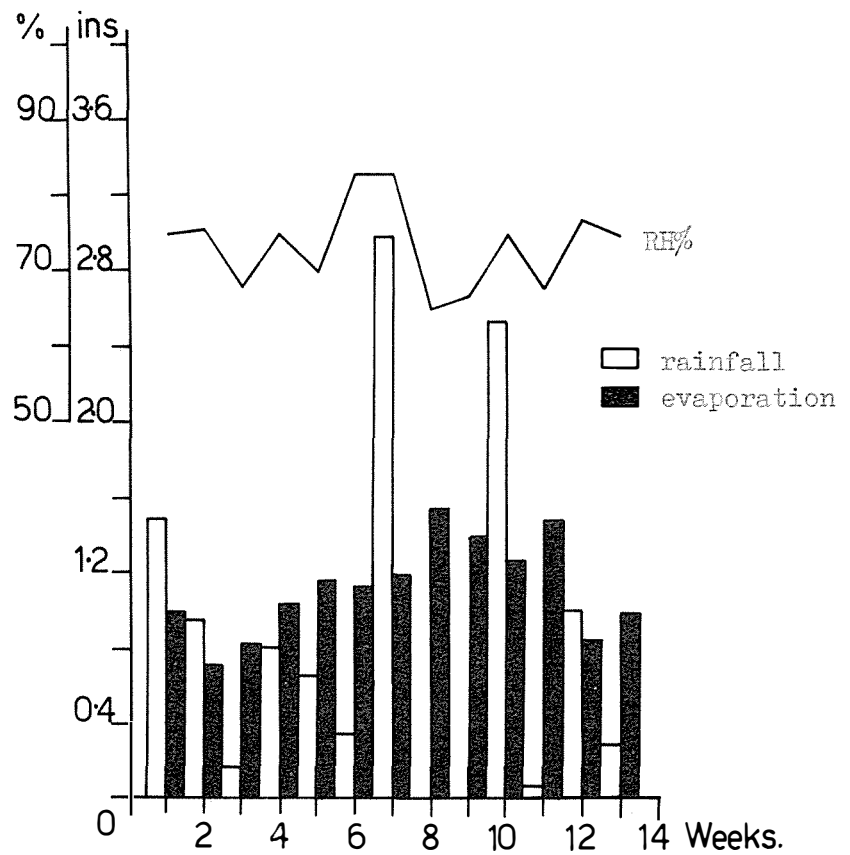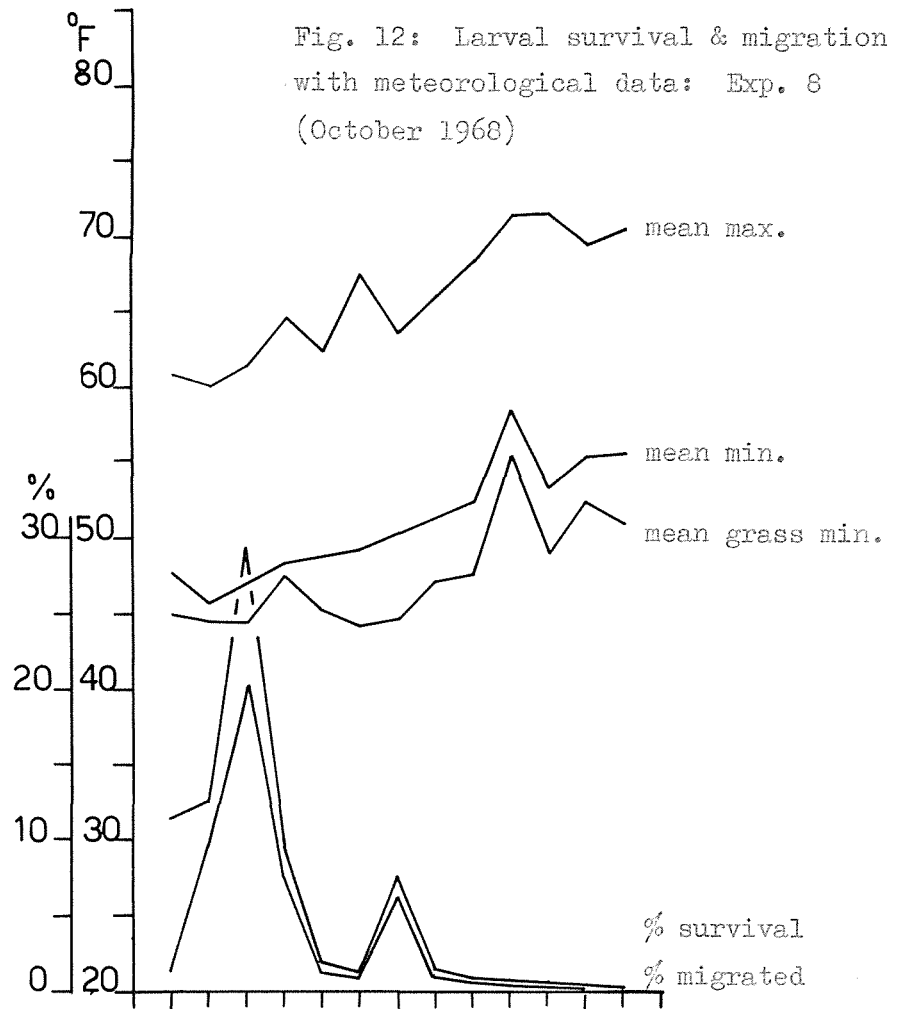
Fig. 13: Larval survival & migration
with meteorological data: Exp. 9
(November 1968)

70 — mean max.

60

%
30 | 50 — mean min.
       — mean grass min.

20 | 40

10 | 30

            % survival
            % migrated
0 | 20

% | ins

90 | 36

70 | 2·8 — RH%
            ☐ rainfall
            ▧ evaporation

50 | 20

1·2

0·4

0   2   4   6   8   10   12   weeks.
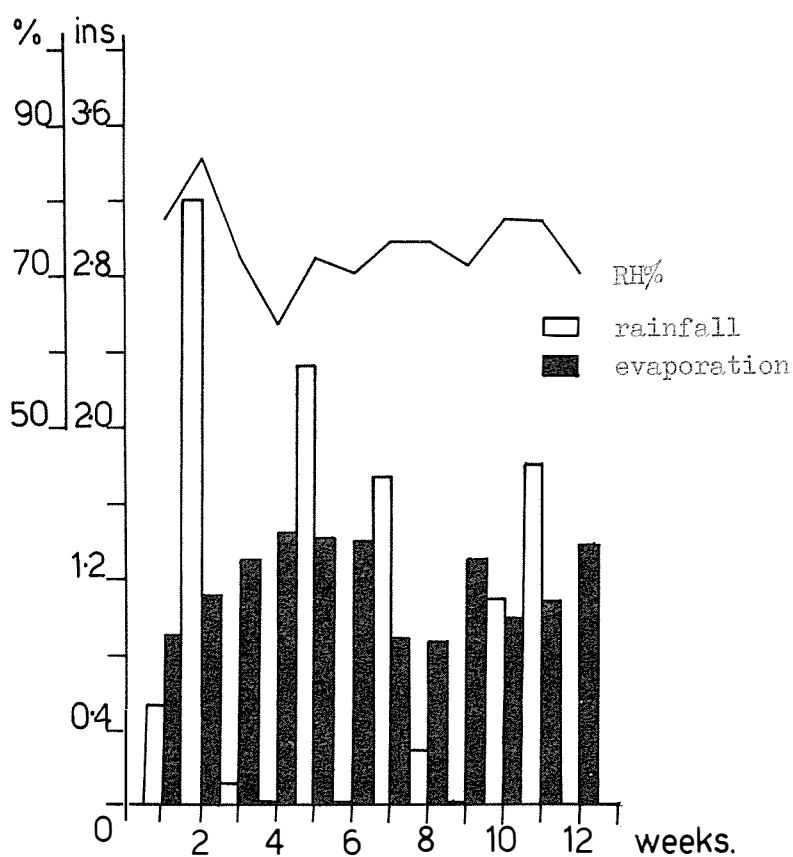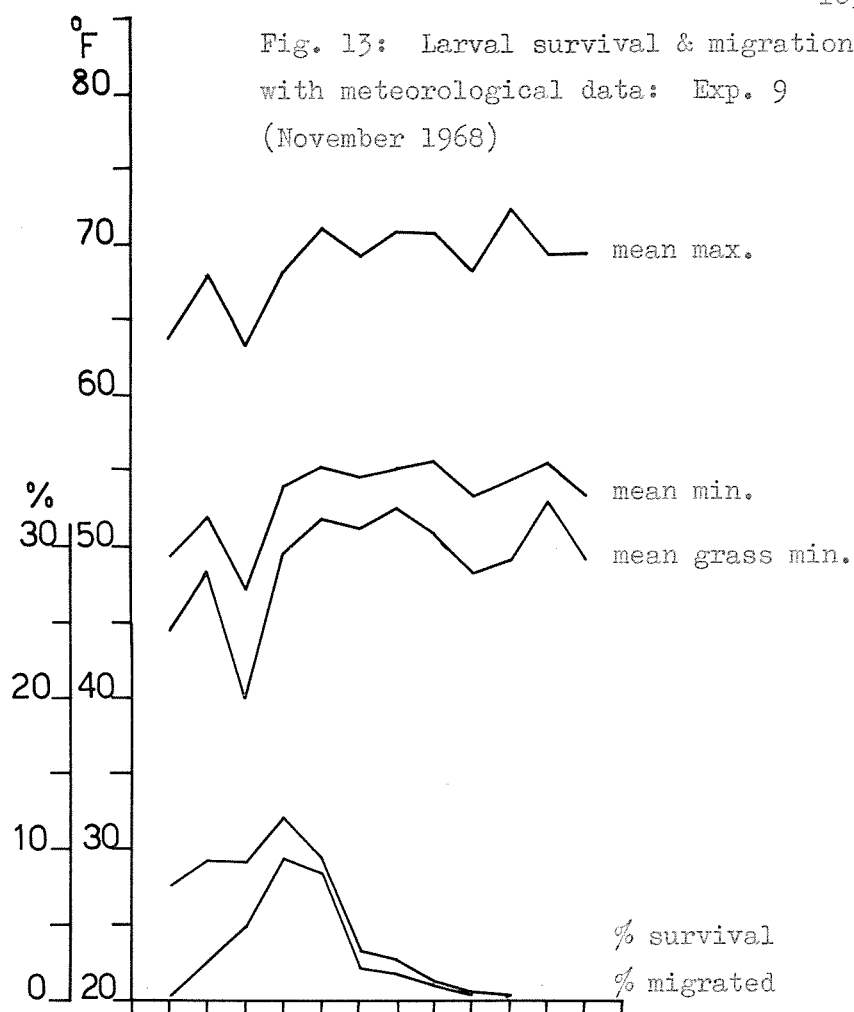
Fig. 14: Larval survival & migration with meteorological data: Exp. 10 (December 1968)
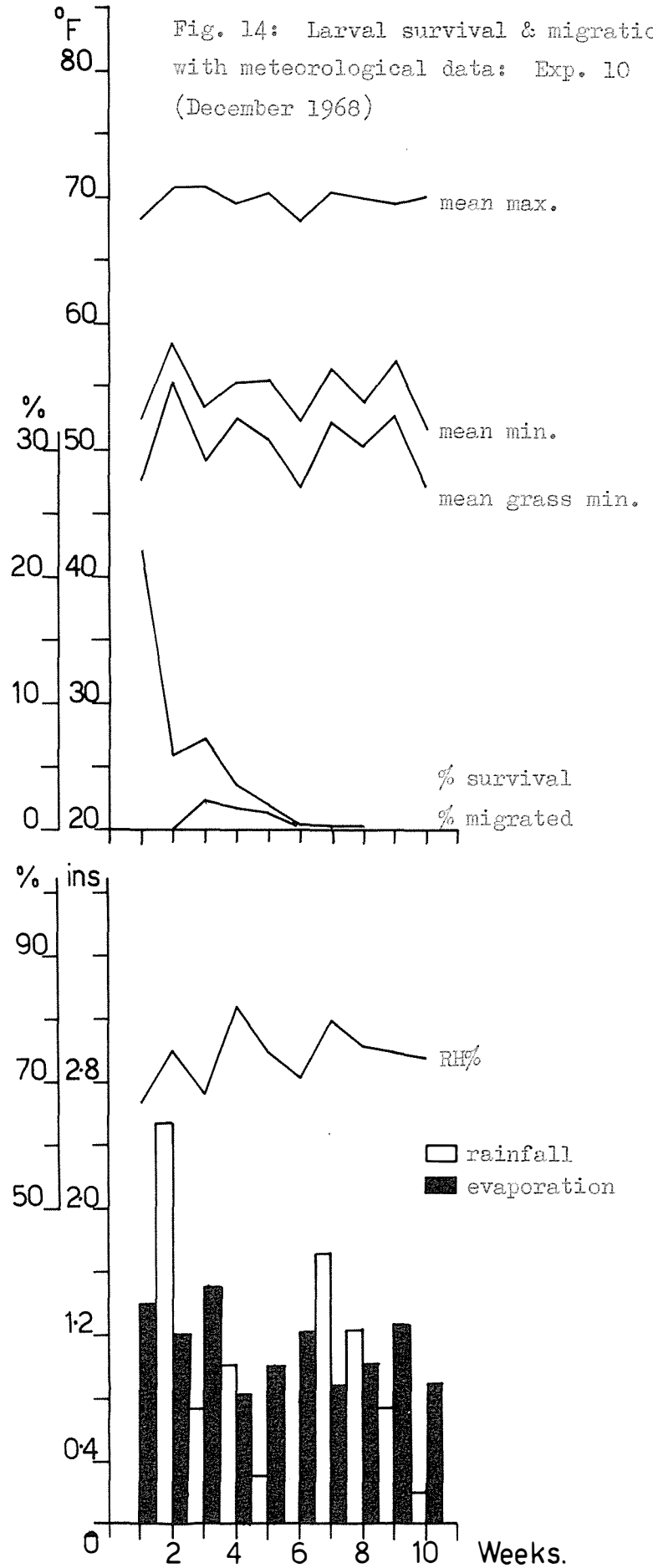
Fig. 15: Larval survival & migration with meteorological data: Exp. 11 (January 1969)
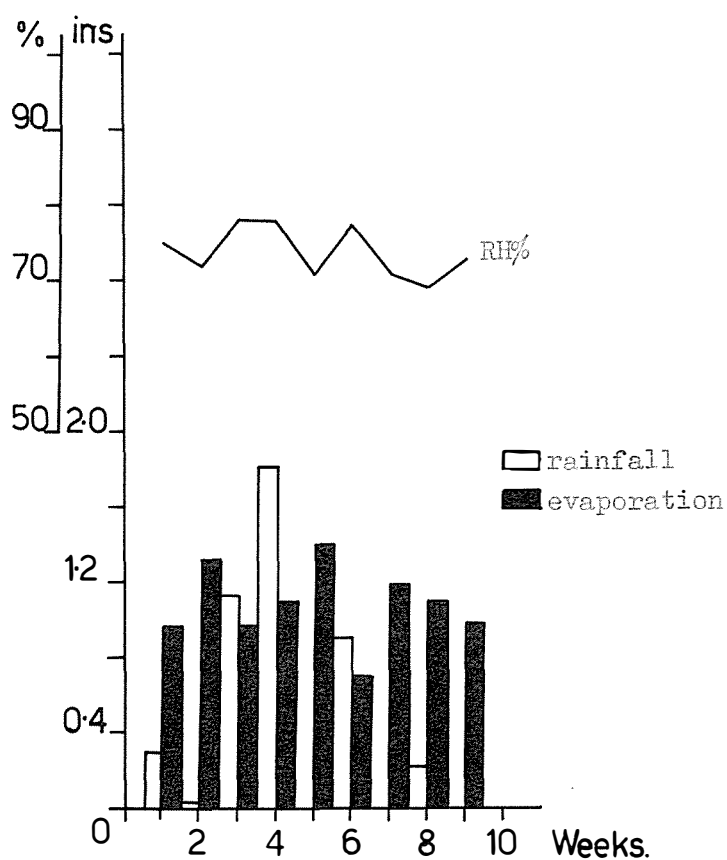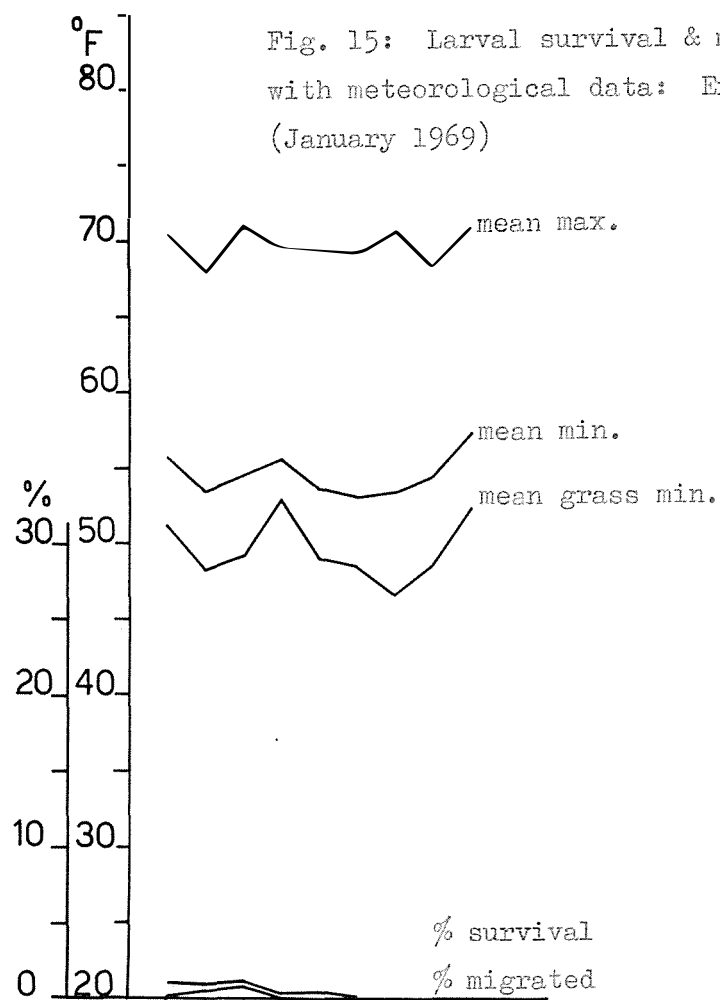
Fig. 16: Larval survival & migration with meteorological data: Exp. 12 (February 1969)

mean max.

mean min.

mean grass min.

% survival
% migrated

RH%

rainfall
evaporation
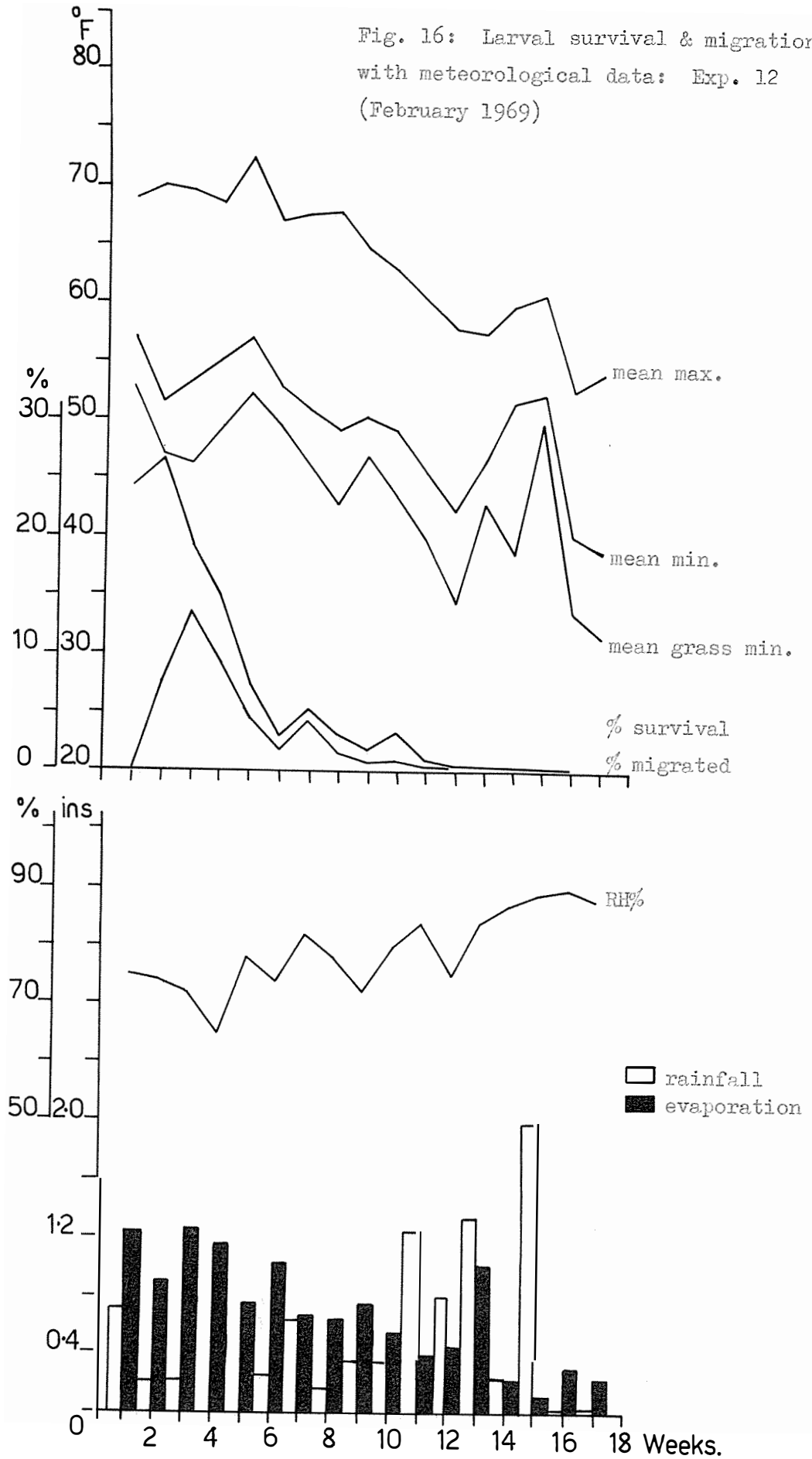
ature strongly affected the maximum survival of the infective larvae of _Cooperia_ _curticei_ and that the apparent correlation between survival and rainfall or relative humidity is spurious.

iii)   <u>Vertical migration of the infective larvae</u>:-   Vertical migration of the infective larvae of <u>Cooperia curticei</u> from 0 to 2 cm and > 2 cm in height was recorded at weekly intervals from duplicate samples throughout the year from March, 1968 to February, 1969 (Experiments 1 to 3 and 7 to 12). Actual numbers of larvae recovered are given in Appendix tables 26 to 34. The numbers of larvae migrated onto the herbage have been converted to a percentage of the numbers of larvae theoretically available i.e. the number of eggs put out. The percentage migration on the herbage in relation to survival for each monthly experiment is given in tables 32 to 40. From experiments 4,5 and 6 (June, July and August, 1968) infective larvae did not develop (refer IV 3 (i)).

From Appendix tables 26 to 34, it appears that in the first 2-3 weeks most of the larvae were on the herbage at 0 to 2 cm in height but after a month or so the number increased on herbage > 2 cm in height. This may be accounted for in part by the growth of the perennial rye grass as well as by meteorological effects.

The relationship between survival, vertical migration and meteorological record is shown in figures 8 to 16. From these figures in most cases it appears that vertical migration was affected by rainfall and evaporation.

Inspection of the data suggested a strong correlation between the percentage migrating and the percentage surviving. To examine the relationship between vertical migration and survival, a Spearman Correlation coefficient was calculated on data given in tables 32 to 40. Calculations are set out in Appendix 36 and correlation coefficient is summarised in Table 44. It indicates that the correlation between the percentage survival and percentage migration was highly significant.

Percentage migration in relation to survival of the infective larvae is given in Table 45. When total survival throughout the year (experiment 1 to 3 and 7 to 12) ranged from 0-0.5%, the mean vertical migration was 0.33%. Similarly when total percentage survival ranged from 0.5 to 1.0; 1.0 to 10.0; 10 to 20 and 20% and above, then mean

Table 44

Correlation coefficient between survival and vertical migration
throughout the year for each monthly experiment

Months	Correlation Coefficient	Results
March 1968	+ 0.83	Significant α = .001
April	+ 0.99	Significant α = .001
May	+ 0.96	Significant α = .001
September	+ 0.62	Significant α = .01
October	+ 0.99	Significant α = .001
November	+ 0.88	Significant α = .001
December	+ 0.89	Significant α = .01
January 1969	+ 0.98	Significant α = .001
February	+ 0.95	Significant α = .001

Table 45

Mean percentage migration in relation to survival of the infective larvae of
Cooperia curticei throughout the year from March, 1968 to February, 1969.

Mean percentage vertical migration

Total percentage survival	Exp. 1 March	Exp. 2 April	Exp. 3 May	Exp. 7 September	Exp. 8 October	Exp. 9 November	Exp.10 December	Exp.11 January	Exp.12 February	Mean percentage migration
1) 0-0.5 =	0.84	0.16	0.18	0.30	0.21	0.14	0.16	0.80	0.16	= 0.33
2) 0.5-1.0 =	0.47	0.62	0.58	-	0.58	0.45	-	0.51	0.32	= 0.50
3) 1.0-10.0 =	0.90	4.23	2.38	2.07	3.09	3.09	1.85	0.79	2.30	= 2.30
4) 10.0-20.0 =	0.81	7.64	4.00	5.89	5.53	9.30	-	-	11.24	= 6.34
5) 20.0-over =	-	16.60	-	-	20.23	-	-	-	7.52	= 14.78

migration on herbage was 0.50%; 2.30%; 6.34% and 14.78% respectively. The relation of migration to survival appears to be logarithmic.

After arcsin transformation, a regression line was fitted on the percentage migration data given in table 45 and the slope of the regression line was 0.688 (refer Figure 17). A Chi-square test was applied to find out the goodness of fit of this regression line. The calculations are given in Appendix 37. The calculated line is not significantly different from the observed data (p < 0.05).
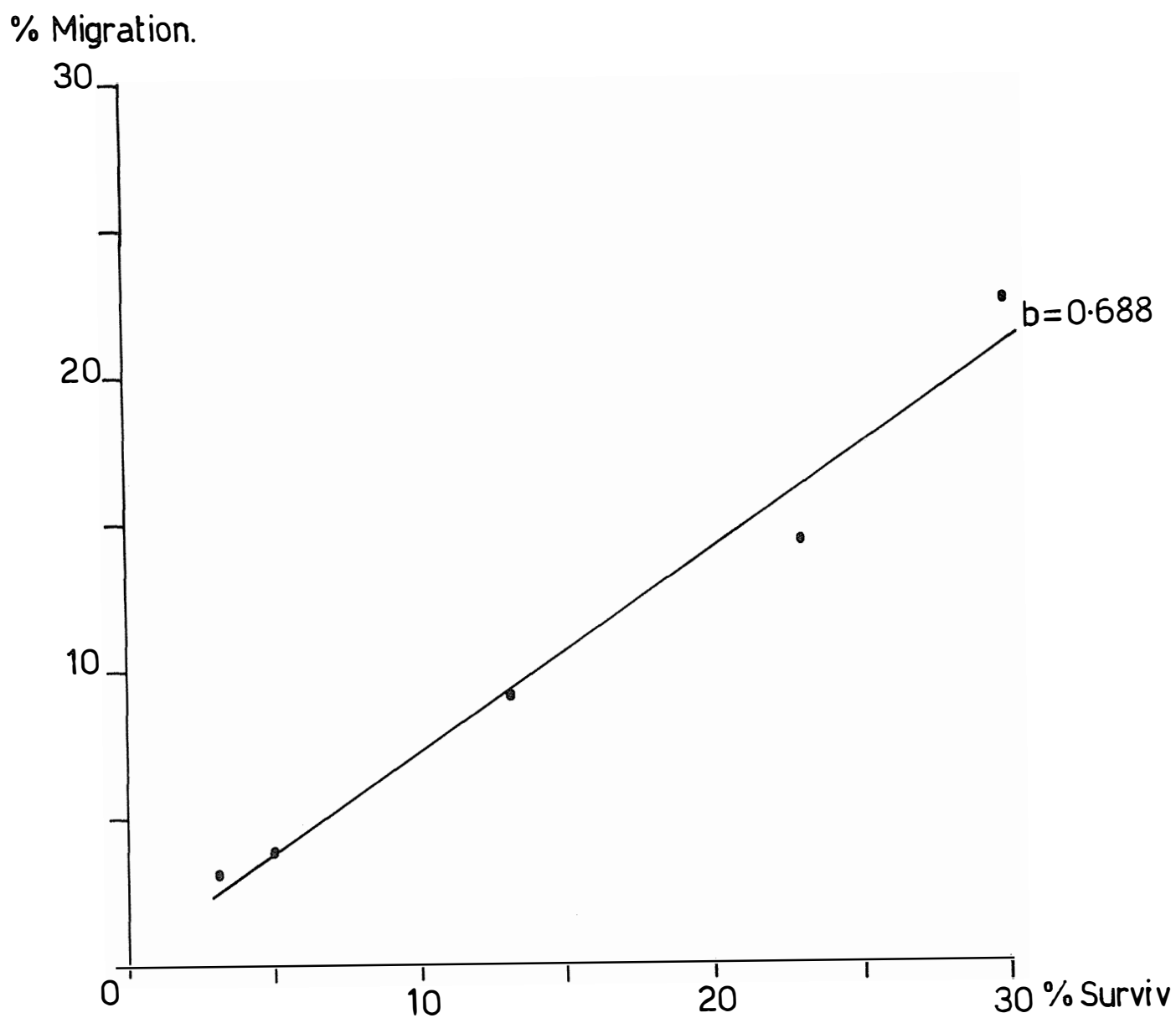
Fig. 17: Arcsin of percentage of larvae recovered from herbage
(migrated) plotted against percentage of larvae
surviving, expressed as class mid-points.

IV 4.   <u>SUMMARY OF EXPERIMENTAL FINDINGS</u>:

Studies on the development, survival and migration of the free living stages of <u>Cooperia curticei</u> were carried out for one year from March, 1968 to February, 1969, under natural conditions.

1.   Techniques are described for the recovery of <u>Cooperia curticei</u> infective larvae from small sample units of pasture, soil and faecal pellets. These techniques do not kill the larvae and counting is therefore greatly facilitated. Tests of the techniques have shown that they will recover 85.6% and 64.5% of the larvae from 1 and 5 gram herbage samples respectively. From soil and faecal pellet samples the recovery will be 64.6% and 58.3% respectively. The overall recovery rate from all samples will be 68.25%. Analysis of variance has shown that the efficiency of this technique is independant of the number of larvae present within the range tested.

2.   Studies on development showed that under natural conditions rate of development is strongly correlated with mean maximum air temperature over the period during which more than 90% develop to the infective third stage. Relative humidity and rainfall appeared to influence the percentage recovery of infective larvae but they did not appear to influence the rate of development.

3.   Development of the infective larvae was not recorded in the winter months (June, July and August) when mean air temperature was below 50°F (10°C) and mean maximum air temperature was between 53 and 56°F (11.7 and 13.3°C) but a few eggs developed to first or second larval stages. Eggs placed outdoors in the months of June and July were found to be disintegrated after 73 and 64 days respectively.

4.   More than 90% of the infective larvae were developed in 15 to 28 days, when mean maximum air temperature was between 57 and 65°F (14 and 18°C) in the months of April, May and September to December, 1968. When mean maximum air temperature was between 65 and 77°F (18 and 25°C) in the months of January, February and March, development was rapid and more than 90%

of the infective larvae developed in 9 to 15 days.

5. Throughout the mean maximum air temperature range of 57 to 77°F (14 to 25°C) the rate of development of the infective larvae was altered by 0.049 log units for each degree centigrade temperature change. This is little different to the rate of change under constant temperatures ·0.033 log units and this difference is statistically not significant.

6. Climatic conditions greatly influenced the percentage development of the infective larvae. Recovery of the infective larvae ranged from 3.0 to 36.2% throughout the year except for the month of January, the poor recovery of 0.23 to 1.34% was due to dry weather when faecal pellets became very hard and dry.

7. The survival of the infective larvae on herbage, soil and faecal pellets throughout the year was influenced by meteorological records. There was significant negative correlation between maximum survival and mean maximum air temperature. It also appeared that there was significant positive correlation between maximum survival and relative humidity and total rainfall. There was no correlation between survival and total evaporation.

8. Throughout the year the maximum survival of the infective larvae was 25 to 26 weeks when minimum and maximum mean air temperature throughout the period was 42.9 to 56.9°F (6.1 to 13.8°C) relative humidity 83 to 86% and total rainfall 21.45 to 21.54 inches. The maximum survival was 17 to 18 weeks when these values were 47.3 to 63.6°F (8.4 to 17.6°C), 75 to 85% and 8.70 to 14.40 inches respectively: the maximum survival was 12 to 13 weeks when these values were 51.1 to 68.9°F (10.6 to 20.5°C), 74 to 75% and 11.48 to 12.07 inches respectively and the maximum survival was 9 to 10 weeks when these values were 54.7 to 70.1°F (12.6 to 21.1°C) 74% and 4.39 to 8.49 inches respectively.

9. The infective larvae of _Cooperia curticei_ survived over winter (June, July and August) when mean minimum temperature on grass

ranged from 34.3 to 38.6°F (1.2 to 3.3°C) and mean air temp-
erature was approximately 50°F (10°C).

10.   The maximum survival of the infective larvae kept in 1 cm
depth of water with each monthly experiment was almost pre-
cisely the same throughout the year as occurring on herbage and
soil. This suggests that, in these observations survival of
infective larvae was only related to temperature and that the
apparent effects of relative humidity and rainfall were not
real.

11.   The maximum percentages of infective larvae surviving was ob-
served for 1 to 4 weeks after development and ranged from 1.02
to 29.4%. Subsequent percentage recoveries showed a regular
decline.

12.   Vertical migration of the infective larvae was affected by
rainfall and evaporation. The percentage of larvae recovered
from herbage was usually increased after rain.

13.   The correlation between percentage survival and percentage
migration was highly significant. The relationship of survival
and migration was not linear but became so after arcsin trans-
formation of the data. The regression line calculated from the
transformed data had a slope of 0.688. When the total survival
throughout the year ranged from 0. to 0.5% the mean percentage
of larvae on grass was 0.33%, similarly when total percentage
survival was 0.5 to 1.0, 1 to 10, 10 to 20 and 20% and above,
the mean percentage of larvae migrating onto herbage was 0.50%;
2.30%; 6.34% and 14.78% respectively.

IV. 5 <u>DISCUSSION</u>

Although there is an extensive literature on the subject, there have been few attempts at a systematic study of the effects of climatic conditions on the development, survival and migration of the infective larvae of a single isolate of one species of strongylate nematode. Even where workers have concentrated on a single species, the observations have been incomplete. Difficulty arises because frequently the methods used and the conditions under which observations have been made have been poorly designed and inadequately defined. Attempts to correlate laboratory and field observations are lacking.

Ecological studies under natural conditions have been carried out by many investigators employing three basic methods i.e. experiments on specially prepared grass plots, field experiments and observations on the seasonal and geographical distribution of species in outbreaks of disease. These several methods each have their attractions and their disadvantages. Precise simulation of pasture conditions is extremely difficult. However, plots or pots sown with grass have frequently been used in an attempt to imitate pasture conditions on a small and manageable scale. A disadvantage of the grass pot is its lack of some features of the microenvironment provided by the 'herbage mat' which is present under natural field conditions. For this reason grass grown in pots is likely to provide a micro-habitat which varies more rapidly with changes in macro-environment than permanent pasture.

However, experiments with specially sown grass plots or pots allow the investigator to exert considerable control over his methods, the type of herbage and the rate of contamination of the herbage, while ensuring exposure to the prevailing weather conditions. The small pot technique, although rather laborious, also requires very little in the way of facilities which is advantageous where resources are limited.

In the present experiments, studies on the free living stages of <u>Cooperia</u> <u>curticei</u> were carried out each month for one year under simulated natural conditions using pots sown with grass. Observations were made in relation to meteorological conditions on the rate of embryonic and larval development, the percentage recovery of the pre-parasitic

stages, maximum survival of the infective larvae and percentage sur-
viving, and the number of larvae migrating on the herbage in relation
to survival.

To minimise variation, the herbage or soil was contaminated once
in each monthly experiment with known numbers of eggs after clipping the
herbage at a height of 4 cm. Thereafter the herbage was allowed to
grow to obtain data on the total percentage recovery and migration of
the infective larvae. The techniques for the recovery of the infective
larvae from herbage, soil and faecal pellets were established and the
efficiency of these techniques was shown to be independant of the
number of larvae present. The results obtained from sequential soaking
of all samples twice for 24 hour intervals showed a high rate of re-
covery i.e. 68.25% (mean). However, more than two soakings were not
justified as it was found that a third soaking resulted in a very small
number of larvae being recovered. It should be noted (see tables 14 to
17) that sometimes quite large numbers of larvae were found in rinsings
of the tubes and pipette, which indicates the importance of this pro-
cedure in the technique.

To the writer's best knowledge, no study has been reported on
the rate of embryonic and larval development of strongylate nematodes
in the detail described in this thesis. Most of the workers (Monnig,
1930; Dinnik and Dinnik, 1958; Rose, 1963; Gibson, 1966; and Gibson
and Everett, 1967) in different countries have only reported the time
when eggs of strongylate nematodes reached the infective stage and have
not recorded the rate and percentage of development of the pre-parasitic
stages under natural conditions. Their weather data is not complete as
they have only recorded the effects of temperatures but other daily
meteorological changes during the period of development are lacking.
Hence detailed comparison of the present results with those of other
workers is difficult.

The results of experiments on the rate of embryonic and larval
development of Cooperia curticei out of doors conformed in general
terms to the pattern which might have been expected from the studies
made under controlled constant temperatures (Chapter III).

Under natural conditions many factors influence the development of the free-living stages of strongylate nematodes but temperature and moisture are the most important limiting factors (Gordon, 1948, 1950, 1953; Silverman and Campbell, 1959; Wallace, 1961; Levine, 1963; Crofton, 1963; Gibson and Everett, 1967). But observations in the present experiments showed that moisture was much less a limiting factor than temperature since, with the regular rainfall and high humidity in New Zealand the ground is generally sufficiently wet and development can take place for most of the time providing the temperature is suitable.

Under laboratory conditions it was found that the minimum temperature at which larvae would develop was in the region of $10^{\circ}C$ ($50^{\circ}F$). In the winter months of June, July and August, the mean temperature was less than $10^{\circ}C$ ($50^{\circ}F$) and little development occurred. Although the mean maximum temperature ranged up to $13^{\circ}C$ ($56^{\circ}F$), there were also frequent frosts and it has already been observed that even infective larvae do not survive long at subzero temperatures (Table 13). It seems likely that the frosts produced a high mortality rate in the developing larval stages.

In spring and early summer (September to December) and autumn (April and May) mean air temperatures were above $10^{\circ}C$ (11.1 to $14.5^{\circ}C$) and mean minimum and maximum temperature ranged from 6.8 to $10.4^{\circ}C$ (44.2 to $50.5^{\circ}F$) and 13.9 to $18.7^{\circ}C$ (57.1 to $65.7^{\circ}F$) respectively; the time for more than 90% of the developing larvae to reach the infective stage ranged from 15 to 28 days. In mid and late summer (January to March) when the mean air temperature ranged from 16.7 to 20.8$^{\circ}C$ (62.1 to 69.3$^{\circ}F$); mean minimum and maximum temperature was 12.5 to 16.7$^{\circ}C$ (54.5 to 62.0$^{\circ}F$) and 21.0 to 24.7$^{\circ}C$ (69.7 to 76.6$^{\circ}F$) respectively, the larval development was rapid requiring only 6 to 10 days ($>$ 90%).

The rate of development to the infective stage (T90) under field conditions was compared with mean maximum, mean minimum and mean air temperatures over the period of development. It was found that the relationship of temperature and development rate approached most closely to the laboratory observations when the mean maximum temperature was considered (tables 31 and Appendix 24). The field data were found to fit a linear regression of log days on mean maximum air temperature (fig. 7).

The slope of the regression line showed that on a log scale for every log unit increase in temperature the rate of development is altered by .049 log units of time. Calculation of intercept of 2 slopes from laboratory and field data showed that these 2 slopes were not significantly different (Appendix 25).

The calculated regression line from field observations was tested for goodness of fit to observed data and no monthly observations deviated significantly (Appendix 23). The point furthest from the calculated line corresponds to the month of May. In this month at the time when faecal pellets were placed outside the temperature for development was favourable, but later the temperature began to fall and hence development was delayed. During May the greatest difference between minimum and T90 development times was recorded. This was probably due to fluctuations in temperature being greater than in any other month (mean max.-mean min. = $20^{\circ}F$). This suggests that temperature fluctuations during the period of development may result in varying degrees of inhibition in development of the free living stages but the extent of this effect will depend on the range of fluctuations in air temperature. There have been no controlled investigations into the effect of temperature fluctuations on larval development.

Gordon (1948, 1950, 1953) and Levine (1959, 1963) introduced bioclimatographs to indicate the times of the year when heavy parasitism from various nematode species may be expected. Gordon (1948) prepared bioclimatographs by plotting the mean monthly maximum temperature against the total monthly rainfall and the resulting points were joined by a closed curve. On these were superimposed lines to indicate the optimum climatic conditions for development of the free living stages of sheep nematodes. Later, Levine (1959, 1963) used mean monthly temperatures in bioclimatographs and concluded that these graphs could not be used to predict the situation for any single year, as they were based on average monthly conditions over a period of years. Soulsby (1965) also noted their unreliability even from the short term point of view.

In the present study the regression slope of temperature on development can be used to predict field events (i.e. rate of development) more accurately than bioclimatographs and even for short term

predictions, the accuracy will not alter appreciably as the regression slopes are based on climatic conditions during the period of development. If this type of study on development were carried out on other strongylate nematodes, the results may also be found to be more accurate in predicting field events. It may be found that strains of a nematode species adapted to different temperature ranges show distinctive regression slopes.

The present study showed that the effect of temperature may accelerate the rate of development but percentage development and recovery of the infective larvae depends both on temperature and moisture. Although the warm period of summer is the one when larval development is most rapid it is not to be expected that high temperatures will inevitably result in more rapid increase of population (Silverman and Campbell, 1959). An increase in temperature has a two-fold effect, the first is direct acceleration of developmental rate and the second is increased mortality through desiccation. The end result will depend on the relative rate at which these two processes progress.

When faecal pellets were deposited outdoors during the summer month of January in the absence of rain, there was a dry spell (Appendix 19). Faecal pellets became dry and very hard in 3-4 days. Development was rapid but most of the free living stages perished before suitably moist conditions returned. After $>$ 90% of the surviving larvae reached the infective stage, the total mean recovery was only 0.7%. In general there was regular rainfall throughout the year and in other monthly experiments the mean percentage recovery of the infective larvae at the $>$90% stage ranged from 10.2 to 24.0%.

Because climatic factors affect the time needed for development of the free living stages of sheep nematodes, the different times required for development of the infective larvae throughout the year will affect their rate of increase of the worm population by affecting the total generation time (Cole, 1954; Crofton, 1963). From the observations made on the rate of development of the infective larvae, it appears that in this locality each generation of Cooperia curticei requires from about 21 to 43 days to complete the life cycle (time required for development of the egg to the infective stage plus prepatent period of

15 days). This observation is in close agreement with the work of Crofton (1963) for Cooperia spp. It means that Cooperia curticei can theoretically produce 9 to 11 generations in a year under local climatic conditions.

Climatic conditions obviously affect not only the rate of development but also the survival of the infective larvae. The survival of the infective larvae of Cooperia and Trichostrongylus spp under various climatic conditions has been studied by a number of workers, (Baker, 1939; Shorb, 1942; Kates, 1943, 1950; Goldberg and Rubin, 1956; Goldberg and Lucker, 1959, 1963; Drudge et al, 1958; Crofton, 1948b, 1957; Bell et al, 1960; Alicata, 1961; Levine, 1963; Anderson et al, 1964, 1965; Schwink, 1963; Rose, 1963; Gordon, 1950; Donald, 1967e; Gibson, 1966; Gibson and Everett, 1967; William and Mayhew, 1967; Tetley, 1949, 1959 a and b; Brunsdon, 1963b). Their findings are conflicting and sometimes contradictory partly due to the species and strain of the parasite studied and the techniques used and partly because their weather data is often not complete. Most of these investigators have studied the survival on monthly or seasonal basis but have recorded on a single chart the weather for the total period of survival and have not compared the maximum survival in relation to meteorological records for the actual period of survival. Without detailed knowledge of the meteorological records in any particular place, it is difficult to compare, in general, the results of the maximum survival of the infective larvae in the present experiment with those of other workers.

The weekly observations on the survival and migration of the infective larvae was carried out on 2 replicate samples. Donald (1967b) found that the variance of his pasture samples was substantially larger than its mean, suggesting that distribution of the infective larvae was statistically highly over-dispersed. Of a number of frequency distribution models, the "truncated log normal" was found to provide the best empirical fit to the sample distributions. In the present experiment as there were limited numbers of flower pots, the samples were chosen at random. In most cases there was great variations between the samples (Appendices 26-34) but Donald (1967b) suggests that because the distribution of infective larvae in pasture departs markedly from a random one, only quite large differences between the means of two random

sample estimates are likely to be significant.

The infective larvae of Cooperia curticei survived throughout the winter months (June, July and August) when mean air temperature was approximately 50°F (10°C) and mean minimum air temperature was 39.0 to 43.0°F (3.9 to 6.1°C) with frost at nights. 50°F (10°C) was found to be the optimum temperature for maximum survival under laboratory conditions (Table 12). This observation on survival of the infective larvae of Cooperia curticei showed agreement with other numerous investigators that infective larvae of Cooperia spp. and trichostrongylids can over-winter on pasture in various climates (Baker, 1939; Goldberg and Lucker, 1959, 1963; Drudge et al, 1959; Rose, 1963; Anderson et al, 1964; and William and Mayhew , 1967). In contrast, Dinaburg (1945), Kates (1950), Levine (1963) and Smith and Archibald, (1965) reported little or no overwinter survival for Cooperia spp. in the U.S.A. and Canada. As infective larvae may survive the winter sometimes in small and at other times in substantial numbers, the epidemiological significance of these larvae will vary with numbers surviving. Kates (1950, 1965) has reviewed a number of reports showing that worm free lambs grazed on pastures in the spring acquired infection with trichostrongylids from the larvae which had survived over winter.

Through the year from each monthly experiment, the maximum survival of the infective larvae of Cooperia curticei was from 9 to 26 weeks. To determine the effect of different climatic factors on maximum survival a Spearman ranking correlation coefficient was used because of its simplicity. No correlation was present between maximum survival and total evaporation. But strong negative correlation was observed between maximum survival and mean maximum air temperature. The correlation between maximum survival and rainfall and relative humidity was significant. However, the maximum survival of the infective larvae kept in 1 cm depth of water was almost precisely the same as that occurring in herbage and soil throughout the year, when mean relative humidity ranged from 74 to 86%. This suggests that in the present experiment the apparent effect of rainfall and relative humidity is spurious, and maximum survival of the infective larvae was dependent almost entirely on temperature.

Studies on maximum survival of the infective larvae in water

at constant temperatures showed longer period of survival than under
natural conditions. This may be due to the absence of death from pre-
dation and of other environmental factors such as the washing of the
larvae into the soil and fluctuating temperatures. However, the relat-
ionship between maximum survival and environmental conditions including
fluctuating temperature is complex and warrants some careful investi-
gation.

Throughout the year the maximum percentage of infective larvae
recovered was observed for 1 to 4 weeks after development and during
this time, the pattern of percentage survival was precisely the same
(Tables 32 to 40; figs. 8 to 16). Thereafter the percentage recovery
of the infective larvae decreased. The results on the percentage survi-
val of infective larvae are of interest in that except in the cooler
months of May and September, less than 1% of the larvae were recoverable
after 9 to 11 weeks and the majority of the larvae died long before the
maximum survival period was reached.

For studies on vertical migration, the samples of the herbage
were obtained in the morning for processing. This time was chosen be-
cause Rees (1950) and Rogers (1940) had suggested that in the majority
of the cases the greatest number of larvae were on the grass blades in
the early morning than at any other time of the day. Vertical migration
of the larvae on herbage, in the present experiment appeared to be
affected by rainfall and evaporation (figs. 8 to 16). Rogers (1940) and
Rees (1950) also demonstrated that moisture on the grass was found to
favour larval migration. Crofton (1948a) reported that during the per-
iods when maximum temperature never exceeded $55^{\circ}F$, little or no migra-
tion of larvae occurred, but when temperature rose above $55^{\circ}F$ migration
increased. However, the rise in temperature was associated with an in-
crease in the rate of evaporation.

In the first 2-3 weeks most of the larvae were on the herbage
at 0-2 cm in height and this observation is in close agreement with the
work of Crofton (1948b) and Rose (1963). After a month or so the num-
ber increased on herbage more than 2 cm in height and this may be
accounted for in part by the growth of the herbage as well as by meteo-
rological effects (Appendices 26 to 34). Largest numbers of larvae

were recovered from the herbage during the first 2-5 weeks. From then
onwards their number gradually declined. Gibson (1966) and Gibson and
Everett (1967) reported that larvae of trichostrongylids persisted on
the herbage in large numbers for as long as 20 weeks. But in their
experiments, the herbage was contaminated for 1 or 2 weeks with faeces
containing eggs. The faeces therefore acted as a store from which lar-
vae were released over a period resulting in their longer persistence
on the herbage in large numbers. In the present experiment faecal
pellets containing eggs of Cooperia curticei were placed in the herbage
once only. In similar experiments with trichostrongylids, Taylor (1938),
Crofton (1948b), and Anderson et al (1965) also contaminated the her-
bage once and the larvae persisted in large numbers for only 5-6 weeks.
Hence the present observations are in close agreement with the work
of these authors and the work of Gibson (1966) and Gibson and Everett
(1967) is not strictly comparable.

The period of survival of the infective larvae on the herbage
was precisely similar to that in soil. Kauzal (1941) also reported
that larvae can survive for long periods in soil and contaminate the
herbage only when there is suitable temperature and moisture.

Crofton (1963) demonstrated that larvae followed existing water
film pathways in soil and that vertical migration of larvae onto the
herbage was due to random movements in all directions independent of
gravity or undescribed special receptors in the larvae. In the present
experiment also the number of larvae recovered from the herbage were
usually increased after rain probably due to the existence of more
extensive and continuous water films during and after rainfall. This
adds support to the theory of the random migration of larvae.

It was also found that there was a strong correlation between
the total number of larvae recoverable from the single pot sample and
the number recoverable from the herbage in it. The relationship of
larvae surviving and larvae migrating was found to be logarithmic, the
proportion of larvae recoverable from the herbage increasing as the
available population increased. This suggests that infective larvae
follow a one way traffic upwards on the grass blades, otherwise the
relationship between number of larvae surviving and migrating should

have been linear. It appears that discontinuity of water film due to desiccation and evaporation hamper their movements, and they get left on the grass blades. But this aspect of the study requires further investigation.

Examination of the summation of the curves of larval survival (figures 8 to 16) and the theoretical 9-11 generations per year, suggests that under New Zealand conditions infective larvae of Cooperia curticei will be available to the grazing sheep throughout the year. This is in agreement with the work of Tetley (1949, 1959 a and b) and Brunsdon (1963b) from New Zealand and Durie (1961) from Australia. Under field conditions they reported that Cooperia curticei has a peak occurrence in autumn and early winter but infection of Cooperia curticei can be acquired throughout the year.

HOST—PARASITE RELATIONSHIPS

OF

COOPERIA CURTICEI

PART TWO

HOST-PARASITE RELATIONSHIP OF COOPERIA CURTICEI AND THE SHEEP

When infective larvae are ingested by the final host, they complete their parasitic stages of development. This comprises exsheathment and development of the adult worm. The relationship between the final host and the adult parasite determines the severity of the disease processes which occur and this varies from species to species. Very little attention has been paid to the relationship of the sheep and Cooperia curticei.

CHAPTER V   REVIEW OF LITERATURE

V 1   EXSHEATHMENT

The first requirement for an infective strongylate larvae to establish itself in the host is the completion of the second moult or exsheathment. Essentially the process of exsheathment consists of two stages, the first is a host stimulus which activates the nematode larvae to release a preformed exsheathing fluid, and second the exsheathing fluid attacks part of the sheath, so that the larva, aided by its own movements, is able to escape from it. The process of exsheathment may take about 3 hours to complete in vitro at $37^{\circ}C$ but the action of the stimulus from the host is complete in a shorter time.

The host stimulus primarily consists of the unionised components of bicarbonate - carbon dioxide buffer, undissociated carbon dioxide and dissolved gaseous carbon dioxide. It has been found that with the larvae of T.axei and H.contortus the activity of the stimulus increases with increasing concentrations of undissociated carbonic acid and dissolved gaseous carbon dioxide. However, these nematodes differ from one another in their precise requirements. Thus, in the presence of 0.02 M sodium dithionite at pH 7.3, 70% of T.axei larvae exsheathed in 3 hours when the total concentration of carbonic acid and dissolved carbon dioxide was about $0.5 \times 10^{-3}M$, while this concentration needed to be $1.5 \times 10^{-3}M$ to achieve the same result with H.contortus (Rogers, 1960).

The exsheathment response of H.contortus larvae to carbon dioxide is enhanced by the presence of 0.1% to 0.8% sodium chloride in the suspending fluid. This action of sodium chloride appears not to be an

osmotic affect since solutions of glucose and lactose with equivalent
osmolarity do not induce or facilitate exsheathment (Taylor and Whitlock,
1960).

It has been suggested that the stimulus provided by CO_2 is also
necessary to set in train a variety of developmental changes (Sommer-
ville, 1964). This suggestion subsequently received experimental support
in the work of Rogers (1966). Treatment of larvae with dilute solutions
of iodine and hydrogen sulphide water caused a reversible inhibition of
exsheathment. The same substances also caused a reversible inhibition
of development in larvae which had previously been artificially exsheath-
ed. It is suggested that CO_2 reacts with the "receptors" in the infect-
ive stage, perhaps in the neurosecretory system, and this causes the
release of substances which effect various target organs and tissues
which are concerned with general developmental processes of parasitic
stage.

Following stimulation of the larvae by the host the completion of
exsheathment requires the presence of a heat-stable low molecular weight
cofactor and Rogers (1963, 1965) demonstrated that H.contortus exsheath-
ing fluid contains an enzyme similar to leucine amino-peptidase. The
optimum activity of this enzyme is affected by pH. The hydrogen ion
concentration has also an important effect on exsheathment and Rogers
(1958, 1960) found that the exsheathment of T.axei and H.contortus larvae
was increased as the pH was raised from 6 to 8. Christie and Charleston
(1965) also reported the decrease in percentage exsheathment of H.contor-
tus larvae as the pH fell from 6 to 3.

The specificity of exsheathing fluid from H.contortus and other
species has been studied by Rogers and Sommerville (1960). It was found
that exsheathing fluid from H.contortus attacked the sheaths of T.axei
to about the same degree but changes in the sheath of T.colubriformis
and Oesophagostomum columbianum did not progress very far. Exsheathing
fluid from T.axei larvae although attacking sheaths from the homologous
species quite markedly, had little affect on the sheaths of H.contortus,
T.colubriformis or O.columbianum.

After receiving the host stimulus in the process of exsheathment,

the exsheathing fluid attacks a small encircling area of the sheath
about 20 μ from the anterior end of the larva. This first appears on
a refractile ring across the sheath which bulges from the surface. Grad-
ually the zone becomes more distinct and finally after this line has
become weakened the movement of the larva causes the anterior end to
become detached as a cap, and the larva then wriggles out of the sheath.
Rogers and Sommerville (1960) consider that the mechanism for the re-
ception of the stimulus to exsheath, for the accumulation of exsheathing
fluid and for its release lies between the base of the oesophagus and
the level of the excretory pore or the nerve ring. Studies with anti-
serum prepared against exsheathing fluid indicate that the excretory
pore is a probable point for the release of exsheathing fluid.

Exsheathment of larvae normally occurs in the part of the alimentary
canal anterior to where the adults are found. Thus T.axei and H.contortus
which mature in the abomasum normally exsheath in the rumen. The optimum
range of pH (7-8) and high carbonic acid concentrations needed for the
stimulus of exsheathment have been confirmed by experimental work and are
not normally to be found outside the rumen. The infective larvae of
T.colubriformis which mature in the small intestine exsheath in the abo-
masum. In this case exsheathment depends upon components in the hydro-
chloric acid secreting region of the stomach. The optimum range of pH
is 1.5 to 2.5 and CO_2 increases the stimulus. Best results are obtained
when the concentrations of undissociated carbonic acid plus dissolved
gaseous CO_2 are about 5×10^{-3}M.

There is no literature on exsheathment of the infective larvae of
Cooperia curticei other than that of Andrews (1939). He induced ex-
sheathment in the antiformin solution used by Lapage (1935) but they
both considered this an abnormal and artificial procedure. As Cooperia
curticei has been recorded both from the abomasum and small intestine of
the host, it is important to know where the stimulus for exsheathment
lies for the infective larvae.

V 2   DEVELOPMENT OF THE PARASITIC STAGES OF COOPERIA CURTICEI, MORPHOLOGY

OF THE ADULT NEMATODE, LOCATION IN THE SMALL INTESTINE AND EGG

PRODUCTION.

i)   Development of the parasitic stages: The life history of Cooperia
curticei was studied experimentally by Andrews (1939) and Sommerville
(1960). The following account is derived from the work of Andrews ex-
cept where indicated.

Two to three days after infection the third stage larvae, having
exsheathed, enter the gland crypts in the anterior part of the small
intestine though they do not penetrate the mucous membrane. The moult
to the fourth stage occurs on the third (Sommerville) or fourth (Andrews)
day after infection. At this stage the larvae can be differentiated in-
to males and females. Following the moult the larvae return to the
lumen of the intestine, the majority have done so by the fifth day and
the larvae are about 1.6 mm long. Little growth occurs over the next
three days and on the eighth (Sommerville) or ninth (Andrews) day the
last moult occurs. Moulting is completed by the tenth day. The worms
grow and mature and reach adult size on the fourteenth day. Eggs first
appear in the faeces of the host on the fifteenth day.

ii)   Morphology of the adult stage: The morphology of the adult nema-
tode has been described by Curtice (1890), Giles (1892), Ransom (1907a),
Lebedev (1929), Ross and Gordon (1936) and Travassos (1935). Their
descriptions are in close agreement one with another.

Typically the anterior part of the body of Cooperia curticei is
spirally coiled and this appears to be characteristic of this species.
At the anterior extremity the cuticle is thickened or dilated and this
region bears transverse striations. There are also pronounced longitud-
inal striations along the length of the body. The buccal capsule is
poorly developed.

The length of the mature male varies from 5.41 to 7.27 mm and
the maximum width anterior to the bursa ranges from 0.078 to 0.090 mm.
The oesophagus is 304 to 344 µ long. The bursa is well developed and
the dorsal ray begins with a fairly thick trunk. The branches of the

dorsal ray are curved to form a lyre-shaped structure with a cleft at
each end. The spicules are yellow in colour, equal and measure 140 to
148 μ in length. Centrally the spicules have a protuberance or fin that
is transversally striated and with small excrescences on the free edge.
On the proximal end of the spicule, where the muscles are attached, is
a rounded disc like structure. The distal end of the spicule is slight-
ly bent and is terminally shaped like a shoe.

The mature female is from 6.57 to 7.38 mm in length and maximum
width in the region of vulva is 0.092 to 0.099 mm. The oesophagus is
315 to 348 μ in length. The vulva is situated at a distance of 1.51 to
2.09 mm from the tail end. The vagina is short, 35 μ in length. The
ovijector is well developed and its length with the sphincter is 290 μ,
the maximal width being 57 μ. The uteri diverge and the width of the
parasite in the anal region is 24 to 27 μ. The anus opens at 99 to
149 μ from the tail end. The body of the parasite is covered with
papillae resembling warts (Lebedev, 1929).

In long established infections the surviving females may be found
to be smaller than normal and to be devoid of eggs. The males may have
colourless spicules. These phenomena may be the result of developing
host resistance. It has also been observed that growth and development
is relatively retarded when large doses of infective larvae (70,000) are
administered to a sheep (Sommerville, 1960).

iii) <u>Location in the small intestine</u>: <u>Cooperia</u> <u>curticei</u> is principally
located in the small intestine although small numbers are not infre-
quently recorded from the abomasum. The nematode population in the
small intestine shows a normal distribution regardless of the duration
of infection (Tetley, 1949). It has been suggested that intestinal nema-
todes take up their position along the intestine in response to stimuli
supplied by the intestinal contents particularly in the duodenum at the
point of entrance of the bile and pancreatic juice (Whitlock, 1966).
Tetley (1935) observed the peak occurrence of <u>Cooperia</u> <u>curticei</u> at
the "lower part" of small intestine but Davey (1938) reported the distri-
bution of this species in small intestine to be approximately 9 to 22
feet from pylorus when infestation was approximately 2,500 worms per

sheep.

iv) Egg production: Little information is available on the number of eggs which are laid per female worm per day. It has been observed that egg production per female worm of Cooperia curticei decreases as the total number of parasite increases (Andrews, 1936). Three lambs received daily dose of 250, 500 and 1000 infective larvae per day respectively, totaling 63,000 to 105,000 for the entire period and Andrews (1936) found that average number of eggs per female for 24 hours period to be 937, 421 and 390 respectively.

While describing the differences between the rate of egg production between trichostrongyle genera, Peters et al (1941) and Nickel (1965) suggested the comparative fecundities were in the order of Nematodirus 1, Cooperia and Trichostrongylus 10, and Haemonchus 100. Kates (1947) observed the eggs per pellet in relation to worms on post mortem probably assuming that each faecal pellet contains constant number of eggs. He reported that Cooperia curticei is a poor layer of eggs or least productive compared with other trichostrongylids. When the size of the infestation was more than 200 worms per lamb, the egg worm ratio was 1:5.8 and when infestation was less than 200 worms per lamb, the ratio was 1:3.5. Later Crofton (1957, 1963) confirmed Kates general conclusions as to the relative low fecundity of Cooperia curticei but suggested that the rates of egg laying are more "impressive" when the total volume of eggs is considered.

However, figures on egg production represent approximate numbers as the output of eggs may be greatly altered by many factors. The variation in egg production within the species may occur because of variation in size of infestation, diet of the host, age of the host, age of the parasite, physical condition of the faeces and technique used to recover the eggs, (Mayhew, 1940; Kates, 1947; Kelly, 1953). The immune response of the host also plays an important part in egg production (Sarles, 1929; McCoy, 1931; Chandler, 1932; Michel, 1967) and in a resistant host egg output of the worm may be reduced to one-thirteenth of the normal (Taylor, 1934).

The output of the egg in an infected animal is also not constant

throughout the day or with each bolus of faeces. On the contrary, a
marked variation has been demonstrated over a 24 hours period whether
expressed on an egg per gram basis or on a total number of eggs over a
collection period (Spedding, 1952). Spedding found no correlation
between egg counts and faecal output, but the total eggs per day shed by
infected animals gave a more uniform estimate of the egg output than an
estimate based on eggs per gram of faeces.

V 3   PATHOGENESIS, PATHOLOGY AND IMMUNE RESPONSE OF COOPERIA INFECTION

The pathogenesis of disease caused by nematodes is very complex,
some exert obvious effects, by ingesting blood for example, whilst with
others the effects are less readily defined. Gastro-intestinal nematodes
commonly affect food intake, digestibility of food, induce diarrhoea
with its consequences, induce hypoproteinaemia and so on. The precise
way in which these changes are produced is, in most instances, unknown.
To some extent they reflect directly the activities of the parasite but
also the immune and reparative responses of the host to the parasite.

i)   Symptoms: Though Cooperia spp. are recognised as causing serious
parasitism in cattle, little detailed observation has been made on their
pathogenicity in sheep. It has been observed that infestations with
(unspecified) Cooperia spp., if present alone, caused no serious ill
effects to the host, and that heavy infestation with these worms became
dangerous in young sheep only when a concomitant infestation with H.
contortus was present; (Taylor, 1935; Ross and Gordon, 1936; Andrews,
1938; Tetley, 1949). Cooperia curticei infection in sheep may produce
no clinical signs (Andrews, 1938) but Edgar (1936) stated that Cooperia
curticei may cause serious disease in goats. Death was reported 5 to
10 days post-infection in all age groups and in both sexes. The first
symptom was diarrhoea and this persisted until the animal died. There
was also a capricious appetite, loss of weight and near death spasmodic
abdominal pain.

There is evidence to suggest that Cooperia oncophora is non
pathogenic in sheep and cannot maintain infection, (Tetley, 1949;
Sommerville, 1963; Smith and Archibald, 1965). The pathogenicity of
C.oncophora in cattle has been described by Baker (1937); Hamoir (1923);

Tunnicliff (1932); Banks and Mifton (1960); and Herlich, 1965b). There can be little doubt that C.oncophora can be a serious pathogen of cattle (Soulsby, 1965) but Herlich (1965 b, c) reported that 350,000 infective larvae of C.oncophora only caused mild symptoms in calves though in high doses (3,000,000) there was severe enteritis.

The clinical signs of Cooperia pectinata in calves were the passage of abnormally soft faeces which became progressively more fluid and foetid, anorexia, dehydration and in some cases pronounced submandibular oedema. Calves receiving a single dose of larvae showed symptoms earlier and to a greater degree than those received multiple doses, (Herlich, 1965 a,b, 1967; Keith, 1967). The clinical signs produced by Cooperia punctata in calves are essentially similar, (Alicata and Lynd, 1961; Bailey, 1949; Herlich, 1962).

ii) Feed intake, body weight and wool production: The gastro-intestinal nematode parasites may affect the nutrition of the host by depression of appetite and a decrease in digestibility of certain food constituents. Lambs experimentally infected with T.axei showed an 8% depression of appetite and with lowered digestive efficiency, this meant an infected animal absorbed 10% less food than the worm free lamb (Spedding, 1954). Similar studies on the effect of parasitism on the digestibility of crude protein were carried out by Stewart (1933), Franklin et al (1946), Spedding (1955), Gordon (1958b) and Spedding et al (1958) and their views agree with the findings of Spedding (1954). However, these findings are based on the balance between dietary and faecal nitrogen and fail to take into account any increase in faecal nitrogen which results from the increased passage of serum protein into the alimentary tract. In view of this it is difficult to be certain that a significant depression of protein digestibility actually occurs.

Inefficient utilization of food results in a poorer rate of weight gain per unit of food than in worm free animals (Goldberg and Lucker, 1960). Heavy infections with parasites early in life may reduce growth and cause permanent stunting, whereas infection of animals when they are 3 or 4 months chiefly affects muscle growth, (Spedding, 1956; Gordon, 1958b).

The level of nutrition also influences the number of parasites which become established in an animal (Whitlock, 1949; Laurence et al, 1951). A high plane of nutrition results in the establishment of fewer parasites or will reduce the ill effects if large numbers become established (Vegors et al, 1955,1956; Gordon, 1964; Brunsdon, 1964 a,b; Gibson, 1963 a,b; Soulsby, 1965; Dobson, 1967a). Studies of the effect of a balanced diet or milk intake on the acquisition of parasitism in lambs have shown that better fed groups carried about half the numbers of worms compared to poorly fed groups (Kates et al, 1962; Spedding et al, 1963).

Parasitism may reduce wool growth and the fleece may be poor in both quality and quantity (Sarles, 1944; Carter et al, 1946; Gordon, 1958b; Spedding and Brown, 1957; Brunsdon, 1963a, 1964b). The amount of wool produced by sheep infected with moderate infection of trichostrongylids may drop to 40% of that of worm free controls (Carter et al, 1946). Studies by Spedding and Brown (1957) on the effect of subclinical worm burdens on wool production showed that worm-free sheep produced 12 to 38% higher fleece weight and 17.3 to 43.2% were clean dry wool per unit area than infected sheep. They further suggested that the effect on wool production depends upon the age of the host when first infected, the level of infection, and the rate of wool growth at the time when the infection is at its maximum. Where infections are severe the effect on fleece weight and quality may be great but in light infections there may be little or no effect.

There is no literature on the effect of feed intake and wool growth in sheep infected with Cooperia curticei or with other species of the genus Cooperia. Andrews (1938) observed that Cooperia curticei decreased the ability to convert food into live weight gain.

The body weights of calves receiving doses of 350,000 larvae of C.oncophora were unaffected but at higher doses, a decreased weight gain was observed, (Herlich, 1965b). In calves given a single large dose (300,000) of C.pectinata larvae lost weight whereas those given multiple doses (total of 300,000 larvae over 10 consecutive days) gained weight but at a slower rate than non-infected controls (Herlich, 1967). The growth rate of calves experimentally infected with C.punctata was also

depressed (Alicata and Lynd, 1961).

iii) <u>Effects on constituents of the blood</u>: Owing to the physiological
functions of blood and its intimate relationship with all body tissues,
it is to be expected that in many, if not all, helminth infections may
produce slight to extensive alterations in the blood picture.

Among the <u>Trichostrongylidae</u>, <u>Haemonchus</u> <u>spp.</u> are the only members
which are capable of producing disease primarily by extraction of the
host's blood, (Fourie, 1931; Boughton and Hardy, 1935; Andrews, 1942;
Baker <u>et</u> <u>al</u>, 1959; Whitlock, 1950; Clark <u>et</u> <u>al</u>, 1962). A study by
Charleston (1964) of the anaemia caused by infection with <u>H.contortus</u>
in sheep showed differences from the anaemia caused by bleeding. The
difference suggested an interference with normoblast maturation in para-
sitised sheep which did not occur in bled sheep. Infections of ruminants
with trichostrongyles which do not suck blood are frequently accompanied
by anaemia and these are considered to be principally due to shortening
of erythrocyte survival time and an inability of the bone marrow to
compensate for this (Baker and Douglas, 1957).

Studies with <u>Cooperia</u> <u>spp.</u> infection have in general indicated no
anaemic changes (Andrews, 1938; Bailey, 1949; Herlich, 1965 b, 1967).
Though Hung (1926) assumed <u>Cooperia</u> <u>punctata</u> to be a blood sucker because
of the occurrence of erythrocytes in the digestive tract of worms,
Bailey (1949) found no red blood cells in worms on the surface of the
mucosa and he considered those in parasites more deeply in the mucosa .
had been engulfed accidently as worms fed on the tissue debris. Evi-
dence against <u>Cooperia</u> <u>spp.</u>being blood suckers is also supported by the
fact that animals may die of severe infections without any evidence of
marked anaemia.

It has been observed that parasitic infections may produce changes
in the total and differential white cell counts. Eosinophilia has been
demonstrated in many parasitic infections, (Archer, 1960). The cellu-
lar elements in the blood of sheep infected with <u>Cooperia</u> <u>curticei</u>
(425 to 25,033 worms) showed slight eosinophilia but no other signifi-
cant change was noticed in total or differential white cell count

haemoglobin content or packed cell volume (Andrews, 1938).

Electrophoretic studies on the serum protein of lambs infected with helminths have shown significant changes (Leland et al, 1958, 1960; Leland, 1961; Kagan and Goodchild, 1961; Geyer, 1965 and Dobson, 1965, 1967 b,c,d). With naturally acquired moderate and light infections of mixed nematodes Kuttler and Marble (1960) noticed that the total serum protein and albumin fraction was decreased while the Alpha 1, Alpha 2, Beta and gamma globulins were increased. Similar changes were found in serum protein of lambs infected with T.axei, (Leland et al, 1959, 1960).

There is no literature on serum protein changes in sheep infected with Cooperia curticei. In calves experimentally infected with Cooperia pectinata and Cooperia oncophora, a decrease in total serum protein has been observed (Herlich, 1965b, 1967). The calves with mixed infections of trichostrongyles and Cooperia punctata showed pronounced hypoprotein-aemia and hypoglycaemia, (Herlich, 1962).

iv)   Gross lesions and histopathology: It has been observed that in-fective larvae of Cooperia curticei do not actually penetrate the wall of the intestine. They come to lie in the crypts with little or no dis-cernible tissue damage. In the animal sensitized by previous infection there is an immediate inflammatory response. The larvae apparently do not penetrate the tissue but the resulting inflammatory response engulfs each larva. Ultimately, a fibrotic capsule is formed and the resulting yellow nodule protrudes through the mucous membrane into the lumen of the intestine, (Otto, 1966). No nodules were found in a lamb from an initial infection of as much as 1,000,000 larvae of Cooperia curticei but 319 to 32,000 nodules were developed from an infection of 63,000 to 400,000 larvae administered to previously infected animals (Andrews, 1939). Parasitic nodules 4 mm in diameter and 2 mm in height were also ob-served in the small intestine of caribao (Bubalus bubalus) infected with Cooperia species, (Schwartz, 1927; Gomez, 1928). Histologically these nodules comprised fibrous connective tissue, lymphocytes and the parasite. Intestine glands were atrophied because of pressure and showed signs of necrosis and lymphocytic infiltration. Local tissue reaction was not seen beyond the muscularis mucosae layer of small intestine.

No gross lesions were noticed on necropsy of sheep infected with single doses of <u>Cooperia</u> <u>curticei</u> infective larvae, (Andrews, 1938) but in naturally acquired infections in goats, irregular haemorrhagic areas, scattered along the mucosa on the first 30 feet of small intestine, were observed, (Edgar, 1936).

There was no evidence of a histotropic phase in calves experimentally infected with <u>C.pectinata</u> and <u>C.oncophora</u> (Herlich, 1965 a,b). Lesions found at necropsy included congestion in the duodenum, petechiae in Payers patches, enteritis and oedema of the abomasum and mesentery. Calves given multiple doses did not harbour more worms at necropsy than calves inoculated with single doses of larvae.

It was observed that <u>C.punctata</u> may produce necrosis and inflammation of the intestinal mucosa (Hung, 1926) and may also penetrate to various depths into the mucosa while encapsulated and degenerate parasites may be seen as deep as the submucosa (Di kmars, 1936). In heavy infections in calves, lesions consisted of numerous small haemorrhages on the mucosal surface of the first 10 feet of the intestine, the lower half of the small intestine was thickened and showed catarrhal exudate. The associated mesenteric lymph nodes were slightly oedematous,(Bailey, 1949). Histologically mucosa and submucosa showed marked lymphocytic and eosinophilic infiltration. In heavy infections the submucosa was thickened with fibrino-necrotic exudate and adult worms and larvae were found in the lower portions of the glandular crypts, even down to the submucosa and the serosa.

v) <u>Resistance and immune response</u>: Resistance to nematode infection may be reflected by an increase in the length of the prepatent period, retarded development of the mature worm, reduced egg production, inhibition of larval development and the number of worms established in the host (Stewart, 1950 a,b,c,d, 1953; Taylor and Michel, 1953; Roberts, 1957; Gordon, 1957; Silverman and Patterson, 1960; Anderson <u>et</u> <u>al</u>, 1965). Older animals may be able to resist parasitic infection while the young animal may suffer severely from it (Bailey, 1949; Gordon, 1950; Goldberg, 1952; Lucker, 1952; Stewart and Gordon, 1953; Rohrbacher <u>et</u> <u>al</u>, 1958; Herlich, 1960). The phenomena of resistance also appear at different stages in the infection (Michel,1952, 1963; Herlich, 1960).

Little information is available on the resistance of sheep to Cooperia curticei. When 4 lambs were given daily doses of Cooperia curticei infective larvae over a period of several months, a reduced number of worms were found at necropsy compared with control animals given single doses (Andrews, 1937, 1939). Under field conditions, Roberts et al (1951, 1952) noted that the majority of cattle became resistant to Cooperia spp. infection at about 5 months of age. Egg counts rose to a peak about at 4 - 5 months of age and following a rapid decline, remained at low levels thereafter. Similar findings were reported by Bailey (1949); Mayhew et al (1958, 1960) in experimental infections of Cooperia punctata in cattle. The faecal egg count of 31 calves was decreased to zero and a challenge infection was resisted as judged by failure to influence the egg count (Mayhew et al, 1960). It has been suggested that resistance to Cooperia punctata may inhibit the exsheathment of larvae, their further development and their passage from the abomasum to the small intestine (Stewart, 1954, 1958). When lambs were given single doses of 27,000 larvae of C.oncophora no signs of resistance were apparent, but after a second dose most of the lambs were resistant to C.oncophora and also to C.pectinata suggesting that a single species of Cooperia is capable of inducing resistance to itself and also to other species. Calves that received multiple doses of C. pectinata larvae also developed resistance. There was expulsion of adult worms and at autopsy large numbers of inhibited fourth stage larvae were found (Herlich, 1965c, 1967).

There is no satisfactory answer as to how the immunological mechanism inhibits the growth and development of nematodes (Soulsby, 1966). Whether antibody alone can affect and ultimately destroy a nematode is by no means clear. The elucidation of the precise role of antibodies in resistance to helminth parasites is rendered difficult by the number and complexity of the potentially antigenic material involved. The worms themselves contain a whole range of antigens, some being structural or somatic materials and others being the result of the metabolic activity of the parasite. The antibody response is therefore correspondingly complex, not only in the range of antibodies produced but also in their relative amount and relative importance from the protective point of view (Urquhart et al, 1962).

It is well known that reactions between immune serum and larval stages can be produced with ease; some of these consist of antigen-antibody precipitates at the orifices of larvae (Hawkins and Cole,1945; Soulsby, 1962; Silverman and Patterson, 1960) a decrease in oxygen consumption (Schwabe, 1957) and a coating phenomena when larvae are grown in immune substances (Douvres, 1962). Precipitates were found on the mouth, anus and excretory pore of the exsheathed larvae of Cooperia curticei when incubated in serum from immune animals (Hawkins and Cole, 1945). However, there is no direct evidence that these reactions are primarily responsible for the immune rejection of the parasite by the host.

The circulating antibody levels of sheep infested with gastro-intestinal nematodes have been examined extensively by complement fixation (Stewart 1950 a,b,c,d, 1953; Stewart and Gordon, 1953,1958; Rice et al, 1966; Dobson, 1967 b,d), by passive haemagglutination (Soulsby, 1956,1957,1960; Soulsby and Stewart, 1960; Dobson, 1967 b,d) and by serum precipitin reactions using the Ouchterlony technique (Soulsby, 1960). There is some evidence to show that antibodies occur in the mucous exudate of the intestine of animals with these infestations (Douvres, 1960, 1962; Dobson, 1966 a,b,c,d; 1967b). They both reported that mucous exudate from the large intestine of sheep infected with Oesophagostomum spp. contain antibodies and, after concentrating the mucous discharge, precipitins were found by gel diffusion. These antibodies were also demonstrated by complement fixation (Dobson, 1966 a) passive haemagglutination and passive cutaneous anaphylaxis (Dobson, 1966 b, 1967 b). The mucus of the gut was found immunologically more potent than serum (Dobson, 1967 b).

The work of Manton et al (1962) and Soulsby (1963) has demonstrated that young sheep may not be able to respond as well, immunologically as adults, and animals must reach an age of 4 - 5 months before they are able to acquire resistance of H.contortus.

There is no literature on the hosts' immunological response to Cooperia curticei or other species of the genus Cooperia.

Apart from the work of Andrews (1937, 1938, 1939) there has been

no experimental study of the relationship of Cooperia curticei and the sheep host. Many aspects of the relationship have never been explored. The conditions under which the infective larvae exsheath have not been defined. Factual information on the pathogenicity of C.curticei and the immune response of the sheep is based entirely on observations on very small numbers of experimentally infected sheep. This is scarcely enough to rule out its possible pathogenicity, particularly in view of the known pathogenicity of other species of the same genus e.g. C.pectinata and C.punctata.

It was, therefore, considered appropriate to extend the studies on the free-living stages of Cooperia curticei to include a more extensive examination of the effects of the parasite on sheep and, to some extent, the pathological and immunological response of the host to the parasite.

CHAPTER VI   EXSHEATHMENT OF THE INFECTIVE LARVAE OF COOPERIA
CURTICEI

Experiments were carried out in vitro to determine the natural stimuli which induce exsheathment of the infective larvae of Cooperia curticei and to confirm the findings, studies were also made in vivo.

I   MATERIALS AND METHODS: Infective larvae were obtained from the donor sheep (refer II 3) and were stored at 10°C after cleaning them with distilled water. Larvae were used between 1 and 3 weeks after collection. For the in vitro observations larvae were suspended in the following solutions.

Hydrochloric acid - sodium carbonate mixture in varying proportions to provide pH from 1 to 10 (pH 5 only with saturated CO_2).

Citric acid - disodium phosphate buffer (McIlvaine) pH 3 to 8 (pH 4 only with saturated CO_2)

Sodium carbonate - bicarbonate - buffer pH 9.2 to 10.0 saturated with CO_2

Normal saline saturated with CO_2

Distilled water saturated with CO_2

Abomasal fluid saturated with CO_2

Abomasal fluid was obtained from the gastric stomach of sheep with a permanent gastric fistula.

The experimental procedure was the same in all cases. Aliquots of approximately 1000 larvae were held in large test tubes containing 5 to 10 ml of suspending agent. Each tube was closed by non-absorbent cotton wool. Tubes were held in a water bath at 38°C for 3 to 4 hours; where carbon dioxide gas was used it was allowed to flow continuously into the solution in the tubes through a Pasteur pipette. The flow was maintained constant by means of a reducing valve attached to the gas cylinder. The bubbling gas served to agitate the solution continuously. Duplicate samples were incubated on each occasion. The material was examined microscopically as frequently as conditions warranted and the hydrogen ion concentration was measured immediately with a Beckman pH meter. After killing the larvae by submerging the sample tubes in boiling water 100 to 200 larvae were counted in each sample and the percentage

of exsheathment was recorded. The criterion for exsheathment was
similar to that used by Sommerville (1957) and Lapage (1935). Larvae
were classified as exsheathed if the refractile ring had commenced to
form.

For experiments in vivo 2 sheep with permanent rumen fistulae were
used. They were fed with a mixture of hay and a concentrate feed (refer
Chaper II 5). Infective larvae were allowed to exsheath in vivo in a
cellophane sac which contained a suspension of larvae in water, and which
was placed in the rumen of the sheep for 3-4 hours. The larvae were
examined for percentage of exsheathment as described for in vitro experi-
ments.

Replicate experiments showed good agreement, and the data reported
are representative of the results obtained.

II   RESULTS: Preliminary attempts to induce exsheathment in different
solutions in the absence of CO_2 was met with failure. These attempts in-
cluded immersion of larvae in HCl-sodium carbonate solutions, at pH 1 to
10 and McIlvaine buffer at pH 3 to 8. Maximum exsheathment occurred
after 4 hours treatment and was only 11%.

The percentage exsheathment in different solutions saturated with
carbon dioxide is summarised in Table 46. When larvae were treated with
abomasal fluid, distilled water, McIlvaine buffer (pH 4) and HCl-sodium
carbonate solution (pH 5), saturated with carbon dioxide, the maximum
exsheathment was only 19% whereas in normal saline solution (pH 6.8)
and sodium carbonate-bi-carbonate buffer (pH 7.3 to 8.0) both saturated
with CO_2, the maximum exsheathment was 88% and 97% respectively. The
results were consistent in carbonate-bi-carbonate buffer, whereas normal
saline solutions gave inconsistent results. The maximum exsheathment
was obtained near pH 7 and carbon dioxide played an important part in
inducing exsheathment.

The concentration of the undissociated carbonic acid in sodium
carbonate-bi-carbonate buffer solution was calculated as:-

Table 46

The effect of different additives and carbon dioxide on
exsheathment of <u>Cooperia</u> <u>curticei</u> infective larvae

Additives	pH after treatment with CO_2	Percentage exsheathed	
		1 hour	3-4 hours
1. Abomasal fluid CO_2 saturated	2.0	8	10
2. Distilled water CO_2 saturated	5.8	5	13
3. HCl-sodium bicarbonate CO_2 saturated	5.0	15	19
4. Standard buffer CO_2 saturated	4.0	10	12
5. Normal saline soln. CO_2 saturated pH 7.9	6.8	56	88
6. Carbonate bi-carbonate carbonate buffer CO_2 saturated pH 9.2	7.3	67	93
7. Carbonate bi-carbonate buffer CO_2 saturated pH 9.5	7.6	71	97
8. Carbonate bi-carbonate buffer CO_2 saturated pH 10.0	8.0	69	95

$$pH = pk + \log \frac{H\,CO_3^-}{H_2CO_3}$$

The maximum exsheathment (97%) was obtained when the carbonic acid concentration was about 7×10^{-3}M.

The refractile ring appeared about 19 μ from the anterior end of the larva. After forming the refractile ring, the anterior cap was detached. Later the remainder of the sheath was also detached from the tail end and the larvae escaped from the sheath aided by their own movements. The process of exsheathment was thus similar to that described by Lapage (1935) and Sommerville (1957) for H.contortus.

In the in vivo experiment when the pH of the rumen fluid was 7.2 97% exsheathment was obtained in 3-4 hours. The results were consistent and showed that under laboratory conditions exsheathment could be obtained in vitro at rates comparable with those observed in vivo.

VI 3 SUMMARY:

1. Under laboratory conditions the maximum exsheathment of the infective larvae of Cooperia curticei was observed near pH 7. The maximum exsheathment of 88 to 97% was obtained in 3-4 hours at 38°C when normal saline solution and sodium carbonate-bi-carbonate buffers (pH 7.3 to 8.0) were saturated with carbon dioxide.

2. The percentage exsheathment in acid media i.e. abomasal fluid (pH 2.0), distilled water (pH 5.8) HCl-sodium bicarbonate buffer (pH 5.0) and McIlvaine buffer (pH 4.0) saturated with CO_2 was negligible.

3. Maximum exsheathment of the larvae was observed when the concentration of carbonic acid in the sodium carbonate-bi-carbonate buffer was about 7×10^{-3}M.

4. In vivo, 97% of the infective larvae exsheathed in the rumen in 3-4 hours, when the pH of the rumen fluid was 7.2; thus under optimum laboratory conditions, the rate of exsheathment was comparable with

those observed _in vivo_.

5. The process of exsheathment of the larvae of Cooperia curticei was similar to that reported for H.contortus.

VI 4 <u>DISCUSSION</u>: Dissolved gaseous carbon dioxide acting at $37^{\circ}C$ is the major factor in the stimulus to exsheathment _in vitro_ of infective larvae of a variety of nematodes which infect the host by ingestion (Rogers, 1960). The action of CO_2 is enhanced by the presence of electrolytes (Taylor and Whitlock, 1960). Another important factor is the hydrogen ion concentration which has an effect independent of its action on the concentration of dissolved gaseous CO_2 (Rogers, 1958,1960, 1963). These factors at appropriate concentrations cause exsheathment _in vitro_ but precise requirements differ according to the species of nematode.

Keeping this view in mind, the stimulus for exsheathment of Cooperia curticei larvae was studied in CO_2 in buffer solutions containing electrolytes both in acidic and alkaline pH ranges (McIlvaine buffer; HCl-sodium carbonate mixture; sodium carbonate-bi-carbonate buffer; normal saline solution). Because C.curticei and other species of Cooperia have been recorded both from the abomasum and small intestine of the host, it was necessary to study the stimulus for exsheathment of larvae of Cooperia curticei both in acidic and alkaline conditions.

When infective larvae were induced to exsheath in the buffer solutions mentioned above in the presence of dissolved gaseous CO_2, the best results were obtained near pH 7. The _in vitro_ experiments showed that acidic media saturated with CO_2 have no effect on exsheathment of C.curticei larvae, whereas slightly alkaline solutions, saturated with CO_2 near pH 7.3 induced exsheathment of 88 to 97% of the larvae.

Experiments _in vivo_ also showed that 97% of the larvae exsheathed in the rumen in 3-4 hours when pH of the rumen fluid was 7.2. Abomasal fluid saturated with CO_2 gave negative results. These results indicate that the stimulus for exsheathment of Cooperia curticei depends on the presence of dissolved CO_2 and electrolytes at a pH in the region of

pH 7 to 7.3 and that exsheathment normally occurs in the rumen. Under appropriate laboratory conditions the rates of exsheathment were comparable with those observed _in vivo._

Exsheathment of strongylate larvae normally occurs in that part of the alimentary canal anterior to that where the adults are found. As _Cooperia curticei_ is primarily found in the small intestine one would expect that the stimulus for exsheathment of _Cooperia curticei_ will lie in the acidic portion of the stomach. But results indicate that _Cooperia curticei_ is unusual in that exsheathment occurs under rumen conditions. The significance of this phenomenon is not clear. It could suggest that in its evolutionary history _Cooperia curticei_ was initially adapted to the abomasum and later became secondarily adapted to the small intestine.

Chapter VII   EFFECT OF COOPERIA CURTICEI IN SHEEP

Although Cooperia curticei is a common helminth of sheep there is no detailed account of its effect on sheep. The present study was undertaken to determine the effect of a pure infections of Cooperia curticei on sheep of various ages and under varying plane of diet. It is reported under the headings of three main experiments:-

VII . 1.   The effect of Cooperia curticei on sheep 6 - 7 months old.

VII . 2.   The effect of Cooperia curticei on sheep 2 - 3 months old.

VII   3.   The effect of diet on Cooperia curticei infections in sheep 9 - 10 months old.

VII. 1. THE EFFECT OF COOPERIA CURTICEI ON SHEEP 6 - 7 MONTHS OLD:-

i)   Materials and methods:-

a)   Experimental design:-   The experiment was carried out with 18 male Romney sheep 6-7 months old. All these animals were raised and maintained indoors under conditions designed to prevent extraneous helminth infection (refer chapter II.5). The animals were randomly allotted to 5 groups arranged according to the size of the infection.

Group A = 4 animals given 10,000 infective larvae. Two were necropsied after 34 days of infection and the other 2 animals were given second dose of 50,000 infective larvae.

Group B = 4 animals given 50,000 infective larvae. Two were necropsied after 34 days of infection and the other two animals were given second dose of 50,000 infective larvae.

Group C = 4 animals given 100,000 infective larvae. Two were necropsied after 34 days of infection and the other 2 animals were given second dose of 50,000 infective larvae.

Group D = 4 worm free animals as controls. Two were necropsied after 34 days and at this time the other two were each given a single dose of 50,000 infective larvae.

Group E = Two worm free animals which were introduced as replacement controls after 34 days of experiment.

Control observations were made on each animal kept in individual pens for 1 - 2 weeks prior to infestation. Throughout the experiment animals were fed approximately 1.5 lb of concentrate feed per day (refer chapter II.5). Each animal to be infected was given a randomly selected dose of infective larvae.

b)   Parasitological techniques:-   Infective larvae were obtained from the faeces of donor sheep and were stored at 10°C (refer chapter II.3).   One to three weeks old larvae were suspended in water and their number per ml was determined using a dissecting microscope (refer chapter II.4).   Doses for infection were measured with a graduated pipette and the required volume of larval suspension was kept in 'Universal' glass bottles.   Checks were made to ensure that the desired number of larvae $\pm$ 5% were in the doses.

The infective larvae were administered before feeding to the experimental animals per os using a plastic pipette.   The 'Universal' glass bottles containing the dose were washed 3 times and these washings were also administered to the experimental animals.   After dosing, the infective larvae left in the pipette and bottle were counted and their numbers were very small ranging from 2 to 47 per dose only.

The infection was allowed to continue for 34 or 56 days during which time the course of the infection was followed at regular intervals.   Clinical observations were made for gastro-intestinal disturbances, faecal consistency, appetite and general physical condition.   Body temperature was recorded in the morning at weekly intervals.

c)   Faecal egg counts:-   Twenty-four hours faecal material was collected by means of faecal collecting bag and after weighing and mixing, the number of eggs per gram was determined by the modified McMaster method (refer chapter II.2). The faecal material from each experimental animal was examined daily after 12 days of infection, until eggs were recovered from the faeces.   From then onwards faecal examination was carried out at intervals varying from 3 to 7 days.

d)   Body weight and wool growth:-   Experimental animals were weighed before feeding at weekly intervals.   For measurement of wool growth, an area 50 mm x 75 mm was clipped from the mid flank region from each experimental animal.   This site was found by Lockhart (1954) to give maximum correlation between the site yield and staple length measurements.   The area was shorn

before necropsy and growth of the wool was recorded on a weight basis.

e)   Blood analysis:-   Blood samples were collected in the morning by
jugular puncture at weekly intervals.   Sodium ethylene-diamine-tetra-acetate
was used as an anticoagulant and approximately 3 ml of blood was taken for
haematological study.   Approximately 25 ml of blood was also collected and
allowed to clot at room temperature in a sterile bottle and the serum produced
was stored at -20°C until used.

Each sample of the blood was treated in the following way.   Packed
cell volume was determined by micro-capillary method.   Haemoglobin concentration
was measured using the cyanmethaemoglobin method in a "Coleman" photohaemo-
globinometer.   Total erythrocyte (RBC) and leucocyte (WBC) counts were made
using a haemacytometer and a bulk dilution technique.   Differential white cell
counts were carried out on smears stained with McNeals tetrachrome.   A total of
200 randomly selected cells were differentiated and differential counts were
expressed in absolute terms.

Total serum protein values were measured by the Biuret technique
(Kabat and Mayor, 1961).   In this test 0.1 ml of serum was diluted with 0.9 ml
of normal saline solution and 4.0 ml of Biuret reagent.   All the absorption
values were obtained with "Beckman" spectrophotmeter at 540 mμ and the protein
values were calculated in grams per 100 ml serum from the standard curve
prepared with the same instrument.   Electrophoretic studies on the serum
proteins were made using "Phoroslide" strips, (Millipore Corporation,
Massachusetts, U.S.A.).   The separation of the serum proteins on Phoroslide
strips was carried out for 20 minutes at 100 volts.   Phoroslides were stained
in Ponceau-S dye fixative solution for 10 minutes and were then rinsed in 3
changes of 5% acetic acid until the background of the strip was free of colour.
The strips were then dried at room temperature and were cleared by immersion for
30 seconds in a solution containing ethyl acetate (30 ml) and glacial acetic
acid (70 ml).   The strips were screened on an electronic densitometer and
actual percentages of protein fractions were calculated by weighing the paper
enclosed by the densitometer curve corresponding to each fraction.

f)   Necropsy procedure:-   The two sheep killed on day 34 were selected
from their group using a table of random numbers.   All the sheep were killed
by exsanguination.   Necropsy procedure included an examination of all the body
organs and tissues for gross lesions.

The alimentary tract was removed. Two ligatures were placed at the pyloric sphincter and the abomasum and small intestine separated. The abomasal contents were collected and formalin added to give a final concentration of 10%. The small intestine was separated from the omentum and mesenteries taking care not to disturb the contents. The small intestine was tied-off at 5 places, first 5 feet then, 10 feet, 15 feet, 20 feet from the pylorus and at the ileocaecal valve. The contents of each section of small intestine were collected separately and formalin added. From each section of intestine approximately 1 inch was removed for histopathological study and approximately 12 inches removed for extracting mucus for immunological studies. Each part of the small intestine was opened separately and the mucosa was scraped in water with the edge of a slide. Scrapings from each section of small intestine were added to the collected contents of the corresponding section. After obtaining the mucous extract for immunological study the intestinal scrapings from these sections were washed in water and kept separately in 10% formalin for worm count.

After thorough mixing, a 10% aliquot from each part of the small intestine and abomasum was examined. Total numbers of males and female worms were estimated from each sample to establish the frequency distribution of Cooperia curticei in the intestine. From the total number of worms present in the small intestine and abomasum, sex ratio and percentage of the administered dose established in the host were calculated.

The following criteria were adopted in classifying the developmental stages of Cooperia curticei; the worms which had not completed the fourth moult were classified as fourth stage larvae. Males with coloured spicules and females which contained eggs in their uteri were described as adult.

g) Histopathology:- Selected pieces of tissue were taken from the small intestine from 5 sites between 0 to 5 feet from the pylorus; 5 to 10 feet; 10 to 15 feet; 15 to 20 feet and 20 feet up to the ileocaecal valve. A piece of tissue was also taken from the fundus of the abomasum together with 2 or 3 mesenteric lymph nodes. Immediately after necropsy the tissue was fixed in Bouins fixative for 48 hours and sections were cut at 5 μ from paraffin embedded blocks and stained with haematoxylin and eosin.

h) Immunological study:- The following procedure was adopted for antigen-antibody precipitation in gel agar.

Source of antibodies:- Serum and mucus extract from the experimental animals were utilised as a source of antibodies. Blood was collected from experimental sheep before necropsy and serum was obtained. Total protein was estimated by the Biuret technique.

To obtain mucus samples each 12" piece of the small intestine was placed in 50 ml of 1:10,000 thiomersal in 0.9% saline at $4^{\circ}C$ for 24 hours. (Dobson, 1966 a, b, 1967 b). This cooling caused expulsion of the mucus from the goblet cells. After washing the mucosa gently in the saline mixture, the mixture of mucus and saline was poured-off. The saline mixture was centrifuged for half an hour at 28,000 g to remove particulate material. The supernatant was concentrated with Carbowax 20 M to a protein value similar to that of serum. The protein determination was made by the Biuret technique and mucus extract was stored at $-20^{\circ}C$ until used.

Preparation of antigens:- The following antigens were used.

1.   Exsheathed third stage larval antigen:- (XL_3).
    Exsheathed larvae were obtained by suspending about 400,000 infective larvae in a dialysis bag for 3 hours in the rumen of a sheep (refer Chapter VI). The exsheathed larvae were recovered by centrifugation, washed twice in phosphate buffer and centrifuged again to sediment the larvae. The exsheathed larvae were homogenized in a Griffith tube for half an hour. Rupture of the larvae was checked under the microscope and material containing ruptured larvae was kept overnight in 3 cc phosphate buffer at $5^{\circ}C$. The larval suspension was then centrifuged at 28,000 g for 30 minutes, and supernatant fluid (antigen) was collected. Total protein was estimated by Lowry's method (cited by Colowick and Kaplan, 1957) and it ranged from 1.10 to 1.25 gm per 100 ml.

2.   Exsheathing fluid and metabolic product antigen (XF):-
    After utilising the exsheathed larvae as described above, the fluid in which exsheathment had occurred was centrifuged for 30 minutes at 28,000 g to remove particulate material. The fluid was concentrated with carbowax M20. Total protein content was 1.06 gm per 100 ml by Lowry's method.

3.   Infective third stage (ensheathed) larval antigen (L_3):-
    About 1 million infective third stage larvae were washed twice in phosphate buffer and disintegrated in a Griffith tube for 30 minutes. The antigen was prepared in a similar way to that described for exsheathed larvae.

Total protein concentration estimated in different samples by Lowry's method
ranged from 1.20 to 2.90 gm per 100 ml.

4.   First and second stage larval antigen (L_1) and (L_2):-
        First and second stage larvae were obtained from 1 and 3 days old
faecal cultures at 27°C (refer Chapter III.2 (i)).   The antigen was prepared as
described for exsheathed larvae.   Total protein concentration for first and
second stage larval antigen was 1.15 and 2.60 gm per 100 ml respectively.

        Serological method:-   Ouchterlony diffusion tests were carried out in
1% agar (Oxoid no. 2) made up in 12% sodium chloride solution (Soulsby, 1960).
The plates were prepared by pouring 20 ml of agar into Petri dishes of 9 cm
diameter.   The wells were made using a No. 5 corkborer and were 10 mm in
diameter and 5 mm in depth with 6 mm distance between wells.

        Antigen was placed in the centre well and was surrounded by wells
containing serum or mucus, extract from the experimental animals.   The plates
were allowed to develop for 5 days at room temperature.   Sketches of the
developing patterns were made at 24 hour intervals.   Three replicates were set
up for each sample of serum or mucus extract.

ii)   Results:-

a)   Clinical observations:-   Sheep showed no obvious sign of infection
when given 10,000 to 150,000 infective larvae of Cooperia curticei except
passage of soft faeces in 2 animals for 1-3 days after the prepatent period.

b)   Egg count and egg production:-   The prepatent period of infection was
found to be 14 days but the eggs appeared infertile on that day.   After 15 days
of infection, the majority of the eggs were fertile.   Peak egg counts ranging
from 600 to 4,600 per gram were recorded 7 days after the infection became
patent (Table 47).   Thereafter egg counts gradually declined in group A (10,000
dose), whereas in groups B and C (50,000 to 150,000) the decline was abrupt.
In all experimental animals after 55 days of infection the egg count ranged from
zero to 150 eggs per gram.

c)   Body weight and wool growth:-   The infection with Cooperia curticei
had no significant effect on either body weight or wool growth (Tables 48, 49;
Appendix 38).

Table 47

Cooperia curticei egg count in sheep when given different doses of larvae:-

Group No:-	A				B			
Sheep No:-	301	319	310	311	324	6078	315	304
Total larval dose:-	10,000	10,000	10,000 + 50,000	10,000 + 50,000	50,000	50,000	50,000 + 50,000	50,000 + 50,000
Days of infection (first dose)				Eggs per gram				
12	nil	nil	nil	nil	nil	nil	nil	nil
13	nil	nil	nil	nil	nil	nil	nil	nil
14	50	50	50	50	100	150	200	100
15	200	200	150	150	350	400	250	450
19	-	-	-	-	350	650	300	700
21	4,300	4,600	600	2,650	1,000	1,800	2,400	-
25	3,200	3,000	650	1,200	150	400	1,900	200
29	2,800	2,200	150	1,100	150	150	200	50
31	-	-	-	-	100	150	350	50
34 (2nd dose 50,000 larvae)	1,550 Killed	2,300 Killed	350	800	150 Killed	nil Killed	1,300	200
42			50	400			900	50
44			50	450			400	-
49			50	400			700	150
52			nil	250			450	100
55			nil	150			-	100
			killed	killed			killed	killed

Table 47   continued

Group No:-		C			D	
Sheep No:-	6079	325	326	303	323	322
Total larval dose	100,000	100,000	100,000	100,000	control	control
			+	+		
			50,000	50,000		
Days of infection:-			Eggs per gram			
(first dose)						
12	nil	nil	nil	nil	nil	nil
13	nil	nil	nil	nil	-	-
14	200	150	200	150	-	-
15	700	300	350	250	-	-
19	1,400	1,000	800	1,100	nil	nil
21	2,000	3,100	-	3,000	-	-
25	1,200	400	500	200	-	-
29	100	200	150	100	-	-
31	100	300	-	-	nil	nil
34 (2nd dose	50	200	100	nil	dosed	dosed
50,000 larvae)	Killed	Killed			50,000	50,000
42			nil	nil	-	-
44			nil	nil	nil	nil
49			50	nil	nil	nil
52			-	-	few eggs	nil
55			50	nil	50	nil
			killed	killed	killed	killed

Table 48

The effects of _Cooperia_ _curticei_ on the weight changes
of sheep given different doses of larvae:-

Group No.	Sheep No.	Total larval dose	Length of exp. period days	Wt. at the beginning of exp. lbs.	Wt. at the end of exp. lbs.	Total wt. gain lbs.
A	301	S10,000	34	48	54	6
	319	S10,000	34	40	42	2
	310	D60,000	56	35	37.5	2.5
	311	D60,000	56	42	45.5	3.5
B	324	S50,000	34	44	50	6
	6078	S50,000	34	44	46	2
	315	D100,000	56	36	39	3
	304	D100,000	56	43	44.5	1.5
C	6079	S100,000	34	42	44.5	2.5
	325	S100,000	34	44	49	5
	326	D150,000	56	39	41	2
	303	D150,000	56	48	53.5	5.5
D	323	S50,000	56	44	49	5
	322	S50,000	56	48	58	10
	6081	Control	34	43	44	1
	302	Control	34	43	44	1
E	307	Control	22	51	53.5	2.5
	318	Control	22	45	46.5	1.5

S:-   Single dose

D:-   Second dose (50,000) after 34 days of infection

Table 49

The effect of _Cooperia_ _curticei_ on wool growth
of sheep given different doses of larvae:-

Group No	Sheep No	Total larval dose (single)	Length of exp. period days	Weight of wool in gms
A	301	10,000	34	2.85
	319	10,000	34	3.10
	310	10,000	34	2.27
	311	10,000	34	1.66
B	324	50,000	34	2.60
	6078	50,000	34	2.47
	315	50,000	34	2.65
	304	50,000	34	2.71
C	6079	100,000	34	-
	325	100,000	34	2.88
	326	100,000	34	2.23
	303	100,000	34	2.53
D	323	Controls	34	2.81
	322	Controls	34	2.88

d)   Blood analyses:-   Haematological data are given in Tables 50, 51, 52
and 53.   The only significant change noted was an increase in the number of
circulating eosinophiles 14 to 21 days after infection.   The mean number of
eosinophiles in worm free sheep was 72 per cubic mm (standard deviation $\pm$ 88).
Frequently the eosinophile count in individual infected sheep exceeded the mean
+ 3 times the standard deviation (i.e. 336) of infected sheep (Table 53).

The effect of Cooperia curticei infection on total serum protein and
serum protein fractions of experimental animals are given in Tables 54 and 55.
Differences in the total serum protein of infected group ranged from +0.2 to
+1.0 gm % whereas in controls the range was +0.4 to +0.7 gm % only.   The
relative percentages of serum protein fractions showed irregular fluctuations
and no significant changes or trends were detected.

e)   Gross lesions, frequency distribution of worms in the intestine, sex
      ratio and percentage of worms established in the host:-   No gross
lesion was present on post-mortem in experimental animals infected with different
doses of Cooperia curticei larvae.   Actual numbers of male and female worms
recovered from the small intestine are given in Appendices 39 and 40.   No worms
were recovered from the abomasa.   Frequency distribution of Cooperia curticei
in small intestine, sex ratio and percentage extablished in the host after
different larval doses are shown in Tables 56 and 57.   Adult worms were present
in all parts of the small intestine but the majority were found between 5 and 10
feet from the pylorus.

Maximum percentages of doses of larvae administered recovered from the
hosts were at 10,000 larval dose i.e. 76 to 79% whereas at higher doses (50,000
to 150,000) it ranged from 0.1 to 4.8% with the exception of one animal (No.
6078) in which 29% were recovered.   It was also noted that a single dose of
50,000 to 100,000 infective larvae not only produced a lower rate of "establish-
ment" but also retarded growth of the adult worm; the worms were smaller than
normal, females contained no eggs and the spicules of males lacked pigmentation.
When experimental animals were given a second dose of 50,000 infective larvae
after 34 days of infection, the development of these larvae was inhibited.   At
necropsy after 56 days of infection varying numbers of larvae were still in
fourth stage (see Table 57).   The female worm population was always more than
that of males except in 3 animals (No. 310, 319 and 311) in which the numbers
of males was slightly greater than that of females.   The predominance of female
worms occurred at all levels of the intestine.

Table 50

Packed cell volumes (%) of experimentally
infected and control sheep

						Days of infection						
Group	Sheep No	First dose	0	7	14	21	28	34	Second dose	41	48	56
A	301	10,000	37	37	35	3415	40	47				
	319	"	40	39	39	40	44	42				
	310	"	37	37	33	38	40	35	50,000	34	37	39
	311	"	29	29	31	33	36	31.5	"	34	37	37.5
B	324	50,000	35	34	36	36.5	42	36.5				
	6078	"	35	34	36	35	34	34				
	315	"	37	36	35	38	40	36	50,000	40	38	37
	304	"	38	37	34	35	37	34	"	34	34	36
C	6079	100,000	39	37	37	39	41	39				
	325	"	35	37	31	30.5	35	37				
	326	"	40	40	37.5	43.5	44	39	50,000	45	42	43
	303	"	38	32.5	33	36	39	33	"	37	36.5	35
D	323	Nil	35	40.5	38	40.5	42	37.5				
	322	"	37	37.5	38	36	38	34				
	6081	"	36.5	41	40	37	43	40	50,000	40	38	35
	302	"	37	39	37.5	37	40	38	"	40	36	36.5
E	307	Nil								43	43	41
	318	"								47	45	44

Table 51

Haemoglobin values (gm %) of experimentally
infected and control sheep

			Days of infection									
Group	Sheep No	First dose	0	7	14	21	28	34	Second dose	41	48	56
A	301	10,000	13.5	12.8	12.3	12.6	13.8	15.2				
	319	"	13.2	12.9	11.9	12.9	14.7	12.0				
	310	"	12.0	11.8	11.5	11.9	12.6	9.9	50,000	9.3	11.1	11.7
	311	"	10.5	10.3	10.8	11.3	11.6	9.3	"	9.8	10.8	12.5
B	324	50,000	12.3	11.3	11.4	11.8	13.8	11.0				
	6078	"	13.0	12.3	12.1	12.0	11.3	10.3				
	315	"	13.0	12.0	12.1	12.5	12.7	11.0	50,000	11.2	12.0	11.8
	304	"	13.2	12.2	11.8	12.3	11.4	10.0	"	9.8	10.3	11.3
C	6079	100,000	13.0	12.3	11.8	13.1	12.6	11.3				
	325	"	12.3	10.4	10.5	11.1	10.9	11.0				
	326	"	14.0	12.3	11.8	13.6	14.3	11.4	50,000	13.4	13.2	14.8
	303	"	13.7	11.8	11.0	12.4	13.5	9.8	"	10.8	11.4	11.2
D	323	Nil	12.5	12.8	11.9	12.9	13.8	10.5				
	322	"	12.5	12.8	12.8	11.2	12.0	10.0				
	6081	"	12.6	12.8	12.8	12.8	13.9	12.6	50,000	11.8	11.4	11.0
	302	"	12.3	12.8	12.0	12.6	13.4	10.9	"	11.9	10.8	11.4
E	307	Nil								12.4	13.6	13.4
	318	"								14.1	14.6	13.8

Table 52

Total leucocyte counts (x 10^3 cu.mm blood)
of experimentally infected and control sheep

Day of infection

Group	Sheep No.	First dose	0	7	14	21	28	34	Second dose	41	48	56
A	301	10,000	12.68	10.33	10.30	9.40	11.90	14.00				
	319	"	10.70	8.65	10.84	7.30	8.70	8.00				
	310	"	8.55	7.84	8.41	8.60	9.70	8.20	50,000	12.30	10.30	3.20
	311	"	7.20	7.30	8.08	5.50	6.40	5.80	"	8.80	7.70	6.20
B	324	50,000	8.50	10.48	10.39	8.60	9.70	3.90				
	6078	"	7.75	7.56	11.50	5.50	13.30	6.80				
	315	"	8.35	9.22	6.00	8.80	8.90	9.50	50,000	9.10	9.10	6.40
	304	"	9.20	8.71	10.56	7.60	9.70	7.80	"	10.90	10.20	5.40
C	6079	100,000	8.20	9.96	9.49	12.20	10.40	10.00				
	325	"	5.95	8.25	9.75	7.90	8.50	2.40				
	326	"	8.75	9.28	10.20	9.30	8.60	9.40	50,000	8.30	13.10	8.90
	303	"	9.05	7.16	8.89	8.60	8.90	9.90	"	14.30	13.10	4.60
D	323	Nil	9.10	8.63	8.66	8.90	9.70	9.10				
	322	"	7.10	9.49	10.50	9.70	8.80	8.90				
	6081	"	10.10	6.58	9.13	10.80	10.10	6.80	50,000	12.90	8.40	9.40
	302	"	9.30	8.95	9.98	9.20	9.60	5.30	"	10.00	9.60	3.20
E	307	Nil								11.40	9.40	2.30
	318	"								11.10	14.80	6.90

Table 53

Eosinophiles in cubic mm of blood in
experimental and control sheep

Group	Sheep No.	First dose	Days of infection 0	7	14	21	28	34	second dose	41	48	56
A	301	10,000	0	0	0	94	0	0				
	319	"	0	0	70	0	261	0				
	310	"	0	132	0	258	97	328	50,000	369	1030	0
	311	"	0	0	67	220	512	58	"	0	616	0
B	324	50,000	85	75	90	86	0	0				
	6078	"	78	0	360	165	399	204				
	315	"	0	190	522	968	445	1330	50,000	1911	1274	192
	304	"	0	0	0	304	388	700	"	1090	1530	162
C	6079	100,000	0	95	296	366	728	500				
	325	"	0	164	0	158	680	0				
	326	"	88	0	0	279	172	470	50,000	415	262	178
	303	"	0	0	95	0	0	594	"	1430	1310	92
D	323	Nil	0	0	0	89	0	182				
	322	"	71	140	0	0	0	178				
	6081	"	0	0	166	108	0	0	50,000	387	2268	0
	302	"	0	160	79	92	96	0	"	0	1056	0
E	307	Nil								114	282	0
	318	"								111	296	0

Table 54

The effect of <u>Cooperia</u> <u>curticei</u> on total serum protein
of sheep 6-7 months old given different doses of larvae:-

Group No.	Sheep No.	Total larval dose	Length of exp. period days	Total serum protein at the start of exp. gm%	Total serum protein at the end of exp. gm%	Difference if any gm%
A	301	S10,000	34	6.4	6.4	0
	319	S10,000	34	7.2	7.4	+ 0.2
	310	D60,000	56	6.4	6.8	+ 0.4
	311	D60,000	56	6.8	6.8	0
B	324	S50,000	34	6.2	6.8	+ 0.6
	6078	S50,000	34	5.8	6.8	+ 1.0
	315	D100,000	56	5.8	6.0	+ 0.2
	304	D100,000	56	6.2	6.6	+ 0.4
C	6079	S100,000	34	6.6	7.0	+ 0.4
	325	S100,000	34	6.8	6.8	0
	326	D150,000	56	6.0	6.8	+ 0.8
	303	D150,000	56	6.2	7.2	+ 1.0
D	323	S50,000	56	6.4	6.4	0
	322	S50,000	56	6.0	8.6	+ 2.6
	6081	Controls	34	6.4	6.8	+ 0.4
	302	Controls	34	6.4	7.0	+ 0.6
E	307	Controls	22	6.0	6.4	+ 0.4
	318	Controls	22	6.1	6.6	+ 0.5

S:-   Single dose

D:-   Second dose (50,000) given after 34 days of infection

Table 55

The effect of <u>Cooperia</u> <u>curticei</u> on serum protein
fractions of sheep 6 - 7 months old
given different doses of larvae:-

Group No.	Sheep No.	Total larval dose	Length of exp period (days)	Sampling time	Percentage protein fractions:-				
					Albumin	Alpha 1	Alpha 2	Beta	Gamma
A	301	10,000	34	B.E.	48.65	3.36	22.65	8.52	16.82
				E.E.	46.71	2.03	31.72	6.60	12.94
	319	10,000	34	B.E.	50.67	3.69	18.46	6.71	20.47
				E.E.	52.53	4.75	14.24	3.16	25.32
	310	60,000	56	B.E.	46.05	5.58	24.65	4.65	19.07
				E.E.	33.27	8.52	20.08	8.32	29.81
	311	60,000	56	B.E.	41.45	8.35	24.73	5.82	19.64
				E.E.	40.24	8.58	25.44	2.66	23.08
B	324	50,000	34	B.E.	39.03	8.55	15.67	17.37	19.37
				E.E.	50.58	6.18	18.24	8.53	16.47
	6078	50,000	34	B.E.	42.86	9.15	20.98	6.03	20.98
				E.E.	49.62	6.77	24.31	2.26	17.04
	315	100,000	56	B.E.	44.04	9.14	21.33	5.82	19.67
				E.E.	39.44	5.16	23.47	7.28	24.65
	304	100,000	56	B.E.	47.75	3.09	27.81	3.65	17.70
				E.E.	44.64	4.92	19.87	8.48	22.09

Table 55   continued

Group No.	Sheep No.	Total larval dose	Length of exp period (days)	Sampling time	Percentage protein fractions:-				
					Albumin	Alpha 1	Alpha 2	Beta	Gamma
C	6079	100,000	34	B.E.	42.86	9.15	20.98	6.03	20.98
				E.E.	49.62	6.77	24.31	2.26	17.04
	325	100,000	34	B.E.	40.05	8.24	22.88	3.66	25.17
				E.E.	49.04	3.38	22.61	5.73	18.79
	326	150,000	56	B.E.	45.33	4.84	20.07	7.27	22.49
				E.E.	31.78	9.96	25.00	6.99	26.27
	303	150,000	56	B.E.	43.85	4.62	23.33	3.58	24.62
				E.E.	34.35	4.57	31.30	4.35	25.43
D	323	50,000	56	B.E.	51.39	4.01	19.48	3.73	21.49
				E.E.	34.52	7.42	32.26	5.16	20.64
	322	50,000	56	B.E.	43.69	7.48	17.52	8.18	23.13
				E.E.	47.48	3.98	27.59	6.63	14.32
	6081	Controls	34	B.E.	45.37	6.65	24.86	3.18	19.94
				E.E.	52.69	6.89	18.57	4.19	17.66
	302	Controls	34	B.E.	35.84	9.02	21.55	10.03	23.56
				E.E.	52.08	6.04	18.10	4.53	19.25
E	307	Controls	22	B.E.	42.75	10.25	21.02	6.92	19.06
				E.E.	40.50	11.00	25.25	5.50	17.75
	318	Controls	22	B.E.	44.26	9.76	23.04	6.76	16.18
				E.E.	42.27	10.87	21.98	4.35	20.53

B.E.:-   Before the start of the experiment.

E.E.:-   At end of the experiment

Table 56

Frequency distribution of _Cooperia curticei_ in the small intestine;
sex ratio and percentage developed in the host:- (single dose)

Group No.	Sheep No.	Total larval dose	Days of in-fection	Aboma-sum	No. of worms recovered small intestine 0-5ft	5-10ft	10-15ft	15-20ft	20 & over	4th stage larvae	Percentage Sex ratio Male	Female	Total No. of worms	Percentage developed in the host	Remarks
A	301	10,000	34	0	350	5,985	850	450	0	0	52.6%	47.4%	7,635	76%	Adults
	319	10,000	34	0	1,190	5,380	1,390	20	0	0	51.2%	48.8%	7,980	79%	Adults
B	324	50,000	34	0	27	72	38	0	0	0	35.7%	64.3%	137	0.3%	Retarded growth
	6078	50,000	34	0	18	8,691	5,449	301	91	0	48.9%	51.1%	14,550	29%	"
C	6079	100,000	34	0	180	504	117	27	27	0	36.8%	63.2%	855	0.8%	Retarded growth
	325	100,000	34	0	45	2,322	1,467	171	27	0	45.0%	54.0%	4,032	4%	"
D	6081	Control	34	0	0	0	0	0	0	0	—	—	—	—	—
	302	Control	34	0	0	0	0	0	0	0	—	—	—	—	—

Table 57

Frequency distribution of Cooperia curticei in small intestine
sex ratio and percentage developed in the host:-
(second dose given after 34 day's of infection)

Group No.	Sheep No.	Total larval dose	Days of infection	Aboma-sum	small intestine 0-5ft	5-10ft	10-15ft	15-20ft	20ft & over	4th stage larvae	Percentage Sex ratio male	female	Total no. of worms	Percentage developed in the host	Remarks
A	310	10,000+ 50,000	56	0	54	117	18	9	0	0	31.8%	68.2%	198	0.3%	retarded growth
	311	10,000+ 50,000	56	0	63	2,511	297	18	9	198	52.4%	47.6%	2,898	4.8%	"
B	315	50,000+ 50,000	56	0	18	612	963	198	27	0	47.0%	53.0%	1,818	1.8%	"
	304	50,000+ 50,000	56	0	72	1,215	630	387	378	90	36.9%	63.1%	2,682	2.7%	"
C	326	100,000 +50,000	56	0	108	711	36	18	0	837	48.4%	51.6%	873	0.5%	"

Table 57   continued

No. of worms recovered

Group No.	Sheep No.	Total larval dose	Days of in-fection	Aboma-sum	small intestine 0-5ft	5-10ft	10-15ft	15-20ft	20ft & over	4th stage larvae	Percentage sex ratio male	female	Total no. of worms	Percentage developed in the host	Remarks
C	303	100,000 +50,000	56	0	108	117	0	0	0	0	16.0%	84.0%	225	0.1%	Retarded growth
D	323	50,000	22	0	18	1,152	18	0	0	24,408	46.9%	53.1%	1,188	2.3%	"
	322	50,000	23	0	18	63	18	0	0	180	36.3%	63.7%	99	0.2%	"
E	307	Controls	22	0	0	0	0	0	0	0	–	–	–	–	–
	318	Controls	22	0	0	0	0	0	0	0	–	–	–	–	–

f)   Histopathology:- Microscopic examination of the sequential sections
of small intestine, abomasum and lymph nodes from infected and control animals
showed no outstanding histopathological changes.   In some sections of intestinal
mucosa from infected sheep, there appeared to be an increase in lymphoid
infiltration and in some others an increased infiltration by eosinophiles and
globular leucocytes but this could not be related to the number or size of
infecting doses or the number of worms recovered.

g)   Immune response:- Total protein concentrations in serum and concen-
trated mucus extract utilised as sources of antibodies are given in Table 54 and
58 respectively.   The gel diffusion reactions of the five antigens prepared
from first stage, second stage, ensheathed third stage and exsheathed third
stage larvae and exsheathing fluid against serum and mucus precipitating anti-
bodies varied with the larval dose given to the sheep.   Replicates were, how-
ever, uniform in their results.

One precipitin line was present between antigens from infective larvae,
exsheathed larvae and exsheathing fluid and serum from animals necropsied after
34 days of infection with single doses of 10,000 to 100,000 larvae (figure 18
(iii), (iv) and (v)) whereas one to 3 precipitating lines were present
between first and second stage larval antigens and serum from animals belonging
to groups B and C (50,000 to 100,000).   Serum from animals with 10,000 larval
dose in group A showed no reaction with first stage larval antigen but one line
of precipitation was present with second stage larval antigen (figure 18 (i)
and (ii)).

Reactions in agar were negative with mucus samples obtained after 34
days of infection against all antigens except exsheathing fluid which gave one
line of precipitation with mucus extract from 3 animals (nos. 6179, 325, 324)
belonging to groups B and C (Fig. 19).

The reaction of all 5 antigens in gel diffusion against serum or mucus
extract of 6 animals necropsied after 56 days of infection with two doses of
larvae (60,000 to 150,000 in total) was positive.   Two to 3 precipitin lines
were present between serum and all antigens (figure 20 (i) to (iv)) except for
exsheathing fluid in which only one line of precipitation was observed (figure
20 (v)).   The precipitating antibodies of mucus extract from animals
necropsied after 56 days of infection showed only one line against all antigens
(figure 21 (i) to (v)).

Table 58

Total protein estimation in concentrated mucus
extract of sheep infected with different
doses of <u>Cooperia</u> <u>curticei</u> larvae:-

Group No.	Sheep No.	Total larval dose	Days of infection	Total protein in mucus extract gm %
A	301	10,000	34	7.8
	319	10,000	34	4.6
	310	10,000 + 50,000	56	3.6
	311	10,000 + 50,000	56	6.7
B	324	50,000	34	6.6
	6078	50,000	34	3.2
	315	50,000 + 50,000	56	5.4
	304	50,000 + 50,000	56	6.3
C	6079	100,000	34	6.0
	325	100,000	34	5.8
	326	100,000 + 50,000	56	5.0
	303	100,000 + 50,000	56	3.9
D	323	50,000	22	5.4
	322	50,000	22	6.1
	6081	Controls	34	5.6
	302	"	34	4.9
E	307	"	22	4.6
	318	"	22	5.4
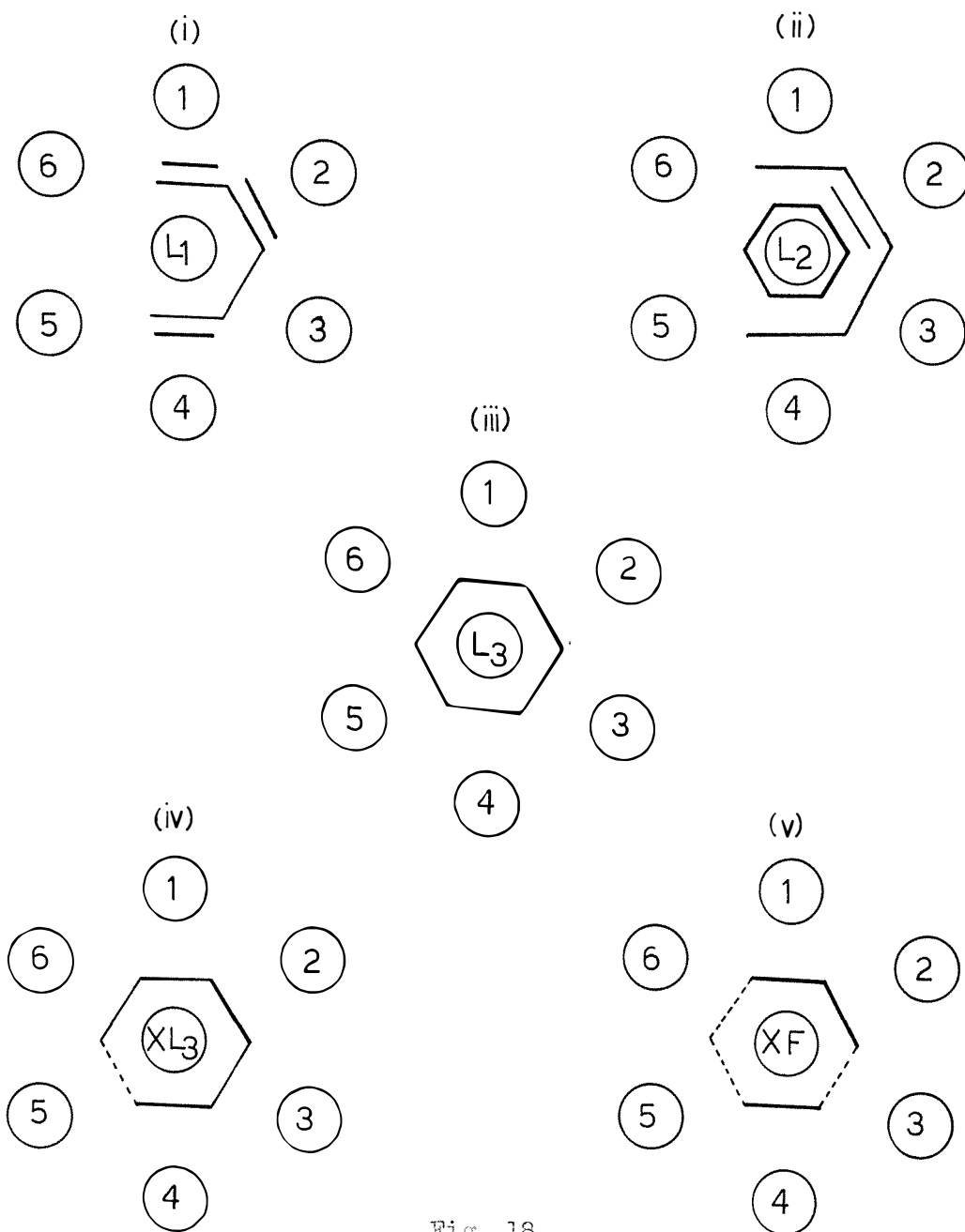
Fig. 18

Gel-diffusion reactions of serum from sheep infected for 34 days against various antigens.

KEY

L_1 = first stage larval antigen

L_2 = second stage larval antigen

L_3 = ensheathed 3rd stage larval antigen

XL_3 = exsheathed 3rd stage larval antigen

XF = exsheathing fluid

Sheep 1 & 2 given 100,000 larvae

Sheep 3 & 4 given 50,000 larvae

Sheep 5 & 6 given 10,000 larvae

Fig. 19

Gel-diffusion reactions of mucus extract from small intestine of
sheep infected for 34 days against various antigens.

KEY

L_1 = first stage larval antigen

L_2 = second stage larval antigen

L_3 = ensheathed 3rd stage larval antigen

XL_3 = exsheathed 3rd stage larval antigen

XF = exsheathing fluid

Sheep 1 & 2 given 100,000
larvae

Sheep 3 & 4 given 50,000
larvae

Sheep 5 & 6 given 10,000
larvae

(i) (ii) (iii) (iv) (v)

Fig. 20

Gel-diffusion reactions of serum from sheep infected for 56 days against various antigens.

KEY

L_1 = first stage larval antigen

L_2 = second stage larval antigen

L_3 = ensheathed 3rd stage larval antigen

XL_3 = exsheathed 3rd stage larval antigen

XF = exsheathing fluid

Sheep 1 & 2 given 150,000 larvae

Sheep 3 & 4 given 100,000 larvae

Sheep 5 & 6 given 60,000 larvae

Fig. 21

Gel-diffusion reactions of mucus extract from small intestine of
sheep infected for 56 days against various antigens.

KEY

L_1 = first stage larval antigen

L_2 = second stage larval antigen

L_3 = ensheathed 3rd stage larval antigen

XL_3 = exsheathed 3rd stage larval antigen

XF = exsheathing fluid

Sheep 1 & 2 given 150,000
larvae

Sheep 3 & 4 given 100,000
larvae

Sheep 5 & 6 given 60,000
larvae

No precipitation was recorded between any antigen and serum or mucus extract from control animals.

VII. 2. <u>EFFECT</u> <u>OF</u> <u>COOPERIA</u> <u>CURTICEI</u> <u>INFECTION</u> <u>IN</u> <u>SHEEP</u> <u>2 - 3</u> <u>MONTHS</u> <u>OLD</u>:-

In order to find out if there is any effect of age on susceptibility of
sheep to <u>Cooperia</u> <u>curticei</u> infection, the present experiment was designed to
determine the effect of this parasite in sheep 2 to 3 months old.

i)   <u>Materials and methods</u>:-   The experiment was carried out with 16 male
Romney sheep 2 to 3 months old.   The design of the experiment and parasitologic
techniques were similar to those described in the previous section, (refer VII
(i) (a) and (b)).   The animals were given single doses of infective larvae and
were arranged in 4 groups according to the size of the infecting dose.

        Group A = 10,000 infective larvae
                    4 animals
        Group B = 50,000 infective larvae
                    4 animals
        Group C = 100,000 infective larvae
                    4 animals
        Group D = Four worm free animals
                    as controls.

The infection was allowed to continue for 20 to 40 days and during this
time the course of the infection was followed at regular intervals.

Each experimental animal was given 1.5 lb of concentrate feed (refer
chapter II.5) and feed intake was recorded daily.   The amount of food eaten was
measured by subtracting the amount of residue from the quantity provided.
Faecal egg counts, body weight and blood analyses were carried out as described
in the previous section (refer VII (i) (c) (d) and (e)).   Total egg output for
24 hour periods was recorded for the two days immediately prior to the day of
slaughter of each animal.   This was compared with the number of adult worms
recovered on post mortem and eggs laid per female worm per day were estimated.

Twenty days after infection two sheep were randomly selected from each
group and killed.   The remaining sheep were killed 40 days after infection.
The necropsy procedure, histopathological examination and immunological studies
were carried out as described in Chapter VII (i), sections (f), (g) and (h).

ii)   Results:- The clinical observations revealed no significant sign of infection in the sheep given 10,000 to 100,000 infective larvae of Cooperia curticei, except the passage of loose faeces for 1-4 days in one animal in group B just after the prepatent period.   Feed intake was not affected in any of the experimental animals throughout the period of infection.

The prepatent period of infection was 14-16 days.   Peak egg counts were recorded 5-7 days after the infection became patent (Table 59) and ranged from 400 to 6,150 eggs per gram.   Thereafter, egg counts declined gradually in the animals given a 10,000 larval dose, and abruptly in those given a 50,000 or 100,000 larval dose.   After 40 days of infection the egg count ranged from zero to 150 eggs per gram in animals given 50,000 or 100,000 larvae, but in those given 10,000 larvae it ranged from 1,910 to 3,440 per gram.

Total egg output of Cooperia curticei per day on 18th and 19th and 38th and 39th days of infection in different dose groups is given in Appendices 41 and 42.   Data expressed as eggs laid per female worm per day are shown in Tables 60 and 61.   In group A (10,000 infective larvae) eggs laid per female worm per day on the 18th and 19th day of infection ranged from 414 to 1048, whereas in groups B and C the range was only 94 to 296 (Table 60).   After the 38th and 39th days of infection eggs laid per female worm per day in group A rose to 807 to 1,958 but in groups B and C the rate of egg production had decreased to 8 to 15 in all animals except one in which the egg production per female worm per day ranged from 460 to 678 (Table 61).

The body weight of sheep recorded at 4 day intervals is given in Appendix 43.   Cooperia curticei infection had no significant effect on body weight after 20 and 40 days of infection (Table 62).

The packed cell volume, haemoglobin, total W.B.C. and total eosinophiles are given in Tables 63, 64, 65 and 66 respectively.   It appears that there was no significant change in P.C.V., haemoglobin or total W.B.C.   No significant change was observed in lymphocyte, monocyte, neutrophil, or basophile numbers. Numbers of circulating eosinophiles in samples from infected animals sometimes after 20 days of infection exceeded the mean count + 3 times the standard deviation of controls (511/cu. mm.).   Total serum protein and serum protein fractions at the start and at the end of the experiment are given in Tables 67 and 68 respectively.   Total serum protein was decreased in all animals including controls and there appears to be no significant difference between

Table 59

Faecal egg count in sheep 2 - 3 months old when given
different doses of _Cooperia_ _curticei_ larvae:-

Group No.	A				B			
Sheep No.	115	105	342	107	108	349	118	341
Total larval:- dose	10,000	10,000	10,000	10,000	50,000	50,000	50,000	50,000
Days of infection	**E g g s**		**p e r**	**g r a m**				
12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
13	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
14	Nil	Nil	150	150	50	200	Nil	50
15	150	50	200	150	150	400	Nil	1,700
16	-	-	-	-	-	-	500	-
18*	1,550	3,860	-	400	550	4,090	-	2,200
19*	2,360	4,460	-	-	910	6,150	400	-
22	Killed	Killed	1,250	2,350	Killed	Killed	400	3,200
25			2,050	2,050			500	800
26			-	-			-	1,450
33			1,675	1,900			425	1,575
37			1,950	2,750			250	100
38*			3,010	3,820			240	Nil
39*			1,910	3,440			140	Nil
			Killed	Killed			Killed	Killed

* = mean of five counts.

Table 59   continued

Group No.			C	
Sheep No.	102	111	103	117
Total larval dose	100,000	100,000	100,000	100,000
Days of infection	E g g s	p e r	g r a	m
12	Nil	Nil	Nil	Nil
13	Nil	Nil	Nil	Nil
14	50	250	100	Nil
15	500	1,650	250	Nil
16	-	-	-	50
18*	532	3,760	-	-
19*	4,970	2,030	-	-
22	Killed	Killed	1,900	200
25			1,000	350
26			1,050	-
33			400	2,650
37			150	4,900
38*			120	5,350
39*			100	6,250
			Killed	Killed

* = mean of five counts

Table 60

Data on eggs laid per female <u>Cooperia</u> <u>curticei</u> per
day in sheep after 18 and 19 days of infection

Group No.	Sheep No.	Total larval dose	Pre-patent period Day	Days of infec- tion	Total no. of eggs/ 24 hours	Sex ratio Male	Female	Total no. of worms	Eggs per female worms per day
A	115	10,000	15	18	720,750	1,510	1,740	3,250	414.2
				19	955,800				549.3
	105	10,000	15	18	1,802,620	1,420	1,893	3,313	952.2
				19	1,984,700				1048.4
B	108	50,000	16	18	291,500	2,550	3,090	5,640	94.3
				19	495,950				160.5
	349	50,000	14	18	1,995,920	6,740	8,830	15,570	226.0
				19	2,613,750				296.0
C	102	100,000	14	18	2,394,000	12,299	14,369	26,668	166.6
				19	2,832,900				197.1
	111	100,000	14	18	1,752,160	11,930	12,210	24,140	143.5
				19	1,258,600				103.0

Table 61

Data on eggs laid per female *Cooperia curticei* per
day in sheep after 38 and 39 days of infection

Group No	Sheep No	Total larval dose	Pre-patent period Days	Days infec-tion	Total no. of eggs/ 24 hours	Sex ratio Male	Female	Total no. of worms	Eggs per female per day
A	342	10,000	14	38	1,495,970	960	1,550	2,510	965.1
				39	1,251,050				807.1
	107	10,000	14	38	1,566,200	840	800	1,640	1,957.7
				39	1,462,000				1,827.5
B	118	50,000	16	38	109,200	5,938	7,054	12,992	15.4
				39	60,200				8.5
	341	50,000	14	38	Nil	1,961	1,555	3,516	-
				39	Nil				-
C	103	100,000	14	38	57,000	3,800	4,630	8,430	12.3
				39	43,000				9.3
	117	100,000	16	38	1,952,750	2,810	4,240	7,050	460.5
				39	2,875,000				678.0

Table 62

The effect of <u>Cooperia</u> <u>curticei</u> on weight gains of sheep
2 - 3 months old given different doses of larvae:-

Group No.	Lamb No.	Total larval dose	Days of infection	Wt. at the beginning of exp.(lbs)	Wt. at the end of exp.(lbs)	Total weight gain(lbs)
A	115	10,000	20	38.5	43.5	5.0
	105	"	20	31.5	39.0	7.5
	342	"	40	26.5	37.0	10.5
	107	"	40	32.5	44.5	12.0
B	108	50,000	20	29.5	36.5	7.0
	349	"	20	30.0	36.5	6.5
	118	"	40	35.5	45.0	9.5
	341	"	40	22.5	36.0	13.5
C	102	100,000	20	31.0	37.5	6.5
	111	"	20	27.5	35.5	8.0
	103	"	40	34.5	45.0	10.5
	117	"	40	33.5	44.0	10.5
D	101	Controls	20	32.0	40.0	8.0
	110	"	20	36.0	41.5	5.5
	112	"	40	40.5	52.0	11.5
	106	"	40	29.0	41.5	12.5

Table 63

Packed cell volumes (%) of experimentally
infected and control sheep

Group No.	Sheep No.	Larval dose	Days after infection					
			0	7	14	20	28	35
A	115	10,000	37	35	39	36		
	342	"	39	37	39	41	40	41
	105	"	33	33	34	39		
	107	"	38	38	38	41	41	40.5
B	118	50,000	39	39	40	42	41	40.5
	341	"	35	33	34	35	38	40
	108	"	33	32	31	33		
	349	"	32	31	32	34		
C	103	100,000	37	34	34	35	35	37
	111	"	34	31	31	36		
	102	"	37	35	36	34		
	117	"	28	31	28	30	30	32
D	106	Nil	29	30	28	33	31	32
	110	"	35	35	34	34		
	112	"	37	35	34	40	39	37
	101	"	32	31	32	31		

Table 64

Haemoglobin values (gms / 100 ml) of
experimentally infected and control sheep

Group No.	Sheep No.	Larval dose	0	7	14	20	28	35
A	115	10,000	12.0	10.6	12.0	11.6		
	342	"	12.8	11.0	12.3	13.6	13.2	13.2
	105	"	10.6	10.8	10.9	13.0		
	107	"	12.0	10.6	11.8	13.5	13.0	13.0
B	118	50,000	12.2	12.4	12.0	14.0	13.3	13.0
	341	"	11.4	10.2	10.7	11.7	12.4	12.4
	108	"	10.6	10.8	9.8	10.8		
	340	"	10.0	9.2	10.0	11.0		
C	103	100,000	12.0	9.2	10.6	11.9	12.4	12.7
	111	"	11.5	8.8	9.8	11.7		
	102	"	11.6	10.6	10.5	11.6		
	117	"	9.2	7.9	8.6	10.0	9.5	10.1
D	106	Nil	9.5	9.0	9.2	11.4	11.0	10.6
	110	"	11.3	10.5	10.4	11.0		
	112	"	12.1	10.6	11.0	13.5	13.4	12.4
	101	"	10.5	9.7	10.2	10.2		

Days after infection

Table 65

Total leucocyte counts (x 10^3 per cu mm blood)
of experimentally infected and control sheep

Group No.	Sheep No.	Larval dose	Days after infection 0	7	14	20	28	35
A	115	10,000	7.1	7.2	6.1	6.6		
	342	"	4.5	4.7	4.5	4.9	8.8	5.3
	105	"	7.2	10.0	8.4	7.6		
	107	"	11.1	10.0	6.8	10.5	9.8	9.7
B	118	50,000	8.3	7.5	9.0	12.8	7.6	8.3
	341	"	6.7	8.7	10.0	7.6	8.1	8.5
	108	"	7.5	6.8	6.3	8.7		
	349	"	8.1	8.1	8.3	9.1		
C	103	100,000	5.3	7.1	8.9	9.2	10.0	10.7
	111	"	9.8	8.8	8.7	4.7		
	102	"	6.6	6.5	6.7	6.8		
	117	"	7.6	13.5	10.8	14.8	11.7	12.2
D	106	Nil	6.9	7.8	5.8	5.6	7.7	5.9
	110	"	5.8	6.9	7.3	5.2		
	112	"	7.9	8.2	9.0	8.6	8.3	7.7
	101	"	6.8	5.7	7.3	5.8		

Table 66

Total eosinophil counts (cells / cu mm blood)
of experimentally infected and control sheep

Group No.	Sheep No.	Larval dose	Days after infection					
			0	7	14	20	28	35
A	115	10,00	0	288	366	660		
	342	"	0	94	0	98	0	0
	105	"	0	0	252	152		
	107	"	0	0	0	315	196	97
B	118	50,000	0	300	540	512	304	498
	341	"	67	348	0	152	324	425
	108	"	0	68	0	522		
	349	"	0	243	332	364		
C	103	100,000	53	0	178	276	300	321
	111	"	0	0	435	0		
	102	"	0	0	0	272		
	117	"	76	270	216	296	702	1220
D	106	Nil	69	390	116	112	154	177
	110	"	232	0	292	416		
	112	"	79	0	0	80	176	0
	101	"	0	0	0	0		

Table 67

The effect of Cooperia curticei on total serum protein of
sheep 2 - 3 months old given different doses of larvae:-

Group No.	Sheep No.	Total larval dose single infection	Length of exp. period days	Total serum protein at the start of exp. gm%	Total serum protein at the end of exp. gm%	Difference if any gm%
A	115	10,000	20	6.8	6.4	- 0.4
	105	"	20	6.6	6.4	- 0.2
	342	"	40	6.8	6.3	- 0.5
	107	"	40	6.7	5.6	- 1.1
B	108	50,000	20	7.0	5.9	- 1.1
	349	"	20	6.4	5.6	- 0.8
	118	"	40	6.6	6.0	- 0.6
	341	"	40	7.0	6.3	- 0.7
C	102	100,000	20	7.0	4.3	- 2.7
	111	"	20	6.9	5.6	- 1.3
	103	"	40	6.8	6.8	0
	117	"	40	6.3	6.3	0
D	101	Controls	20	6.8	6.0	- 0.8
	110	"	20	6.8	5.8	- 1.0
	112	"	40	6.7	6.6	- 0.1
	106	"	40	6.3	6.1	- 0.2

Table 68

The effect of Cooperia curticei on serum protein
fractions of sheep 2 - 3 months old
given different doses of larvae:-

Group No.	Sheep No.	Total larval dose	Days of in-fection	Sampling time	Percentage protein fractions				
					Albumin	Alpha 1	Alpha 2	Beta	Gamma
A	115	10,000	20	B.E.	41.09	10.85	18.99	8.53	20.54
				E.E.	48.33	7.43	29.00	3.71	11.53
	105	10,000	20	B.E.	47.16	9.61	27.07	5.24	10.92
				E.E.	43.76	7.81	24.37	7.50	16.56
	342	10,000	40	B.E.	42.16	8.58	24.63	8.58	16.05
				E.E.	52.85	3.25	21.95	2.00	19.95
	107	10,000	40	B.E.	48.55	6.88	19.93	18.84	5.80
				E.E.	50.27	3.78	30.28	3.24	12.43
B	108	50,000	20	B.E.	45.15	5.02	24.45	10.00	15.38
				E.E.	41.69	6.02	29.89	9.80	22.60
	349	50,000	20	B.E.	37.03	13.58	25.62	7.10	16.67
				E.E.	31.50	7.49	41.19	12.55	7.27
	118	50,000	40	B.E.	45.86	5.05	22.02	4.13	22.94
				E.E.	41.86	8.14	23.26	6.40	20.34
	341	50,000	40	B.E.	38.27	8.66	27.08	6.50	19.49
				E.E.	42.70	4.86	35.68	1.60	15.14
C	102	100,000	20	B.E.	40.83	4.58	25.83	11.25	17.50
				E.E.	52.59	5.17	15.52	3.45	23.27

Table 68   continued

Group No.	Sheep No.	Total larval dose	Days of in- fection	Sampling time	Percentage protein fractions				
					Albumin	Alpha 1	Alpha 2	Beta	Gamma
C	111	100,000	20	B.E.	43.49	7.78	21.00	9.03	18.70
				E.E.	51.49	4.48	26.21	2.24	5.67
	103	100,000	40	B.E.	34.19	10.03	38.69	12.30	4.79
				E.E.	38.47	11.92	20.00	17.69	11.92
	117	100,000	40	B.E.	40.81	4.93	26.46	7.62	20.18
				E.E.	45.19	9.21	23.01	4.60	17.99
D	101	Controls	20	B.E.	37.15	8.57	28.83	18.18	7.27
				E.E.	47.90	5.50	32.68	10.68	3.24
	110	"	20	B.E.	43.93	12.14	18.21	10.98	14.74
				E.E.	50.72	9.42	22.46	7.25	10.15
	112	"	40	B.E.	44.41	9.67	21.75	8.46	15.71
				E.E.	51.03	8.16	20.00	5.71	15.10
	106	"	40	B.E.	42.09	8.54	21.20	8.23	19.94
				E.E.	44.49	5.51	24.58	8.05	17.37

B.E.:-   Before the start of experiment

E.E.:-   At end of the experiment

groups.   The percentages of serum protein fractions was again found to vary
between the beginning and end of the experiment but no significant trends could
be detected.

No gross lesions were present on post mortem examination.   Actual
numbers of male and female worms recovered from the small intestine after 20
and 40 days of infection are given in Appendices 44 and 45.   No worms were
recovered from the abomasa.   The distribution of worms along the intestine,
sex ratio and percentage established in the host after 20 and 40 days of .
infection are given in Tables 69 and 70.   The worms were present in all parts
of the small intestine but majority were found in 5 to 10 feet from the pylorus.
The percentage of worms recovered in all experimental animals after 20 days of
infection ranged from 11.2% to 33.5% of the infecting larval dose.   After 40
days of infection the percentage of worms recovered in group A ranged from 16.4
to 25.1 whereas in groups B and C the range was 0.7 to 7.0 with the exception of
one animal (no. 118) in which the percentage was 25.8.   It was also noticed
that besides the low rate of establishment in high dose groups (B and C) many
adult worms were stunted, the females devoid of eggs and the males with colour-
less spicules.   The female worm population was always more than that of males
except in 2 animals (No. 107 and 341) in which the numbers of males was slightly
greater than that of females.   The predominance of female worms occurred at all
levels of the intestine.

Histopathological changes in the small intestine were similar to those
described in the previous section (refer VII, (ii) (f)).

The total protein content of sera and concentrated mucus extracts of
experimental animals necropsied after 40 days of infection are given in Tables
68 and 71 respectively.   The reactions of the 5 antigens derived from first
stage larvae, second stage larvae, third stage ensheathed larvae, exsheathed
larvae and exsheathing fluid against serum and concentrated mucous extracts
were completely negative in gel diffusion tests.

Table 69

Frequency distribution of Cooperia curticei in small
intestine; sex ratio and percentage established in
the host after 20 days of infection

No. of worms recovered

Group No.	Sheep No.	Total larval dose	Days of infection	Abomasum	small intestine 0-5ft	5-10	10-15	15-20	20 & over	Total worms	Percentage sex - ratio Male	Female	Percentage established in the host	Remarks
A	115	10,000	20	0	0	3,050	130	70	0	3,250	46.5%	53.5%	32.5%	Adults
	105	"	20	0	200	2,850	223	40	0	3,313	42.8%	57.2%	33.1%	"
B	108	50,000	20	0	130	2,870	2,140	480	20	5,640	45.2%	54.8%	11.2%	"
·	349	"	20	0	1,940	9,670	3,450	320	190	15,570	43.3%	56.7%	31.0%	"
C	111	100,00	20	0	510	3,640	8,270	10,610	1,110	24,140	46.1%	53.9%	24.1%	Retarded growth
·	102	"	20	0	1,173	10,040	5,365	1,250	8,840	26,668	49.4%	50.6%	26.6%	"
D	101	Control	20	-	-	-	-	-	-	-	-	-	-	-
	110	"	20	-	-	-	-	-	-	-	-	-	-	-

Table 70

Frequency distribution of Cooperia curticei in small
intestine; sex ratio and percentage established in
the host after 40 days of infection

No. of worms recovered

Group No.	Sheep No.	Total larval dose	Days of infection	Aboma-sum	small intestine 0-5ft	5-10	10-15	15-20	20 & over	Total worms	Percentage sex - ratio Male	Female	Percentage established in the host	Remarks
A	342	10,000	40	0	530	1,950	30	0	0	2,510	38.2%	61.8%	25.1%	Adult
	107	"	40	0	310	1,290	40	0	0	1,640	51.2%	48.8%	16.4%	"
B	118	50,000	40	0	80	715	6,237	5,780	180	12,992	45.7%	54.3%	25.8%	Retarded growth
	341	"	40	0	10	1,850	960	66	630	3,516	55.7%	44.3%	7.0%	"
C	103	100,000	40	0	260	6,540	1,410	200	20	8,430	45.0%	55.0%	0.84%	"
	117	"	40	0	60	6,910	30	50	0	7,050	39.9%	60.1%	0.70%	"
D	106	Control	40	-	-	-	-	-	-	-	-	-	-	-
	112	"	40	-	-	-	-	-	-	-	-	-	-	-

197.

Table 71

Total protein estimation in concentrated mucus
extract of sheep given different doses of larvae:-

Group No.	Animal No.	Total larval dose	Days killed	Total protein per 100 ml
A	342	10,000	40	5.8 gms
	107	10,000	40	7.2 gms
B	341	50,000	40	6.2 gms
	118	50,000	40	6.0 gms
C	117	100,000	40	5.2 gms
	103	100,000	40	7.0 gms
D	106	Control	40	5.4 gms
	112	Control	40	6.3 gms

VII. 3.   EFFECT OF DIET ON COOPERIA CURTICEI INFECTION IN SHEEP 9 - 10 MONTHS
          OLD:-

The present experiment was designed to find out whether or not a
lowered plane of nutrition influenced the effect of Cooperia curticei on sheep.

i)   Materials and methods:-   The experiment was carried out with 16 Romney
sheep 9-10 months old.   The design of the experiment and parasitological
techniques were similar to those described at the beginning of this chapter
(refer VII   (i) (a) and (b)).   The animals were arranged in 4 groups according
to the diet provided and number of doses of the infective larvae of Cooperia
curticei.

              Group A = 10,000 infective larvae daily for 5 days,
                          4 animals given 1.5 lb of hay per day.
              Group B = 50,000 infective larvae as single dose,
                          4 animals given 1.5 lb of hay per day.
              Group C = 50,000 infective larvae as single dose,
                          4 animals given 1.5 lb of concentrate feed per day
                          (refer II. 5).
              Group D = Four worm free animals as controls.   Two animals
                          were given 1.5 lb of hay and the other 2 1.5 lb
                          concentrate feed.

The infection was allowed to continue for 35 days and during this time
the course of the infection was followed at regular intervals.   Feed intake was
recorded daily.   The amount of food eaten was measured by subtracting the amount
of residue from the quantity provided.   The animals were weighed and faecal egg
counts, and blood analyses carried out as previously described (refer VII (i)
(c), (d) and (e)).   No electrophoretic or immunological studies were made in
this experiment.

After 35 days of infection animals were killed.   The necropsy
procedure was similar to that already described (refer VII (i) (f)) except
that male and female worms were estimated from the abomasum and the whole of
the small intestine.   Sex ratio and percentage of worms established in the
host were also calculated.

ii)   Results:-   There were no significant clinical signs referable to the Cooperia curticei infection in the sheep given hay or concentrate feed.   The feed intake was not affected in any of the experimental animals throughout the period of infection.

The prepatent period of infection was again 14-16 days.   Peak egg counts were recorded 7 days after the infection became patent (Table 72) and ranged from 50 to 10,050 eggs per gram.   Thereafter egg counts abruptly declined and after 35 days of infection the egg count ranged from zero to 650 eggs per gram only.

The weekly records of body weight of experimental animals are given in Appendix 46.   The effect of Cooperia curticei infection on body weight after 35 days of infection is shown in Table 73 and appeared not significant in different diet groups when compared with controls.

The packed cell volume, haemoglobin, total W.B.C. and total eosinophiles are given in Table 74, 75, 76 and 77 respectively.   It appears that there was no significant change in P.C.V., haemoglobin and total W.B.C.   No significant change was observed in lymphocytes, monocytes, neutrophils, and basophile numbers.   In some animals the numbers of circulating eosinophils were significantly increased when compared with the mean count + 3 x standard deviation of controls (428 per cu. mm).   The effect of Cooperia curticei infection on total serum protein is given in Table 78 and no significant changes were observed.

No gross lesions were present on post mortem examination.   The number of worms recovered from the abomasa and small intestine with sex ratio and percentage established in the host after 35 days of infection are given in Table 79.   No worms were recovered from the abomasa.   The percentage of worms established in hosts given concentrate feed ranged from 0 to 17.4% whereas in experimental animals given hay the range was 0 to 12.5%.   It was also observed that in all groups many adult worms were stunted, the females devoid of eggs and the males with colourless spicules.   The female worm population was always more than that of males.

Table 72

Cooperia curticei egg count in sheep 9 - 10 months old when given different doses of larvae:-

Group No.	Sheep No.	Total larval dose	Feed 1.5 lbs	Days of infection / Eggs per gram.						
				14	15	16	21	28	30	35
		Daily								
A	320	10,000x5	Hay	Nil	100	–	550	2,150	600	50
	346	= 50,000	"	Nil	150	–	3,600	–	700	100
	330	"	"	Nil	50	–	3,150	–	150	Nil
	10	"	"	50	–	–	9,100	500	550	650
B	345	50,000	Hay	Nil	Nil	50	150	50	50	50
	339	(single	"	100	–	–	7,500	–	500	Nil
	344	dose)	"	50	–	–	1,950	–	100	Nil
	347	"	"	250	–	–	10,050	11,000	200	Nil
C	113	50,000	Conc.feed	Nil	100	–	5,250	2,800	3,000	400
	336	(single	"	Nil	150	–	2,250	–	Nil	Nil
	350	dose)	"	150	–	–	150	–	100	100
	328	"	"	Nil	Nil	–	50	50	100	50
D	332	Controls	Hay	Nil	–	–	Nil	–	–	Nil
	348	"	"	Nil	–	–	Nil	–	–	Nil
	120	"	Conc.feed	Nil	–	–	Nil	–	–	Nil
	119	"	"	Nil	–	–	Nil	–	–	Nil

Table 73

The effect of Cooperia curticei on weight changes
of sheep when given different feed.

Group No.	Sheep No.	Total larval dose	Feed 1.5 lbs	Days of infection	Wt. at the beginning of exp. lbs	Wt. at the end of exp. lbs	Total weight gain lbs
		Daily					
A	320	10,000x5	Hay	35	50.0	50.5	0.5
	346	= 50,000	"	"	63.0	63.0	nil
	330	in total	"	"	45.0	46.5	1.5
	10	"	"	"	74.0	75.5	1.5
B	345	50,000	Hay	35	55.0	57.5	2.5
	339	(single	"	"	50.5	50.5	nil
	344	dose)	"	"	44.0	45.0	1.0
	347	"	"	"	65.0	67.5	2.5
C	113	50,000	Feed conc.	35	47.0	50.0	3.0
	336	(single	"	"	65.0	69.0	4.0
	350	dose)	"	"	87.5	87.5	nil
	328	"	"	"	61.5	63.5	2.0
D	332	Controls	Hay	35	60.0	62.0	2.0
	348	"	"	"	54.5	56.0	1.5
	120	"	Feed conc.	"	52.5	58.5	6.0
	119	"	"	"	65.5	72.0	6.5

Table 74

Packed cell volume (%) of experimentally
infected and control sheep

Group No.	Sheep No.	Total larval dose	Feed 1.5 lb	Days after infection		
				0	16	35
		Daily				
A	320	10,000 x 5	Hay	42	37.5	38.0
	346	= 50,000	"	45.5	41.0	39.0
		total				
	330	"	"	44.5	38.5	38.0
	10	"	"	36.5	36.5	38.0
B	345	50,000	Hay	37.5	40.0	35.5
		(single dose)				
	339	"	"	40.0	38.0	37.0
	344	"	"	38.0	35.5	37.5
	347	"	"	42.5	38.5	38.0
C	113	50,000	Conc. feed	38.0	33.0	34.5
		(single dose)				
	336	"	"	42.0	41.0	46.0
	350	"	"	43.5	46.0	44.0
	328	"	"	39.0	39.0	43.5
D	332	Controls	Hay	43.5	39.5	38.0
	348	"	"	45.0	39.0	37.0
	120	"	Conc. feed	38.0	40.0	38.0
	119	"	"	40.0	39.0	38.0

Table 75

Haemoglobin values (gm / 100 ml) of
experimentally infected & control animals

Group No.	Sheep No.	Total larval dose	Feed 1.5 lbs	Days of infection 0	16	35
		Daily				
A	320	10,000 x 5	Hay	12.4	11.6	12.0
	346	= 50,000	"	14.2	13.3	12.7
		in total				
	330	"	"	13.2	12.2	13.0
	10	"	"	11.2	13.2	13.0
B	345	50,000	Hay	11.2	12.7	11.6
		(single dose)				
	339	"	"	13.0	12.2	11.4
	344	"	"	11.2	12.4	11.9
	347	"	"	13.2	14.0	12.0
C	113	50,000	Conc. feed	11.2	10.9	11.2
		(single dose)				
	336	"	"	13.8	14.8	13.7
	350	"	"	14.7	15.9	15.0
	328	"	"	11.8	13.3	13.0
D	332	Controls	Hay	13.7	13.0	11.5
	348	"	"	12.3	12.8	12.0
	120	"	Conc. feed	12.0	13.4	12.7
	119	"	"	12.3	13.0	12.0

Table 76

Total leucocyte counts (x 10^{-3} per cubic m.m. blood)
of experimentall infected & control sheep

Group No.	Sheep No.	Total larval dose	Feed 1.5 lbs	Days of infection		
				0	16	35
		Daily				
A	320	10,000 x 5	Hay	6.8	7.4	6.7
	346	= 50,000	"	8.7	6.5	6.3
	330	in total	"	7.2	6.0	7.5
	10	"	"	6.8	9.1	7.0
B	345	50,000	Hay	6.0	6.3	8.5
	339	(single dose)	"	8.6	7.1	8.0
	344	"	"	7.2	6.2	6.3
	347	"	"	7.3	5.1	7.8
C	113	50,000	Conc. feed	6.3	5.5	6.5
	336	(single dose)	"	6.2	7.2	7.6
	350	"	"	4.6	5.9	6.4
	328	"	"	6.2	6.9	6.1
D	332	Controls	Hay	5.9	6.0	5.7
	348	"	"	6.0	6.5	7.4
	120	"	Conc. feed	5.0	5.6	6.7
	119	"	"	5.3	6.7	5.7

Table 77

Total eosinophil counts (cells / cubic m.m. blood)
of experimentally infected & control sheep

Group No.	Sheep No.	Total larval dose	Feed 1.5 lbs	Days of infection 0	16	35
		Daily				
A	320	10,000 x 5	Hay	204	0	67
	346	= 50,000	"	174	65	0
	330	in total	"	72	80	525
	10	"	"	136	180	140
B	345	50,000	Hay	240	126	510
	339	(single dose)	"	258	71	640
	344	"	"	144	183	378
	347	"	"	73	50	624
C	113	50,000	Conc. feed	0	55	520
	336	(single dose)	"	62	144	380
	350	"	"	138	360	576
	328	"	"	186	690	366
D	332	Controls	Hay	177	120	114
	348	"	"	240	325	294
	120	"	Conc. feed	150	168	134
	119	"	"	106	134	0

Table 78

The effect of Cooperia curticei on total serum protein of sheep 9-10 months old given 50,000 larvae with different feeds

Group No.	Sheep No.	Total larval dose	Feed 1.5 lb	Day of infection	Total serum protein at the start of exp. gm%	Total serum protein at the end of exp. gm%	Difference if any gm%
		Daily					
A	320	10,000x5	Hay	35	6.2	6.2	−
	346	= 50,000	"	"	6.0	6.0	−
	330	in total	"	"	6.2	6.2	−
	10	"	"	"	5.6	5.6	−
B	345	50,000	Hay	35	5.6	5.8	+ 0.2
	339	(single	"	"	5.8	5.8	−
	344	dose)	"	"	5.6	5.8	+ 0.2
	347	"	"	"	6.3	6.4	+ 0.1
C	113	50,000	Conc. feed	35	7.0	7.0	−
	336	(single	"	"	5.8	5.8	−
	350	dose)	"	"	5.7	5.8	+ 0.1
	328	"	"	"	5.5	5.8	+ 0.3
D	332	Controls	Hay	35	6.0	6.0	−
	348	"	"	"	6.0	6.0	−
	120	"	Conc. feed	"	5.1	5.2	+ 0.1
	119	"	"	"	5.5	5.6	+ 0.1

Table 79

Sex ratio and percentage of Cooperia curticei
established in the host after 35 days of infection

Group No.	Sheep No.	Total larval dose	Feed 1,5 lb	Aboma-sum	Small intestine Male	Female	Total	Sex ratio Male	Female	P.c. estab-lished in the host
		Daily								
A	320	10,000x5	Hay	0	20	20	40	50%	50%	0.008%
	346	= 50,000	"	0	0	0	Nil	-	-	-
	330	in total	"	0	0	40	40	-	100%	0.008%
	10	"	"	0	2,780	3,490	6,270	44.3%	55.7%	12.5%
B	345	50,000	Hay	0	1,310	2,410	3,720	35.2%	64.8%	7.4%
	339	(single	"	0	1,540	2,770	4,310	36.0%	64.0%	8.6%
	344	dose)	"	0	60	70	130	46.1%	53.9%	0.3%
	347	"	"	0	100	170	270	37.0%	63.0%	0.5%
C	113	50,000	Conc.feed	0	3,900	4,800	8,700	44.8%	55.2%	17.4%
	336	(single	"	0	3,680	4,750	8,430	43.6%	56.4%	16.9%
	350	dose)	"	0	3,190	4,380	7,570	42.1%	57.9%	15.1%
	328	"	"	0	0	80	80	-	100%	0.016%
D	332	Controls	Hay	-	-	-	-	-	-	-
	348	"	"	-	-	-	-	-	-	-
	120	"	Conc.feed	-	-	-	-	-	-	-
	119	"	"	-	-	-	-	-	-	-

VIII. 4. SUMMARY OF THE EXPERIMENTAL FINDINGS:-

The effect of Cooperia curticei infection was investigated in sheep at different ages, on different diets, and with various sizes of infection.

1.   The prepatent period of infection was found to be 14-16 days in all age groups.

2.   Peak egg counts were recorded 5 - 7 days after the infection became patent.   Thereafter egg counts gradually declined in 10,000 dose groups, but in 50,000 to 150,000 dose groups showed an abrupt decline and after 40 to 55 days of infection the egg counts of experimental animals ranged from zero to 150 eggs per gram only.

3.   Total egg output of Cooperia curticei per female per day on the 18th and 19th day of infection in sheep 2 - 3 months old receiving 10,000 larvae, ranged from 414 to 1048 whereas the range was 94 to 296 in higher dose groups (50,000 to 150,000).   After 38 and 39 days of infection eggs laid per female worm per day were 807 to 1,958 in the 10,000 larval dose group but in all animals except one, with higher larval doses the rate of egg production was o to 15 only.

4.   No clinical signs referable to Cooperia curticei infection were observed when sheep 2-3, 6-7 and 9-10 months old were given 10,000 to 150,000 infective larvae, which resulted in the establishment of worm numbers ranging up to approximately 26,000.   Feed intake was not affected and repeated doses and low plane of nutrition produced no ill effect.

5.   Cooperia curticei infections had no significant effect on body weight or wool growth of sheep regardless of age, diet and size of infection.

6.   Blood analysis of experimental animals showed no significant change in packed cell volume, haemoglobin and total leucocyte counts regardless of age, diet and size of infection.   No significant change was observed in lymphocyte, monocyte, neutrophiles, and basophile numbers but after 14 - 21 days of infection a few experimental animals showed increased number of circulating eosinophiles when compared with controls.   The relative percentage of serum protein fractions showed irregular fluctuations and no significant changes or trends were detected.   Differences in the total serum protein before infection

and at necropsy were also not significant.

7.   Experimental animals showed no gross lesions on post mortem when given different doses of <u>Cooperia</u> <u>curticei</u> larvae (10,000 to 150,000) regardless of age, diet and number of worms recovered.

8.   Histopathological examination of sequential sections of small intestine, abomasum and lymph nodes showed no evidence of significant change regardless of age, diet, size of infection or number of worms recovered from the experimental animals.

9.   Adult worms were present in all parts of the small intestine but the majority were recovered from 5 - 10 feet from the pylorus. No worms were recovered from abomasa.

10.   Maximum recovery of <u>Cooperia</u> <u>curticei</u> from experimental sheep 6 - 7 months old, after 34 days of infection was 76 to 79% at a 10,000 larval dose whereas at higher doses (50,000 to 100,000) it ranged from 0.3 to 29.0%. After 56 days of infection in all animals with doses of 50,000 to 150,000 infective larvae the percentage recovery ranged from 0.1 to 4.8% only.

In sheep 2 - 3 months old the percentage recovery of <u>Cooperia</u> <u>curticei</u> in all experimental animals after 20 days of infection ranged from 11.2 to 33.5%. After 40 days of infection, the rate of recovery in the low dose group (10,000) was 16.4 to 25.1%, whereas in the high dose groups (50,000 and 100,000) it ranged from 0.7 to 7.0% (except in one animal).

The percentage of worms recovered from sheep 9 - 10 months old after 35 days of infection ranged from 0 to 17.4% only in spite of a poor plane of nutrition.

11.   Irrespective of age and diet, higher doses of larvae (50,000 or 100,000) resulted in inhibited and retarded growth of adult worms. Female worms were devoid of eggs and males had colourless spicules. After a second dose of 50,000 larvae, varying numbers of larvae were still in the fourth stage 22 days later.

12.   The female worm population was always more than that of males except in a few animals in which the number of males were slightly greater than that

of females.   The predominance of female worms occurred at all levels of the small intestine.

13.   The gel-diffusion reactions of five antigens prepared from first stage, second stage, ensheathed third stage, exsheathed third stage larvae and exsheathing fluid gave positive reactions with sera from sheep 6 - 7 months old (10,000 to 150,000 larval dose), bled after 34 and 56 days of infection.   One to 3 precipitin lines were present against all 5 antigens except with the serum of 2 animals (10,000 larval dose) which showed no reaction with first stage larval antigen.

Reactions in agar with intestinal mucus samples obtained after 34 days of infection were negative against all antigens except exsheathing fluid.   But mucus samples obtained after 56 days of infection showed positive reactions; one precipitin line being present between the mucus extracts and all 5 antigens.

Forty days after infection, serum or mucus extract from sheep 2 - 3 months old failed to give any reaction in gel diffusion tests with the 5 antigens.

VII. 5. DISCUSSION:-

Host resistance to nematodes may be reflected by reduced egg production inhibition of larval development, retarded development of the mature worm and the number of worms established in the host (Stewart 1950a, b, c, d, 1953; Taylor and Michel, 1953; Roberts, 1957; Gordon, 1957; Silverman and Patterson, 1960; Anderson et al., 1965).

In the present study the prepatent period of infection was 14 to 16 days and is in general agreement with the work of Andrews (1939) and Sommerville (1960). Peak egg counts were observed in all animals 5 to 7 days post infection and they then invariably declined. In 10,000 larval dose groups, the decline was gradual but in 50,000 to 150,000 dose groups the decline was more abrupt and after 40 to 55 days the egg counts ranged from zero to 150 eggs per gram only (Table 47, 59). This suggests that the higher doses of Cooperia curticei larvae produced greater resistance in animals than single doses of 10,000 larvae.

The differences recorded in the pattern of egg output between different dose groups is paralleled by differences in the number and condition of worms recovered. Without serial killing of large numbers of animals during the course of infection it is not possible to recognise changes in worm populations in animals and in the present experiment it is only possible to compare the numbers of worm recovered at varying times after patency of the infection had been reached.

In 6 - 7 month old sheep killed 34 days after infection a much higher percentage of the infecting dose was recovered in animals given 10,000 larvae than in those given larger doses (Table 56). At the same time the worms from sheep in the larger dose groups showed evidence of stunting and retardation of sexual development. A second dose of 50,000 larvae in six sheep resulted in a variable but low recovery of worms 22 days later and varying numbers of larvae were still in the fourth stage (Table 57).

In the 2 - 3 month old sheep killed only 20 days post-infection, there were no marked differences in percentage of larvae recovered as worms (Table 69) but 40 days post infection the animals which had received 100,000 larvae had a much reduced worm burden (Table 70) and in both the higher dose groups the worms were retarded.

In the oldest group of sheep (9 - 10 months) all were given 50,000 infective larvae. Contrary to what might have been expected, the worm recovery 35 days post infection was lower in animals fed on hay than those on concentrates. In three of the four animals given daily doses of 10,000 larvae and fed on hay the recovery was very low (Table 79).

A decreased percentage recovery and interference with worm development after increasing doses of nematode larvae has been commonly reported (Andrews 1937, 1939; Bailey, 1949; Stewart, 1954; Sommerville, 1960; Herlich, 1958, 1960, 1965c, 1967) and is considered to result from an immune reaction on the part of the host. From the present results it appears that in the sheep more than 6 months old doses of 50,000 larvae and more, rapidly resulted in the establishment of resistance which failed to allow normal development of the worm population.

It has been suggested that sheep under the age of 4 - 5 months are incapable of making an immune response to nematodes (Soulsby, 1963). Although the 2 - 3 months old sheep failed to develop precipitating antibodies to Cooperia curticei, they undoubtedly showed sign of the development of resistance to the nematode. There is a suggestion in the results however, that they required exposure to a higher level of infection than did older animals.

It was anticipated that the animals fed on a limited amount of hay would be more susceptible to infection than those on the concentrate feed. The results were contrary to expectations in terms of both worms recovered and clinical effects. The result is difficult to explain although other workers have found a poorer establishment of nematode populations in some instances where the hosts were on a deficient diet, (Vegors et at, 1955, 1956).

There is no satisfactory answer as to how the resistance mechanism inhibits the growth and development of nematodes (Soulsby, 1965). The precise role of circulating antibodies in serum and antibodies in mucus from the alimentary tract, in resistance to helminth parasites has been studied by many investigators (Stewart and Gordon, 1953 1958; Soulsby and Stewart, 1960; Soulsby, 1956, 1957, 1960; Rice et al, 1966; Dobson, 1966 a, b, c, d, 1967b, d,). Most have utilised antigens from ensheathed third stage larvae, exsheathed third stage larvae and exsheathing fluid. In the present study, besides the above mentioned antigens, first and second larval stage antigens were also used.

Sera obtained after 56 days of infection from sheep 6 - 7 months old showed positive reactions against all antigens on gel diffusion regardless of size of infection (total of 60,000 to 150,000 larvae); one to 3 precipitin lines were present between sera and 5 antigens (figure 20). Some precipitin lines were stronger than others but there appeared to be no consistent pattern. In all animals only one precipitin line formed against exsheathing fluid. Mucus samples obtained after 56 days of infection from the same experimental animals also showed one strong precipitin line between mucus extract and all 5 antigens (figure 21).

This suggests that antibodies against helminth parasites can be formed locally in the alimentary tract. The mucus of the gut may be immunologically more potent than serum (Dobson, 1967b, d) but the present experiment showed that circulating antibodies of serum were apparently directed at a wider range of antigens than were the antibodies in mucus.

Serum samples from animals necropsied after 34 days of infection after single doses of 10,000 to 100,000 larvae, mostly showed 1 - 2 precipitin lines against all antigens. The precipitin lines were stronger in higher dose groups (50,000 and 100,000) than in the 10,000 larval dose group (figure 18). Mucus samples obtained after 34 days of infection from the same animals gave negative reactions with all antigens except exsheathing fluid which gave one line of precipitation in animals which had a 50,000 or 100,000 larval dose (figure 19). This suggest that antibodies are detectable on gel agar diffusion, earlier from serum samples than from mucus samples from the small intestine.

In young sheep 2 - 3 months old, the reaction of the 5 antigens against serum or mucus samples obtained after 40 days of infection (10,000 to 100,000 larval dose) were completely negative, in spite of the fact that the animals with higher larval doses (50,000 and 100,000) showed retarded growth of the adult worms at necropsy. This suggest that the precipitating antibodies in serum or mucus may not be connected to actual resistance and is in contrast to the work of Manton et al. (1962) and Soulsby (1963, 1966) that animals must reach an age of 4 - 5 months before they are able to show resistance. It also suggests that certain antibodies may take a longer time to be synthesized in an adequate manner in young lambs and such a situation would provide an explanation for the inability of lambs to show an immune reaction detectable by serology until they are 4 - 5 months old.

The antibody response is complex not only in the range of antibodies produced but also in their relative amount and relative importance from a protective point of view, and there is no direct evidence that the reactions on gel diffusion in the present experiment were responsible for the resistance of the host to the parasite.

Whitlock (1966) stated that intestinal nematodes take up their position along the intestine in response to stimuli supplied by the intestinal contents particularly in the duodenum at the point of entrance of the bile and pancreatic juice. This suggests that intestinal parasites have a skewed distribution in the intestine. Present observations confirm this.

In the present study Cooperia curticei was present in all parts of the small intestine in higher dose groups (50,000 to 100,000) and 0 - 20 feet from the pylorus in low dose groups (10,000) but the majority of the worms were recovered from 5 - 10 feet from the pylorus in all animals regardless of age, diet or level of infection. Caudal to this region the numbers of worms progressively declined. Worm numbers increased sharply between the first and second 5 feet sections of intestine and the overall distribution appears skewed, with the tail of the distribution directed caudally. In some animals killed after receiving 50,000 or 100,000 larvae larger numbers of worms were recovered from the last section of the intestine than from sections immediately cranial to it. This could mean that those worms in the caudal section were being eliminated.

Davey (1938) observed the peak occurrence of Cooperia curticei between 9 and 22 feet from the pylorus and Tetley (1935) observed the peak occurrence in the "lower part" of the small intestine. But their observations were based on mixed infestations under field conditions.

In most cases, in the present experiment, the female worm population was greater than that of males and this predominance occurred at all levels of the small intestine.

Precise comparison of various workers data on egg production of different species of nematodes is difficult. For instance, varying techniques have been used to estimate egg numbers, varying doses of infective larvae have been administered to hosts of varying age, species or breed and history. However, it is possible to make semi-quantitative comparisons between nematode

species, (Crofton 1963).

In the present experiments it was notable that the egg output per worm decreased with increasing size of the nematode population and that this difference became more marked with the passage of time.   Thus, in lambs 2 - 3 months old with populations of female worms ranging from 3,250 to 3,313, 20 days after infestation, the daily egg output per female estimated for the previous two days ranged from 414 to 1048.   In lambs of similar age with female worm counts ranging from 5,640 to 26,668, the output per worm was estimated as 94 to 296 eggs per day (Table 60).   Forty days after infection when the number of female worms in the 10,000 larval dose group ranged from 800 to 1,550 the output on the 38th and 39th days was estimated as 807 to 1,958 eggs per day.   In lambs given 50 to 100 thousand larvae the output ranged from 0 to 678 eggs per day, when the numbers of female worms ranged from 1,555 to 7,054 (Table 61).   Clearly some care is needed in the interpretation of these findings.

It has generally been agreed that species of Cooperia are comparatively poor producers of eggs (Peter et al, 1941; Kates, 1947; Crofton, 1963; Nickel, 1965).   From the observations recorded here it appears that egg production of Cooperia curticei can be as high as 2000 eggs per female per day i.e. about 1/5th the fecundity of H.contortus (Crofton 1963).   This conclusion is in general agreement with that of other workers (Kates, 1947; Nickel, 1965).

In the present study on Cooperia curticei, the establishment of worm numbers ranging up to approximately 26,000 with a maximum faecal output of 10,050 e.p.g., produced no ill effect.   No clinical sign of infection was observed in experimental animals regardless of age, diet, size of infecting dose and number of worms present in the small intestine.   Feed intake was normal throughout the period of infection and body weight, wool growth and blood analysis showed no significant change apart from a mild eosinophilia.   At necropsy no gross lesion was observed and histopathological study showed no significant changes.

These observations are in close agreement with the experimental work of Andrews (1938) on 4 pairs of lambs when the range of infestation was 458 to 25,033 worms per animal and also with the work of Taylor (1935), Ross and Gordon (1936), Tetley (1949) and Gordon (1950) under field conditions.   It appears that Cooperia curticei is an instance of a nematode species very well adapted to the sheep.

General Discussion

&

Summary

CHAPTER VIII   GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

VIII I   GENERAL DISCUSSION

The interactions of living organisms and their environment
are very complicated. With free living microorganisms, such as larval
nematodes, "meteorological" environmental effects are the most important.
The main components of these meteorological effects are temperature,
relative humidity, insolation, rainfall and evaporation. These compon-
ents are interrelated and the relative importance of each component
to the organism can only be evaluated by experiments under controlled
conditions. However, to completely understand the epidemiology and
population dynamics of nematodes, studies under natural conditions are
also necessary. Field conditions provide a natural environment but
there is no control over the conditions under which observations are
made. Therefore, experiments under simulated field conditions are im-
portant as they allow some control. Laboratory, plot and field obser-
vations are all necessary and complementary for the proper understanding
of the parasite and no one approach can be substituted for any of the
others.

In ecological studies on nematode larvae many different methods
have been adopted. For the proper interpretation and comparison of
data from different sources the use of standardised methods would be
preferable.

It is surprising that no complete study on a single isolate of
a species of strongylate nematode has been carried out employing all
the three methods mentioned above. Although the present work is by no
means complete, many aspects of the ecology of Cooperia curticei have
been studied. And it has been shown that the influence of different
climatic conditions on larval ecology can be expressed and analysed
mathematically. With a few notable exceptions, such as Crofton and
Whitlock (e.g. Crofton and Whitlock, 1965), workers have failed to
make full use of mathematical concepts for the expression of their
data. This is important for the objective comparison of data from
different workers on different strains and species of nematode and for
the comparison of laboratory and field observations.

Of all the meteorological effects temperature is the most important for larval development and under laboratory conditions it is the most readily controlled. Therefore, the present experiments and those of other workers have concentrated most on temperature. The laboratory studies on Cooperia curticei provided highly repeatable results and the relationship to development rate was readily converted to a linear regression. The observations made under simulated field conditions were likewise convertible to a linear regression. In this condition the two sets of data could be easily and quantitatively compared. It is difficult to see how one might quantitatively compare laboratory and field data or extrapolate from one to the other without the use of such a mathematical analysis. Although observations made under "field" conditions may easily be expressed mathematically, the interpretation of meteorological effects under these conditions requires data from laboratory experiments.

The examination of other worker's data on development of strongylate nematodes has also shown an exponential relationship between the rate of development and temperature. As has been pointed out earlier, this form of relationship is what might be expected within the range of temperatures that permits normal larval metabolism. Because of this exponential relationship, it seems probable that with other nematode species the best correlation may also be found between laboratory and field developmental rates when mean maximum air temperature is considered rather than the mean air temperature.

The constant proportion of the development time occupied by each larval stage at different temperature, as demonstrated in the present study, further simplifies the exponential relationship between temperature and development.

The effect of desiccation (i.e. low humidity) on larval development and survival needs careful examination. It is obvious that low humidity adversely affects survival though there is little quantitative information concerning the effects of humidity on nematode larval stages. In the year in which the present experiments were carried out, the daily mean atmospheric relative humidity was usually between 60 and 90% and one would expect that the humidity in soil or on herbage would be higher than that of the atmosphere. Within the

range of climatic conditions occurring during the course of the experimental studies it was found that the relative humidity had no great effect on the maximum survival of larvae. It would be interesting to know if the same result would be obtained in areas, such as parts of Australia, where much lower relative humidities are experienced.

Most studies on larval survival have been concerned with maximum survival times; however, these are largely of academic interest. From the epidemiological point of view, the number of larvae surviving with time is much more significant. In the present experiment the percentage survival appeared to be affected by temperature and rainfall i.e. water balance. This suggests that desiccation may be important in determining the percentage of larvae surviving.

Studies on percentage survival are rendered difficult by the differential ability of various developmental stages to survive under natural conditions. It appears that pre-infective stages are more susceptible to desiccation than infective larvae. It is, therefore, necessary to distinguish between the ability of eggs to reach the infective stage and the ability of the larvae to survive thereafter. If larval survival under field conditions is being studied by the deposition of eggs on pasture, the weather in the first few days of development may have a disproportionate effect on the larval survival. Therefore, to determine the factors affecting the survival of the infective larvae under field conditions, known numbers of infective larvae need to be deposited on the pasture plots. In this way a quantitative estimation of the larval survival can be obtained. Studies of this kind were commenced but time did not permit their completion. In such an experiment it would again seem advisible to compare larval survival in water in an attempt to distinguish the role of desiccation,

Under natural conditions larval ecology is further complicated by the migration of larvae onto herbage into areas where the microclimate fluctuates more rapidly than it does at soil level. Numbers of factors (e.g. negative geotropism, phototropism) affecting vertical migration have been described, but much of the evidence of their importance is circumstantial. There is very little quantitative work under controlled conditions other than that of Crofton (1954b).

Water is very necessary for vertical migration as it provides moisture films for movements of the infective larvae. The observed association between precipitation and the appearance of the larvae on the grass is, therefore, not surprising. The exponential relationship between numbers of larvae surviving and larval populations on herbage supports the theory that the random movements of the larvae are largely responsible for their appearance on the herbage. It also indicates that larvae follow a one way traffic upwards onto the grass blades presumably because the continuity of water films on herbage is temporary. Hence larvae are left on the grass blades when the water film becomes discontinuous due to desiccation and evaporation. With the sophisticated equipment now available for climate control on a laboratory scale it should be possible to examine the factors affecting larval migration more critically.

The epidemiology of strongylate nematode infections does not depend only on the ecology of the larval stages on pasture. The host plays an important part in controlling worm populations particularly through its ability to acquire resistance to infection.

In the present experiments sheep given more than 50,000 infective larvae of Cooperia curticei showed signs of development of resistance. But how the larval doses given relate to the size of infection acquired under field conditions is not clear. Although C. curticei has a short generation time and can theoretically complete 9-11 generations in a year, the female worm has a comparatively low fecundity. This means that the build-up of larval infection is comparatively slow throughout the year and peak numbers of larvae are available on pasture in autumn, (Tetley, 1949; Brunsdon, 1963b). Present experiments clearly show that infective larvae are available all the year round. However field observations (Tetley, 1949) suggest that the worm population in the sheep reaches a maximum in the second year of life, after the occurrence of peak numbers of larvae on pasture. This suggests that Cooperia curticei is a comparatively poor stimulator of an immune response and long exposures to infection with comparatively large numbers of larvae are required to establish a high level of resistance. This is compensated for by the extraordinarily low pathogenicity of Cooperia curticei for sheep.

If this may be taken to indicate a well balanced host-parasite relationship, the question still arises as to why comparatively large numbers of Cooperia curticei are found in New Zealand sheep. The New Zealand sheep flock is mainly of one breed i.e. Romney, and it might, therefore, be suspected that this is a breed characteristic. However, with the variation of genotype occurring within the breed, this seems rather unlikely. More probably the explanation lies in the adaptation of Cooperia curticei to local climatic conditions.

VIII 2 <u>GENERAL SUMMARY AND CONCLUSIONS</u>

1. Studies on the development and survival of the free living stages
    of <u>Cooperia</u> <u>curticei</u> were carried out both under controlled
    temperatures and under natural conditions.

2. Hatching did not occur below $10^{o}C$ ($50^{o}F$). Throughout the
    temperature range of 10 to $37^{o}C$ each larval stage occupied the
    same proportion of the total development time taken to reach the
    third stage. The relationship between the rate of development in
    log days and temperature was found to be linear. For each degree
    centigrade change of temperature, the rate of development ($>$ 90%)
    of the infective larvae was altered by 0.033 log units of time.

3. Under natural conditions the rate of development to the infective
    stage was most strongly correlated with mean maximum air tem-
    perature over the period of development. Infective larvae did
    not develop in winter when the mean air temperature was below
    $50^{o}F$ ($10^{o}C$) and mean maximum air temperature was between $53^{o}F$
    ($11.7^{o}C$) and $56^{o}F$ ($13.3^{o}C$). Development occurred throughout
    the rest of the year. The relationship of the development time
    in log days and the mean maximum air temperature was linear.
    The slope of the regression line showed that for each degree
    centigrade change in mean maximum air temperature the rate of
    development was changed by 0.049 log units of time.
    This relationship of development to mean maximum air temperature
    was not significantly different to that observed under controlled
    temperature conditions.

4. The percentage of eggs which completed development to the in-
    fective larvae varied with temperature. In 'normal' cultures at
    constant temperatures of 10, 27 and $37^{o}C$, the mean percentage
    recovery of the infective larvae was 44.22, 92.16 and 7.14%
    respectively. In cultures of intact faecal pellets the mean
    percentage recovery of the infective larvae was 35.24%. Under
    natural conditions, the percentage of eggs developing to in-
    fective larvae ranged from 0.23 to 36.2% and was influenced by
    weather conditions, particularly rainfall.

5. Submergence of eggs and first and second stage larvae in
    water inhibited their further development. Survival was
    better at lower than at higher temperatures. Thus eggs stored
    in water survived for up to 65 days at $5^{o}C$ and up to 25 days
    at $27^{o}C$. Eggs in faecal pellets survived up to 50 days at $5^{o}C$.
    In the temperature range $5^{o}C$ to $45^{o}C$ first stage larvae survived
    longest at 5 to $10^{o}C$ (21-23 days) and second stage larvae sur-
    vived longest at 8 to $15^{o}C$ (21 - 35 days).

6. The survival of the infective larvae was examined at temperatures
    ranging from $-6^{o}C$ to $52^{o}C$. The maximum survival of 312 days was
    obtained at $10^{o}C$.

7. Techniques are described for the recovery of <u>Cooperia</u> <u>curticei</u>
    larvae from sample units of pasture, soil and faecal pellets
    and an overall recovery rate of 68.25% was obtained from all
    samples.

8. Under natural conditions the survival of infective larvae on her-
    bage, in soil and faecal pellets was influenced by meteorologi-
    cal effects. Maximum survival times were found to be predomin-
    antly influenced by temperature and the correlation of maximum
    survival and mean maximum air temperatures for the survival
    period was highly significant. The maximum periods of survival
    ranged from 9 to 26 weeks throughout the year. Infective larvae
    survived through the winter.

9. The maximum number of infective larvae surviving on herbage,
    soil and faecal pellets was observed for 1 to 4 weeks after
    development and subsequent recoveries showed a regular decline.

10. There was a highly significant correlation between the percen-
    tage of larvae surviving and the percentage recovered from the
    herbage. The percentage migrating onto the herbage increased
    exponentially as the percentage survival increased.

11. The migration of larvae onto the herbage was found to be
    primarily affected by rainfall and evaporation.

12. Studies on exsheathment of the infective larvae of <u>Cooperia</u> <u>curticei</u> were carried out both <u>in vitro</u> and <u>in vivo</u>. <u>In vitro</u> the maximum exsheathment of up to 97% was observed between pH 7.0 and 8.0 in normal saline solution and sodium carbonate-bi-carbonate-buffers saturated with gaseous carbon dioxide. A negligible proportion of larvae exsheathed in acid media (pH 2.0 to 5.8) saturated with CO_2. <u>In vivo</u> 97% of the infective larvae exsheathed in the rumen in 3-4 hours when the pH of the rumen fluid was 7.2.

13. The process of exsheathment of the larvae of <u>Cooperia</u> <u>curticei</u> was similar to that of <u>H.contortus</u>.

14. The effect of <u>Cooperia</u> <u>curticei</u> infection in sheep was investigated using animals of different ages, on different diets and with various sizes of infection. The prepatent period of infection was 14-16 days in all age groups. Peak egg counts were recorded 5 - 7 days after infections became patent. Thereafter egg counts gradually declined in low (10,000) dose groups but in high dose groups (50,000 to 150,000) the decline was more abrupt.

15. Daily egg outputs per female worm were estimated and found to range up to 1,958 eggs per day.

16. No clinical sign of infection was recorded in sheep when given 10,000 to 150,000 infective larvae regardless of host age, or number of worms recovered. Neither repeated doses of larvae nor a low plane of nutrition influenced the clinical picture. Body weight, wool growth and blood analysis showed no significant changes and no gross lesions or significant histopathological changes were observed.

17. Adult worms were present in all parts of the small intestine but the majority were recovered from 5 - 10 feet from the pylorus. A predominance of female worms was observed at all levels of the small intestine.

18.   Maximum percentage recovery of the Cooperia curticei was
      observed in low dose groups (10,000). Experimental animals
      with higher doses of larvae (50,000 to 150,000) besides lower
      rates of recovery also showed inhibited and retarded growth of
      the worms.

19.   Five antigens were prepared from first stage, second stage,
      ensheathed third stage, exsheathed third stage larvae and ex-
      sheathing fluid. Gel diffusion reactions were observed between
      antigens and serum or intestinal mucus samples from infected
      sheep. The reactions were positive from sheep 6 - 7 months old
      but serum and mucus samples from experimental animals 2 - 3
      months old showed completely negative results. In spite of the
      lack of precipitating antibodies in these sheep, those given
      50,000 to 100,000 infective larvae showed retarded growth of
      adult worms.

20.   The results of experiments on the biology of the free living
      stages of C.curticei are discussed. It is stressed that more
      quantitative information is needed for further understanding of
      the ecology of nematode larvae. It is also very desirable that
      results of such studies be expressed in mathematical terms to
      allow objective comparison of data from different sources, and
      extrapolation from laboratory observations to field conditions.
      The results are discussed in relation to the epidemiology of
      infection of Cooperia curticei. It is concluded that the
      parasite is available to the grazing sheep throughout the year
      under local environmental conditions.

21.   The observation that exsheathment of the infective larvae occurs
      in the rumen suggests that in its evolutionary history Cooperia
      curticei was initially adapted to the abomasum and later became
      secondarily adapted to the small intestine.

22.   The low pathogenicity of Cooperia curticei suggests that this
      nematode species is very well adapted to sheep. The large numbers
      of C.curticei which are found in New Zealand sheep could be a
      characteristic of the Romney breed. However, it seems more

likely to be due to the suitability of the climate for _Cooperia curticei_ or, more correctly, the adaptation of this nematode species to local climatic conditions.

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A P P E N D I C E S

Appendix 1

X Temperature °C	Time (days) of development of infective larvae (T90)	Y Log time
10	40	1.6021
15	23	1.3617
20	13	1.1390
27	8	0.9031
32	6	0.7782
37	5	0.6990

$b = -.0\ 33$

Equation of the regression is

$Y = 1.785 - .033X$

The slope indicates that the log rate
of development of infective larvae (T90)
per degree centigrade is 0.033 log units of
time.

Appendix 2

Analysis of variance on the rate of
development of the larval stages
* (T90) of _Cooperia_ _curticei_ at
different temperatures.

Source of variation	Degree of freedom	Sum Squares	Mean Squares	F
Development between temperatures	5	0.026	0.005	0.0009 n.s.
Errors (within stages of development) L_1, L_2, L_3	12	610.474	50.87	
Total	17	611.500		

* After arcsin transformation

n.s. Not significant

Appendix 3

Analysis of variance on percentage
* recovery data in Table 14
(one gram of herbage)

Source of variation	Degree of freedom	Sums of Squares	Mean Squares	F
Between groups	3	21.09	7.03	1.15 n.s.
Replications (Errors)	16	97.62	6.10	
Total	19	218.71		

*   Arcsin transformation

n.s.   not significant

Appendix 4

Analysis of variance on percentage
* recovery data in Table 5
(5 gram of herbage)

Source of variation	Degree of freedom	Sums of Squares	Mean Squares	F
Between groups	3	15.94	5.31	2.39 n.s.
Replications (Errors)	16	35.62	2.22	
Total	19	151.56		

*   Arcsin transformation

n.s.   not significant

Appendix 5

Analysis of variance on percentage
* recovery data in Table 16
(soil surface)

Source of variation	Degree of freedom	Sums of Squares	Mean Squares	F
Between groups	3	49.40	16.46	1.60 n.s.
Replications (Errors)	16	164.40	10.27	
Total	19	213.80		

* Arcsin transformation

n.s. not significant

Appendix 6

Analysis of variance on percentage
* recovery data in Table 17
(faecal pellets)

Source of variation	Degree of freedom	Sums of Squares	Mean Squares	F
Between groups	3	4.48	1.49	0.19 n.s.
Replications (Errors)	16	120.18	7.51	
Total	19	124.66		

*   Arcsin transformation

n.s.   not significant

Appendix 7

Analysis of variance for four treatment groups
(herbage one gram, and 5 gram, faecal pellets, and soil)
on * percentage recovery data given in
in Tables 14, 15, 16 and 17

Source of variation	Degree of freedom	Sums of Squares	Mean Squares	F
Between Treatment groups	3	3858.82	1286.27	21.7 **
Replications (Errors)	12	708.21	59.02	
Total	15	4567.03		

* Arcsin transformation

** Significant

Appendix 8

Analysis of variance between three treatment groups
(herbage 5 gm, faecal pellets and soil) on *percent-
age recovery data given in Tables 15, 16 and 17.

Source of variation	Degree of freedom	Sums of Squares	Mean Squares	F
Between treatment groups	2	185.54	92.77	1.21 n.s.
Replications (Errors)	9	689.50	76.61	
Total	11	875.04		

* Arcsin transformation

n.s. not significant

Appendix 9

Daily meteorological data for the period up to
maximum development of infective larvae of
Cooperia curticei for the month of March, 1968.

Period of Development (Days)	Date	Air temperature		Minimum temp.on grass ^{o}F	Relative humidity %	Total rainfall inches	Total evaporation inches
		Minimum ^{o}F	Maximum ^{o}F				
1	6.3.68	57.0	80.6	49.9	71	-	0.051
2	7.3.68	60.6	81.2	54.7	61	0.01	0.211
3	8.3.68	67.2	78.9	64.2	80	0.13	0.060
4	9.3.68	66.5	79.9	62.0	82	0.11	0.149
5	10.3.68	65.4	73.3	65.3	91	-	0.085
6	11.3.68	64.5	76.8	63.0	76	0.08	0.085
7	12.3.68	60.5	71.3	57.8	84	0.23	0.049
8	13.3.68	58.2	74.1	52.5	80	0.09	0.172
9	14.3.68	58.2	73.3	54.2	79	-	-
						Total	Total
	Means:-	62.0	76.6	58.2	78	0.65	0.86
		$(16.7^{o}C)$	$(24.7^{o}C)$	$(14.5^{o}C)$			

Appendix 10

Daily meteorological data for the period up to
maximum development of infective larvae of
Cooperia curticei for the month of April, 1968.

Period of Development (Days)	Date	Air temperature Minimum °F	Maximum °F	Minimum temp.on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	7.4.68	62.0	77.6	59.4	70	-	0.094
2	8.4.68	54.5	75.9	49.7	69	-	0.085
3	9.4.68	54.0	76.0	49.5	68	0.97	0.109
4	10.4.68	53.6	54.7	52.9	81	0.98	0.082
5	11.4.68	45.8	58.2	45.2	93	0.01	0.065
6	12.4.68	48.1	62.5	45.2	86	-	0.101
7	13.4.68	51.3	63.2	45.4	78	0.02	0.042
8	14.4.68	48.5	56.5	41.2	94	0.32	0.001
9	15.4.68	40.6	62.8	40.0	88	0.80	-
10	16.4.68	52.2	60.6	46.0	66	-	0.142
11	17.4.68	42.8	61.3	35.4	72	0.11	0.075
12	18.4.68	47.8	60.9	43.6	85	-	0.092
13	19.4.68	45.5	60.7	37.9	85	0.06	0.035
14	20.4.68	45.0	58.5	40.3	80	-	0.046
15	21.4.68	50.3	63.0	47.5	87	-	0.038
16	22.4.68	51.0	65.1	43.6	83	0.01	0.075
17	23.4.68	49.8	69.1	41.2	97	0.55	0.032
18	24.4.68	54.0	62.7	50.2	78	0.02	0.110
19	25.4.68	48.8	59.4	44.0	80	0.23	0.004
20	26.4.68	48.3	60.2	45.6	89	0.01	0.100
21	27.4.68	45.3	58.4	40.6	84	-	0.055
						Total	Total
	Means:	49.5	63.2	45.0	82	4.09	1.41
		(9.7°C)	(17.3°C)	(7.2°C)			

Appendix 11

Daily meteorological data for the period up to
maximum development of infective larvae of
Cooperia curticei for the month of May, 1968.

| Period of Development (Days) | Date | Air temperature | | Minimum temp. on grass $^\circ$F | Relative humidity % | Total rainfall inches | Total evaporation inches |
		Minimum $^\circ$F	Maximum $^\circ$F				
1	3.5.68	57.3	64.7	57.0	79	-	0.153
2	4.5.68	57.0	66.3	55.6	78	-	0.126
3	5.5.68	53.0	62.5	60.0	75	-	0.122
4	6.5.68	53.1	66.2	51.0	73	-	0.103
5	7.5.68	48.1	55.9	59.4	70	-	0.094
6	8.5.68	47.3	56.3	49.7	69	-	0.085
7	9.5.68	48.8	56.2	49.5	68	0.97	0.109
8	10.5.68	40.4	56.2	52.9	81	0.98	0.082
9	11.5.68	40.2	58.8	45.2	93	0.01	0.065
10	12.5.68	36.3	59.6	45.2	86	-	0.101
11	13.5.68	37.4	60.2	45.4	78	0.02	0.142
12	14.5.68	40.0	63.9	41.2	94	0.32	0.001
13	15.5.68	42.8	61.5	40.0	88	0.80	0.080
14	16.5.68	43.0	62.4	46.0	66	-	0.142
15	17.5.68	46.9	65.6	35.4	72	0.11	0.175
16	18.5.68	50.4	60.6	43.6	85	-	0.192
17	19.5.68	50.3	64.1	37.9	85	0.06	0.035
18	20.5.68	53.5	61.7	40.3	80	-	0.046
19	21.5.68	46.3	54.1	47.5	87	-	0.038
20	22.5.68	47.0	57.6	43.6	83	0.01	0.075
21	23.5.68	34.6	55.6	41.2	97	0.55	0.032
22	24.5.68	34.7	59.6	50.2	78	0.02	0.110
23	25.5.68	33.8	60.2	44.0	80	0.23	0.004
24.	26.5.68	39.3	60.7	45.6	89	0.01	0.100
25	27.5.68	44.7	61.5	40.6	84	-	0.055
26	28.5.68	50.0	60.4	38.2	88	-	0.046
27	29.5.68	52.6	60.7	37.1	91	0.08	0.009
28	30.5.68	48.8	59.2	52.8	94	0.31	-
						Total 4.66	Total 1.14
	Mean:-	45.6 (7.5°C)	65.1 (18.3°C)	46.3 (8.0°C)	82		

Appendix 12

Daily meteorological data for the period up to
maximum development of larval stages of
Cooperia curticei for the month of June, 1968.

Period of Development (Days)	Date	Air temperature Minimum °F	Air temperature Maximum °F	Minimum temp. on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.6.68	40.4	57.2	31.6	94	0.23	0.052
2	2.6.68	49.2	59.1	44.7	94	0.22	-
3	3.6.68	44.0	57.2	38.2	91	0.28	0.035
4	4.6.68	48.6	57.7	48.0	84	0.02	0.065
5	5.6.68	44.2	55.8	39.0	95	0.02	-
6	6.6.68	38.2	56.5	31.0	96	0.62	0.018
7	7.6.68	43.2	58.2	41.8	92	0.32	0.042
8	8.6.68	48.9	55.6	46.8	96	0.78	0.036
9	9.6.68	43.8	51.2	38.0	73	-	0.056
10	10.6.68	38.8	50.5	24.7	96	-	0.048
11	11.6.68	29.8	50.6	21.2	87	-	0.033
12	12.6.68	32.4	52.8	28.2	81	-	0.048
13	13.6.68	33.5	44.0	28.8	79	0.03	0.017
14	14.6.68	47.8	56.3	46.1	77	-	0.046
15	15.6.68	51.9	58.7	47.8	78	0.18	0.026
16	16.6.68	42.2	57.6	40.4	96	0.02	-
17	17.6.68	42.8	59.7	36.1	93	0.01	0.001
18	18.6.68	46.0	53.3	41.2	88	-	0.033
19	19.6.68	44.0	53.8	37.6	92	-	0.051
20	20.6.68	43.1	54.1	37.0	87	-	0.025
21	21.6.68	45.3	57.6	43.2	80	0.22	0.058
22	22.6.68	52.0	56.3	49.9	80	0.13	0.012
23	23.6.68	48.9	51.4	48.2	96	0.38	-
24	24.6.68	45.7	53.4	40.8	95	0.37	0.011
25	25.6.68	46.3	58.8	43.3	86	0.04	0.037
26	26.6.68	42.9	55.1	34.6	86	0.30	0.014
27	27.6.68	39.0	50.6	37.2	87	0.28	0.045
28	26.6.68	41.0	65.2	37.1	82	-	0.048
29	29.6.68	44.1	55.6	35.7	55	0.78	0.018
30	30.6.68	42.6	51.1	41.2	87	0.79	0.022
						Total	Total
Mean		43.2	56.2	38.6	87	6.02	0.898

Appendix 13

Daily meteorological data for the period up to
maximum development of larval stages of
Cooperia curticei for the month of July, 1968.

Period of Development (Days)	Date	Air temperature Minimum °F	Air temperature Maximum °F	Minimum temp. on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.7.68	42.8	51.8	41.5	78	0.52	0.015
2	2.7.68	35.0	49.0	29.4	96	0.06	0.040
3	3.7.68	36.1	49.0	31.2	95	-	0.023
4	4.7.68	31.0	49.2	23.5	85	-	0.043
5	5.7.68	33.0	49.6	28.1	90	trace	0.022
6	6.7.68	34.3	51.3	27.9	81	trace	0.034
7	7.7.68	42.0	54.0	38.9	76	-	0.003
8	8.7.68	50.6	56.3	46.4	79	0.04	0.016
9	9.7.68	51.8	55.2	46.8	79	0.11	-
10	10.7.68	48.5	54.9	48.6	98	trace	0.030
11	11.7.68	45.1	52.2	40.0	69	0.12	0.041
12	12.7.68	38.1	43.1	31.9	96	0.23	0.039
13	13.7.68	33.5	53.4	28.3	94	-	0.023
14	14.7.68	33.6	53.6	25.3	81	-	0.039
15	15.7.68	30.6	54.0	25.0	92	0.12	0.008
16	16.7.68	33.7	52.3	34.0	98	0.13	0.015
17	17.7.68	38.0	48.3	30.4	89	trace	0.014
18	18.7.68	32.3	49.2	27.6	93	-	0.027
19	19.7.68	31.3	51.8	25.3	98	-	0.015
20	20.7.68	35.0	57.0	32.0	96	-	0.073
21	21.7.68	35.3	55.0	29.1	91	0.01	-
22	22.7.68	40.3	59.2	39.5	90	0.21	-
23	23.7.68	49.4	59.9	46.1	84	0.23	-
24	24.7.68	44.4	54.3	38.4	91	0.46	-
25	25.7.68	48.0	49.8	45.3	92	0.39	0.006
26	24.7.68	30.8	49.8	34.6	94	-	0.039
27	27.7.68	31.5	54.0	25.2	84	-	0.036
28	28.7.68	36.2	61.1	31.8	80	-	0.086
29	29.7.68	41.9	55.4	32.5	72	0.11	0.032
30	30.7.68	45.0	55.4	44.2	87	0.22	-
31	31.7.68	50.5	57.0	46.9	80	0.06	0.032
						Total	Total
Means:		39.0	53.1	34.3	87	2.93	0.779

Appendix 14

Daily meteorological data for the period up to
maximum development of larval stage of
Cooperia curticei for the month of August, 1968.

Period of Development (Days)	Date	Air temperature Minimum $^\circ$F	Air temperature Maximum $^\circ$F	Minimum temp.on grass $^\circ$F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.8.68	46.2	54.3	43.1	90	0.09	0.030
2	2.8.68	41.6	55.1	36.8	92	0.14	0.029
3	3.8.68	41.8	55.4	45.6	74	-	0.052
4	4.8.68	45.8	56.7	37.9	89	0.25	0.034
5	5.8.68	48.9	55.0	47.8	75	0.14	0.055
6	6.8.68	38.6	54.6	30.3	86	-	0.065
7	7.8.68	37.9	56.7	30.4	82	-	0.012
8	8.8.68	36.5	57.8	29.4	93	-	0.044
9	9.8.68	41.8	57.2	38.7	75	-	0.037
10	10.8.68	37.4	58.4	30.1	94	-	0.045
11	11.8.68	38.8	61.9	30.8	88	-	0.051
12	12.8.68	36.1	64.7	29.0	84	0.05	0.010
13	13.8.68	41.6	50.7	35.2	96	0.50	-
14	14.8.68	44.5	53.6	37.8	94	-	0.024
15	15.8.68	40.0	53.8	32.2	85	-	0.059
16	16.8.68	40.3	56.2	34.6	86	0.01	0.066
17	17.8.68	46.7	52.2	45.1	90	0.01	0.047
18	18.8.68	44.6	52.6	42.3	78	0.01	0.086
19	19.8.68	43.4	51.4	39.0	78	-	0.058
20	20.8.68	34.5	52.6	27.2	82	-	0.042
21	21.8.68	31.6	52.3	25.2	80	0.08	0.014
22	22.8.68	41.3	56.3	39.1	84	0.11	0.048
23	23.8.68	43.8	58.0	42.3	96	0.11	0.027
24	24.8.68	48.8	56.2	46.0	91	trace	0.068
25	25.8.68	44.2	55.3	38.0	88	-	0.044
26	26.8.68	41.2	60.5	35.9	78	-	0.065
27	27.8.68	38.5	59.7	32.1	79	-	0.063
28	28.8.68	37.1	65.0	29.2	84	-	0.047
29	29.8.68	42.2	60.3	34.1	88	0.35	0.050
30	30.8.68	45.1	51.9	40.0	95	0.66	0.055
31	31.8.68	43.0	52.6	40.0	82	trace	0.034
						Total	Total
	Means:-	41.4	56.1	36.3	86	2.51	1.361

Appendix 15

Daily meteorological data for the period up to
maximum development of infective larvae
of Cooperia curticei for the month of September
1968.

Period of Development (Days)	Date	Air temperature		Minimum temp. on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
		Minimum °F	Maximum °F				
1	1.9.68	38.0	52.0	31.0	95	-	0.050
2	2.9.68	35.9	54.5	29.3	86	0.02	0.028
3	3.9.68	46.4	58.8	43.0	92	-	0.068
4	4.9.68	41.4	58.4	33.4	80	0.01	0.054
5	5.9.68	43.4	53.6	38.0	88	0.18	0.019
6	6.9.68	45.7	55.7	44.0	85	-	0.083
7	7.9.69	38.1	58.2	31.7	87	-	0.062
8	8.9.69	46.0	59.9	40.6	84	0.47	0.142
9	9.9.68	43.8	52.5	40.6	68	0.27	0.122
10	10.9.68	42.5	55.5	37.8	72	-	0.183
11	11.9.68	35.7	56.7	29.0	85	1.02	0.074
12	12.9.68	48.0	56.8	43.0	86	trace	0.094
13	13.9.68	48.4	56.0	47.0	90	0.05	0.020
14	14.9.68	49.0	55.2	47.0	92	0.42	-
15	15.9.68	47.2	58.0	40.4	78	-	0.061
16	16.9.68	46.2	58.0	43.0	84	0.12	0.025
17	17.9.68	41.1	59.0	32.5	77	trace	0.133
18	18.9.68	46.8	54.2	38.0	91	0.03	0.087
19	19.9.68	35.1	58.9	28.2	70	-	0.056
20	20.9.68	43.1	55.5	37.3	92	0.01	0.055
21	21.9.68	49.1	59.0	44.9	88	-	0.011
22	22.9.68	47.9	65.5	42.1	79	0.23	0.053
23	23.9.68	51.0	59.6	49.5	80	0.01	0.082
24	24.9.68	47.2	57.0	49.0	79	0.15	0.100
25	25.9.68	47.6	59.3	47.3	73	0.07	0.008
						Total	Total
Means:-		44.2 (6.8°C)	57.1 (13.9°C)	39.5 (4.1°C)	83	2.06	1.67

Appendix 16

Daily meteorological data for the period up to
maximum development of infective larvae
of *Cooperia curticei* for the month of October,1968.

Period of Development (Days)	Date	Air temperature		Minimum temp.on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
		Minimum °F	Maximum °F				
1	1.10.68	36.0	57.9	32.0	97	-	0.119
2	2.10.68	46.9	64.6	41.4	83	0.08	0.051
3	3.10.68	54.5	60.6	49.2	81	0.05	0.100
4	4.10.68	46.2	58.9	42.8	76	0.12	0.106
5	5.10.68	50.0	59.6	45.4	90	0.61	0.053
6	6.10.68	41.8	54.5	41.3	78	-	0.156
7	7.10.68	46.8	58.1	40.5	85	0.10	0.132
8	8.10.68	52.6	63.0	50.0	91	0.92	-
9	0.10.68	54.0	58.2	52.8	97	0.06	0.030
10	10.10.68	48.0	53.4	44.0	76	0.04	0.277
11	11.10.68	42.6	54.2	39.7	72	0.15	0.089
12	12.10.68	41.1	55.0	36.3	82	-	0.108
13	13.10.68	34.9	54.9	30.9	75	0.01	0.176
14	14.10.68	47.2	55.6	43.0	74	0.21	0.040
15	15.10.68	36.8	55.8	31.7	60	0.18	0.095
16	16.10.68	41.5	54.9	40.5	73	trace	0.122
17	17.10.68	38.1	57.0	32.0	73	0.05	0.087
18	18.10.68	51.0	58.2	51.0	93	0.07	-
19	19.10.68	52.0	62.0	49.0	88	0.02	0.109
20	20.10.68	48.5	59.2	49.8	90	0.12	0.101
21	21.10.68	49.4	61.6	48.6	96	0.09	0.020
22	22.10.68	50.0	59.4	45.8	86	0.27	0.115
23	23.10.68	51.0	58.8	47.2	95	0.36	0.051
						Total	Total
	Means:-	46.1 (7.8°C)	58.1 (14.4°C)	42.8 (6.0°C)	83	3.51	2.15

Appendix 17

Daily meteorological data for the period up to
maximum development of infective larvae
of _Cooperia curticei_ for the month of November, 1968.

Period of Development (Days)	Date	Air temperature Minimum $^{\circ}F$	Maximum $^{\circ}F$	Minimum temp.on grass $^{\circ}F$	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.11.68	42.6	60.2	41.0	75	-	0.119
2	2.11.68	48.5	62.3	49.0	71	0.01	0.037
3	3.11.68	45.1	59.8	43.4	70	0.25	0.125
4	4.11.68	49.2	59.4	49.9	77	trace	0.088
5	5.11.68	39.5	57.8	38.3	77	0.01	0.124
6	6.11.68	42.9	57.9	39.1	66	-	0.163
7	7.11.68	37.0	63.2	33.8	54	trace	0.150
8	8.11.68	47.7	62.9	47.6	83	0.17	0.090
9	9.11.68	49.6	59.4	46.2	66	-	0.242
10	10.11.68	49.1	60.3	45.3	69	-	0.066
11	11.11.68	50.0	62.0	47.0	73	-	0.094
12	12.11.68	52.4	65.3	51.4	66	-	0.027
13	13.11.68	42.0	65.0	39.8	79	-	0.102
14	14.11.68	46.9	69.8	44.8	81	-	0.145
15	15.11.68	47.9	67.1	47.8	83	0.01	0.167
16	16.11.68	51.0	62.1	49.2	69	0.02	0.195
17	17.11.68	51.9	65.7	50.2	71	0.58	0.052
18	18.11.68	49.0	60.0	51.4	65	0.20	0.204
19	19.11.68	49.1	61.0	49.3	74	-	0.200
20	20.11.68	48.2	62.1	44.5	63	-	0.200
21	21.11.68	49.7	64.0	47.5	63	0.10	0.160
22	22.11.68	50.0	58.1	44.5	67	0.02	0.202
23	23.11.68	48.4	60.6	44.7	63	-	0.148
						Total	Total
	Means:-	47.3 $(8.4^{\circ}C)$	62.0 $(16.7^{\circ}C)$	45.5 $(7.5^{\circ}C)$	71	1.37	3.10

Appendix 18

Daily meteorological data for the period up to maximum development of infective larvae of *Cooperia* *curticei* for the month of December, 1968.

Period of Development (Days)	Date	Air temperature Minimum °F	Air temperature Maximum °F	Minimum temp.on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.12.68	48.5	65.2	40.3	75	-	0.110
2	2.12.68	52.0	63.5	47.5	86	0.29	0.217
3	3.12.68	54.0	71.1	53.2	98	0.06	0.079
4	4.12.68	55.5	68.3	52.4	77	0.24	0.259
5	5.12.68	52.4	65.7	52.9	98	0.33	0.327
6	6.12.68	56.5	69.1	54.5	95	2.29	-
7	7.12.68	54.4	60.8	34.6	87	0.14	0.071
8	8.12.68	54.0	60.9	52.9	91	-	0.178
9	9.12.68	42.0	59.8	33.5	72	-	0.190
10	10.12.68	37.1	60.8	31.6	60	-	0.180
11	11.12.68	41.9	63.0	37.0	75	-	0.191
12	12.12.68	49.8	67.8	44.2	68	-	0.244
13	13.12.68	51.1	69.2	46.2	61	-	0.282
14	14.12.68	56.2	69.5	51.2	58	-	0.263
15	15.12.68	56.8	69.6	52.2	64	0.02	0.235
						Total	Total
	Means:-	50.7 (10.4°C)	65.7 (18.7°C)	45.6 (7.5°C)	78	3.37	2.83

Appendix 19

Daily meteorological data for the period up to
maximum development of infective larvae
of _Cooperia_ _curticei_ for the month of January, 1969.

Period of Development (Days)	Date	Air temperature Minimum °F	Maximum °F	Minimum temp. on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.1.69	52.7	70.0	49.6	67	–	0.210
2	2.1.69	52.1	70.3	48.8	74	–	0.128
3	3.1.69	53.4	70.8	48.0	68	–	0.232
4	4.1.69	51.7	76.1	45.9	75	–	0.209
5	5.1.69	57.1	79.5	52.8	57	0.72	0.136
6	6.1.69	60.8	73.2	60.2	84	0.68	0.066
7	7.1.69	55.8	66.5	55.8	86	0.19	0.182
8	8.1.69	51.0	64.5	48.9	67	0.17	0.121
9	9.1.69	54.0	67.2	51.0	71	–	0.032
10	10.1.69	57.5	69.0	53.9	88	–	0.117
						Total	Total
	Means:-	54.6 (12.5°C)	70.7 (21.4°C)	51.5 (10.9°C)	74	1.76	1.43

Appendix 20

Daily meteorological data for the period up to
maximum development of infective larvae
of _Cooperia_ _curticei_ for themonth of February, 1969.

Period of Development (Days)	Date	Air temperature Minimum °F	Air temperature Maximum °F	Minimum temp.on grass °F	Relative humidity %	Total rainfall inches	Total evaporation inches
1	1.2.69	57.1	67.9	55.0	83	0.43	0.103
2	2.2.69	55.6	70.0	53.8	65	0.17	0.148
3	3.2.69	57.0	70.7	56.0	90	0.05	0.118
4	4.2.69	52.0	74.8	45.1	76	-	0.114
5	5.2.69	56.6	75.7	51.1	78	1.18	0.125
6	6.2.69	58.2	65.6	58.0	79	-	0.235
7	7.2.69	54.0	62.9	52.5	75	-	0.266
8	8.2.69	43.0	69.0	34.8	60	trace	0.146
9	9.2.69	57.0	70.9	50.9	75	-	0.109
10	10.2.69	53.3	73.3	49.2	73	-	0.271
						Total	Total
Means:-		54.5 (12.5°C)	69.7 (21.0°C)	50.6 (10.4°C)	75	1.83	1.36

Appendix 21

Analysis of variance between the *time of development of the
infective larvae (> 90%) under natural conditions and on
predicted time from regression line under constant temperatures
(data in Table 31)

Source of variation	Degree of freedom	Sum of Squares	Mean Squares	F
Between developmental time in nature and predicted time	1	17202	17202	0.682 n.s.
Errors (within months)	16	40321	25213	
Total	17	420623		

*   after log transformation

n.s. not significant

Appendix 22

Month	X Mean maximum air temperature	Days of development of infective larvae (> 90%)	Y Log days
March 1968	24.7°C (76.6°F)	9	0.9542
April	17.3°C (63.2°F)	21	1.3222
May	18.3°C (65.1°F)	28	1.4472
June, July & August	—	No development	—
September	13.9°C (57.1°F)	25	1.3979
October	14.4°C (58.1°F)	23	1.3617
November	16.7°C (62.0°F)	23	1.3617
December	18.7°C (65.7°F)	15	1.1761
January 1969	21.4°C (70.7°F)	10	1.0000
February	21.0°C (69.7°F)	10	1.0000

$b = -.049$

Equation of the regression is

$$Y = 2.1315 - .049X$$

The slope indicates that the log rate
of development of infective larvae
(> 90%) per degree Centigrade is
0.049 log units of time.

Appendix 23

Calculations for goodness of fit of the regression line:-
(data in Appendix 22)

Months	Log days y_i	Predicted log days $\hat{y}$	Actual days y_i	Predicted days $\hat{y}$	$\dfrac{(y_i - \hat{y})^2}{\hat{y}}$
March 1968 =	0.9542	0.9112	9	8.15	0.88
April =	1.3222	1.2838	21	19.23	0.16
May =	1.4472	1.2348	28	17.18	6.81
September =	1.3979	1.4504	25	28.18	0.36
October =	1.3617	1.3259	23	21.18	0.15
November	1.3617	1.3132	23	20.56	0.28
December =	1.1761	1.2152	15	16.41	0.12
January 1969 =	1.0000	1.0829	10	12.02	0.33
February =	1.0000	1.1035	10	12.74	0. 8

Total = 9.67

The Chisquare for 8 degree
freedom and
 p < 0.05 = 9.67
Therefore calculated line
not significant different
from observed data.

X	Y	Y
Temperature °C	Predicted log days of development of infective larvae (> 90%) from regression line under natural conditions Y = 2.1315 - (.049)X Appendix 22	Predicted log days of development of infective larvae T90 from regression line under constant conditions (Y = 1.785 - (.033)X Appendix 1
15	1.396	1.290
20	1.151	1.125
25	0.906	0.960
Total 60	3.453	3.375

Sum of Squares accounted for by the best single line
with 1 degree of freedom =

$$\frac{[\Sigma(x - \bar{x})(y - \bar{y})]^2}{\Sigma(x - \bar{x})^2} \qquad = .1681$$

Sum of Squares due to regression line under natural
conditions = .1200

Sum of Squares due to regression line under laboratory
conditions = .0544

Appendix 25

Anova table for testing the slopes of the two regression
lines on development of infective larvae under laboratory
and natural conditions*   (data on Appendix 24)

Source of variance	Degree of freedom	Sums of Squares	Mean Squares	F. Variance ratio
1. Common regression	1	.1681	.1681	
2. Difference between common regression line and the separate lines	1	.0063	.0063	
3. Difference in intercepts	1	.0003	.0003	0.545 n.s.
4. Residual	2	.0011	.00055	
5. Total	5	.1755		

*   after log transformation

n.s.   not significant

Appendix 26

Survival and vertical migration of the infective larvae of Cooperia curticei under natural conditions from Experiment I
(March, 1968)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample = 5000 (flower pot)		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	38	16	799	5	860	1.00	17.00
	R2	21	11	460	4	496	0.62	10.00
2	R1	21	14	325	5	365	0.70	7.30
	R2	65	10	130	4	209	1.5	4.2
3	R1	12	11	238	51	312	0.46	6.24
	R2	0	0	21	2	23	0.0	0.5
4	R1	19	6	242	52	319	0.5	6.4
	R2	6	3	34	8	51	0.18	1.0
5	R1	34	28	158	56	276	1.24	5.5
	R2	45	39	72	63	219	1.68	4.3
6	R1	51	20	60	12	143	1.4	3.0
	R2	54	10	21	9	99	1.28	2.0
7	R1	38	12	54	22	126	1.00	2.52
	R2	21	9	41	18	89	0.6	1.8
8	R1	53	20	6	12	91	1.46	1.82
	R2	31	2	0	4	37	0.66	0.74
9	R1	22	31	1	8	62	1.06	1.24
	R2	6	0	1	23	30	0.0	0.6
10	R1	19	0	3	24	46	0.4	0.92
	R2	14	0	4	17	35	0.3	0.7
11	R1	19	0	0	14	33	0.66	0.66
	R2	17	0	3	4	24	0.42	0.48
12	R1	14	0	0	2	16	0.32	0.32
	R2	7	0	1	3	11	0.20	0.22
13	R1	2	0	0	2	4	0.05	0.1
	R2	3	0	5	6	14	0.07	0.35
14	R1	5	0	0	1	6	0.02	0.12
	R2	4	0	0	1	5	0.01	0.12
15	R1	2	0	0	0	2	0.05	0.05
	R2	3	0	0	0	3	0.06	0.06

Period in weeks	Samples (Replicates)	No. of larvae recovered from each flower pot				Eggs per sample = 5000 (flower pot		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
16	R1	0	0	5	1	6	-	0.12
	R2	0	0	2	2	4	-	0.08
17	R1	0	0	0	2	2	-	0.005
	R2	0	0	0	0	Nil	-	-
18	R1	0	0	0	4	4	-	0.08
	R2	0	0	0	0	Nil	-	-
19	R1	0	0	0	0	Nil	-	-
	R2	0	0	0	0	Nil	-	-
20	R1	0	0	0	0	Nil	-	-
	R2	0	0	0	0	Nil	-	-

Appendix 27

Survival and vertical migration of the infective larvae of Cooperia curticei under natural conditions from Experiment 2 (April, 1968)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample =20000 (flower pot)		
		Herbage 0.2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	91	29	3760	796	4776	0.6	23.4
	R2	302	159	1075	994	2530	2.3	12.7
2	R1	748	2368	715	339	4170	15.58	20.85
	R2	776	2367	405	435	3983	15.7	19.9
3	R1	1227	1248	504	763	3743	12.37	18.7
	R2	216	2435	481	476	3608	13.25	18.0
4	R1	462	2971	507	287	4227	17.16	21.13
	R2	1420	2172	214	115	3901	17.96	19.5
5	R1	1840	820	240	576	3476	13.3	17.35
	R2	440	454	108	406	1408	4.47	7.04
6	R1	1060	592	210	360	2222	8.26	11.11
	R2	520	796	76	405	1797	6.58	9.0
7	R1	805	336	64	127	1332	5.70	6.66
	R2	406	164	48	178	796	2.85	4.0
8	R1	710	307	113	210	1340	5.08	6.70
	R2	677	209	71	66	1023	4.43	5.10
9	R1	551	717	Disintegrated	260	1528	6.34	7.64
	R2	448	508	"	383	1301	4.59	6.5
10	R1	318	205	"	82	605	2.61	3.02
	R2	169	277	"	120	566	2.23	2.83
11	R1	22	103	"	25	150	0.63	0.75
	R2	41	76	–	18	135	0.60	0.68
12	R1	13	38	–	19	70	0.26	0.35
	R2	18	65	–	9	92	0.41	0.46
13	R1	35	41	–	18	94	0.38	0.47
	R2	24	34	–	6	64	0.29	0.32
14	R1	6	89	–	8	103	0.48	0.52
	R2	16	52	–	7	75	0.34	0.38
15	R1	36	104	–	8	148	0.70	0.74
	R2	15	31	–	6	52	0.23	0.26

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample = 20,000 (flower pot)		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
16	R1	6	40	−	9	55	0.23	0.27
	R2	7	20	−	10	37	0.13	0.18
17	R1	9	30	−	3	42	0.19	0.21
	R2	6	17	−	2	25	0.11	0.12
18	R1	9	19	−	3	31	0.14	0.15
	R2	5	8	−	9	22	0.06	0.11
19	R1	5	9	−	2	16	0.07	0.08
	R2	2	10	−	0	12	0.06	0.06
20	R1	2	8	−	0	10	0.04	0.05
	R2	2	6	−	0	8	0.03	0.04
21	R1	0	9	−	0	9	0.04	0.04
	R2	1	3	−	0	4	0.02	0.02
22	R1	0	13	−	0	13	0.06	0.06
	R2	0	2	−	1	3	0.01	0.01
23	R1	0	8	−	1	9	0.04	0.04
24	R1	0	7	−	0	7	0.03	0.03
25	R1	0	2	−	0	2	0.01	0.01
26	R1	0	0	−	0	Nil	−	−
27	R1	0	0	−	0	Nil	−	−

Appendix 28

Survival and vertical migration of the infective larvae of Cooperia curticei under natural conditions from Experiment 3
(May, 1968)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot Herbage 0.2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Eggs per sample = 4000 (flower pot) Total recovery	Percentage migrated	Percentage survival
1	R1	37	171	151	186	545	5.2	13.6
	R2	9	48	71	145	273	1.63	6.82
2	R1	106	176	84	190	556	7.05	14.0
	R2	44	41	72	171	328	2.12	8.2
3	R1	92	75	92	221	480	4.0	12.0
	R2	39	45	46	178	318	2.10	8.0
4	R1	53	240	145	52	490	7.32	12.25
	R2	34	172	30	76	332	5.15	8.05
5	R1	85	235	71	35	426	8.0	10.65
	R2	62	105	31	41	215	4.17	5.37
6	R1	30	54	140	93	317	2.10	7.93
	R2	56	35	56	42	189	2.28	4.73
7	R1	46	95	104	112	357	3.52	9.0
	R2	38	106	105	76	325	3.57	8.1
8	R1	41	61	54	105	261	2.55	6.52
	R2	32	76	36	85	229	2.70	5.72
9	R1	36	107	59	54	256	3.57	6.4
	R2	21	78	41	36	176	2.50	4.40
10	R1	8	62	15	34	119	1.75	3.0
	R2	2	75	10	31	118	1.23	3.0
11	R1	26	111	27	44	208	4.42	5.20
	R2	13	73	24	36	146	2.15	3.65
12	R1	19	86	14	30	149	2.62	3.72
	R2	15	99	12	16	142	2.85	3.55
13	R1	8	55	Disintegrated	25	88	1.56	2.20
	R2	13	49	-	21	83	1.55	2.15
14	R1	12	40	-	20	72	1.30	1.80
	R2	5	23	-	25	53	0.70	1.32
15	R1	19	41	-	18	78	1.25	1.95
	R2	9	44	-	13	66	1.32	1.65

Period in weeks	Samples (Replicates)	No. of larvae recovered from each flower pot				Eggs per sample = 4000 (flower pot)		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
16	R1	19	67	-	26	112	2.15	2.80
	R2	11	45	-	25	81	1.40	2.02
17	R1	20	46	-	20	86	1.65	2.15
	R2	24	40	-	19	83	1.60	2.07
18	R1	13	42	-	39	94	1.37	2.40
	R2	16	34	-	26	76	1.24	1.90
19	R1	8	15	-	37	60	0.57	1.50
20	R1	11	21	-	10	42	0.80	1.05
21	R1	12	19	-	9	40	0.77	1.0
22	R1	12	15	-	5	32	0.67	0.80
23	R1	8	12	-	5	25	0.50	0.62
24	R1	2	12	-	0	14	0.35	0.35
25	R1	1	6	-	0	7	0.17	0.17
26	R1	0	2	-	0	2	0.02	0.02
27	R1	0	0	-	0	Nil	-	-
28	R1	0	0	.	0	Nil	-	-

Survival and vertical migration of the infective larvae of
Cooperia curticei under natural conditions from Experiment 7
(September, 1968)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample =2500 (flower pot)		
		Herbage 0-2 cm	Herbage 2 &over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	14	10	315	118	457	1.0	18.3
	R2	3	1	60	48	112	trace	4.5
2	R1	5	9	105	64	183	0.6	7.30
	R2	2	7	39	15	63	0.4	2.52
3	R1	142	93	73	45	353	8.6	14.12
	R2	79	34	68	30	211	5.88	8.44
4	R1	63	144	15	34	256	8.28	10.24
	R2	32	57	21	25	135	3.56	5.70
5	R1	97	162	80	63	402	10.4	16.08
	R2	94	104	43	52	293	8.0	11.72
6	R1	91	121	49	71	332	8.48	13.28
	R2	45	79	37	66	227	5.0	9.08
7	R1	27	102	19	28	176	5.16	7.04
	R2	24	41	15	21	101	2.6	4.04
8	R1	22	78	15	5	116	4.0	4.64
	R2	15	56	13	9	93	2.84	3.73
9	R1	12	35	14	17	78	1.9	3.1
	R2	10	30	17	18	75	1.8	3.0
10	R1	14	30	22	5	71	1.76	2.84
	R2	12	23	11	2	48	1.4	1.92
11	R1	12	40	18	15	85	2.1	3.4
	R2	10	24	8	5	46	1.4	1.84
12	R1	11	30	22	20	83	1.64	3.32
	R2	4	20	5	7	36	1.0	1.44
13	R1	4	30	4	2	40	1.4	1.6
	R2	0	10	0	0	10	0.4	0.4
14	R1	3	31	2	3	39	1.36	1.56
	R2	4	10	1	2	17	0.6	0.7
15	R1	2	26	Disintegrated	37	65	1.12	2.6
	R2	0	15	-	2	17	0.6	0.7

Period of weeks	(Replicates)	No.of larvae recovered from each flower pot				Eggs per sample = 2500 (flower pot)		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
16	R1	5	58	—	4	67	2.52	2.62
	R2	3	25	—	0	28	1.12	1.12
17	R1	0	8	—	0	8	0.3	0.3
	R2	0	0	—	0	Nil	—	—
18	R1	0	0	—	0	Nil	—	—
	R2	0	0	—	0	Nil	—	—
19	R1	0	0	—	0	Nil	—	—
	R2	0	0	—	0	Nil	—	—

Appendix 30

Survival and vertical migration of the infective larvae of _Cooperia curticei_ under natural conditions from Experiment 8
(October, 1968)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample = 5000 (flower pot)		
		Herbage 0.2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	22	27	273	248	570	1.0	11.40
	R2	46	44	364	84	538	1.8	10.76
2	R1	102	442	107	93	744	10.88	14.88
	R2	86	337	56	47	526	8.46	10.52
3	R1	106	1108	56	202	1472	24.30	29.44
	R2	86	1022	94	267	1469	22.16	29.38
4	R1	282	208	12	28	530	9.8	10.6
	R2	72	218	32	104	426	8.52	5.8
5	R1	20	46	29	21	116	1.32	2.32
	R2	21	44	16	12	93	1.30	1.86
6	R1	15	35	42	18	110	1.14	2.20
	R2	18	26	21	20	85	0.88	1.7
7	R1	29	331	15	25	400	7.2	8.0
	R2	22	248	47	54	371	5.4	7.42
8	R1	15	72	12	7	106	1.74	2.12
	R2	10	36	10	5	61	0.92	1.22
9	R1	9	42	5	5	61	1.0	1.22
	R2	5	24	13	4	46	0.58	0.92
10	R1	4	24	2	14	44	0.56	0.88
	R2	0	10	4	6	25	0.20	0.50
11	R1	2	16	0	6	24	0.36	0.48
	R2	12	34	0	6	52	0.90	1.04
12	R1	15	47	Disintegrated	0	62	1.24	1.24
	R2	0	10	—	4	14	0.20	0.28
13	R1	0	12	—	3	15	0.24	0.30
	R2	0	9	—	0	9	0.18	0.18
14	R1	0	0	—	0	Nil	—	—
	R2	0	0	—	0	Nil	—	—
15	R1	0	0	—	0	Nil	—	—
	R2	0	0	—	0	Nil	—	—

Appendix 31

Survival and vertical migration of the infective larvae of *Cooperia* *curticei* under natural conditions from Experiment 9 (November, 1968)

Period in weeks	Samples (Replicates)	No. of larvae recovered from each flower pot				Eggs per sample = 11000 (flower pot)		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	24	20	488	452	984	0.2	8.95
	R2	22	16	270	403	711	0.3	6.46
2	R1	105	92	345	544	1086	1.80	8.87
	R2	239	132	213	390	974	3.4	8.85
3	R1	264	602	192	215	1273	7.87	11.57
	R2	28	201	140	402	771	2.10	7.01
4	R1	105	910	156	221	1392	9.20	12.65
	R2	242	792	172	69	1275	9.40	11.59
5	R1	260	970	48	64	1342	11.20	12.20
	R2	122	520	36	55	733	5.90	6.70
6	R1	41	301	65	85	492	3.11	4.50
	R2	32	125	32	64	253	1.43	2.30
7	R1	26	177	12	110	325	1.85	3.0
	R2	32	201	24	40	297	2.12	2.70
8	R1	21	106	17	41	185	1.16	1.70
	R2	12	99	16	28	155	1.0	1.4
9	R1	2	52	12	18	84	0.50	0.77
	R2	5	36	9	12	62	0.4	0.57
10	R1	4	36	2	2	44	0.37	0.40
	R2	0	24	0	5	29	0.22	0.26
11	R1	0	8	0	0	8	0.07	0.07
	R2	0	2	0	0	2	0.01	0.01
12	R1	0	2	0	0	2	0.01	0.01
	R2	0	0	0	0	Nil	-	-
13	R1	0	0	0	0	Nil	-	-
	R2	0	0	0	0	Nil	-	-
14	R1	0	0	0	0	Nil	-	-
	R2	0	0	0	0	Nil	-	-

Appendix 32

Survival and vertical migration of the infective larvae of Cooperia curticei under natural conditions from Experiment 10
(December,1968)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample = 12500 (flower pot)		
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	8	0	910	2255	3173	—	25.24
	R2	8	0	188	2136	2332	—	18.65
2	R1	7	0	782	282	1071	—	8.56
	R2	18	2	254	100	374	—	3.0
3	R1	240	162	76	475	953	3.22	7.63
	R2	104	71	64	602	841	1.4	6.73
4	R1	60	223	101	220	604	2.26	4.84
	R2	45	126	42	122	335	1.37	2.68
5	R1	42	170	24	58	294	1.70	2.35
	R2	21	122	12	46	201	1.14	1.61
6	R1	2	14	4	36	56	0.13	0.45
	R2	4	17	0	32	53	0.17	0.43
7	R1	7	28	2	20	57	0.28	0.45
	R2	4	22	2	4	30	0.20	0.25
8	R1	3	36	0	12	51	0.31	0.42
	R2	2	14	4	8	28	0.13	0.22
9	R1	4	17	3	7	31	0.17	0.25
	R2	2	14	0	2	18	0.13	0.14
10	R1	0	9	2	2	13	0.05	0.10
	R2	0	6	0	3	9	0.04	0.05
11	R1	0	0	0	0	Nil	—	—
12	R1	0	0	0	0	Nil	—	—

Appendix 33

Survival and vertical migration of the infective larvae of Cooperia curticei under natural conditions from Experiment 11 (January, 1969)

| Period in weeks | Samples (Replicates) | No.of larvae recovered from each flower pot | | | | Eggs per sample =10800 (flower pot) | | |
		Herbage 0-2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage Survival
1	R1	4	0	124	0	128	–	1.19
	R2	5	0	64	0	69	–	0.64
2	R1	76	24	36	9	145	0.92	1.34
	R2	10	3	12	0	25	0.10	0.23
3	R1	65	66	24	16	171	1.30	1.59
	R2	16	14	12	6	48	0.28	0.44
4	R1	3	19	0	5	27	0.21	0.25
	R2	1	8	0	6	15	0.09	0.14
5	R1	2	32	2	4	40	0.31	0.40
	R2	5	12	0	6	21	0.14	0.20
6	R1	2	4	0	4	10	0.06	0.09
	R2	0	3	0	5	8	0.03	0.08
7	R1	0	4	0	2	6	0.04	0.06
	R2	0	0	0	0	Nil	–	–
8	R1	0	2	0	3	5	0.02	0.05
	R2	0	2	0	0	2	0.02	0.02
9	R1	0	2	0	2	4	0.02	0.04
	R2	0	0	0	0	Nil	–	–
10	R1	0	0	0	0	Nil	–	–
	R2							
11	R1	0	0	0	0	Nil	–	–

Appendix 34

Survival and vertical migration of the infective larvae of Cooperia curticei under natural conditions from Experiment 12 (February,1969)

Period in weeks	Samples (Replicates)	No.of larvae recovered from each flower pot				Eggs per sample = 13000 (flower pot)		
		Herbage 0-2 cm	Herbage 2 &over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
1	R1	2	0	2916	1388	4706	–	36.2
	R2	16	9	888	629	1543	trace	11.9
2	R1	836	342	1124	1512	3814	9.06	29.34
	R2	544	232	853	1744	3373	5.97	25.95
3	R1	1216	710	308	358	2592	14.82	19.91
	R2	987	612	384	254	2337	12.30	17.98
4	R1	896	1604	121	96	2717	11.54	20.90
	R2	309	510	110	182	1111	6.30	8.55
5	R1	202	422	221	232	1077	4.80	8.28
	R2	168	379	83	152	782	4.21	6.02
6	R1	72	241	48	179	540	2.41	4.15
	R2	26	117	42	86	271	1.10	2.08
7	R1	104	501	32	136	773	4.65	5.95
	R2	92	409	12	74	587	3.85	4.52
8	R1	21	157	44	267	489	1.37	3.76
	R2	26	202	12	106	346	1.75	2.66
9	R1	15	91	29	108	243	0.82	1.87
	R2	12	76	11	92	191	0.68	1.47
10	R1	41	146	231	294	712	1.44	5.48
	R2	2	54	24	46	126	0.43	0.97
11	R1	4	31	32	106	173	0.27	1.33
	R2	2	21	21	11	55	0.18	0.42
12	R1	2	48	0	4	54	0.38	0.42
	R2	0	12	0	16	28	0.09	0.22
13	R1	21	72	2	22	117	0.72	0.90
	R2	4	12	8	2	26	0.12	0.20
14	R1	9	51	Disintegrated	2	62	0.46	0.48
	R2	0	4	–	2	6	0.03	0.05
15	R1	12	36	–	12	60	0.37	0.46
	R2	0	4	–	4	8	0.03	0.06

Appendix 34 continued...

Period in weeks	Samples (Replicates)	No. of larvae recovered from each flower pot				Eggs per sample = 13000 (flower pot)		
		Herbage 0–2 cm	Herbage 2 & over	Faecal pellets	Soil surface	Total recovery	Percentage migrated	Percentage survival
16	R1	2	14	–	0	16	0.12	0.12
	R2	3	4	–	0	7	0.05	0.05
17	R1	0	1	–	0	1	0.01	0.01
	R2	0	0	–	2	2	–	0.02
18	R1	0	0	–	0	Nil	–	–
	R2	0	0	–	0	Nil	–	–
19	R1	0	0	–	0	Nil	–	–
	R2	0	0	–	0	Nil	–	–

Spearman Rank correlation coefficient between maximum survival
and meteorological data given in Table 42 (Tied observations)

Maximum survival (weeks) X_1	Mean max. air temp. $^{\circ}F$ X_2	Total rainfall inches X_3	Mean relative humidity % X_4	Total evaporation inches X_5	=	Ranks X_1	Ranks X_2	Ranks X_3	Ranks X_4	Ranks X_5
9	70.1	4.39	74	9.7		1	8.5	1	2	3
10	70.1	8.49	74	11.4		2	8.5	2	2	5
12	68.9	11.46	75	14.5		3	7	4	4.5	7
13	66.0	12.07	74	14.6		4	6	5	2	8
17	63.6	15.80	75	17.0		5.5	4.5	6	4.5	9
17	63.6	8.70	79	11.8		5.5	4.5	3	6	4
18	61.4	17.40	85	6.9		7	3	7	8	1
25	56.5	21.45	86	7.9		8	1	8	9	2
26	56.9	21.54	83	12.0		9	2	9	7	6

$$\rho\ X_1 X_2 = \frac{\Sigma X_1{}^2 + \Sigma X_2{}^2 - \Sigma d_1{}^2}{2\sqrt{\Sigma X_1{}^2\ \Sigma X_2{}^2}}$$

$$\rho\ X_1 X_2 = \frac{59.5 + 59.0 - 234.5}{2\sqrt{(59.5)(59.0)}} = -0.98$$

This correlation coefficient suggests perfect negative
correlation between maximum survival and mean maximum
air temperature.

$$\rho\ X_1 X_3 = \frac{59.5 + 60.0 - 4.5}{2\sqrt{(59.5)(60.0)}} = +0.97$$

The correlation coefficient indicates perfect positive
correlation between maximum survival and total rainfall

$$\rho \ X_1 X_4 \ = \ \frac{59.5 - 59.0 - 14.5}{2\sqrt{(59.5)\ (59.0)}} \ = \ + \ 0.88$$

This correlation coefficient suggests a high positive correlation between maximum survival and mean relative humidity.

$$\rho X_1 X_5 \ = \ \frac{59.5 + 60.0 - 137.5}{2\sqrt{(59.5)\ (60.0)}} \ = \ - \ 0.14$$

This correlation coefficient suggests no correlation between maximum survival and total evaporation.

Appendix 36

Spearman rank correlation coefficient between survival and
vertical migration throughout the year for each monthly
experiment data given in Tables 32 to 40

Months	March 1968		April		May		September		October	
Weeks	s.	v.m.	s.	v.m.	s.	v.m.	s.	v.m.	s.	v.m.
1	1	5	4	10	2	5	2	15	3	5
2	2	3	1	2	1	3	6	15	2	2
3	5	12	3	3	4	7	3	2	1	1
4	4	10	2	1	3	1	5	4	4	3
5	3	1	5	4	6	2	1	1	6	7
6	6	2	6	5	7	11	4	3	8	8
7	7	6	9	8	5	4	7	5	5	4
8	8	4	8	7	8	10	8	6	7	6
9	9	8	7	6	9	8	9	7	9	9
10	10	9	10	9	12	12	11	10	12	12
11	11	7	11	11	10	6	10	9	10	11
12	12	11	14	14	11	9	11	11	10	10
13	13	13	15	14	14	15	16	13	13	13
14	14	15	13	13	18	18	15	12	————	
15	16	14	12	12	17	17	14	14		
16	15	16	16	16	13	13	13	17		
17	17	17	17	17	16	14	17	17		
18	18	18	18	18	15	16	————			
19	————		19	19	19	22				
20			20	20	20	19				
21			22	23	21	20				
22			21	21	22	21				
23			23	23	23	23				
24			24	22	24	24				
25			25	25	25	25				
26			————		26	26				

Months	November		December		January		February	
Weeks	s.	v.m.	s.	v.m.	s.	v.m.	s.	v.m.
1	5	10	0	0	0	0	0	0
2	3	4	0	0	2	2	1	3
3	4	3	1	1	1	1	2	1
4	1	1	2	2	4	4	3	2
5	2	2	3	3	3	3	4	4
6	6	5	4	6	5	5	8	6
7	7	6	5	5	7	6	5	5
8	8	7	6	4	6	6	7	7
9	9	8	7	6	8	8	9	9
10	10	9	8	8			6	8
11	11	11					10	13
12	12	12					12	12
13							11	10
14							13	11
15							14	14
16							15	15
17							16	16

s = survival

v.m. = vertical migration on herbage

$$\rho = 1 - 6\ \frac{\Sigma d_i^2}{N^3 - N}$$

Appendix 37

Arcsin transformation of data on percentage migration
(data in Table 45)

Survival Class mid points X	Arcsin	% migration Y	Arcsin
0.25	2.87	0.33	3.29
0.75	4.97	0.50	4.05
5.0	12.92	2.30	8.72
15.0	22.79	6.34	14.60
25.0	30.00	14.78	22.61

Calculation of regression - transformed data

		Deviations	
X	Y	x	y
2.87	3.29	−11.84	−7.364
4.97	4.05	−9.74	−6.604
12.92	8.72	−1.79	−1.934
22.79	14.60	+8.08	+3.946
30.00	22.61	+15.29	+11.956
Σ 73.55	53.27	0	0
Mean 14.71	10.654		

$$\text{Regression coefficient } b = \frac{\Sigma xy}{\Sigma x^2} = 0.68797$$

Calculation of $\hat{Y} = \bar{y} + b \ (x - \bar{x})$

Y	$\hat{Y}$	Deviation	$\dfrac{(Y_i - \hat{Y})^2}{\hat{Y}}$
3.29	2.51	0.78	0.427
4.05	3.95	0.10	0.005
8.72	9.42	0.70	0.108
14.60	16.21	1.61	0.337
22.61	21.17	1.44	0.190
			1.113

The Chisquare for 4 degree freedom & $p <$ 0.05 = 9.49

Therefore calculated line not significantly different from observed data.

Appendix 38

The effect of <u>Cooperia</u> <u>curticei</u> on the weekly
weight changes of sheep 6-7 months old given
different doses of larvae:-

Group No.	Sheep No.	Total larval dose	Before in- fection	7	14	21	28	34	42	49	56
								Weight in lbs, days infection			
A	301	10,000	48	49	52	51	50	54 =	killed		
	319	10,000	40	41	41	41	41	42 =	killed		
	310	60,000	35	38	41	40	39	38	40	42	37.5
	311	60,000	42	44	46	48	44.5	49	48	48.5	45.5
B	324	50,000	44	48	46	49	50	51 =	killed		
	6078	50,000	44	46	43	44	45	46 =	killed		
	315	100,000	36	40	39	39	39	40	41	40	39
	304	100,000	43	46	45	45	46	44.5	45	43	44.5
C	6079	100,000	42	44	47	45	43	44.5 =	killed		
	325	100,000	44	48	53	50	50	49	= killed		
	326	150,000	39	40	45	45	41	42	42	43	41
	303	150,000	48	50	54	53	52.5	53.5	54	54	53.5
D	323	50,000	44	45	48	49	47	46.5	48	49	49
	322	50,000	48	49	54	55	54	55	57	57	58
	6081	Control	43	45	48	46	43	44	= killed		
	302	Control	43	44	44	42	41	44	= killed		
E	307	Control	--	--	--	--	--	--	51	51	53.5
	318	Control	--	--	--	--	--	--	45	45	46.5

Appendix 39

Frequency distribution of *Cooperia curticei* in small intestine
and sex ration in experimental animals:- (single dose)

Group No.	Sheep No.	Total larval dose	Days of in-fection		Aboma-sum	S. intestines: 0-5	5-10	10-15	15-20	20 & over	4th stage larvae	Total Male worms	Total female worms	Total no.of worms	Percentage developed worms in the host
				M	0	150	3,321	370	180	0	0				
A	301	10,000	34	F	0	200	2,664	480	270	0	0	4,021	3,614	7,635	76%
				T	-	350	5,985	850	450	-	-				
				M	0	830	2,710	540	10						
	319	"	"	F	0	360	2,670	850	10	0	0	4,090	3,890	7,980	79%
				T	-	1,190	5,380	1,390	20						
				M	0	9	27	13							
B	324	50,000	"	F	0	18	45	25	0	0	0	49	88	137	0.3%
				T	-	27	72	38							
				M	0	9	4,341	2,578	149	44					
	6078	"	"	F	0	9	4,350	2,871	152	47	0	7,121	7,429	14,550	29%
				T	-	18	8,691	5,449	301	91					

M = Male   F = Female   T = Total

Appendix 39   continued

Group No	Sheep No	Total larval dose	Days of infection		Abomasum	S. Intestines 0-5	5-10	10-15	15-20	20 & over	4th stage larvae	Total male worms	Total female worms	Total no.of worms	Percentage developed in the host
				M	0	72	180	36	9	18					
C	6079	100,000	34	F	0	108	324	81	18	9	0	315	540	855	0.8%
				T	-	180	504	117	27	27					
				M	0	27	1,008	675	90	18					
	325	"	"	F	0	18	1,314	792	81	9	0	1,818	2,214	4,032	4%
				T	-	45	2,322	1,467	171	27					

M = Male   F = Female   T = Total

Appendix 40

Frequency distribution of Cooperia curticei in small intestine
and sex ratio in experimental animals:-
(second dose given after 34 day's of infection.)

Group No.	Sheep No.	Total larval dose	Days of in-fection	Aboma-sum		S. Intestine 0-5	5-10	10-15	15-20	20 & over	4th stage larvae	Total male worms	Total female worms	Total no.of worms	Percentage developed in host
A	310	10,000 + 50,000	56	M		18	36	9	0						
				F	0	36	81	9	9	0	0	63	135	198	0.3%
				T		54	117	18	9						
	311	"	"	M		45	1,440	9	18	9					
				F	0	18	1,071	228	0	0	198	1,521	1,377	2,898	4.8%
				T		63	2,511	297	18	9					
B	313	50,000 + 50,000	"	M		9	252	477	108	9					
				F	0	9	360	486	90	18	0	855	963	1,818	1.8%
				T		18	612	963	198	27					
	304	"	"	M		9	495	252	153	81					
				F	0	63	720	378	234	297	90	990	1,692	2,682	2.7%
				T		72	1,215	630	387	378					

M = Male   F = Female   T = Total

Group No.	Sheep No.	Total larval dose	Days of infection		Aboma-sum	S. Intestine 0-5	5-10	10-15	15-20	20 & over	4th stage larvae	Total male worms	Total female worms	Total no. of worms	Percentage developed in host
C	326	100,000 + 50,000	56	M		27	378	18	0						
				F	0	81	333	18	18	0	837	423	450	873	0.5%
				T		108	711	36	18						
	303	"	"	M		18	18								
				F	0	90	99	0	0	0	0	36	189	225	0.1%
				T		108	117								
D	323	50,000	"	M		9	540	9							
				F	0	9	612	9	0	0	24,408	558	630	1,188	2.3%
				T		18	1,152	18							
	322	"	"	M		9	27	0							
				F	0	9	36	18	0	0	180	36	63	99	0.2%
				T		18	63	18							

M = Male   F = Female   T = Total

Appendix 41

Total egg output of <u>Cooperia</u> <u>curticei</u> in sheep
necropsied after 19 days of infection.

1 8   D a y s   o f   i n f e c t i o n

Group No.	Animal No.	Total 24 hours faeces gms.	I	II	III	IV	V	Total	Average egg per gram	Total eggs 24 hours
A	115	465	31	24	38	33	29	155	1,550	720,750
"	105	467	78	76	68	79	85	386	3,860	1,802,620
B	108	530	8	10	11	12	14	55	550	291,500
"	349	488	90	82	83	70	83	409	4,090	1,995,920
C	111	466	79	77	65	75	70	376	3,760	1,752,160
"	102	450	90	123	118	101	109	532	5,320	2,394,000

1 9   D a y s   o f   i n f e c t i o n

Group No.	Animal No.	Total 24 hours faeces gms.	I	II	III	IV	V	Total	Average egg per gram	Total eggs 24 hours
A	115	405	39	38	50	56	53	236	2,360	955,800
"	105	445	89	90	90	85	92	446	4,460	1,984,700
B	108	545	17	20	23	14	17	91	910	495,950
"	349	425	100	120	117	151	127	615	6,150	2,613,750
C	111	620	45	43	41	36	38	203	2,030	1,258,600
"	102	570	98	105	120	77	97	497	4,970	2,832,900

Total egg output of <u>Cooperia</u> <u>curticei</u> in sheep
necropsied after 39 days of infection.

3 8   D a y s   o f   i n f e c t i o n

Group	Animal	Total 24 hours faeces gms.	I	II	III	IV	V	Total	Average egg per gram		Total egg's 24 hours
A	342	497	71	62	60	55	53	301	3,010	=	1,495,970
"	107	410	75	69	79	78	81	382	3,820	=	1,566,200
B	118	455	4	6	5	4	5	14	240	=	109,200
"	341	570	0	0	0	0	0	Nil	Nil	=	Nil
C	103	475	3	2	3	3	1	12	120	=	57,000
"	117	365	112	109	89	110	115	535	5,350	=	1,952,750

3 9   D a y s   o f   i n f e c t i o n

Group	Animal	Total 24 hours faeces gms.	I	II	III	IV	V	Total	Average egg per gram		Total egg's 24 hours
A	342	655	36	42	38	36	40	191	1,910	=	1,251,050
"	107	425	86	50	73	75	60	344	3,440	=	1,462,000
B	118	430	4	1	5	2	2	14	140	=	60,200
"	341	665	0	0	0	0	0	Nil	Nil	=	Nil
C	103	430	2	4	1	1	2	10	100	=	43,000
"	117	460	114	134	118	135	114	625	6,250	=	2,875,000

The effect of <u>Cooperia</u> <u>curticei</u> on weight
changes of sheep 2-3 months old given
different doses of larvae:-

Group No. & Sheep No.	Before in-fection	4	8	12	15	19	23	27	32	36	40
A											
115	38.5	39.5	40.0	40.5	42.5	43.5 =	killed				
105	31.5	32.0	34.0	36.0	37.7	39.0 =	killed				
342	26.5	26.5	27.0	27.5	29.0	30.5	30.5	32.5	33.2	35.0	37.0
107	32.5	34.0	35.3	37.5	37.5	38.2	39.0	40.5	41.5	42.5	44.5
B											
108	29.5	29.5	32.5	35.0	36.0	36.5 =	killed				
349	30.0	31.5	33.0	35.5	36.0	36.5 =	killed				
118	35.5	36.5	39.0	41.0	41.0	41.2	41.2	42.0	43.5	44.2	45.0
341	22.5	25.0	25.8	27.5	30.0	30.5	31.5	33.5	34.5	35.2	36.0
C											
102	31.0	32.5	32.5	35.0	36.0	37.5 =	killed				
111	27.5	28.5	31.5	33.5	35.0	35.2 =	killed				
103	34.5	34.5	36.5	37.5	37.5	39.5	41.0	42.0	42.5	44.5	45.0
117	33.5	34.5	35.5	38.0	38.0	38.5	39.5	41.0	41.5	43.0	44.0
D											
101	32.0	33.5	35.0	37.5	39.0	40.0 =	killed				
110	36.0	37.5	39.5	40.0	41.5	41.5 =	killed				
112	40.5	41.5	44.0	46.5	47.0	48.0	48.0	49.0	50.0	51.0	52.0
106	29.0	31.0	33.0	35.0	35.5	36.0	37.2	37.5	39.0	40.5	41.5

Appendix 44

Frequency distribution of Cooperia curticei in small intestine and sex ratio in sheep after 20 days of infestation

Group No.	Sheep No.	Total larval dose	Aboma-sum		small intestine 0-5ft	5-10	10-15	15-20	20ft & over		Sex ratio Male	Female	Total worms	Percentage established
A	115	10,000	M=			1,470	30	10						
			F=	0	0	1,580	100	60	0	=	1,510	1,740	3,250	32.5%
			T=			3,050	130	70						
	105	"	M=			100	1,210	90	20					
			F=	0	0	100	1,640	133	20	=	1,420	1,893	3,313	33.1%
			T=			200	2,850	223	40					
B	108	50,000	M=		70	1,590	670	210	10					
			F=	0	60	1,280	1,470	270	10	=	2,550	3,090	5,640	11.2%
			T=		130	2,370	2,040	480	20					
	349	"	M=		790	4,240	1,480	160	70					
			F=	0	1,150	5,430	1,970	160	120	=	6,740	8,830	15,570	31.0%
			T=		1,940	9,670	3,450	320	190					
C	102	100,000	M=		316	4,770	2,583	630	4,000					
			F=	0	857	5,270	2,782	620	4,840	=	12,299	14,369	26,668	26.6%
			T=		1,173	10,040	5,365	1,250	8,840					
	111	"	M=		230	1,680	4,300	5,280	440					
			F=	0	280	1,960	3,970	5,320	670	=	11,930	12,210	24,140	24.1%
			T=		510	3,640	8,270	10,610	1,110					

M = Male   F = Female   T = Total

Appendix 45

Frequency distribution of <u>Cooperia</u> <u>curticei</u> in small intestine

and sex ratio in sheep after 40 days of infestation

Group No.	Sheep No.	Total larval dose	Aboma-sum		small intestine 0-5ft	5-10	10-15	15-20	20 & over		Sex ratio Male	Female	Total worms	Percentage established
A	342	10,000	M= F= 0 T=		230 300 530	730 1,220 1,950	6 30 30	0	0	=	960	1,550	2,510	25.1%
	107	"	M= F= 0 T=		140 170 310	660 630 1,290	40 0 40	0	0	=	840	800	1,640	16.4%
B	118	50,000	M= F= 0 T=		10 70 80	335 380 715	2,783 3,454 6,237	2,740 3,040 5,780	70 110 180	=	5,938	7,054	12,992	25.8%
	341	"	M= F= 0 T=		10 0 10	1,180 670 1,850	480 480 960	11 55 66	280 350 630	=	1,961	1,555	3,516	7.0%
C	103	100,000	M= F= 0 T=		180 80 260	2,870 3,670 6,540	650 760 1,410	90 110 200	10 10 20	=	3,800	4,630	8,430	0.84%
	117	"	M= F= 0 T=		10 50 60	2,790 4,120 6,910	10 20 30	0 50 50	0	=	2,810	4,240	7,050	0.70%

M = Male   F = Female   T = Total

Appendix 46

The effect of Cooperia curticei on the weekly
weight change of sheep 9-10 months when given
different type of feeds:-

				Days of infection / weight in lbs.					
Group No.	Sheep No.	Total larval dose	Feed 1.5lb	Before in-fection	7	14	21	28	35
		Daily							
A	320	50,000x5	Hay	50.0	51.0	50.0	50.5	50.5	50.5
	346	= 50,000	"	63.0	63.5	64.0	64.5	63.5	63.0
	330	in total	"	45.0	46.5	47.5	48.5	48.5	46.5
	10	"	"	74.0	75.5	74.0	73.5	74.0	75.5
B	345	50,000	Hay	55.0	56.5	56.5	56.5	57.0	57.5
	339	(single	"	50.5	51.0	50.0	50.0	49.5	50.5
	344	dose)	"	44.0	45.0	45.5	45.0	46.0	45.0
	347	"	"	65.0	66.0	66.5	67.5	67.0	67.5
C	113	50,000	Feed conc.	47.0	46.0	48.0	48.0	50.0	50.0
	336	(single	"	65.0	64.5	67.0	68.0	69.0	69.0
	350	dose)	"	87.5	87.0	87.0	87.0	86.0	87.5
	328	"	"	61.5	60.0	62.5	62.5	63.5	63.5
D	332	Controls	Hay	60.0	61.5	61.5	61.5	61.5	62.0
	348	"	"	54.5	54.5	55.0	55.0	55.5	56.0
	120	"	Feed conc.	52.5	52.5	54.0	54.0	57.0	58.5
	119	"	"	65.5	67.0	67.5	70.0	68.0	72.0