THE BIOLOGY

OF

ASPICULURIS TETRAPTERA SCHULTZ

(NEMATODA, OXYURIDAE)

Thesis

for the

Degree of Doctor of Philosophy

in the

University of London

by

Jerzy Marian Behnke

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Nie depczcie przeszłości ołtarzy,
Choć macie sami doskonalsze wznieść,
Tam się jeszcze Święty Ogień żarzy
I miłość ludzka stoi tam na straży
I wy winniście im cześć!

(A. Asnyk.)

ABSTRACT

The following results of field observations and laboratory experiments conducted during a study of the biology of Aspiculuris tetraptera are presented:

a) Sex-resistance and age resistance were demonstrated in a survey of the incidence of A.tetraptera in wild populations of its natural host, Mus musculus. Concurrent infection with Syphacia obvelata was not detrimental to either parasite.

b) Soon after infection the larvae of A.tetraptera became established in the Crypts of Lieberkuhn in the mid-colon, undertook an anterior emigration on day 7 and thereafter persisted in the anterior colon, where the infection became patent on day 26.

c) It was discovered that mice resisted infection by rejecting up to 80% of the worm burden before day 20.

d) This response was stronger in female mice than in males, consequent (it is suggested) to the immunosuppressive properties of testosterone.

e) It is proposed that a different response accounted for the earlier termination of infections in rats and Apodemus sylvaticus.

f) Mice aquired immunity to A.tetraptera, which could be elicited by the crypt phase alone.

g) Hydrocortisone was effective in suppressing immunity. It was suggested that drug-induced tolerance may have been involved.

h) Methotrexate delayed immunity, but retarded worm growth.

i) Specific precipitating antibody was studied and it was proposed that two classes of antibody were involved,

tentatively identified as IgG and IgM.

j) A procedure for transplanting worms from donor to recipient mice was developed and the following observations were made:

i/ 7 day larvae evoked immune expulsion suppressible by hydrocortisone and methotrexate.

ii/ Larvae were expelled faster from immune mice than from controls.

iii/ "Damaged" larvae were also more rapidly rejected but survived in hydrocortisone treated or in irradiated recipients.

iv/ Mature worms survived for 85 days, senescence accounting for their eventual disappearance.

v/ Mature worms survived in immune recipients.

vi/ Mature worms evoked antilarval immunity but were themselves not affected by it.

k) Concurrent infection with Trichuris muris was detrimental to A.tetraptera.

l) The results are discussed in the context of current research in parasitology.

m) It is suggested that A.tetraptera would prove a good model for future work.

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SECTION 1

CHAPTER ONE
General Introduction

It is a generally accepted principle in biology, that in order to survive in nature, an organism must be adapted to the environment in which it is destined to live, the process of natural selection functioning somewhat like a sieve, eliminating from the population those individuals which are inferior, preserving only the best adapted organisms to perpetuate the species. Not only does this fundamental law hold true for free living organisms, but it is equally valid for those species which depend on some form of interspecific association for survival. Such associations range from mutualism and symbiosis, where both organisms derive a benefit from the association to obligate parasitism where one organism is totally dependant on the other, the host, and may cause pathological changes which result in death (Faust, Russel and Jung, 1970). A well adapted parasite, on the other hand, derives benefit at the host's expense, without causing severe clinical manifestations as a result of its presence, Not surprisingly, therefore, such organisms are in the main, not the concern of the veterinary or the medical parasitologist. Rather, the attention of these workers is drawn to the parasites which cause suffering, mortality and serious economic loss, in an attempt to elucidate some of the factors underlying the detrimental aspects of the relationship, and discover ways of reversing the balance in favour of the host.

Lapage wrote, "Parasitic animals not only cause, in man and other animals, incalculable suffering and kill great numbers of people, but they may also, if they are not controlled make it impossible for man to live in certain parts of the world. They

can seriously disrupt man's industrial organizations and they inflict on his domesticated animals, upon which man depends for his supplies of meat, milk, eggs and other important foods, losses the extent of which is rarely fully realized."

The United States Bureau of Animal Industry estimated in 1942 that 69% of the 418 million dollars lost as a result of various animal diseases was attributable directly to parasitic infections. Hagan in 1947 gave a higher estimate (1,000 million dollars) and Stoll (1947) found that the total annual incidence of helminth infections was almost equal to the contemporary world population.

Clearly an understanding of all the factors involved in host-parasite relationships is of paramount importance, if their harmful effects are to be eradicated without upsetting the ecological balance and bringing about other undesirable phenomena. It was to this end, in an effort to contribute some useful information to present knowledge, that the study reported in this thesis was embarked upon.

The relationship of the parasite to its host is basically no different from that of any other living thing to the ecological niche it occupies. As with any other organism, the parasite must change in response to changes in its environment, namely the host. In long associations between particular parasites and their hosts, evolution has proceeded in parallel, the parasite adapting to the successive evolutionary changes in the host physiology. Because of this long association parasites in general are host specific and can survive only in a limited number of host species, in which the environment is not too dissimilar from that in the pri-

mary host. To the investigator of parasites of veterinary or medical importance, this specificity poses great difficulties, since the hosts concerned (i.e. man and farm-stock) are not the easiest of animals to study experimentally. One approach has been to study closely related parasites in easily maintained laboratory animals, and this, to a large extent, has formed the basis of parasitological research.

In this context, nematode parasites have been intensively studied, but present knowledge is primarily based on a few model systems. Nippostrongylus brasiliensis, the strongyloid parasite of rats is perhaps the prime example of such a "favourite" system and present knowledge of this host-parasite relationship exceeds that in any other laboratory model (recent reviews, Ogilvie and Jones 1971,1973). Trichinella spiralis in rats and mice and Trichostrongylus colubriformis in guinea-pigs have also been intensively studied and more recently, interest has been renewed in the murine-parasites Trichuris muris and Nematospiroides dubius. How far these parasites represent the situation in Man and his animals is yet unknown, but with the exception of N.dubius, all stimulate immunological responses in the host, which elicit an abrupt termination of the primary infection (spontaneous self cure). Spontaneous self-cure is not a universal phenomenon and certainly does not occur in many parasites of man and domestic animals, where parasitic infections frequently persist for months and even years.

The original aim of this thesis was to study in detail the interactions of three species of nematodes in the caecum and the colon of the mouse; namely the trichuroid Trichuris muris and the oxyuroids Aspiculuris tetraptera and Syphacia

obvelata. The primary objective was to investigate the existence of cross-immunity between the species and its effects on the stages in each life cycle; direct competition of the species for space and food forming an integral part of the study. This aim, however, proved difficult to achieve when it was discovered that little was known about the biology of Aspiculuris tetraptera. Of the several papers already published, the two concerned with the life-history of the parasite and its position in the intestine during infection, were not in agreement. During initial experiments, carried out primarily to determine which of the two hypotheses (Chan,1955 or Anya,1966) was correct, a number of interesting observations were made and these formed the basis of the final objectives of the thesis. The experiments were carried out in low grade mice (one or two star) which contained moderate natural infections with this parasite. The animals were cleared of pinworms with piperazine citrate and were reinfected with embryonated eggs of A.tetraptera. These attempts frequently failed to establish an infection, suggesting that in spite of previous reports, acquired resistance may have been preventing re-infection. The use of specific pathogen free (S.P.F.) mice solved this problem and gave repeatable results with reasonable worm recovery during the first two weeks of infection. The discovery of a spontaneous self-cure response in female mice (and to a lesser extent in males) necessitated detailed analysis of the response in order to evaluate the potential usefulness of this host-parasite system in further studies on the mechanisms underlying immunological protection of the host from parasitic invasion.

A particularly interesting aspect of the biology of

A.tetraptera is the fact that it does not normally penetrate host-tissue, unless the infection is unusually heavy, when damage may occur (Mullink,1970). In this respect A.tetraptera is unique among the laboratory model systems studied, where there is a greater degree of host-parasite contact.

The second section of the thesis is an attempt to analyse infections with A.tetraptera in wild host population made as a basis on which laboratory experiments could be designed. Section three, includes thirteen chapters, each concerned with a particular aspect or aspects of the biology of A.tetraptera. Each chapter incorporates a review and a discussion of the pertinent literature. It was decided to arrange the thesis in this way because a separate chapter dealing solely with a review of the literature would have necessitated too many cross-references, hindering the integrity of the thesis. Finally in section four, the implications of the findings are discussed in the general context of parasitology, problems encountered are considered and scope for further work is proposed.

SECTION 2

CHAPTER TWO

A survey of infection rates with Aspiculuris tetraptera in wild populations of small mammals.

INTRODUCTION

The Literature contains a number of reports concerning the relationship of Aspiculuris tetraptera with laboratory strains of its host the mouse, but there have been relatively few attempts made to determine whether the principles derived by experimental work, also apply in naturally occuring populations of the host.

Two of the earliest lists of hosts for A.tetraptera were given by Hall (1916) and Schultz (1924). Both authors include Mus musculus and Apodemus sylvaticus in the list of 15 species although it is possible that some of the species named by Hall may be synonymous with the latin names given by Schultz. Mus musculus was mentioned as a host for this parasite by Yorke and Maplestone (1926), who also included Cricetus species. The earliest British record was given by Baylis (1928) who found A.tetraptera in Clethrionomys glareolus in Oxford. In addition Elton et al (1931) recorded this vole as a host, but his examination of Apodemus sylvaticus (Elton 1934) did not reveal A.tetraptera. Twort and Twort (1932) found that 75% of 75 laboratory mice were infected with A.tetraptera.

Yamaguti (1935), Vanni (1937) and Chin (1939) reported A.tetraptera from Rattus rattus and another record of wild house mice infected with the parasite was given by Erickson (1938). During this same period Harkema (1936) failed to find

A.tetraptera in Mus musculus and Baylis (1939) did not include the species in his addition to the British record of parasites from A.sylvaticus. Elton (1934) failed to detect it in the three A.sylvaticus which he examined.

A.tetraptera was, however, again found in wild Mus musculus in the U.S.A.in 1949 by Rausch and Tiner. Rankin (1945), who only looked at one house mouse in his survey of 10 other species of small mammals (total 131 animals), did not report a single case of this pinworm.

A second study of laboratory mice (Li and Hsu,1951) revealed that 74.6% (100 of 134) mice contained A.tetraptera, which is in very close agreement with the previous work by Twort and Twort (1932). Roman (1951) gave more information than previous authors about the rate of infection in Mus musculus by considering male and female mice separately. He discovered that whereas 48% of male mice were infected, only half this number (24.4%) of female mice were similarly infected. Akhtar (1955) found a higher level of infection in Mus musculus (57%) and a similarly high rate in Rattus rattus (60%). Albino laboratory rats, Rattus norvegicus were noted to contain the parasite by Stahl 1959 (in a private communication to Mathies). Voles, Clethrionomys glareolus were again reported as hosts by Thomas (1953) who found 3/13 (23%) voles, on Ulva in the Inner Hebrides, infected; each animal had 10-20 worms. He examined 45 Apodemus sylvaticus (15 from Raasay and 30 from Ulva), but found none to contain this worms. During this time, Grundmann (1957) found Peromyscus maniculatus sonoriensis infected with A.tetraptera, but the two animals, of a total of 115 laboratory reared mice most probably acquired their infections from laboratory house mice kept in the same animal room. Wild populations of this species did

not contain A.tetraptera, (Grundmann and Frandsen 1959). Lubinsky (1957), who examined M.musculus and thirteen other species of rodents, failed to record the parasite.

In 1960, Grundmann and Frandsen again mentioned Peromyscus maniculatus sonoriensis as a host, possibly referring to their earlier account. Bernard (1963) found A.tetraptera in 10-16% of M.musculus, one gravid female worm and several juveniles in five infected Rattus rattus alexandrinus and on one occasion he found the worm in Apodemus sylvaticus. In 1964 he recorded A.tetraptera from Rattus norvegicus; but Leon (1964) who examined 116 of these rats and four Rattus rattus, in Puerto Rico, did not mention A.tetraptera. One hundred and thirty five Rattus norvegicus from Haifa and Tel Aviv were examined by Wertheim (1963), again with no mention of A.tetraptera. Quentin (1966) gives a list of exotic hosts from Africa, which include Mastomys sp (32 chromosome variety), Praomys jacksoni, Praomys tullbergi and Thamnomys ratilans. Fahmy et al (1969) found A.tetraptera in Mus musculus in Kharga Oasis, but failed to report the worm from the 296 Rattus norvegicus which they examined in another work published in the same year. Dyer (1969) records Peromyscus maniculatus as a host, but it is doubtful whether this was an original reference. Both Quentin (1965) and Talbot (1968) failed to find A.tetraptera in wild rodents, the first author in Washington,USA and the second in Papua and New Guinea. Threlfall (1969) did not refer to A.tetraptera in connection with the three M.musculus which were included in his survey. Another species of Aspiculuris, Aspiculuris africana was reported from Acomys cahirinus and from Acomys rusatus, by Greenberg (1969); but no Aspiculuris tetraptera.

Three surveys of the parasites of British rodents were published in the 1960's. Sharpe (1964) found a 6.4% infection rate in the 472 Clethrionomys glareolus which he examined. Analysing his results according to sex and age, he found 3.6% of mature females, 9.7% of immature females, 5.3% of mature males and 7.0% of immature males to be infected with A.tetraptera. Sharpe analysed the infection rates with the other parasites recorded from these mice and he attempted to correlate the degree of infection with the habits and the biology of the host. In this respect Lewis's work (1968 a,b,) is more informative, but he did not find A.tetraptera in A.sylvaticus, C.glareolus, and other Welsh small mammals.

Lewis and Twigg (1972) were no more successful in their work on Surrey rodents, nor was Whitaker (1970) who looked at 503 M.musculus and Ow Yang (1971) who did not report A.tetraptera from his examination of the gut contents of 2,500 individual rats of 15 species living in Malasia. Erhardova-Kotrla and Daniel (1970,1971) could not find A.tetraptera, but they recorded Aspiculuris dinniki from Alticola argentata, A.arianica from Marmota caudata and A.rysavyi from Alticola argentata in the Eastern Hindu Kush of West Pakistan.

Thus although for other parasites, several studies have tried to relate infection rates to the biology of the parasite and host involved (Lewis 1968 a,b, Whitaker 1970, Sharpe 1964) for A.tetraptera most reports have been only quantitative. Roman's work in Mus musculus and Thomas's and Sharpe's in C.glareolus remain as the only qualitative studies involving this parasite. Since Aspiculuris tetraptera is very commonly encountered in laboratory mice, it seems somewhat strange that so few workers have been interested in this parasite in wild populations of host animals. It was with the intention of providing information to fill this gap

in our present knowledge of the parasite in wild situations, that this study was embarked upon.

The present chapter will descibe an analysis of the levels of infection in wild M.musculus. Three factors which may have influenced infection rates were investigated; namely, the sex of the host, age and concurrent infection with Syphacia obvelata, the only other helminth recorded from these mice.

TABLE I A list of the reported hosts for *Aspiculuris tetraptera*

Author	Year	Host	Details
Hall M.C.	1916	Mus musculus Apodemus sylvaticus Cricetus cricetus Jaculus jaculus Jaculus sagitta	
Schultz R.E.	1924	M.musculus M.sylvaticus C.frumentarius Hipdaeus arvalis Dipus jaculus Dipus sagitta Apodemus sylvaticus Cricetus cricetus Hipudaeus amphibius Arctomys citellus	
Yorke W. Maplestone P.A.	1926	M.musculus Cricetus sp.	
Baylis	1928	Clethrionomys glareolus	
Elton	1931	Clethrionomys glareolus	
Twort & Twort	1932	Mus musculus (Laboratory mice)	75% infected.
Yamaguti	1935	Rattus rattus alexandrius M.molossinus molossinus	
Vanni V.	1937	Rattus rattus rattus	(cited by Bernard 1963)
Erickson A.B.	1938	Mus musculus	
Chin T.G.	1939	Rats	
Rausch & Tiner	1949	Mus musculus	
Roman E.	1951	Mus musculus	48% ♂ 24% ♀ infected
Li S.Y.& Hsu H.F.	1951	Mus musculus	74.6% laboratory mice infected.
Thomas R.J.	1953	Clethrionomys glareolus	23% infected A.sylvaticus not infected.

Author	Year	Host	Details
Akhtar S.A.	1955	Rattus rattus rufescens Mus musculus	60% infected 57% infected
Grundmann A.W.	1957	Peromyscus maniculatus sonoriensis	2/115 lab-reared infected
Stahl	1959	Rattus norvegicus albinus	
Grundmann A.W. & Frandsen J.C.	1960	Peromyscus maniculatus sonoriensis	(mentioned)
Bernard J.	1963	Mus musculus Rattus rattus alexandrinus Apodemus sylvaticus	10-16% infected 5 rats infected 1 mouse infected
Bernard J.	1964	Rattus norvegicus	
Sharpe G.I.	1964	Clethrionomys glareolus	6.4% infected
Quentin J.C.	1966	Mastomys sp. Praomys jacksoni Praomys tullbergi Thamnomys rutilans	
Dyer W.G.	1969	Peromuscus maniculatus	(mentioned)
Fahmy M.A. Rifaat M.A. Arafa M.S.	1969	Mus musculus	3 mice examined

THE METHODS AND THE PROCEDURES USED

House mice.

The population.

Two populations of house mice were examined. One population lived in the basement of a private house in the Bayswater District of London. The methods used, the results and the conclusions have been reported else where (Behnke & Wakelin, 1973).

The second population sampled was that existing in the corridors, service-ducts and passages, behind and in the cages in the Charles Clore Small Mammals Pavilion at the London Zoo, Regents Park. These mice seemed to live mainly inside the cages with the animals on exibit, but they had free movements in and out of the cages, in the corridor, passages, ventilation system and in the central heating complex. The temperature in the Pavilion was kept relatively constant at 18-22°C., throughout the year, to ensure the well being of the many tropical animals housed in the Pavilion.

The house-mouse population fed by stealing food from the food trays in the cages. There was always an adequate quantity of food available, so the mice were presumed to be well fed.

As far as could be determined, there were only three sources of danger to the mice. Occasionally a mouse was found to be suffering from an undetermined disease. The environment was not such as to exclude any inherent disease agents among the mice, although precautions were taken by the keepers by thoroughly cleaning the service areas with detergents. The artificially created habitats inside the cages, simulating the natural environment of the animals, could not be cleaned

very often as this would have meant altering the landscape design, uprooting the trees, shrubs, rocks etc., also because of the danger of unsettling some of the more timid animals.

It is possible that the animals (cats,dogs,marmosets etc.)housed in the Pavilion, actually disposed of any mice which were foolish enough to approach too near. (I witnessed on one occasion a mongoose eating a mouse.)

Thirdly, the keepers periodically washed out the cages and flooded likely hiding places of the mice in an effort to keep the population down, but as already stated this could not be achieved frequently enough to be of much value in exterminating the mice. An effort had been made previously to poison the mouse population but again this met with little success and at the time of the survey, healthy mice abounded. Any effect which the poison used may have had on the parasite was ignored, it being presumed that the effect was equal on all the groups of mice and thus hardly affected the relative susceptibility of the animals to infection with A.tetraptera.

Trapping

Mice were caught in break-back traps, baited with cheese, bread or both. The traps were set up on Friday evening every week for a period of 3 months between May and July 1971. Traps were inspected and collected early on Saturday morning and the mice were taken to the laboratory where they were weighed and sexed.

Treatment of Captured Animals.

All the mice were deep frozen at -18°C until examination. In each case the colon and the caecum were examined in detail. Every fifth mouse was also examined for the presence of other helminth parasites, but only the two species of pin-

worm were recovered during the study.

Recovery of Worms.

On thawing, the guts were opened under water in petri-dishes, the mucosa was scraped with the edge of a glass slide, and examined under a low power binocular microscope. Worms were recovered with forceps and a pipette. A distinction was made between mature worms (which were then sexed) and larvae. The number of worms and the position of all the parasites in the gut, was recorded.

The allocation of mice to different age groups.

The choice of age categories was not based on a detailed knowledge of the growth rate of the mice. It was decided to use six categories because it was thought that just the two used by previous authors were not sufficient to give a precise picture of the effect age has on infection with A.tetraptera. Whitaker (1970) who examined 270 male house mice and 210 female mice, divided the population into four groups, based on weight. His groups were mice under 10 gm, 10.0-14.9 gm, 15.0 gm -19.9 gm, and 20 + gm. The distinction between the categories in the present study was purely one of weight rather than one suggesting any major developmental differences between the two groups. It is after all an accepted characteristic of mammals that ageing is accompanied by growth and increase in body weight and that this proceeds until after the animal has reached maturity. Since category A was the largest, it was assumed that this corresponded to the plateau region of growth when the rate of increase in weight begins to decline. It is fairly safe then, to suggest that the few heavier animals were the older mice, perhaps the dominant individuals in the colony

The smallest female found pregnant weighed 14 gms., so this weight was chosen as the dividing line between mature and non-mature mice. Previous authors have gone to the trouble of analysing the gonads of every animal examined, in order to determine the state of maturity, but it was decided that such a practice would be far too laborious and time consuming. The same weight distinction (14 gms.) was made between mature and non-mature males. The choice of the dividing line between the various categories was, therefore, one of convenience, corresponding to succesive increments in weight, rather than to morphological and physiological steps in development. It is known, that the availability of food, concurrent infection, injury, social pressure etc., can all exert their influence on the total body weight of an animal and this must be taken into account when interpreting the results.

Field Mice.

Field mice, Apodemus sylvaticus, were obtained from three sources.

1/ Regents Park.

Longworth and break-back traps were laid out in the gardens of Bedford College, Regents Park. Eight field mice were caught in this location.

2/ Palmers Green.

Longworth and break-back traps were also used to catch field mice in the authors garden.

3/ Trent Park College Estate.

Permission was obtained from the Chief gardener (Mr. Matthews) on Trent Park College Estate to lay Longworth Traps in the woods on the estate. Thirteen Longworth traps were used in all. These were laid out on Thursday evenings.

An orange thread was tied in the branches of the shrubs surrounding the trap for easy identification of the exact location of each trap. A mixture of gerbil & hamster food (purchased commercially) was used as bait. A small amount was placed inside the box of the trap and a trail was arranged in the tunnel. Some seeds were also scattered on the ground surrounding the trap.

The traps were examined every evening and every morning until Sunday evening, when they were taken back to the laboratory. During this time, traps which were unsuccessful during the preceeding 12 hrs., were left untouched. A few more seeds were sprinkled in the vicinity of the trap entrance and it was left for a further twelve hours.

Trapped animals were transferred to jars after the trap had been opened inside a polythene bag, whilst the trap itself, was cleaned, rebaited and replaced. All the traps were scrubbed and cleaned with boiling water before another trapping session was attempted.

The animals were killed by an overdose of chloroform and air, weighed, sexed and frozen until an examination of their parasite burden could be undertaken.

RESULTS.

The population of house mice.

A total of 102 mice was caught, of which 45 were males and 57 were female mice. Since the 57 females included 9 pregnant mice, female mice were studied in three groups which consisted of the pooled results from all female mice, non-pregnant mice and pregnant mice (Table 2).

A histogram was constructed to show the number of mice of each sex in every weight range (Fig.1.) Most weight range

groups were reasonably sampled but it was found that there was some disparity between the sexes. Thus whereas 50.8% female mice weighed less than 15 gms., only 37.7% male mice fell into the same category. Although this difference should be considered when evaluating the results it is not thought to have altered the pattern of results significantly.

Infection rates in male and female mice.

The analysis of infection rates is shown in table 3. More male mice were infected than female mice (84.4% and 70.8% respectively, $P<0.05$), but the difference was only just valid. A more significant difference was obtained when the infection rate with mature worms was examined. Whereas 62.2% of male mice were infected, only 41.7% females were similarly infected, a statistically significant difference ($P<0.01$). In general pregnant mice were more heavily infected than non-pregnant mice but the figures were not compared since the sample contained only nine pregnant mice, too small a number to compare accurately with the much larger group of non-pregnant female mice.

A comparison of the mean worm recovery from male and female mice.

There was no significant difference between the worm burdens of male and female mice. The mean worm recovery of mature worms from male mice was numerically almost twice that in females (9.0 ± 18.8 and 5.4 ± 10.7) but there was no significant difference here either.

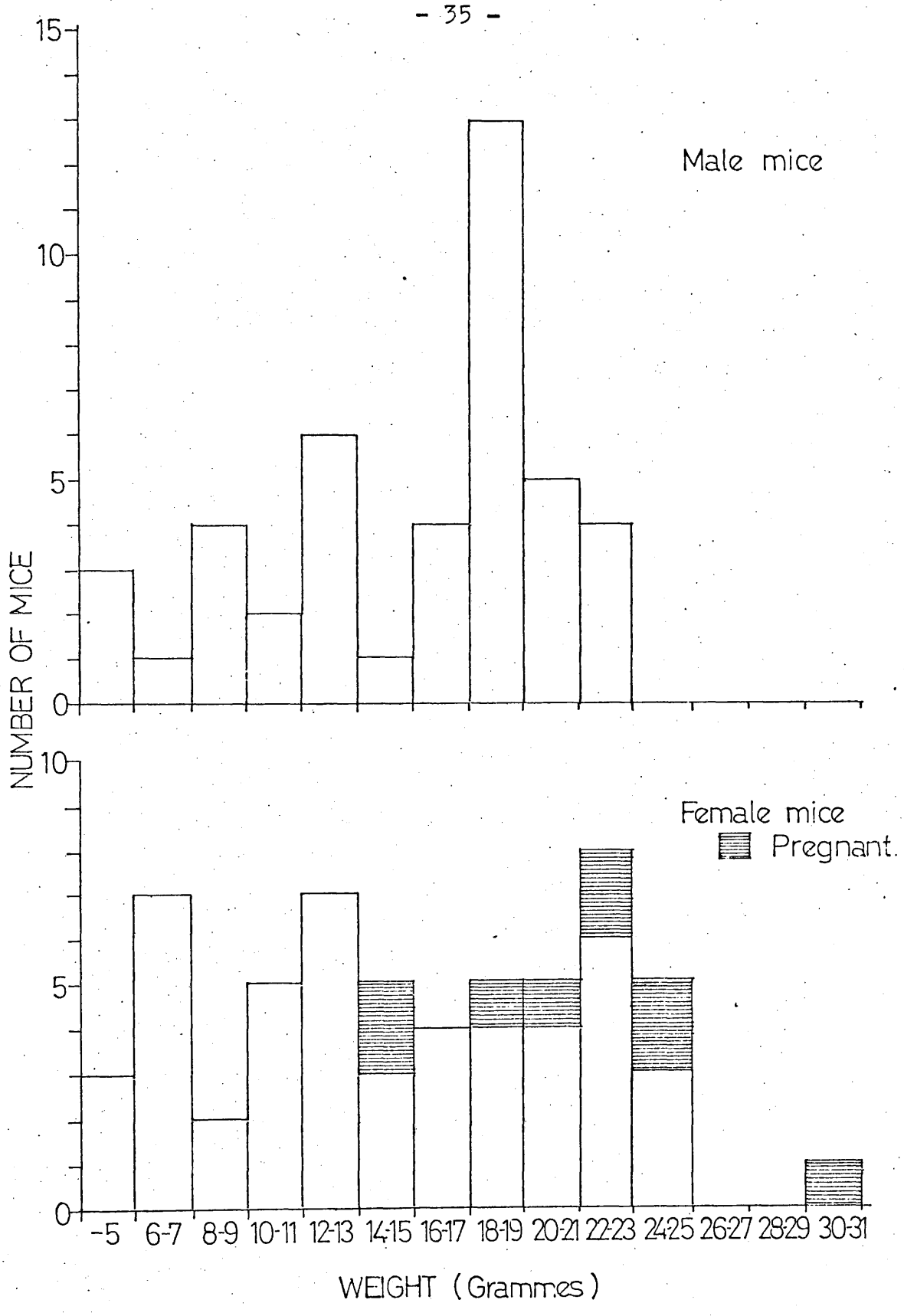


FIG. 1 The weight distribution of mice examined in the survey at the London Zoo.

TABLE 2 DETAILS OF THE HOST POPULATION

Total number of mice caught	102
Number of male mice caught	45
Number of female mice caught	57
Number of female mice, non-pregnant	48
Number of pregnant female mice	9

TABLE 3 THE MEAN WORM RECOVERY AND THE MEAN MATURE WORM RECOVERY IN MALE AND FEMALE MICE

	Male mice	Female mice		
		Total	Non-pregnant	pregnant
Number of mice infected. / Number of mice caught.	38/45	42/57	34/48	8/9
% mice infected	84.4(1)	73.7	70.8(1)	88.9
Mean worm burden ± s.d.	12.4(3) ±19.2	13.1 ±19.9	11.4(3,5) ±18.0	21.9(5) ±27.4
Mean worm burden in infected mice only ± s.d.	14.7 ±20.1	17.7 ±21.3	16.1 ±19.6	24.6 ±27.9
Number of mice infected with mature worms.	28	27	20	7
% of mice infected with mature worms	62.2(2)	47.4	41.7(2)	77.8
Mean mature worm burden ± s.d.	9.0(4) ±18.8	6.8 ±12.2	5.4(4,6) ±10.7	14.3(6) ±17.2
Mean mature worm burden in infected mice only ± s.d.	14.5 ±22.3	14.3 ±14.5	12.9 ±13.4	18.4 ±17.6

Statistical analysis.

1. $P < 0.05$
2. $P < 0.01$
3. P= N.S.
4. P= N.S.
5. P= N.S.
6. P= N.S.

The distribution of infection levels in male and female mice.

Male and female mice were arranged into groups, according to their worm burden as shown in Table 4 & 5 and Figures 2 & 3. There was an almost equal percentage of mice infected with more than eleven worms in both sexes but more female mice were not infected ($P<0.05$) and more males were infected with 1 - 10 worms ($P<0.05$). The distribution of mature worms was similar in that again, there was no significant difference in the number of animals with more than 11 worms in both sexes. Further, the differences in the percentage of mice of both sexes not infected with mature worms and the percentage of males and females infected with 1 - 10 mature worms, was enhanced ($P<0.01$ and $P<0.02$ respectively).

The distribution of the mouse population in six weight range categories.

Pregnant female mice were not considered in this study. The remaining 93 male and female mice were arranged into six categories corresponding to:

Group	W	-	under 5 gms.
Group	Y	-	6 - 9 gms.
Group	J	-	10- 13 gms.
Group	M	-	14 -17 gms.
Group	A	-	18 -21 gms.
Group	O	-	22 + gms.

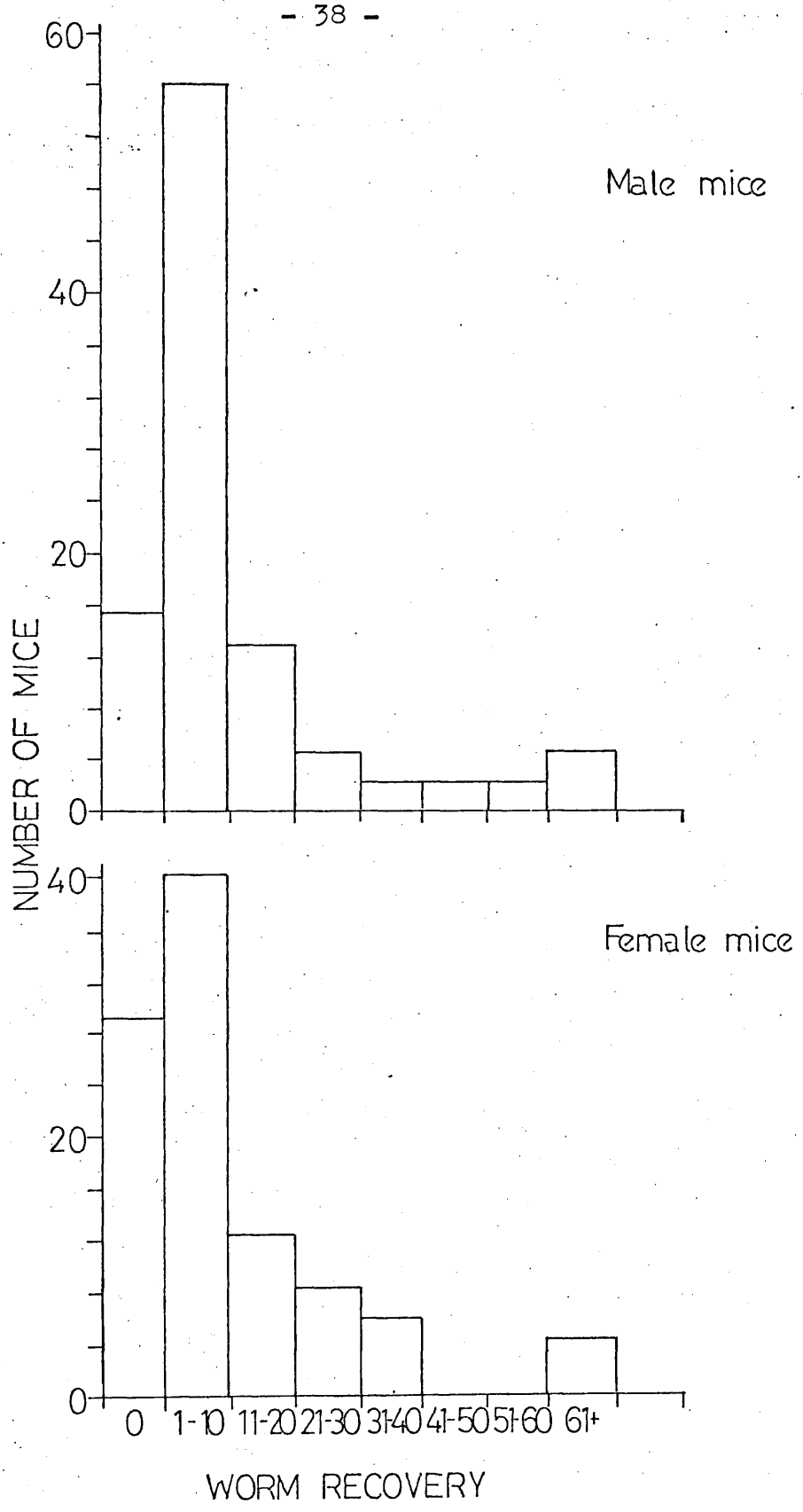


FIG. 2 The percentage distribution of infection levels with A.tetraptera.

Table 4 The distribution of infection levels in the population of house mice

			Number of worms							
			0	1-10	11-20	21-30	31-40	41-50	51-60	61+
Male mice		No mice	7	25	6	2	1	1	1	2
		% mice	15.6(1)	55.6(2)	28.7(3)					
					13.3	4.4	2.2	2.2	2.2	4.4
Female mice	Total female mice	No mice	15	23	6	6	3	-	1	3
		% mice	26.3	40.4	10.5	10.5	5.3	-	1.8	5.3
	Pregnant female mice	No mice	1	4	-	2	-	-	1	1
		% mice	11.1	44.4	-	22.2	-	-	11.1	11.1
	Non-pregnant female mice	No mice	14	19	6	4	3	-	-	2
		% mice	29.2(1)	39.6(2)	31.3(3)					
					12.5	8.3	6.3	-	-	4.2

Statistical analysis: (1) $P < 0.05$ (2) $P < 0.05$ (3) P = N.S.

Table 5 The distribution of mature worm infection levels in the population of mice

			Number of mature worms							
			0	1-10	11-20	21-30	31-40	41-50	51-60	61+
Male mice		No mice	17	19	3	2	1	1	-	2
		% mice	37.8(1)	42.2(2)	6.7	4.4	2.2	2.2	-	4.4
					19.9(3)					
Female mice	Total female mice	No mice	30	16	4	4	1	-	2	-
		% mice	52.6	28.1	7.0	7.0	1.8	-	3.5	-
	Pregnant female mice	No mice	2	4	-	2	-	-	1	-
		% mice	22.2	44.4	-	22.2	-	-	11.1	-
	Non-pregnant female mice	No mice	28	12	4	2	1	-	1	-
		% mice	58.3(1)	25.0(2)	8.3	4.2	2.1	-	2.1	-
					16.7(3)					

Statistical analysis: (1) $P < 0.01$ (2) $P < 0.02$ (3) P = N.S.

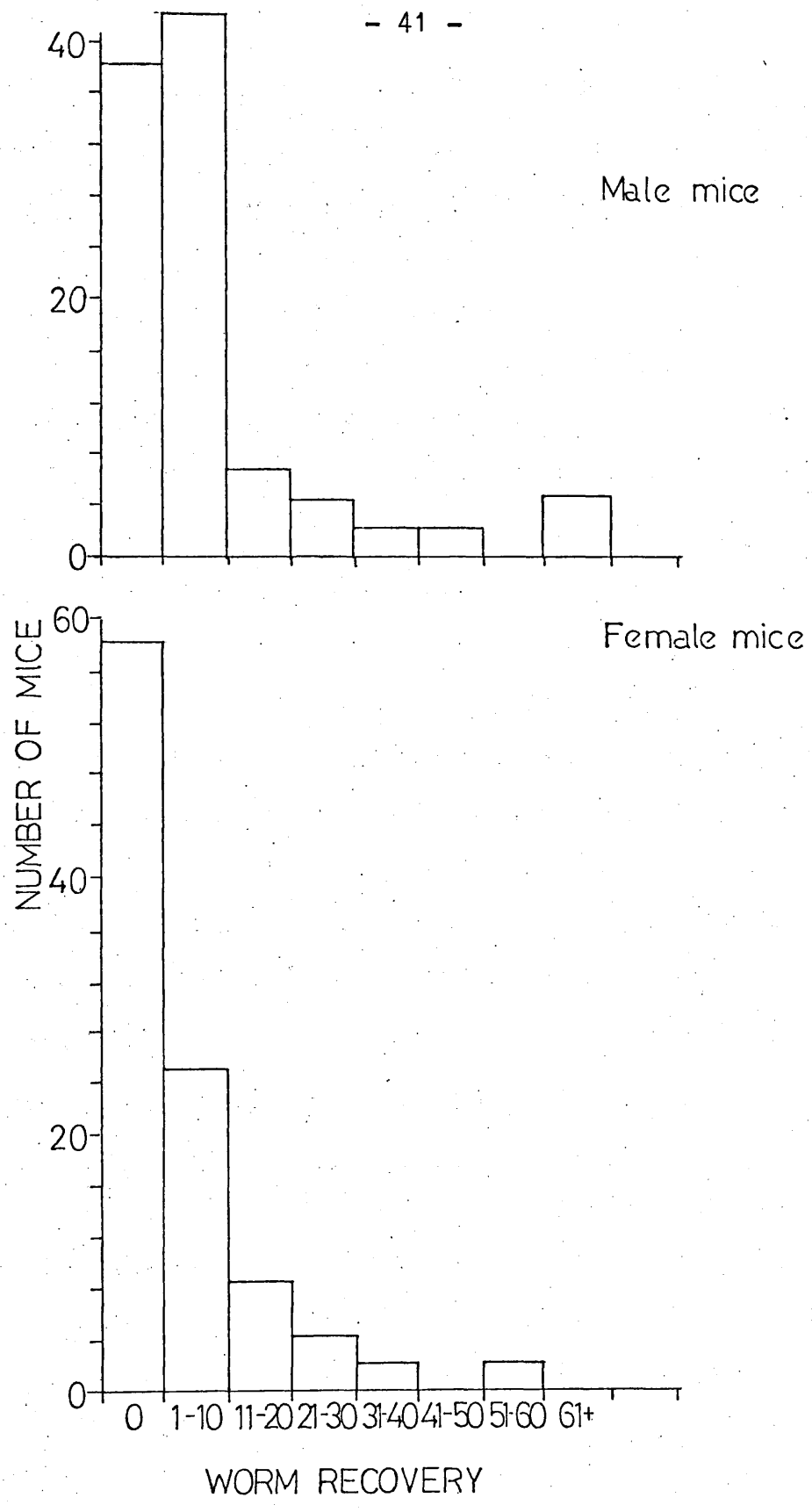


FIG. 3 The percentage distribution of infection levels with mature A.tetraptera.

The intention of this study was to examine the relationship between age and level of infection with A.tetraptera. The groups consisted of 4 gm weight range categories, above and below the 14 gm borderline. For the purpose of this study the following assumptions were made:

1) The six groups represented mice of different ages
2) The lightest mice were the youngest and the heaviest (non-pregnant) mice were the oldest.

With the exception of group W the groups were based on an examination of 13 or more mice (group W-6). Each group, however, was further subdivided into male and female mice, resulting in a rather smaller number of mice (group O-4, group Y and M males - 5 mice each). It is therefore perhaps more significant to base conclusions on the overall infection levels, rather than on infection levels in male and female mice separately. Nevertheless since sex does appear to influence the progress of parasitic infection, all the data pertaining to infection levels in both sexes is included.

The infection rates of mice in the different weight range groups.

Overall, the group with the fewest infected mice was, not surprisingly, group W in which most mice were weanlings, under 5 gms. (Table 6 & 7, figures 4 & 5). Only two of the six mice were infected (1 & 9 larvae respectively) with larval worms. Mice of this age would not be expected to carry patent infections, since, assuming the situation is comparable, laboratory mice can be infected only after 14 days of age and A.tetraptera infections become patent 24 days post infection. The overall infection rate in the remaining categories was high, 57.1% or more in all groups. The infection

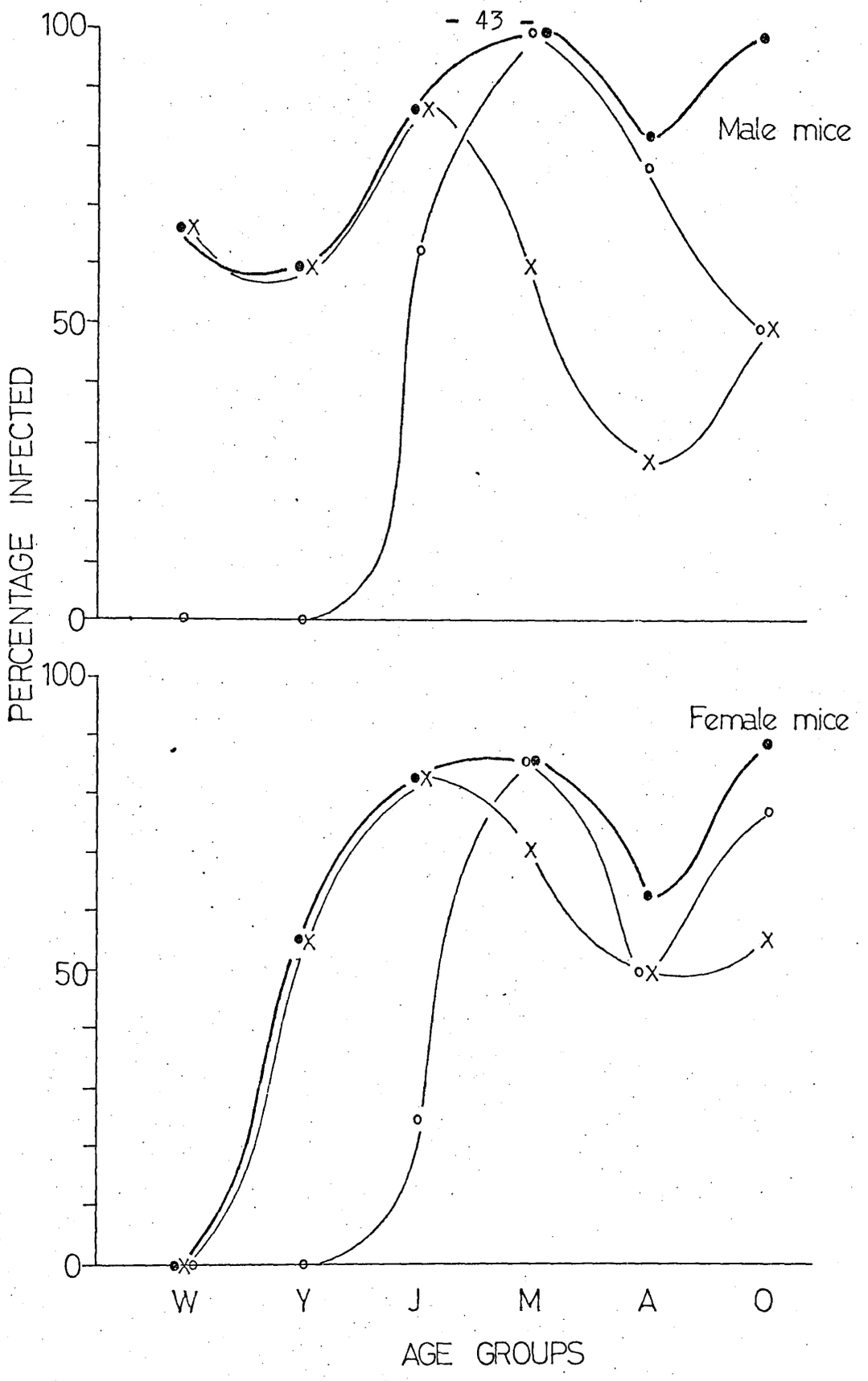


FIG. 4 A comparison of the overall percentage infection rate (•—•), the percentage infection rate with mature (∘—∘) and juvenile (x-x) worms of mice in different age groups in male and female mice.

TABLE 6 THE EFFECT OF AGE OF HOST ON THE RATE OF INFECTION WITH ASPICULURIS TETRAPTERA

Group	W	Y	J	M	A	O
No.female infected	0	5/9	10/12	6/7	5/8	8/9
No.males infected	2/3	3/5	7/8	5/5	15/18	4/4
% female infected	0	55.6	83.3	85.7	62.5	88.8
% male infected	66.7	60.0	87.5	100.0	83.3	100.0
% female infected juvenile worms	0	55.6	83.3	71.4	50.0	55.5
% male infected juvenile worms	66.7	60.0	87.5	60.0	27.8	50.0
% female infected mature worms	0	0	25.0	85.7	50.0	77.8
% males infected mature worms	0	0	62.5	100.0	77.8	50.0

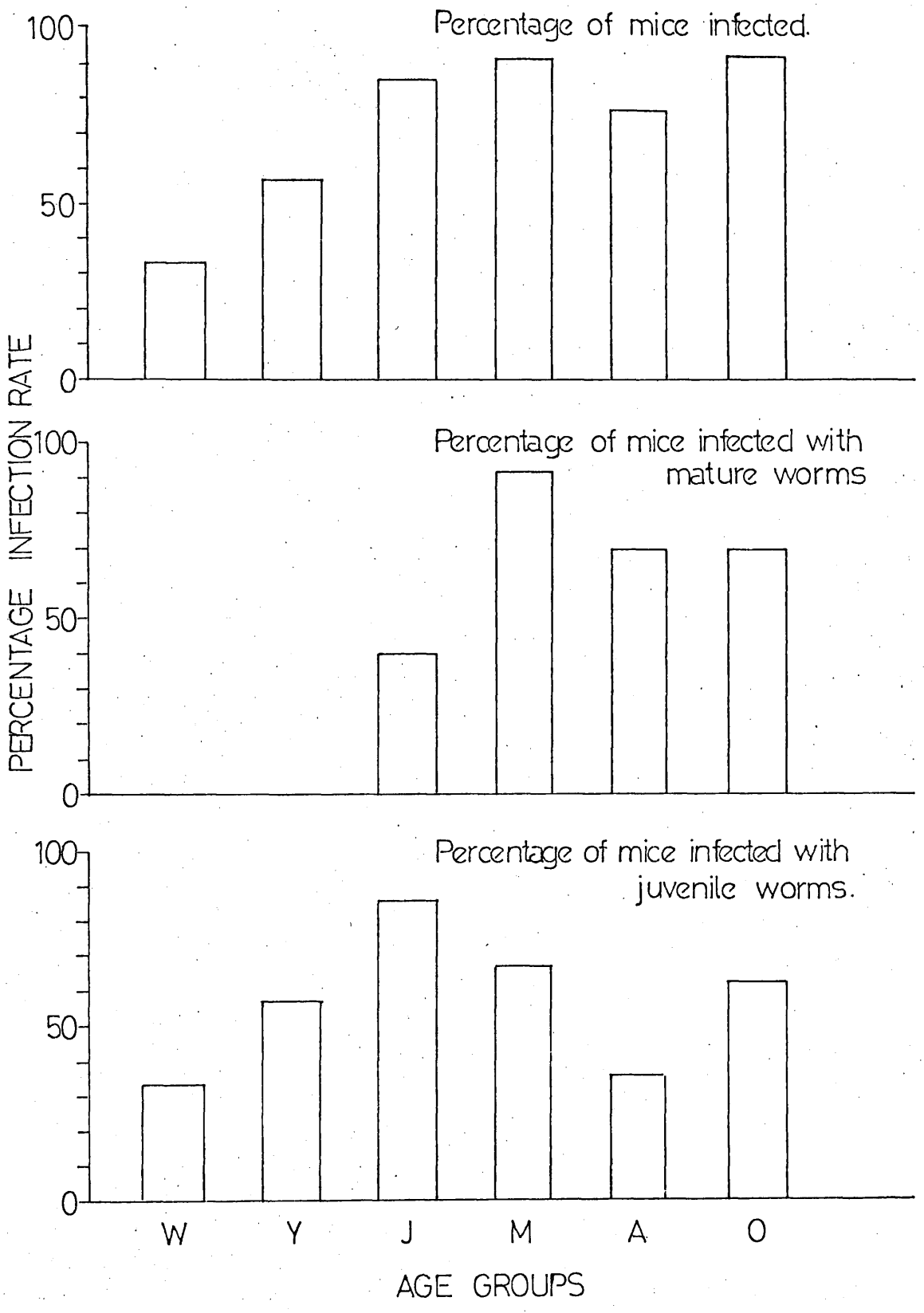


FIG. 5 A comparison of the overall percentage infection rate, the percentage infection rate with mature and juvenile worms of mice in different age groups.

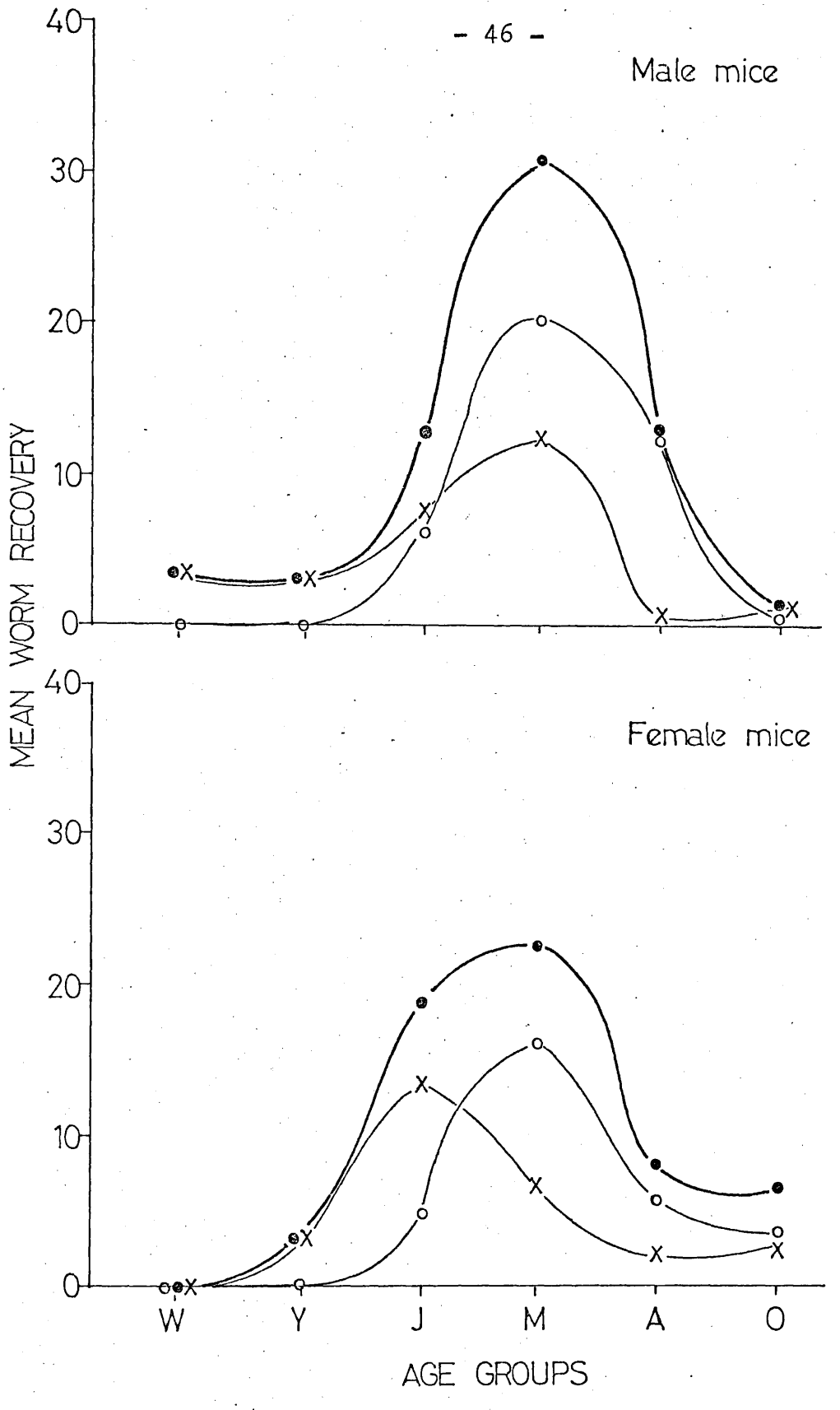


FIG. 6 A comparison of the overall (•—•), the mature (○—○) and juvenile (x—x) mean worm recovery of mice in different age groups in male and female mice.

TABLE 7 THE EFFECT OF AGE ON THE OVERAL INFECTION RATE AND THE RATE OF INFECTION WITH JUVENILE AND MATURE WORMS

Group	W	Y	J	M	A	O
Number of mice	6	14	20	12	26	13
No.male mice	3	5	8	5	18	4
No.female mice	3	9	12	7	8	9
% mice infected	33.0	57.14	85.0	91.7	76.9	92.3
% mice infected mature worms	0	0	40.0	91.7	69.2	69.2
% mice infected juvenile worms	33.0	57.14	85.0	66.7	34.6	61.5

rate with juvenile worms reached a peak in groups J and M, which, as mentioned before, correspond to mice only just sexually mature. There is a slight drop in the percentage infection rate with juvenile worms in older mice and this is interpreted as the result of resistance to super-infection in older mice.

The mean worm recovery (m.w.r.) in mice from the different weight range groups.

The tendency to age resistance is clearly exemplified by an examination of the mean worm burden. (Table 8, Figures 6 & 7) Before such an examination is undertaken, however, the statistical approach must be explained.

In table 4, it was shown that in wild house mice, only 28.7% males and 31.3% of non-pregnant females were infected with more than 10 worms. There were individual mice with as many as 60 worms and more, but the majority of the population was either non-infected or carried a very small infection. The distribution of mice, based on the infection levels, cannot therefore be a normal distribution since the mode of this distribution is bound to be nearer to the zero level of infection rather than to the higher infection levels. Special methods are available to analyse such a skewed distribution. If a Students t test is used, a generous allowance must be made in the critical value of t, before assigning significance to a comparison. As can be seen in Table 8 the standard deviations are greater than the means in most groups indicating that besides the mice with low infection levels, there were individuals with much higher m.w.r.s.

As expected, the highest m.w.r. came in groups J and M. This trend was consistent in all the m.w.r. analyses undertaken,

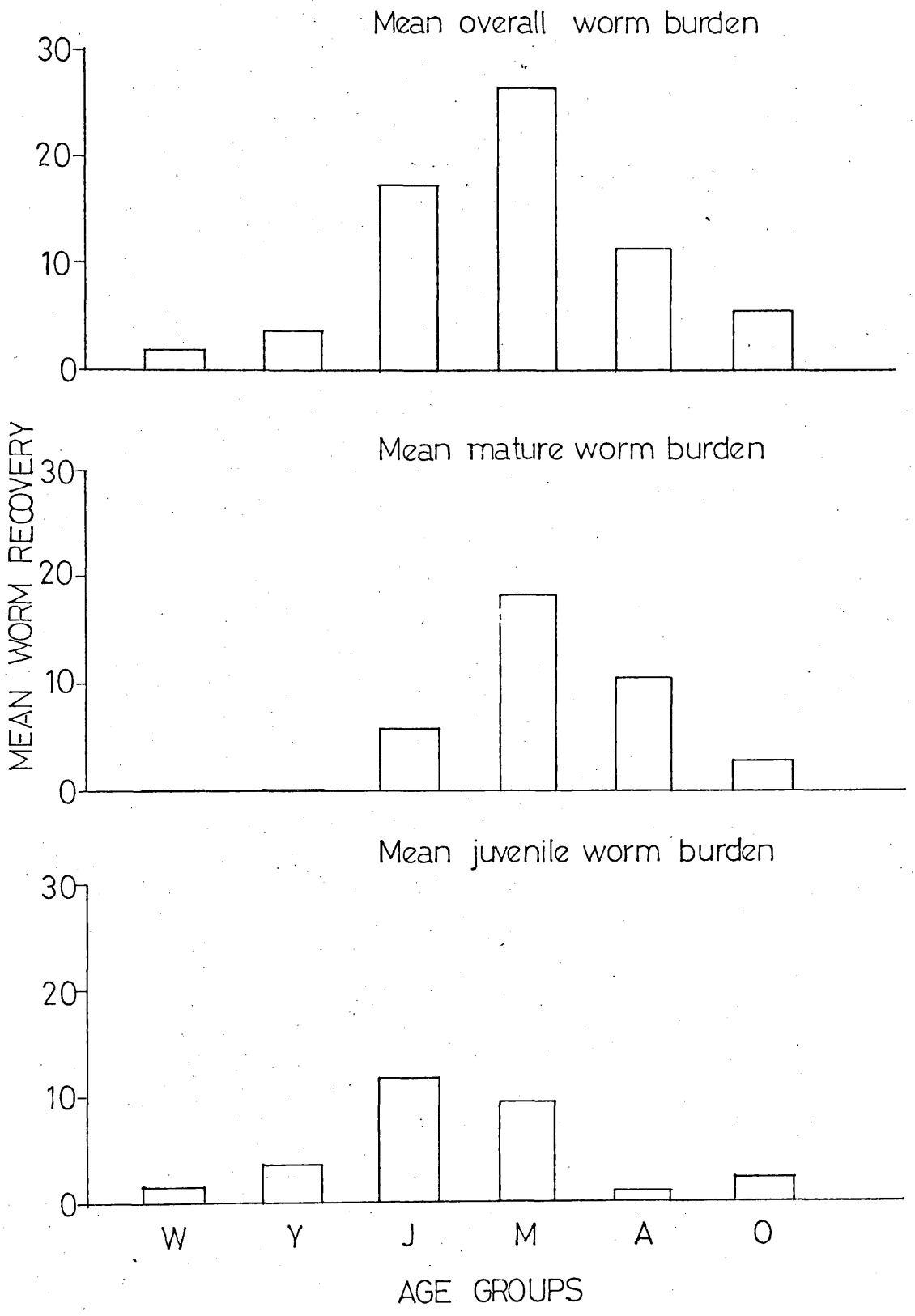


FIG. 7 A comparison of mean overall, mature and juvenile worm burden of mice in six different age groups.

TABLE 8 THE EFFECT OF AGE ON THE MEAN WORM RECOVERY IN MALE AND FEMALE MICE

Group	W	Y	J	M	A	O
Mean worm burden	1.7 ±3.6	3.4 ±4.1	17.1 ±17.3	26.5 ±30.5	11.5 ±19.1	5.1 ±8.3
Mean mature worm burden	0 0	0 0	5.6 ±10.2	18.1 ±25.3	10.4 ±18.8	2.9 ±5.7
Mean juvenile worm burden	1.7 ±3.6	3.4 ±4.1	11.5 ±10.1	9.3 ±18.6	1.1 ±2.8	2.2 ±3.3
Mean worm burden in female mice	0 0	3.7 ±4.6	18.9 ±21.6	23.1 ±29.9	8.1 ±12.1	6.8 ±9.7
Mean worm burden in male mice	3.3 ±4.9	3.0 ±3.7	14.3 ±8.1	31.2 ±34.2	13.0 ±21.7	1.3 ±0.5
Mean mature worm burden in females	0 0	0 0	5.1 ±11.5	16.3 ±17.8	5.9 ±8.1	4.0 ±6.6
Mean mature worm burden in males	0 0	0 0	6.4 ±8.5	20.6 ±35.7	12.4 ±21.8	0.5 ±0.6
Mean juvenile worm burden in females	0 0	3.7 ±4.6	13.8 ±11.4	6.9 ±13.0	2.3 ±4.8	2.8 ±3.8
Mean juvenile worm burden in males	3.3 ±4.9	3.0 ±3.7	7.9 ±6.9	12.6 ±26.0	0.6 ±1.1	0.8 ±0.9

whether the m.w.r. in male and female mice, overall m.w.r., juvenile m.w.r.or mature m.w.r. was examined. The lightest (youngest ?) groups had lower m.w.r.'s as did the heaviest (Oldest?) groups. Thus in the case of the overall, m.w.r., a value of 26.5 ± 30.5 was recorded in group M and a corresponding drop to 5.1 ± 8.3 was found in group O. A similar drop was observed in all the other groups studied, the most spectacular being the m.w.r. in male mice, where group M had a m.w.r. of 31.2 ± 34.2 which subsequently fell to 1.3 ±0.5 in group O.

Another feature of interest was the weight (age) of mice acquiring a peak level of infection with juvenile worms. The maximum value for the overall juvenile m.w.r. was in group J, one group earlier than the maximum for mature worms. This trend was similar in female mice considered alone, but it was not so in male mice.

The effect of concurrent infection with Syphacia obvelata.

The house mice in this population were not only infected with Aspiculuris tetraptera, but also with Syphacia obvelata. Stahl (1966 b) reported that in experimental concurrent infections, neither parasite survived as well as in single infection controls. It was decided to test the validity of his finding in a wild population of the host, known to contain both parasites. Four tests were used to examine the situation.

Mean Aspiculuris tetraptera recovery in mice infected with various levels of S.obvelata.

The mice were arranged into seven groups corresponding to their infection level with A.tetraptera (Table 9). These groups were 0, 1-5,6-10,11-20,21-30,31-40, and 41 + S.obvelata worms. For each group the mean A.tetraptera recovery was cal-

culated. Table 9 shows that there is no obvious relationship, indicative of an antagonistic effect between the two species.

X^2 test with 2 x 2 contingency table analysis.

The number of S.obvelata infected mice, infected and uninfected with A.tetraptera was compared to the number of mice (uninfected with S.obvelata) infected and uninfected with A.tetraptera.

		Syphacia obvelata		
		Present	Absent	Total
Aspiculuris tetraptera	Present	48	32	80
	absent	14	8	22
	Totals	62	40	102

$X^2 = 0.3 =$ Non - significant.

Calculation of the correlation coefficient.

The coefficient of correlation was calculated as

$r = 0.114 =$ Non - significant.

The coincidence, and the presence and absence of one or other of the two parasites was found to be random.

TABLE 9. The relationship between Aspiculuris tetraptera and Syphacia obvelata in concurrently infected mice.

Aspiculuris tetraptera	No mice	Syphacia obvelata mean worm recovery Mean ± s.d.
0 worms	40	12.2 21.7
1-5 worms	18	9.4 14.9
6-10 worms	13	19.2 24.5
11-20 worms	10	12.2 24.5
21-30 worms	4	3.8 2.5
31-40 worms	5	14.8 9.8
41+- worms	12	16.3 17.1

Calculation of the degree of convergence (Sab).

(Kisielewska, 1970c; Tarwid, 1960.)

$Sab = \frac{Nab}{Nt}$ where Sab = Index of convergence
Nab = No.animals concurrently infected
Nt = Expected No.animals concurrently infected.

$Nt = \frac{Na \times Nb}{N}$ where Na = No.infected species a
Nb = No.infected species b
N = total No.animals

Thus

$$Nt = \frac{62 \times 80}{102}$$

$$Sab = \frac{102 \times 48}{62 \times 80}$$

$$Sab = 0.987$$

$$= 1$$

Sab < 1 indicates both species do not occur together
Sab =1 distribution is random, i.e. independant.
Sab > 1 both species occur together more often than individually.

Antagonistic effects can be found when Sab < 1, one species completely excluding the other or in situations where one parasite is reduced quantitatively in favour of the other, whence the index Sab can be positive.

Field mice.

1) Regents Park.

A total of eight male *Apodemus sylvaticus* were caught in Regents Park. The only parasite, besides the very common *Syphacia stroma*, recorded from the caecum and colon and *Nematospiroides dubius* from the small intestine, was one female *Trichuris muris* found in the caecum.

2) Palmers Green.

Five *Apodemus sylvaticus* were caught in the garden of

a private household in Palmers Green, Aspiculuris tetraptera was not recorded.

3) Trent Park College Estate.

Most field mice caught at Trent Park were used for experimental work reported in Behnke & Wakelin (1973) and also in Chapter twelve. A total of 26 mice was examined for the presence of Aspiculuris tetraptera and Trichuris muris. Neither parasite was found in these mice.

Several C.glareolus were also studied. One vole which was used in Expt.14 was found to contain three mature worms which could not have originated from the experimental infection given three days previously or from infective eggs picked up in the laboratory, since the voles were caught at Trent Park a week earlier. It was concluded that these three worms must have represented a natural infection from the Trent Park College Estate. One other vole, C.glareolus, was found to have five mature worms, also presumed to have originated at Trent Park.

DISCUSSION.

The fact that the progress of a parasitic infection can be influenced by the sex of the host is well documented in the Literature, (Solomon,1969; Stahl,1962; Mathies,1959 b; Haley,1958). Whitaker (1970), considering the overall parasite burden in house mice, irrespective of the species involved, found that there was no difference in the percentage of male and female mice infected. When he examined the mean number of parasites per mouse, however, there were more parasites in male than in female mice. Unfortunately Aspiculuris tetraptera was not identified in Whitaker's survey. Lewis,1968, reported that male Apodemus sylvaticus were more susceptible

than females to infection with Nematospiroides dubius, Syphacia stroma and Corrigia vitta. In the case of N.dubius, the percentage infection was high and not dissimilar in both sexes, but the mean worm recovery from male mice was higher. A sex difference in the incidence of A.tetraptera in wild hosts has been reported twice. Roman (1951) found that more male mice were infected and Sharpe (1964) found that the incidence of this parasite in mature Clethorionomys glareolus was greater in male voles than in females, but the actual figures show only a minimal difference to support this conclusion.

These last two reports are in agreement with the present work since the sex difference in the incidence of the parasite in house mice was found to be statistically significant ($P<0.05$). Moreover a greater percentage of male mice were infected with mature worms. The mean worm recovery nevertheless, was very similar for both sexes. The most informative analysis is presented in Table 4 & 5, where the frequency distribution of overall and mature worm-burdens is compared. The worm burden was greater than 11 in an equal percentage of both sexes suggesting that the similar mean worm recoveries for male and female mice were due to an equal distribution, between the sexes, of individual mice with high worm burdens. After all, a single heavy worm burden is more likely to noticeably alter the mean worm recovery, than a low one. In other words the m.w.r. is more an expression of the incidence of high worm burdens in the population, than a true representation of the typical worm burden encountered in most of the mice. Since this incidence was similar in both sexes it follows that the m.w.r could not be expected to dif-

fer significantly. Whitaker (1970), who found that male mice had more Heligmosoides polygyrus (1.79) than female mice (0.78), attributed this difference primarily to three males with particularly heavy infections (40, 58, and 100 worms).

When the remaining two categories on the frequency distribution graph were compared, it was discovered that there were more female mice with no infection (in both the overall m.w.r. and mature m.w.r. comparisons)and conversely there were more males in 1-10 worms group (in both overall m.w.r. and mature m.w.r. comparisons). This was interpreted as signifying that more male mice became infected and more worms matured in male mice, providing that the total worm burden did not exceed 10 worms. Although the difference in percentage of non-infected male and female mice (considering overall m.w.r.) was significant, when the mature m.w.r.'s were examined the difference was exaggerated suggesting that not only fewer female mice became infected with juvenile worms, but that more of these infected mice overcame the parasitic infection and prevented the worms from reaching maturity. The presence of heavy worm burdens in almost a third of male and female mice indicated that these particular individuals were affected equally in both sexes by either exogenous or endogenous factors which enhanced their susceptibility to infection.

Although Lewis could find no substancial difference in the incidence of parasites in pregnant mice, and Dunn and Brown (1962) failed to demonstrate a difference in the worm burden of pregnant, lactating and control mice infected with A.tetraptera, it is known that lactation has a profound influence on the immune mediated spontaneous self-cure in some parasitic infections (Dineen and Kelly,1972; O'Sullivan and

Donald 1970; Connan 1967, 1968,1970,1972,1973). Behnke and Wakelin (1973) attributed the high Trichuris muris worm burden found in female mice to the effects of lactation. In the present study only nine mice were pregnant but of these nine, eight were infected (88.9%), 4 heavily and m.w.r. was greater than in either males or non pregnant females (21.9±27.4; 12.4 ± 19.2; 11.4 ± 18.0 respectively). If lactation is one factor affecting infections in female mice, then others may include social stress, hunger, concurrent infection, or debilitation resulting from any one or more of these conditions.

Age resistance, like sex resistance, is not a new concept in medical and parasitological literature (Africa,1931; Ackert et al,1939; Mathies,1959 a; Stahl,1962; Murray et al, 1971; Gray,1972).

Whitaker (1970) subdivided the population of house mice into four age groups. He found that an increase in the incidence of internal parasites correlated positively with an increase in weight (age). For Heligmosomoides polygyrus, for instance, the percentage infestation altered from 9.1% to 16.5%, 23.5% and 47.8% in the oldest mice. Sharpe (1964) on the other hand, identified an opposite trend in C.glareolus infected with A.tetraptera. The incidence of infection was greater in immature animals (male -7.0%, female -9.7%) than in mature voles (male - 5.3%, female 3.6%). A similar tendency was identified in voles infected with Catenotaenia although the reverse was true for A.sylvaticus infected with the same parasite.

Here, the method of estimating age by weight, was equivalent to that used by Whitaker, but the population was subdivided into six categories whereas Whitaker only used four.

TABLE 10. THE INCIDENCE OF VARIOUS PARASITES IN CLETHRIONOMYS GLAREOLUS AND APODEMUS SYLVATICUS (Sharpe 1964)

	Percentage infection				
	Mean	Immature animals		Mature animals	
C.glareolus		♂	♀	♂	♀
A.tetraptera	6.4	7.0	9.7	5.3	3.6
Heligmosomoides	4.5	3.0	4.4	4.7	5.9
Catenotaenia	18.6	26.0	30.9	8.8	13.1
A.sylvaticus					
Syphacia	54.6	56.9	57.4	62.5	40.8
Heligmosomoides	29.5	18.0	12.7	39.8	39.4
Catenotaenia	6.8	2.8	2.1	9.1	12.7

It was found that only in group W, was the incidence of infection low. The youngest mice infected with mature worms were in group J, although both younger groups (W and Y) were to a lesser extent infected with juvenile worms. The maximum levels of infection were noted in groups J and M, corresponding to mice just before and after maturity. There was a fall subsequently in the infection levels in older mice, groups A and O. This trait was confirmed by an examination of the mean worm recoveries for all six groups. The highest m.w.r. was recorded in group M, and there was a corresponding drop in m.w.r in group A. and O. Not at all surprising was the discovery that the juvenile m.w.r. was higher in younger mice, than the mature m.w.r. Juvenile worms were acquired by the young mice, reaching a peak level in group J, before falling to a lower value in the older mice, groups M, A and O. The peak for mature worms (group M) followed closely that for the

larvae but it took longer to fall to the low level of larval worms in groups A and O.

If the interpretation for the relationship between individual heavy worm burdens and m.w.r. is correct, then it would follow that one might expect all the heavy worm burdens to exist in those groups with the highest m.w.r. and indeed, such is the case, since almost invariably the highest individual worm recoveries were recorded in groups J and M.

Mathies (1959 b) suggested that age resistance may be associated with a greater secretion of oestrogenic hormone at the time of maturity and thereafter. It is equally feasible that immune acquired resistance may be the critical factor, but previous authors have rejected the possibility that such a response exists in this host-parasite relationship. (Mathies,1962, Stahl,1966 a). The theory of acquired resistance could favourably account for the phenomena observed in this study. Thus juvenile worms were acquired by young mice until a critical level (a threshold value) was arrived at in group J, when mice were becoming sexually mature. The presence of mature worms and large numbers of larvae in these mice conceivably triggered events which ensured that in all the older mice the quantity of incoming juvenile worms was reduced. This could have been accomplished by the generation of an immune mediated, or possibly innate natural acquired resistance. The persistance of mature worms for a longer time suggests that these worms were resistant to the host response, senescence, conceivably accounting for their final disappearance from the host. The presence of mature worms in mice apparently resistant to reinfection in the older groups, suggests that concomitant immunity may be of importance here or alternatively non immune age resistance may be preventing the establish-

ment of juvenile worms.

Kisielewska (1970 a,1970 b,1970 c) described competition between different species of parasites in the same host. She found that two species which 'avoided' one another were Capillaria muris sylvatici and Mastophorus muris in C.glareolus (Kisielewska 1970 c). The index of the degree of convergence was only 0.3. Kisielewska reported further examples of interspecific competition, mainly between nematodes of the genus Heligmosomum and the Cestoda in the small intestine. Earlier authors had already noted some of these observations (Rupes,1964; Mozgovcj et al,1966; Tenora,1967; Kisielewska, 1970 a,1970 b). One species which appeared not to compete with any of the other parasites was Syphacia obvelata. This is probably due to the absence of other species in the caecum and the colon of the voles analysed during the survey. Stahl, 1966 b, however, showed that under experimental conditions, an antagonistic effect could be demonstrated between S.obvelata and A.tetraptera, the parasite which established first being the more successful. Keeling,1961, reported antagonism between Trichuris muris and A.tetraptera, but no one has as yet examined the possibility that A.tetraptera may be competing with other parasites in nature. Since the population of house mice at the London Zoo was infected with A.tetraptera and S.obvelata it was considered of interest to examine the worm burden for evidence of inter-action between the two species.

Four tests were carried out in an attempt to clarify this situation, but all failed to detect any relationship other than a random coincidence of the parasites in the host population. Stahl used fairly heavy worm infections and it

could be that precise, accurate infection levels are necessary to detect antagonism between these species. If neither species is dominant then the acquisition of both parasites by mice, at an equal rate, would prevent either from excluding the other. Whatever the inconsistency between this survey and the experimental work of Stahl, it is clear that in nature, neither of the two pinworm parasites of mice suffers at the expense of the other. Both appear to be equally successful in the same host population.

SECTION 3

CHAPTER THREE

Materials and methods

PROCEDURES INVOLVED IN ANIMAL HUSBANDRY

Housing and strain of mice.

Unless otherwise stated three strains of mice were used throughout this work. CFLP mice were obtained commercially from Carworth Europe Ltd., Huntingdon. A small randomly breeding colony of mice, originally CFLP was maintained in the animal room. These animals will be referred to as CFLPa and CFLPb mice. Naturally infected TFI male mice were killed to provide worms from which eggs were obtained for the preparation of infective material. These mice were purchased from A.Tuck & Son Ltd., Rayleigh,Essex.

CFLP and CFLPb mice were kept in MBI cages (North Kent Plastic Cages Ltd., Dartford, Kent) on an MBI Rack. The maximum number of animals kept in one cage did not exceed 12. Two groups of breeding CFLPb mice were kept in RCI cages. M 1 cages were used to keep smaller groups of mice (not exceeding 5 animals).

TFI mice were housed in a single RCI cage which was kept in a corner of the main laboratory. Because these mice were naturally infected with *Syphacia obvelata*, *Hymenolepis nana nana* and *Aspiculuris tetraptera*, it was important to ensure that they did not come into contact with the helminth free and the experimentally infected mice maintained in the animal room.

Mice were kept on sawdust litter and were given access to pellet food (modified diet 41 B - Oxoid) and water *ad libitum*. Two drugs were given to mice via the drinking water.

When dimetridazole (May and Baker) was used to treat trichomaniasis. 10 litres of a 0.1% solution were made up twice weekly and the drinking water of the entire mouse colony was changed for the freshly made solution on each occasion.

Terramycin (Pfizer of Kent), an antibiotic given to mice on immunosuppressive treatment, was dissolved in a concentration of about 0.04% in the drinking water and was changed every 2 days.

The sawdust was changed daily, except on Sundays and Bank Holidays when the cages were cleaned at least on every second day. On the completion of an experiment the cages were washed with detergent and warm water and were allowed to dry before being used again. Food from disbanded cages was rejected.

On various occasions during the course of this study, experimental locusts, cockroaches, stick-insects, midges and other insects were present in the same animal room. Rabbits, gerbils and rats were also housed in the animal room at various times.

The lighting arrangements were connected to an automatic photoperiod switch which gave 12 hours of light and 12 of dark.

Maintenance of the breeding colony.

A small breeding colony of CFLPb mice was maintained in the animal room. Two large cages were used to keep male and female mice together. Once weekly (Usually on Mondays) these cages were examined for pregnant females, which were removed and housed individually in small M1 cages. The female and the young mice were transferred to the larger cages soon after the birth of the litter. If two or three litters were born within 24 hours of each other they were pooled in

groups of two-three. The number of litters kept together never exceeded three.

Eighteen to twenty day old mice were separated into groups of 12 animals of each sex, and larger groups of young mice, born within the space of a week, were pooled at this stage. Mothers were kept with the young mice until they were 4 weeks old, before being, themselves, returned to the breeding cages. Female mice which destroyed their litters for various reasons were eliminated from the colony.

Problems connected with husbandry.

One of the major problems, encountered during this study was the maintenance of non experimental animals free from infection.

Since the eggs of *Aspiculuris tetraptera* become infective a week after leaving the host, particular care was taken to ensure that no sawdust remained in the cages after cleaning. The eggs of *Syphacia obvelata*, however, are infective within hours of appearing on the anal surface of the mouse and once established, it was very difficult to eliminate this parasite from the colony.

TFI mice were almost invariably infected with *S.obvelata* and because of this, there was a constant possibility that infective eggs might find their way into the animal room. On a number of occasions, the parasite was detected in young mice. The procedure adopted was to dose all the non-experimental mice in the colony with piperazine citrate (500 mg/kg b.w-t.) *per os*. However, if the parasite persisted, these mice were dosed regularly until all the experiments were completed.

Hymenolepis nana appeared sporadically in the colony. It was most probably introduced from the TFI mice but it

never persisted in the animal room for very long. No particular treatment was used specifically to remove this parasite.

Procedure involving the host.

Injections.

Subcutaneous - Hydrocortisone acetate was injected subcutaneously under the dorsal loose skin. 23 g 1" sterile disposable syringe needles were used for this procedure.

Intra-peritoneal - Freund's complete adjuvant was injected intra-peritoneally with a 23 g 1" sterile disposable syringe needle. Serum was injected in the same manner using a 27 g ½" permanent syringe needle.

Intra-dermal - Intra dermal injections of experimental serum in PCA tests was achieved using a size 3-Record syringe needle and Record 0.5 ml syringe.

Intra-muscular - Sodium methotrexate was injected intra muscularly with a 27 g ½" permanent syringe needle.

-Oral - A blunted 1g 35 cm syringe needle was used to treat mice with Indocid suspended in milk and also to administer infective material.

For subcutaneous, intra muscular and oral injections mice were firmly held by the loose neck skin with the thumb and 1-st finger. The third finger was used to clamp the tail to the palm of the hand in order to restrain the animal.

Intra muscular injections into the hind limb,were carried out while the animals were under a light ether anaesthesia.

Anaesthesia.

Ether anaesthesia - Laboratory mice were anaesthetised by placing them in a jar containing cotton wool soaked in diethyl ether. The animals recovered very quickly when

the source of the anaesthetic was removed.

Apodemus sylvaticus, Clethrionomys glareolus and Microtus agrestis were anaesthetised by placing cotton wool soaked in ether directly into the cage, whilst preventing the dispersal of the anaesthetic by enclosing the cage temporarily in a plastic bag. The mice soon became unconscious. They were then removed, treated and transferred to a fresh cage. This procedure was also adopted when cleaning the cages of wild animals since the usual methods could not be used.

Trilene anaesthesia - Trichloroethylene (Trilene - British Drugs House) was used to anaesthetise mice during laparatomy, gonadectomy and splenectomy operations.

Irradiation.

Mice for irradiation were placed individually into 50 ml polythene centifuge tubes with screw-on tops. These tubes were specially prepared, and contained a series of holes small enough to prevent the escape of the animals but large enough to allow air to pass inside and not to prevent the passage of electromagnetic waves. Tubes containing mice were placed on the target table of the source at a distance calculated to give the mice a dose of 25 Rads/min. The required dose was then administered by exposure to the radioactive source for the corresponding time. The machine used to carry out this procedure was housed in the medical physics department of the Middlesex Hospital Medical School in Mortimer Street, London W.1. The source of irradiation was Cobalt 60.

Drugs and agents used.

Methotrexate - was used as sodium methotrexate obtained from Lederle Laboratories Division, American Cyanamid Company, in 5 mg/vial, reconstituted with 2 ml. of distilled,

sterile,water.

Hydrocortisone - was purchased as Hydrocortisyl from Roussel Laboratories.

Indomethacin - was purchased in 200 ml bottles as Indocid, from Merck Sharp and Dohme Limited, in a 25 mg/5 ml suspension.

The ALS - used, was Wellcome Anti-Mouse Lymphocyte Serum, raised in horses by repeated injection of mouse thymocytes.

Freund's complete adjuvant - was obtained in vials from Difco laboratories in Surrey.

Rabbit anti Mouse IgG - was obtained from Dr.Wakelin (Glasgow) originally from Cappel Laboratories, Downingtown, P.A.19335.

Rabbit anti Mouse immunoglobulin serum - was also obtained from Dr.Wakelin, (originally from Wellcome Reagents Limited).

Histology.

Worms for histological examination were fixed either in Bouin's fixative or in buffered formol-saline. Pieces of intestine were cut to no more than ½ cm., lengths before being placed in the fixative.

The tissue was dehydrated in ethyl alcohol, cleared in toluene or xylene, embedded in paraffin wax and sectioned at 6-8μm . The sections were then stained in Haematoxylin and Eosin.

PROCEDURES INVOLVING THE PARASITE .

Preparation of Infective material.

Aspiculuris tetraptera - As explained before, worms for the preparation of infective material were obtained from

TFI male mice. These mice were treated once weekly with 2.5 mg. hydrocortisone acetate to reduce the development of resistance to the parasite (Chapt.nine). The drinking water of these mice contained Terramycin.

Eggs for the infection of experimental animals were obtained by crushing gravid female worms on a metal grid. The liberated eggs were incubated in petri-dishes, at 25°C, for one week. When the eggs became infective, 7-9 days later (Anya,1966 a) they were transferred to a small conical flask and the concentration of embryonated eggs was adjusted to give the required dose in 0.1 ml., of suspension. A magnetic stirrer was used to ensure an even distribution of eggs in the suspension and egg counts on sample doses showed that the variation in the number of eggs in subsequent doses varied by no more than 10%. The inoculum was given orally to the mice through a blunted hypodermic needle.

Nematospiroides dubius. - Fresh fecal pellets were removed from the sawdust of cages containing infected mice. The droppings were broken up in cold tap water and were then filtered through a 250 micron grid to remove the larger pieces. The filtrate was centrifuged at 1,000 R.P.M. for 5 minutes in 50 ml., tubes. The supernatant was discarded and the sediment was emptied onto filter paper pads in petri-dish. These were then incubated at room-temperature (20-25°C). A week later the infective larvae were washed out of the petri-dishes with tap water and transferred to a conical flask with a Pasteur pipette. The concentration was adjusted as for A.tetraptera eggs. The infective material was kept at 0-4°C., for up to a year without significantly losing infectivity .

Trichuris muris - The preparation of infective T.muris eggs was the same as A.tetraptera. Gravid female worms were obtained from hydrocortisone treated mice infected with 500 eggs of T.muris. Three doses of 1.25 mg., of hydrocortisone acetate were given to these animals in the second week of infection (Wakelin and Selby,1974). The mice were killed after 5 weeks, the worms were removed, crushed to separate eggs and left to incubate at room temperature (20-25°C) for up to three months.

Preparation of Whole-Worm Antigen.

Antigen from whole worms was prepared identically for each of the three species involved in this study. The worms were either alive or deep-frozen in phosphate buffered saline. They were resuspended in cold P.B.S.(0°C) and allowed to settle. The supernatant was rejected and the worms were resuspended. They were washed in this way 3-5 times. Finally the worms were transferred to a glass hand-homogeniser and processed in the apparatus for about 15 minutes. The homogeniser was placed in ice occassionally to cool the contents. Antigen was stored at -18°C in glass tubes. Seven separate A.tetraptera antigen preparations were made, these being labelled and referred to as Ag1-Ag7. All of these preparations were made from mature worms with some larval contamination, except Ag6 which was an entirely larval antigen preparation. Two N.dubius antigen preparations were made (N.d.Ag1 and N.d.Ag2) and a single T.muris antigen preparation (T.m.Ag1)

The protein content of antigen preparations was measured by the Biuret test in which the reading for the test sample, at 555 A°. was compared to a standard curve (Kabat and Mayer, 1961). The usual final concentration was 3-4 µg/ml of antigen preparation.

Autopsy, treatment of guts and recovery of worms.

Animals were killed either by an overdose of chloroform and air or by cervical dislocation and the caecum, colon and rectum were removed immediately after death. In some experiments the intestines were deep frozen at -18°C until they could be examined. On thawing the guts were opened under water in a petri-dish and scraped clean with the edge of a glass slide. The petri-dish was then examined with a binocular microscope and any worms found were removed with a pipette or if larger with forceps. A record was kept of total number of worms of each sex recovered.

Baerman extraction. - The Baerman method of recovery was adapted as follows: 3 x 1 in. glass tubes were filled with Hanks's saline and a small bag made from silk Swiss Bolting Cloth (120 meshes to the inch) was suspended from the rubber top. The centre of the plastic top was cut out converting it into a ring, by means of which the silk bag could be suspended from the top of the tube. The tubes were placed in an oven kept at 37-38°C and were given time to reach the same temperature. Mice were killed by cervical dislocation, the colon was removed, slit open longitudinally and placed into the silk bag. The incubation period was 2 hours. After this time the contents of the bag were removed from the tube and discarded. The saline was stirred to re-suspend any worms on the bottom and transferred to petri-dishes. These were examined for worms. This method of worm recovery was found to work efficiently for larvae under 12 days old.

A similar method was used to recover mature worms for the preparation of antigen. The silk used, however, was a 25 meshes to the inch, silk Swiss bolting cloth. This cloth lined

a medium size glass funnel, to the outlet of which was connected rubber tubing, clamped on the end. The funnel was filled with saline and incubated in an oven at 37-38°C. Guts were placed in the top of the funnel, immediately after removal from the mice. After 2 hours incubation saline containing worms was drawn from the bottom of the tubing.

Recovery of worms after piperazine treatment - In a number of experiments piperazine citrate was given to mice to remove all the worms present in the colon (Keeling,1961). Several small experiments were carried out, which have not been reported else where, by means of which it was determined whether the drug was effective or not. It was found that mature worms, from a patent infection, could be recovered in the faeces of the host, about 2-3 hours after piperazine treatment, which corresponded closely to the time of the first appearance in the faeces of a non absorbable marker (2.8 ± 1.1 hours; EDTA,Cr^{51} - Dawson,1972), after oral inoculation. It was similarly found that piperazine was 100% effective and that 91% of the worms could be recovered from the faeces collected over the twenty four hours following treatment. Presumably the difference between the 100% effective clearance of the parasite and the 91% recovery (calculated by comparison to control mice given water and autopsied to determine worm burden) can be accounted for by worms which disintegrated after leaving the host or worms which were eaten by the mice (Coprophagy).

Egg counts.

Egg counts were carried out by a modified method of McMaster. All the fecal pellets produced in a 24 hours period were collected, and mixed; 3 gms were weighed out and broken

up in 20 ml. of water. The mixture was then filtered on a 250 micron grid. The filtrate was centrifuged at 3,000 R.P.M. for 5 minutes in 50 ml.tubes. The sediment was pooled from both tubes resuspended and centrifuged. It was then transferred to a 10 ml.tube and saturated salt solution was added to make up 10 ml. 5ml. of even suspension was now tranferred to another 10 ml.tube. Both tubes were refilled to the 10 ml., mark with more saturated salt solution. A McMaster slide was used to count the eggs. Five counts were made on each tube, and the mean was converted to eggs per gm., faeces using the formula:-

$$\text{EPG} = \frac{\text{Mean count X 20}}{\text{0,15 X 3 (wt of faeces)}}$$

Measurement of worms.

Worms were measured with a camera lucida arrangement. They were drawn on to white typing paper, together with a 1 mm scale. A calibrated map measurer was then used to measure the length and the reading was converted to mm by the appropriate conversion factor. Worms for measuring were kept in Hanks's saline at 4°C for no more than two days before measurement.

Direct measurements via the microscope were rarely used

PROCEDURES USED IN SEROLOGICAL STUDIES.

Bleeding and serum separation.

Mice were killed by an overdose of chloroform and air. The thoracic cavity was opened as quickly as possible. The right lung was then removed and the blood collecting in the cavity was removed with a clean Pasteur pipette, and transferred to a 10 ml.tube. Blood samples from the animals in the same experimental group were pooled together. The blood was allowed

to stand at room temperature for about 6-8 hours. When the blood was clotted, the tubes were cooled to 0°C in an ice bucket. They were then centrifuged at 3,000 R.P.M. for 5 min. The supernatant serum was transferred to labelled glass bottles and was frozen at -18°C.

Electrophoresis.

Cellulose acetate - Blood for electrophoresis was obtained by cutting the tip of the tail of mice kept under ether anaesthesia. About 1 cm length of blood was taken directly into the end of a heparinized capillary tube. The other end was sealed and the tube was centrifuged for 2 mins., in a microhaematocrit centrifuge. After centrifugation the tube was broken at the junction of the packed cells and the serum.

The electrophoresis tank was filled with a barbitone buffer (ph 8.6 0.07 M) and 25 x 100 mm strips of cellulose acetate were placed across the bridges. The serum sample was applied one third of the distance from the anode, leaving a 5 mm margin along each edge. The current was adjusted to 1 M A per strip and was maintained for 2 hours.

After this time the strips were transferred to a 0.2% Ponceau S in 3% trichloroacetic acid for 10 mins. They were differentiated in 3 changes of 5 % acetic acid and rinsed in water. The strips were blotted on filter paper and left overnight pressed between two sheets of filter paper.

Immuno-electrophoresis - A 1 % Ion agar No 2 (Oxoid) suspension in veronal buffer (pH 8.2) was prepared, heated to melting and then poured onto clean, 1" x 3" glass slides (2½ ml/slide). Merthiolate in a final concentration of 1:10,000 was added to the agar to retard microbial growth. The slides were cooled and then a small well was punched in

the centre with a cork borer. Prepared slides were placed in the electrophoretic chamber and were allowed to equilibrate in the system for 30 minutes. A current of 4 milliamps/slide was maintained. After 30 minutes, the power was disconnected, the serum was added to the centre well, the current was re-applied and continued for 1½-2 hours (sometimes longer).

After the required time, the power was again disconnected, the slides were removed and the side troughs were cut according to the patterns shown in Fig.57. These side troughs were filled with antigen, before being placed in a humid chamber for 24-72 hours. After this time, the slides were washed for 48 hours with 8 changes of 0.14 M saline, and then allowed to dry overnight under a single layer of Whatman No 1 filter paper. The stain used to examine the precipitates was Amido Black 10 B. Two hundred ml. of a 0.1 M sodium acetate solution were mixed with 200 ml. of 1.0 M acetic acid, to which were added 0.25 gms. of Amido Black 10 B and 25 ml. of glycerol. Slides were differentiated in 1.0% acetic acid for 5 baths and 1.0% acetic acid + 2 drops glycerol/20ml. of acetic acid, in final bath.

Control slides were stained in a 0.2% Ponceau S solution in 3% Trichloroacetic acid (TCA). These slides were differentiated in 5% acetic acid.

Precipitation tests in agar.

The procedure here was essentially similar to that outlined above, with the exception that the electrophoretic step was omitted. Preparation of agar, incubation in a moist chamber and staining procedures were identical.

In some experiments Hyland Immuno-plates were used (Hyland,Div.Travenol Laboratories,INC.Los Angeles,Calif.90039, U.S.A.)

Passive cutaneous anaphylaxis tests (PCA)

Mice were shaved on both flanks and 0.01 ml. of the experimental serum was injected, intradermally in to the left flank. The right flank was similarly injected with either saline or noninfected mouse serum. One ml. of a 50% mixture of antigen and 1% Evans Blue was injected into the caudal vein after either 2-4 hours or 76-96 hours. Twenty minutes later the mouse was killed, skinned and examined for lesions on the under surface of the skin

Hemagglutination tests.

Fresh Wellcome sheep blood in Alsever's solution was washed three times in PBS. Three ml of the blood giving a packed cell volume of 0.6 ml. were resuspended in 10 ml of PBS and a further 10 ml of tannic acid in PBS (concentration 5 mg/50 ml PBS) was added for 15 minutes. The cells were then centrifuged down, washed in PBS and resuspended in 10 ml of PBS. At this stage the suspension was divided into two halves, to one of which ten ml. of antigen (diluted 1 in 10) was added and it was then left for 30 minutes after which the cells were again centrifuged and resuspended in 10 ml.of 0.4% guinea pig serum (GPS) in PBS. Pretreatment of the guinea-pig serum included complement inactivation by heating to 56°C for half an hour and absorbtion with SRBC by mixing with packed SRBC for half an hour, before centrifugation to eliminate the cells. The tanned cells were washed twice in GPS before being suspended in 20 ml. of GPS to give a final concentration of 2.5% suspended cells. The experimental serum was then titrated out in GPS by halving the concentration at each step, the tanned cells were added and the preparation was allowed to incubate until a reaction could be detected. Control titrations consisted of, nonantigen conjugated tanned cells, and serum from

A.tetraptera free mice. A positive titration using BSA antigen and antiserum was also incorporated in the test.

Separation of serum components by Sephadex Column.

A 2.5 cm. x 12 cm. column, containing Whatman ion exchange DEAE 52 gel was set up. The starting buffer was a 0.005 M K_2HPO_4 + KH_2PO_4 at pH. 8.0. The final gradient was from 0.30 M K_2HPO_4 + KH_2PO_4 at pH 8.0 to 1M. NaC1. The fractions were collected on a LKB Minirak fraction collecter, after passing through a Uvicord II detector unit, linked to a LKB pen recorder giving a continous trace of protein concentrations. The 45-minute fractions were pooled as described in Chapter twelve and concentrated either using "Lyphogel" (Gelman Instrument Co.) or an Amicon positive pressure membrane filter unit, with a membrane of cut-off value 10,000 MW. Further treatment of the fractions is described in Chapter twelve.

PROCEDURES IN EXPERIMENTS INVOLVING SURGICAL OPERATION.

General methods.

Operating table - An operating table was constructed from a sheet of metal measuring 27.5 cm. x 30 cm. and 1mm in thickness. The metal sheet was curved in the middle, as shown in the diagram (Fig.1) to form a 30° angle. The legs of the table were 12.5 cm long, hollow with an external diameter of 9 mm. They were fixed to a wooden platform 29 cm. x 34 cm. and a 25 w. lamp was attached 6½ cm. below the platform to warm the metal surface.

Bench incubator - A bench incubator was purchased from Townson & Mercer Ltd., Croydon for £ 60. It was adjusted to give a temperature of 37 $\pm$ 1°C. This was not altered throughout the experimental period. The mains switch was used

to switch the incubator on or off.

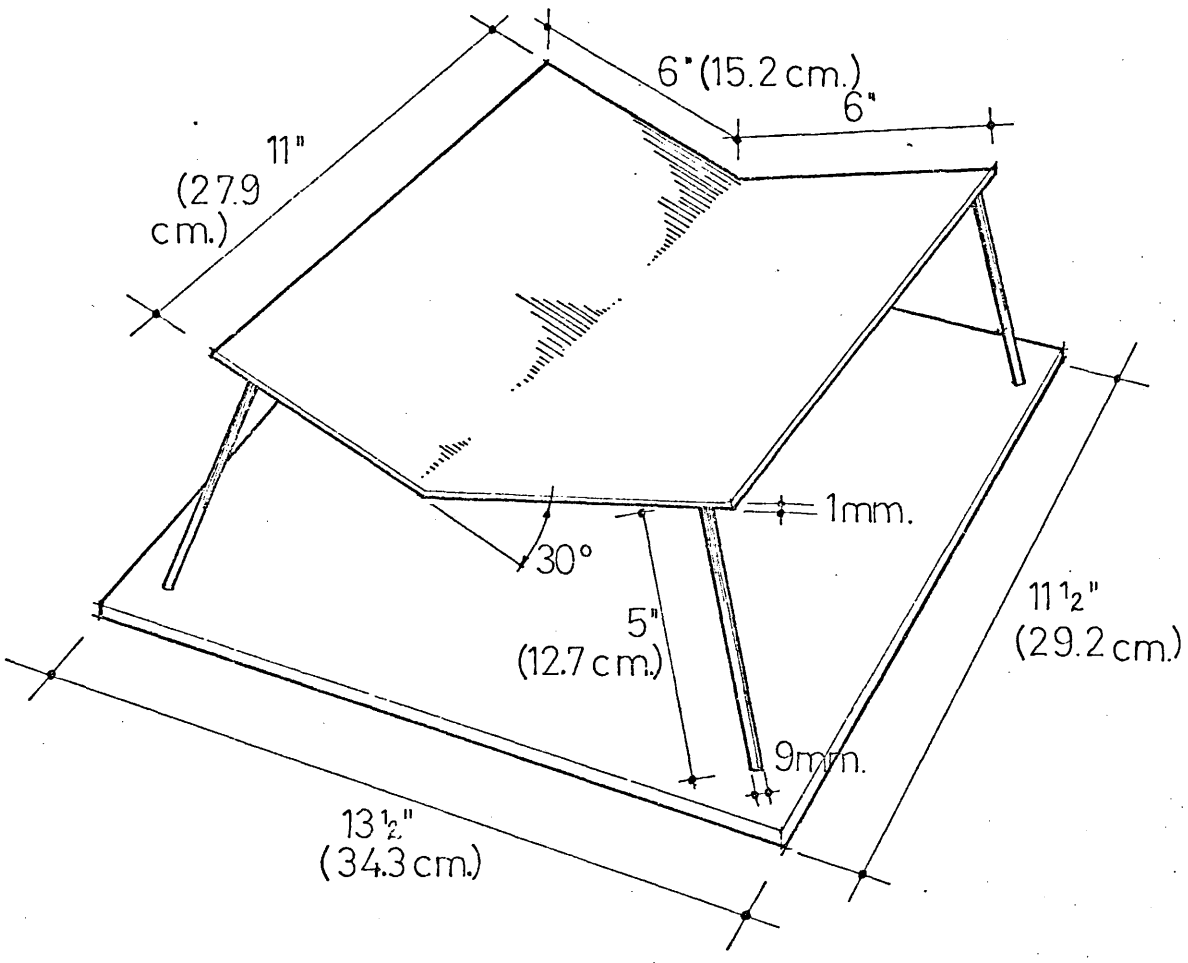
Anaesthesia - Animals for operation were anaesthetised with 'Trilene' as described previously. A small shallow glass tube, half filled with cotton wool, soaked in 'Trilene', was used to keep the animals anaesthetised during the operation. It was placed over the animals head and maintained in this position until the animal showed signs of over exposure to the anaesthetic. The tube was then removed to allow the animal time to partially recover before continuing with the administration of the anaesthetic.

Operative procedure. - The operating table lamp was switched on at least 5 minutes before the beginning of any treatment to the first experimental animal. The surface of the table was then cleaned with tissue paper soaked in 70% alcohol and the mouse was placed into the trilene jar. As soon as the mouse was unable to maintain upright balance it was transferred to the operating table and the trilene was placed over its head to maintain the anaesthesia. The legs of the mouse were fixed to the table with four pieces of adhesive tape (Sellotape products).

The fur of the animal was wetted with a Cetavlon P.C. (ICI Ltd) diluted solution (1:10), applied with a piece of cotton wool, the hair was removed with a razor blade and the skin was further cleaned with Cetavlon solution. This procedure was used for all the operations carried out before April 1973. Thereafter the fur was wetted with ordinary soap, the animal was shaved on the left side and the naked skin surface was sterilized with a piece of cotton wool soaked in 70% alcohol.

An incision was made with a pair of fine scissors while the skin was held in a pair of ordinary forceps. The length

(a)



(b)

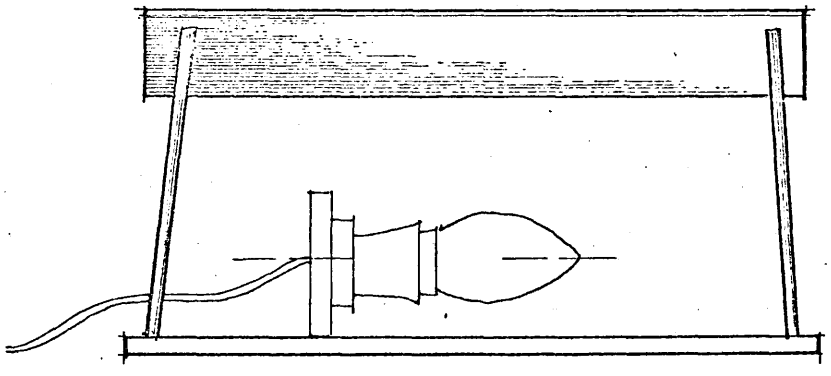


FIG. 8 (a) Perspective view of the operating table.
(b) Side view.

of the incision was usually about 1-1½ cm. Any fat below the skin was cut and pushed aside to expose the peritoneum. Forceps were used to pinch a part of this muscle layer while a very small cut was made with the scissors. This opening was enlarged by tearing the muscle layer apart by the tip of the scissors, placed inside the cut and then opened wide.

An antibiotic spray, Preocin (Wellcome), was used to sterilise any part of the body cavity which had been exposed through the opening in the peritoneum. This part of the gut and the associated organs were replaced into into the body cavity with forceps and the edges of the peritoneum were sewn together with sterilised braided silk sutures and a 12 mm 3/8 circle cutting atraumatic needle (Davis & Geck Cyanamid of Gt.Britain Ltd.) held in pair of Martin, stainless suture holders. A single loop was double knotted and the remaining silk cut away. The peritoneum was again sprayed with the antibiotic spray and the edges of the skin incision were connected in the same way, with a 19 mm 3/8 circle cutting atraumatic needle. The wound was then covered with a transparent plastic dressing (BDH) Nobecutane Spray. The ears of the mouse were punched with a code arranged to identify the individual mice. The anaesthetic was removed, the tape was detached from the legs and the animal was transferred to a fresh cage, one end of which was maintained at 30-34°C on an ordinary laboratory hot plate. Mice usually recovered from the anaesthetic within 5-10 minutes. Cages containing post-operative animals were returned to the animal room towards the evening of the same day.

Throughout the course of a series of operations the instruments used were kept in jars containing 70% alcohol. Immediately before use these instruments were taken out of

the jars and shaken quickly to remove droplets of alcohol. All the instruments were returned to the jars immediately after use.

When sutures were required to ligature either blood vessels or sections of the intestine, these were sterilised an hour before the begining of the operations by boiling in distilled water for at least 30 minutes. They were then transferred to jars containing 70% alcohol. They were taken out of the alcohol immediately before use, dried quickly and used as quickly as possible to minimise the length of exposure to the atmosphere.

On the completion of each operation the operating table was cleaned with 70% alcohol in preparation for the next mouse. The instruments were rinsed in cold water to remove blood and pieces of tissue, dried and replaced in 70% alcohol.

A note was made of any relevant details which might be important in interpreting the progress of infection in the mouse at a later date.

Methods in splenectomy.

The mice were prepared for operation as already described. The incision in the skin was made directly over the spleen which could normally be seen through the naked skin of albino mice. The spleen was exposed through the incision in the peritoneum and the blood vessels leading to it were ligatured. It was then cut away, the ligatures and blood vessels were sprayed with the antibiotic spray, replaced inside the body cavity. The remaining details were as described above.

Methods in gonadectomy.

Mice were placed on the operating table on their backs and their limbs were fixed to the table on either side. The skin around the scrotal sac was moistened and cut on one side

only. The testes were pushed down by applying slight pressure to the lower abdomen. The scrotal skin was cut and the testes were drawn through. Each testis was ligatured independently before being cut away. The blood vessels and fat were replaced inside the scrotum and the skin was sealed by two discontinuous sutures with the 19 mm needle. Sterilisation and treatment of the wound was as described above.

Methods in laparotomy.

Preparation of worms for transfer - All petri-dishes and watch glasses were washed in tap water and rinsed in distilled water on the day of the operations. The glassware was dried with clean paper towels and arranged on the bench incubator.

Hanks's saline was prepared from two stock solutions A & B (Oxoid), kept in a refrigerator at 0-4°C. The stock solutions were made in 1 litre quantities with de-ionised water and auto-claved at 15 lbs/sq in for 15-20 minutes before use. Thereafter appropriate quantities were measured out and diluted with distilled water in the following proportions - - 1 vol solution A; 1 vol solution B; 8 vols distilled water.

The clean petri-dishes were filled with Hanks's solution and were allowed to equilibrate for 10-15 minutes.

A beaker of distilled water was heated to boiling and maintained at 100°C throughout the operation-time. All Pasteur pipettes were kept in this beaker when not in use.

When the above preparations were completed the first donor mouse was killed, the colon was removed (usually within 1 minute) and transferred to one of the petri-dishes containing Hanks's saline. It was cut into two sections, each of which was opened in a separate petri-dish. The contents were washed out and the tissue was discarded.

Petri-dishes containing worms were now transferred individually to a dissecting microscope and worms were counted, as they were picked out by Pasteur pipette and transferred to covered watch glasses, containing Hanks's saline. The procedure adopted consisted of adding 10 worms to each of the watch glasses in turn. This was to ensure that in a case where more than one donor mouse was used, each watch glass contained equal proportions of worms from all the donors.

When the required number of worms was distributed in the watch glasses they were topped up with fresh Hanks's saline and left on the bench incubator until required for transfer.

The transfer of worms - Mice were prepared for laparotomy as described in the section on operative procedure. The caecum was exposed through the incision in the peritoneum. As soon as this was completed, one of the pipettes kept in boiling water was attached to a rubber tube connected to a 2 ml syringe clamped in a bench retort stand. The watch glass containing worms was placed on the microscope stand and the worms were drawn up into the pipette. The stand with the syringe and the pipette containing the worms was transferred to the operating bench. The end of the pipette was forced through the tip of the caecum while the caecal tissue was held with a pair of forceps in the left hand. The elbow of the right hand could now be used to push the plunger down and so effect the transfer of the worms from the pipette to the caecum. The pipette was withdrawn and the forceps in the left hand were used to clamp the opening in the caecum, until a ligature could be tied around the wound. This was a particularly difficult procedure but with practice a system was evolved whereby a loop was made in the suture and tigh-

tened to form a knot under the forceps, so sealing the wound completely before the removal of the forceps. Any tissue which was pinched out by the ligature, was cut away.

The caecum, at this stage much bloated because of the additional contents, was now sprayed with the antibiotic spray and returned to the body cavity. The remaining procedures have been described.

It was important to know the exact number of worms transferred to each mouse, in order to assess the "take" and the subsequent expulsion of worms. The pipette used for worm transfer was, therefore, washed out twice and the washings were examined for worms which might have adhered to the glass. These worms were then deducted from the number originally in the watch glass and the total number of transferred worms was recorded beside the code of each individual animal.

Problems associated with worm transfer. - In a number of experiments worms of different ages were transferred to recipient mice, so the problems associated with these procedures will be discussed separately according to the age of the donor infection.

The transfer of 7-10 day larvae - The usual number of 7-10 day larvae transferred was 100. This was only so, however, when donor mice had an infection capable of providing this number of larvae. When the donor mice were weakly infected the procedure by which the larvae were distributed evenly among the petri-dishes ensured that the total donor worm population was equally distributed amongst the recipient mice.

The pipette used in the transfer of 7-10 day larvae was drawn out in a bunsen flame until it was about 0.25 mm in external diameter. The larvae could easily pass through

this size of pipette end so consequently it was rarely that a blockage resulted. The total volume of transferred saline was never more than 0.5 ml. and was usually about 0.25 ml.

The transfer of 14-day larvae - These worms could also be easily transferred, but this time a slightly wider pipette was necessary. In spite of this, however, the pipette was blocked on several occasions. Mice which had received unknown numbers of worms because of inaccurate transfer were either rejected from the experiment or noted as such, so that care could be taken when interpreting the results.

The transfer of mature worms - The accurate transfer of large numbers of mature worms was found to be an extremely difficult procedure. The main difficulty was the frequency with which these worms became lodged in the pipette necessitating the laborious procedure required to free them.

Pipettes used for the transfer of these worms were fairly large, usually external diameter 1-2 mm. As these pipettes could not be forced through the ceacal wall, a cut had to be made first, and then the pipette was pushed in, consequently the tissue did not adhere tightly to the glass and as soon as an attempt was made to inject saline, some would leak out. In order to carry out these transfers accurately, it was found that the assistance of another person was of great value, since a third hand was required not only to push down the syringe plunger to expel the worms into the caecal lumen but also because an upward movement of the plunger was required to dislodge the worms when they became lodged in the pipette. It can be appreciated that the use of an elbow as a "third" hand could not be of much value where an upward movement of the plunger was required.

This, then was the greatest difficulty associated with the transfer of mature worms. Frequently the leakage of saline, caecal contents and blood resulted in a gruesome scene, but this was cleared up with sterile cottonwool and the use of the antibiotic spray rarely resulted in death from contamination once the technique had been mastered.

PROCEDURES USED TO ANALYSE THE RESULTS STATISTICALLY.

A number of tests were employed to analyse the experimental results for significance.

The Wilcoxon test - This test was used as described in Sokal and Rohlf, 1969, Biometry (Freeman & Co.)

Student's t-test - as described in Bishop, 1971, Statistics for Biology 2-nd Edition (Longman)

Chi squared test with a 2 x 2 contingency table analysis - as described in Bishop, 1971.

Correlation co-efficient - as described in Bishop,1971.

Calculation of the degree of convergence (Sab) - as described by Tarwid, 1960 and Kisielewska, 1970 a and b.

CHAPTER FOUR

The movement and the distribution of the larvae of *Aspiculuris tetraptera* during the course of a primary infection.

INTRODUCTION

A number of previous authors (Philpot,1924; Hsu,1952; Chan,1955; Fisher,1958; Anya,1966 b) have studied the life cycle of *A.tetraptera*, but there still remained a degree of uncertainty about the exact distribution of the larvae and their position in the colon throughout the course of a primary infection.

Chan's report (1955) of "a preliminary downwards migration followed by an upwards migration of the immature stages" was reinterpreted by Anya (1966 b). Anya stated, "Some of the second stage larvae, however, do not leave the caecum, or if they do they are liable to 'lose their way' during the migration. The former are still found in the caecum after 72 hours, while the latter may be released with the hosts faeces. If there is a heavy infection, such as Chan (1955) used, then large numbers of these larvae may be recovered along the length of the colon, thus giving the impression of a migration to the anal region". The first migration Anya mentioned referred to the entry of the larvae into the Crypts of Lieberkuhn. The second migration was that reported by Chan which Anya claimed resulted from the passage of non-viable larvae with the faeces.

Anya and Chan used different methods by which to assess the distribution of larvae in the colon, but neither of these two methods was sensitive enough to establish conclusively which of their theories was valid. The experiments reported

in this section were carried out in an attempt to elucidate the life-history of this parasite, to clarify previous hypotheses and to discover how these hypotheses fit into the biology of A.tetraptera.

RESULTS.

The distribution of worms on days 7,14,21 and 28 after infection.

Experiment 1 - Eighty four, 4-wk old, male CFLP mice were divided into 4 groups of 33, 7, 39 and 5 mice respectively. The first group was given 450 embryonated eggs/mouse and the second group was left uninfected to monitor the rate of natural reinfection. The third group was given 120 embryonated eggs/mouse and the fourth group formed a second control uninfected group.

Six to ten mice were killed from the first and the third groups at weekly intervals. One to two mice from the second and the fourth group were killed at the same time.

Mice were killed by cervical dislocation and the caecum, colon and rectum were removed to a labelled petri-dish. The colon was ligatured with a light loop at the ileo-caecal sphincter and then every 2 cm., to the rectum. The petri-dishes were then transferred to a deep freeze at -18°C. until examination. (The procedure for worm recovery has been described previously, Chapter three). On examination the number of worms recovered from each section was recorded individually the worms were sexed and measured by camera-lucida. The results are presented in Table 11, 12 and 13 and in Figs.9 and 10.

It can be seen that in both the heavy and in the light infection, the majority of worms were recovered from sections

4, 5 and 6, seven days after infection. Worms were found throughout the colon at this time, but in each case the preferred site appeared to be between section 4 and 6.

Fourteen days after infection, larvae were still found throughout the length of the colon, but the preferred site (the region in which the majority of worms were found) was more anterior to that identified seven days earlier. In both groups 1 and 3 section 2 and 3 harboured more worms than any other section. By day 21, the change was even more pronounced. The preferred site was still section 2 and 3, but there were no worms in the distal colon. The worm burden was confined to sections 1 and 4 from day 21 to the end of the experiment.

The second point of interest was the mean worm recovery during the course of the infection, there being a very clear loss of worms between day 7 and day 28 in both groups.

No worms were recovered from the control groups indicating that there was no infection taking place in the animal room and that all the worms recovered from the experimental group most probably originated from the experimentally induced infection.

The results obtained from the measurement of worms are presented in the section on growth in Chapter five.

There can be at least two possible explanations to account for the change in the distribution of worms, observed between day seven and twenty-eight. Firstly, it could be that the worms became established in the mid-colon (sections 4-7) before day seven, and that after this time they emigrated anteriorly to the proximal region (sections 1-3) this emigration lasting until day fourteen or even later. The distribution on day fourteen represented the majority of worms already in the proximal colon, and the remnants still completing the emigra-

TABLE 11
EXPT. 1

The percentage distribution of the larvae of A. tetraptera in the colon of mice infected with 450 and 120 eggs.

Day killed	Infection with 450 eggs/mouse Percentage of larvae recovered Regions of the Colon										
	C	1	2	3	4	5	6	7	8	9	10
7			5.0	8.1	25.0	30.5	22.9	7.6	0.9		
14		6.2	37.3	25.8	6.7	7.6	5.6	4.0	2.5	4.4	
21		1.7	47.6	44.9	5.7						
28		8.8	51.9	35.6	3.7						

Day Killed	Infection with 120 eggs/mouse Percentage of larvae recovered Regions of the Colon										
	C	1	2	3	4	5	6	7	8	9	10
7		0.5	4.5	5.0	20.1	36.9	27.6	4.7	0.7		
14		1.0	30.4	23.0	5.7	3.0	5.7	9.1	11.1	10.8	
21		4 0.9	50.6	7.3	0.6						
28		3.7	55.9	36.7	3.7						

TABLE 12 The distribution of the larvae of A.tetraptera in
EXPT 1 the mouse colon after infection with 450 eggs.

Days post infection	Number of mice	Mean worm burden	Mean number (±s.d.) of larvae recovered — Regions of the colon										
			C	1	2	3	4	5	6	7	8	9	10
7	6	109.0 ± 15.1			5.5 (4.0)	8.8 (5.7)	27.2 (19.7)	33.2 (21.4)	25.0 (17.9)	8.3 (12.8)	1.0 (2.0)		
14	8	103.3 ± 26.7		6.4 (9.5)	38.5 (23.6)	26.6 (17.8)	6.9 (5.4)	7.9 (6.9)	5.8 (4.0)	4.1 (4.7)	2.6 (3.3)	4.5 (7.1)	
21	10	63.0 ± 55.5		1.2 (1.9)	33.3 (39.8)	31.4 (28.1)	4.0 (8.8)						
28	9	21.6 ± 19.7		1.9 (3.0)	11.2 (14.3)	7.7 (6.0)	0.8 (1.9)						

TABLE 13 The distribution of the larvae of A.tetraptera in
EXPT. 1 the mouse colon after infection with 120 eggs.

Days post infection	Number of mice	Mean worm burden	Mean number (±s.d.) of larvae recovered — Regions of the colon										
			C	1	2	3	4	5	6	7	8	9	10
7	10	40.3 ± 11.5		0.2 (0.6)	1.8 (1.9)	2.0 (3.0)	8.1 (6.8)	14.8 (11.5)	11.1 (6.6)	1.9 (3.5)	0.3 (1.0)		
14	10	29.7 ± 9.6		0.3 (0.5)	9.0 (10.2)	6.8 (5.5)	1.7 (2.8)	0.9 (1.3)	1.7 (2.2)	2.7 (2.4)	3.3 (3.6)	3.2 (6.2)	
21	9	16.4 ± 16.3		6.7 (10.4)	83 (7.1)	1.2 (2.6)	0.1 (0.3)						
28	10	10.9 ± 9.3		0.4 (0.7)	6.1 (6.0)	4.0 (5.2)	0.4 (1.0)						

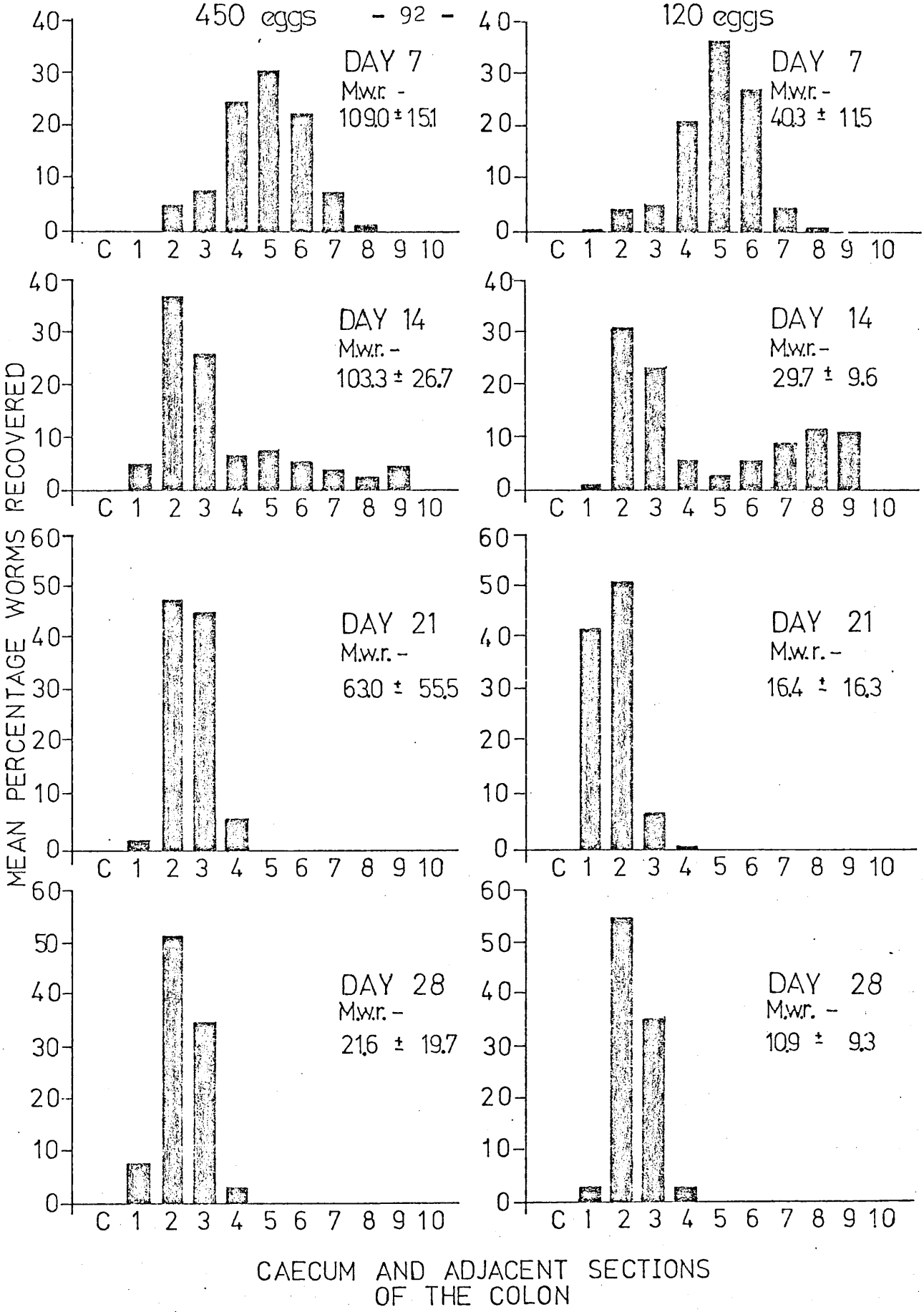


FIG 9 EXPT. 1 The distribution of the larvae of A.tetraptera in the mouse colon after infection with 120 and 450 eggs.

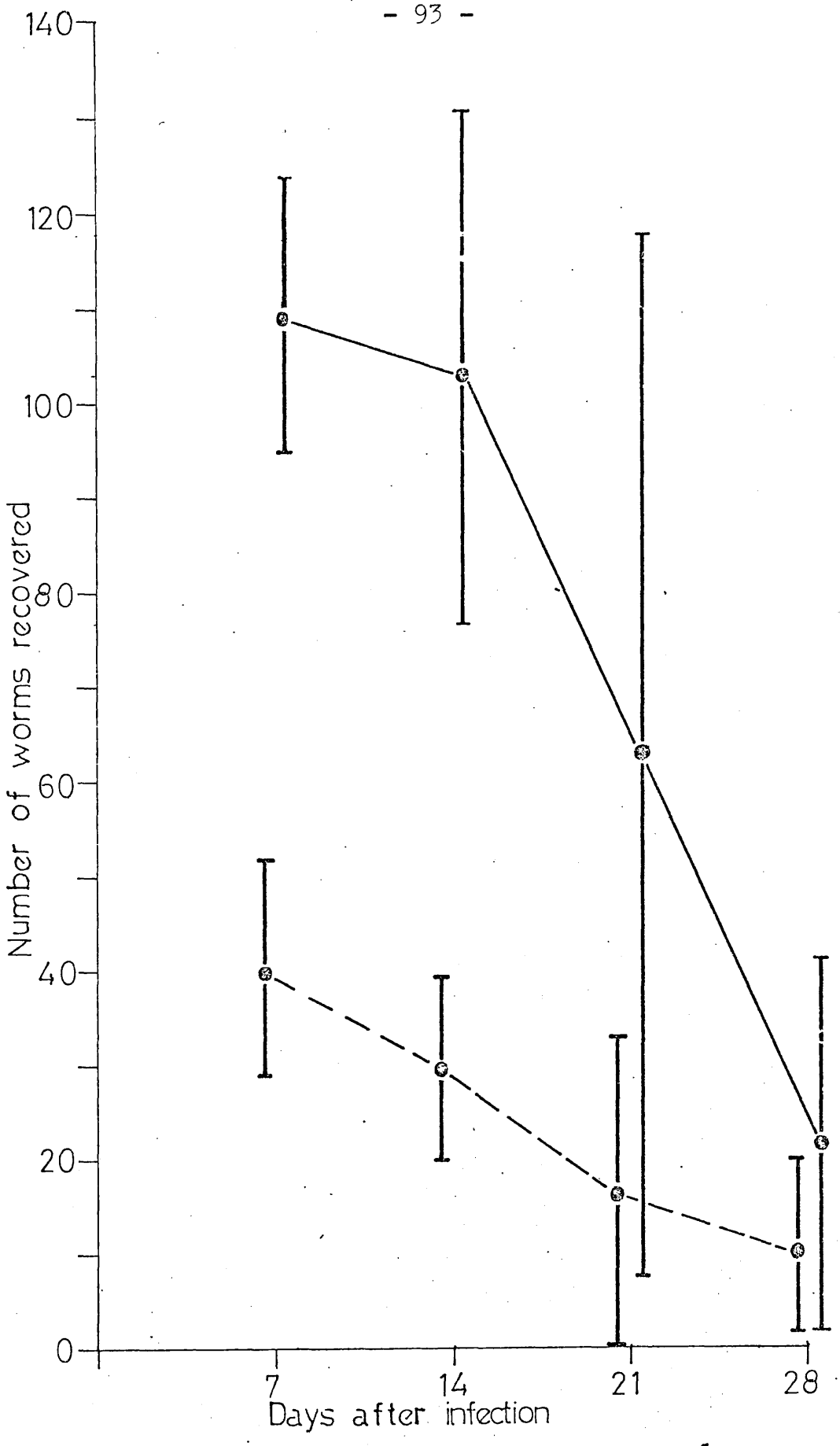


FIG. 10 EXPT. 1 Mean worm recovery from mice infected with 450 eggs (———) and 120 eggs (- - - -) of A. tetraptera

tion. By day twenty-one, this emigration had been completed and the worms were confined to sections 1-4. The inconsistency in this explanation is that it does not account for the continual loss of worms. The second possibility is that the anterior emigration was completed soon after day seven and that the distribution on day fourteen represented the major expulsion phase, during which a proportion of the worm burden was rejected. Worms, presumably damaged by a host response and passing out of the host with the faeces were recovered along the length of the colon only during the critical phase Thereafter it was more difficult to detect the few worms still to be found in the mid and the distal colon.

The next experiment was carried out in order to clarify this situation and to distinguish between the possibilities outlined above.

The distribution of larvae during the first two weeks of infection.

Experiments 2 and 3 - Fifty-six male CFLP mice were infected with 250 embryonated eggs and then divided into five groups. The first group contained 25 animals which were killed in pairs every 12 hours after infection until day 7. The 11 mice in group 2 were killed on day 10 (3), day 14 (4) and day 17 (4). Group 3 (6 mice), group 4 (7 mice) and group 5 (7 mice) were killed on days 21, 28 and 35 respectively. The colon was removed immediately after death, ligatured, stored and examined as described previously.

In Experiment 3, 12, 4-week old male CFLP mice were given 1,000 infective eggs of A.tetraptera. The times at which these mice were killed are shown in Table 15. Worms were recovered by incubating the infected intestines as described

in Chapter three.

The results of experiment 2 are presented in Table 14 and in Figures 11 and 12. The larval worms were confined almost exclusively to sections 5 & 6 during the first six days of the infection. As early as 12 hours post infection 20 worms (44% of the mean worm burden for days 1-7) were recovered from this region. No worms were found in the caecum at any time during the experimental period. After the sixth day, the worms began to move anteriorly and by day 7 this emigration was clearly evident. On day 10 the worms were found in the first 4 sections of the colon.

On day 14, however, worms were once again distributed throughout the colon, and it was at this time that the greatest loss of worms took place. This phase was completed by day 17, when the remaining larvae could only be found in the proximal colon and it was from this region that the mature worms were recovered 4 wks. after infection.

Experiment 3 - In this infection (Table 15,Fig.13) the sequence of events appeared to be very similar to that in the previous experiment. Thus here also the mid colon was the preferred region. It was noted that whereas in the first experiment larvae were seldom found in more that two adjoining sections between days 1 and 5, they seemed to be more widely distributed in the heavier infection and were frequently recovered from three or four sections, although the maximum number was always in sections 5,6, or 7.

No mouse was killed on day 6, but it was clear that on day 7 the worms were moving anteriorly and the majority were recovered from the proximal region (section 1-4) on day 8. There was no further change in distribution on either day 9 or 10, but it was observed that a small number of larvae

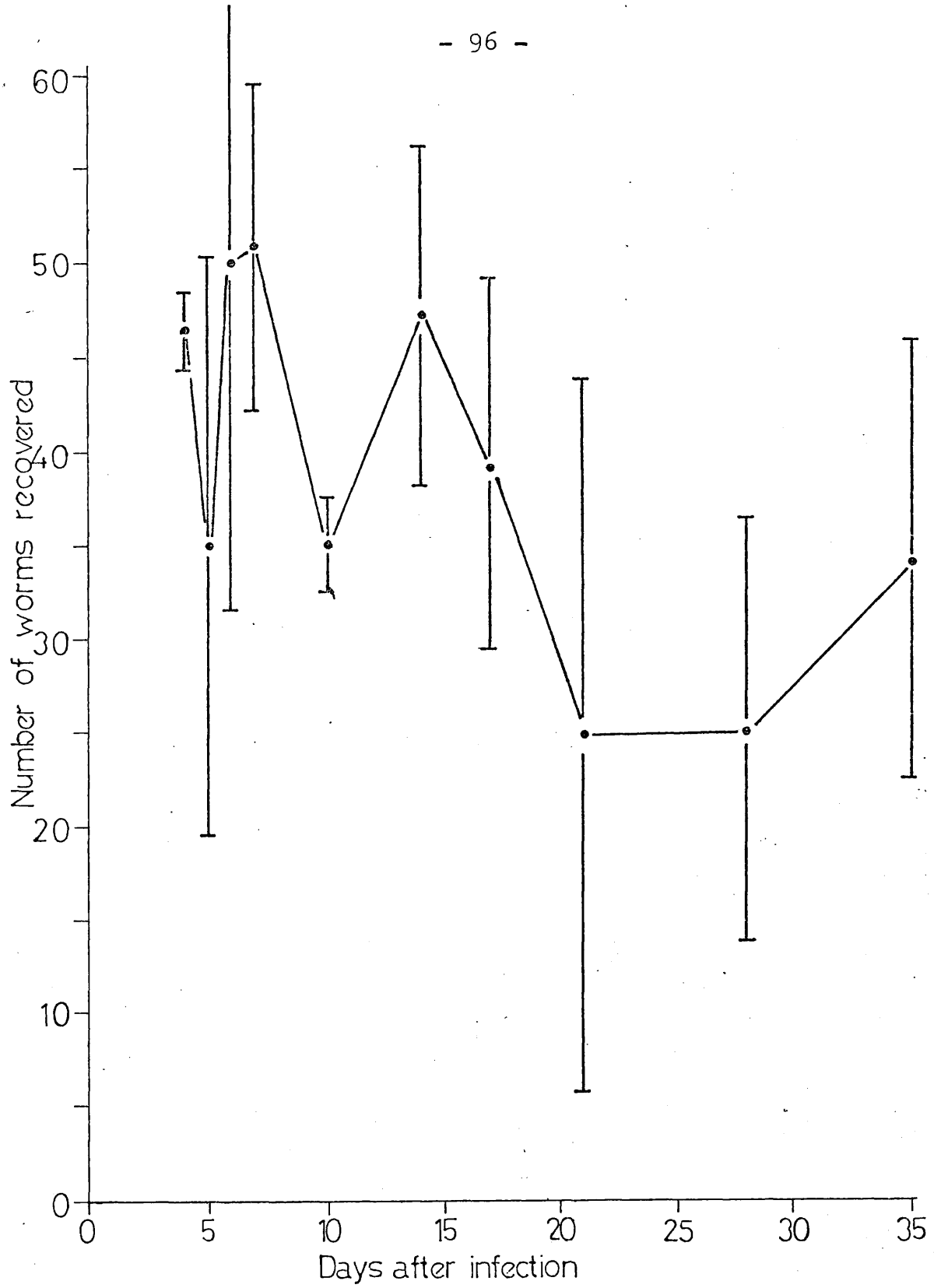


FIG. 11 EXPT. 2 Mean worm recovery from mice infected with 250 eggs of Aspiculuris tetraptera.

TABLE 14
EXPT. 2

The distribution of the larvae of A.tetraptera in the mouse colon after infection with 250 eggs.

Hours post infection	Number of mice	Mean worm burden	Percentages of larvae recovered — Regions of the colon										
			C	1	2	3	4	5	6	7	8	9	10
12	1	20						80.0	20.0				
24	2	36.5 ± 7.8						61.6	38.5				
36	2	52.5 ± 0.7						40.0	60.0				
48	2	5.0 ± 1.4						90.0	10.0				
60	2	32.0 ± 1.4						57.8	42.2				
72	2	16.5 ± 17.7						33.3	57.6	9.1			
84	2	45.5 ± 2.1					1.1	26.4	65.9	6.6			
96	2	46.5 ± 2.1					1.1	58.1	39.8		1.1		
108	2	43.0 ± 11.3					1.2	77.9	20.9				
120	2	35.0 ± 15.6						55.7	37.1	7.1			
144	2	50.0 ± 18.4					3.0	30.0	65.0	2.0			
156	2	44.0 ± 9.9					13.6	22.7	44.3	17.0	2.3		
168	2	51.0 ± 8.5		2.9	6.9	9.8	32.4	44.1	3.9				
10 Days	3	35.3 ± 2.5	0.9	34.0	48.1	16.0	0.9						
14 "	4	47.3 ± 9.0		21.2	38.6	23.3	5.8	0.5	2.1	3.2	5.3		
17 "	4	39.3 ± 9.9		36.3	54.8	8.9							
21 "	6	24.7 ± 19.1		21.1	62.5	18.3							
28 "	7	25.1 ± 11.4		9.1	63.6	26.1		0.5	0.5				
35 "	7	34.1 ± 11.8		7.1	37.2	46.9	3.7	3.0	2.0				

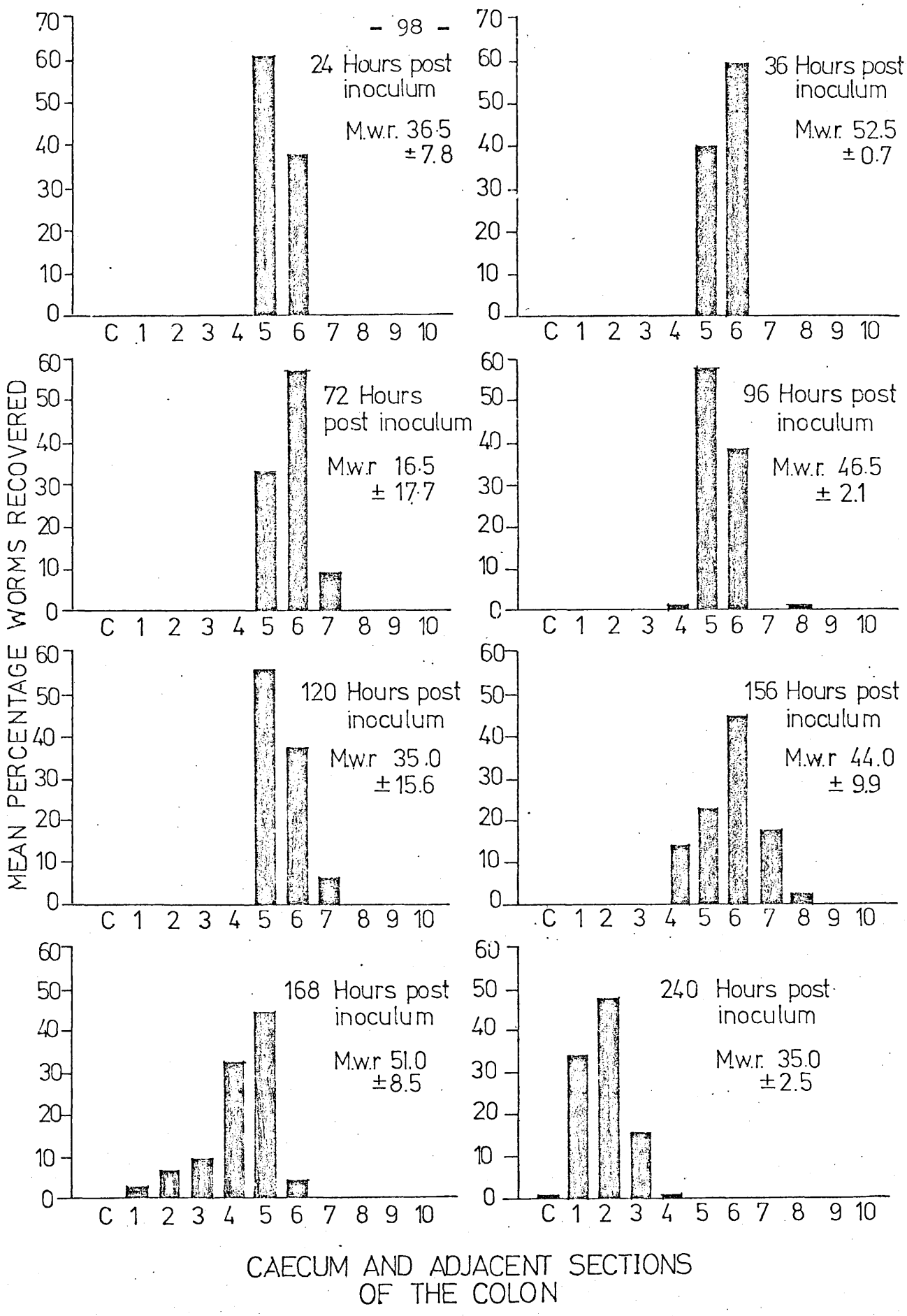


FIG. 12 EXPT. 2 The distribution of the larvae of A. tetraptera in the mouse colon after infection with 250 eggs.

still remained in the mid-colon and the distal colon (Sections 8-10) on day 10.

Experiments 1-3 confirmed Chan's hypothesis (1955) that the lifecycle of A.tetraptera includes an emigration of the larvae, to the anterior colon, during day 7 after infection. There is however, no record in the literature of a further redistribution of the larvae during the second week, associated with an expulsion of a percentage of the worm burden, although Stahl (1962), noted that female mice lost larvae before day 21.

The distribution of larvae between days 10 and 20 after infection.

Experiment 4 - Thirty nine male and forty female 4 wk old CFLP mice were infected with 250 embryonated eggs of A.tetraptera. Two to four mice of each sex were killed every 24 hours from day 10 to day 20 and the distribution of worms in the colon was recorded as described previously. The results are recorded in Tables16,17,18 and 19 and in Figs. 14, 15, 16, and 17.

Worms were first observed outside the proximal colon on day 12, when a few larvae were recovered from the mid-colon. By day 13 larger numbers of worms were found more posteriorly and this movement continued until day 16. On day 17 and thereafter worms were once again confined solely to the proximal region of the colon. Figure 14 shows that the worm burden was reduced by more than half in female mice. In some mice the entire worm burden was eliminated, in others the residual worm burden was still comparable with the pre 14 day mean worm recovery. Female mice lost larger numbers of worms than male mice, since 61.5% (8 out of 13) males were still infected after

TABLE 15
EXPT. 3

The distribution of the larvae of A. tetraptera in the mouse colon after infection with 1000 eggs.

Hours post infection	Number of mice	Mean worm burden	Percentages of larvae recovered — Regions of the colon										
			C	1	2	3	4	5	6	7	8	9	10
24	1	218					0.5	47.7	45.4	6.4			
48	2	168.5					1.2	81.9	15.4	1.5			
72	2	180.0±9.9						19.7	70.3	8.9	1.1		
96	2	125.0±17.0						16.4	42.8	32.8	6.4	0.8	0.8
120	1	202.0						0.5	24.8	36.6	31.7	4.5	2.0
168	1	204.0		1.0	0.5	12.7	30.4	26.5	17.2	11.3			0.5
192	1	305.0			3.9	46.2	37.4	9.5	2.6	0.3			
216	1	357.0		4.2	31.7	34.7	9.2	7.0	5.6	6.7	0.8		
240	1	254.0		5.9	13.4	44.5	20.1	13.8	0.8	0.4	0.8	0.4	

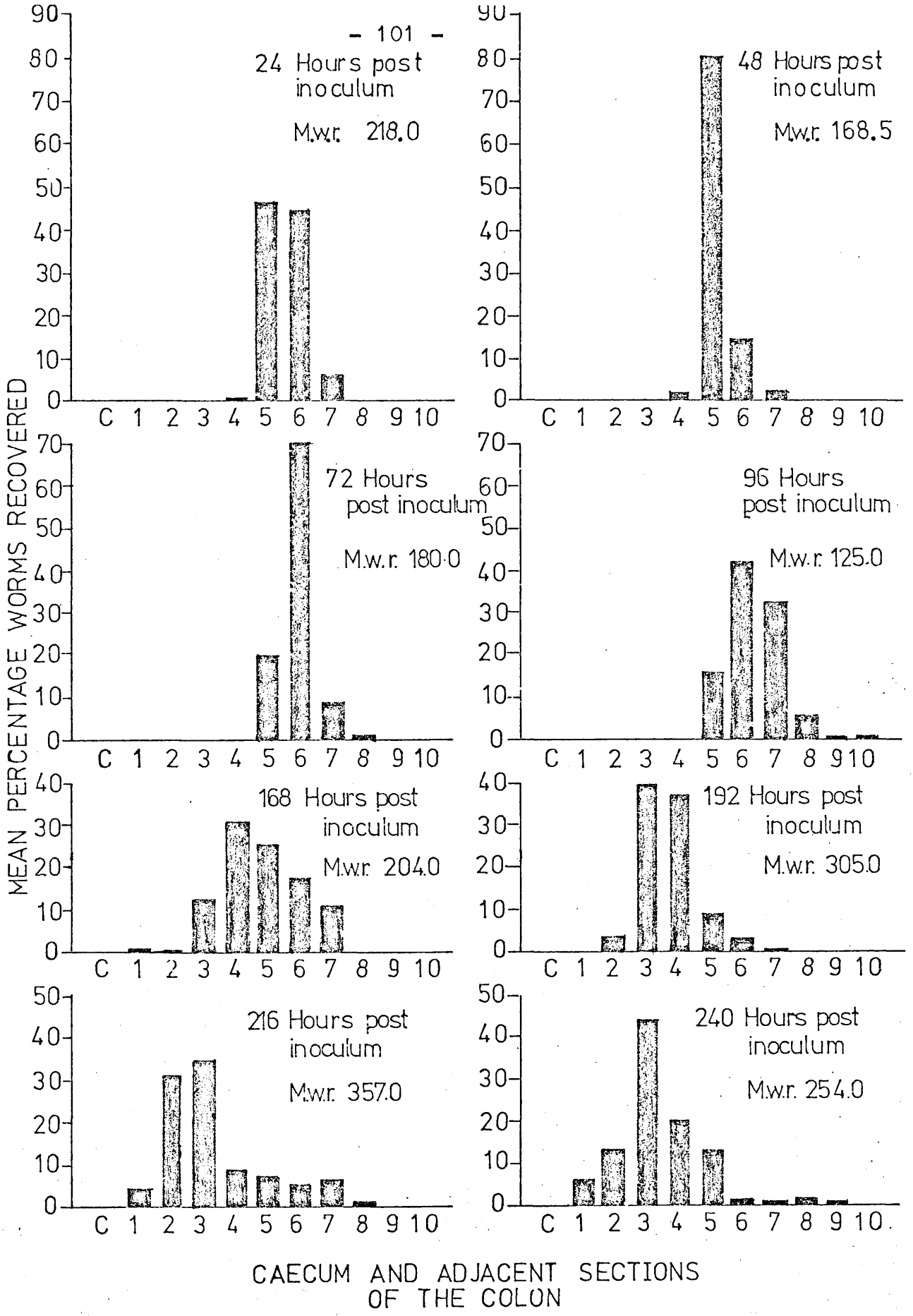


FIG. 13 EXPT. 3 The distribution of the larvae of A. tetraptera in the mouse colon after infection with 1000 eggs.

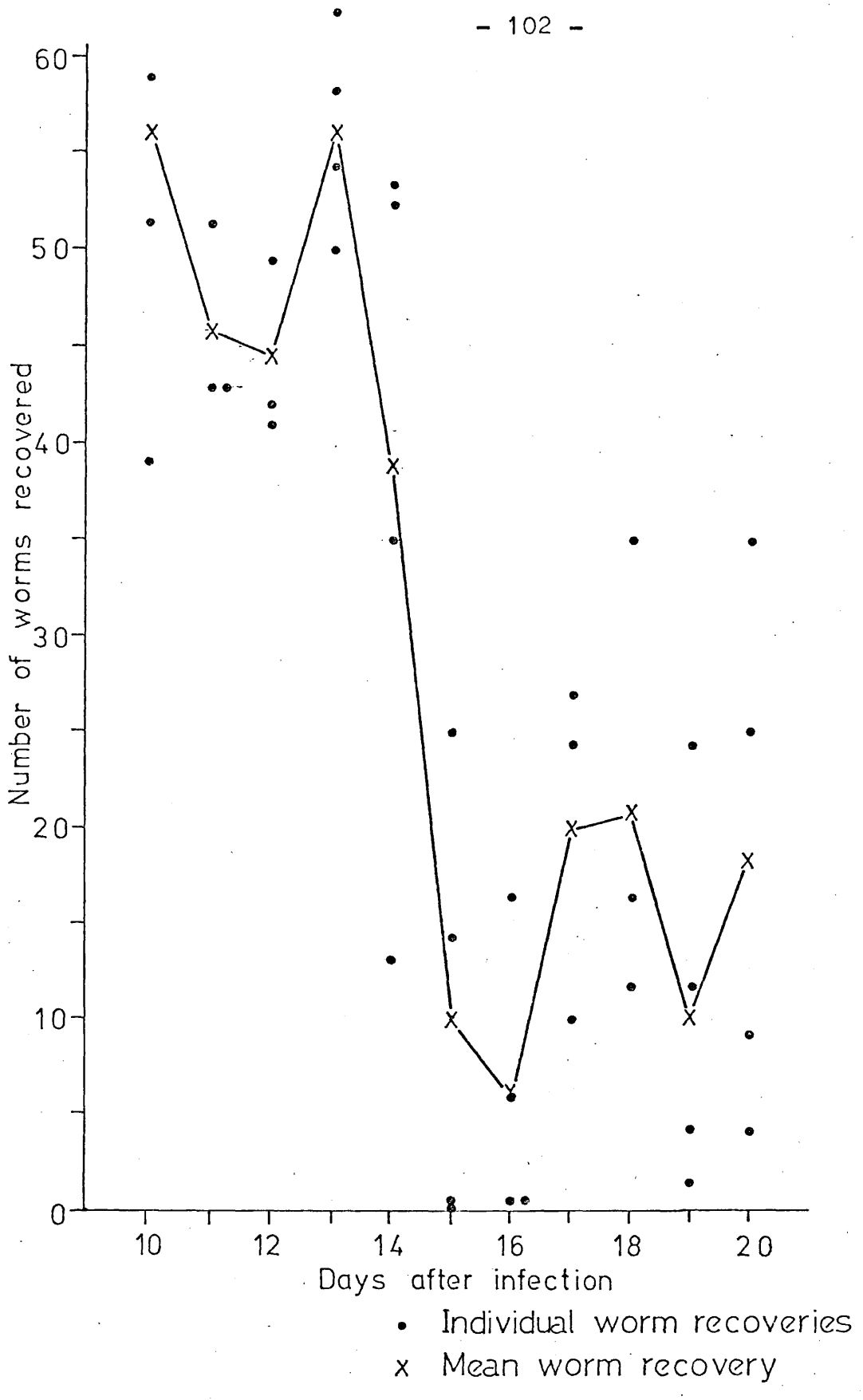


FIG. 14 EXPT. 4 Mean worm recovery from female mice infected with 500 eggs of A.tetraptera.

TABLE 16 EXPT. 4 The distribution of the larvae of A.tetraptera in the colon of female mice infected with 500 eggs.

Mean no.(s.d.)of larvae recovered

Day killed	No. mice	Mwr	Regions of the colon C	1	2	3	4	5	6	7	8	9	10
10	4	56.3±15.0		3.0 (3.6)	22.3 (14.8)	24.5 (14.0)	6.5 (7.3)						
11	3	46.0± 5.2		6.0 (2.0)	27.3 (2.5)	12.3 (5.5)	0.3 (0.6)						
12	3	44.0± 4.4		17.0 (5.3)	17.3 (7.8)	8.7 (4.2)	0.7 (1.2)			0.3 (0.6)			
13	4	56.3± 5.6		8.8 (4.9)	26.3 (10.9)	10.0 (3.7)	4.3 (3.8)	2.0 (1.8)	1.5 (0.6)	1.0 (1.4)	1.3 (2.5)	1.0 (1.2)	0.3 (0.5)
14	4	38.3±18.8		5.5 (4.1)	19.3 (12.7)	3.0 (3.5)	2.3 (2.6)	2.8 (4.3)	1.5 (2.4)	0.8 (1.0)	0.8 (1.0)	1.0 (0.8)	1.5 (1.9)
15	4	10.0±11.9		1.5 (1.7)	3.0 (4.2)	0.3 (0.5)	0.3 (0.5)	0.5 (1.0)	0.5 (0.6)	1.8 (2.1)	1.0 (1.2)	0.5 (1.0)	0.8 (1.5)
16	4	6.3± 7.5		2.8 (4.9)	1.5 (1.3)	1.5 (1.7)					0.3 (0.5)	0.3 (0.5)	
17	3	20.3± 9.1	0.3 (0.6)	10.7 (9.0)	7.3 (4.2)	2.0 (3.5)							
18	3	21.3±12.1		11.3 (10.7)	9.3 (6.7)	0.7 (0.6)							
19	4	10.5±10.0		7.0 (7.1)	3.3 (3.0)	0.3 (0.5)							
20	4	18.3±14.3		3.8 (7.5)	12.0 (8.7)	2.5 (2.4)							

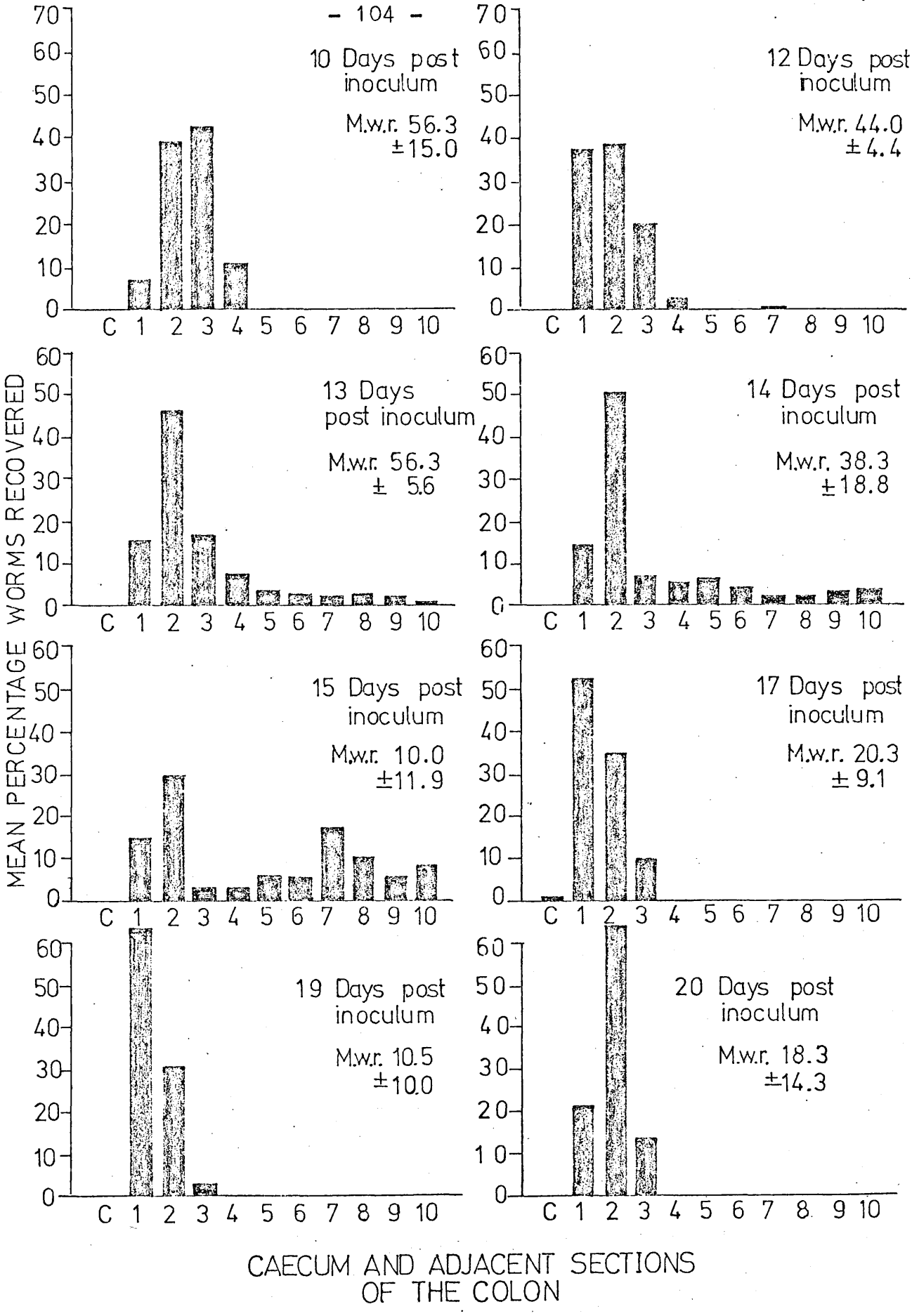


FIG. 15 EXPT. 4 The distribution of the larvae of A. tetraptera in the mouse (female) colon after infection with 500 eggs.

TABLE 17
EXPT. 4

The percentage distribution of the larvae of A. tetraptera in the colon of female mice infected with 500 eggs.

Day killed	Percentage of larvae recovered — Regions of the colon										
	C	1	2	3	4	5	6	7	8	9	10
10		5.3	39.5	43.6	11.6						
11		13.0	59.3	26.7	0.7						
12		38.6	39.4	19.7	1.5		0.8				
13		15.6	46.7	17.8	7.6	3.6	2.7	1.8	2.2	1.8	0.4
14		14.4	50.3	7.8	5.9	7.2	3.9	1.9	1.9	2.6	3.9
15		15.0	30.0	2.5	2.5	5.0	5.0	17.5	10.0	5.0	7.5
16		44.4	23.8	23.8					4.7	4.7	
17	1.5	52.7	36.0	9.9							
18		53.1	43.7	3.3							
19		66.7	30.9	2.4							
20		20.8	65.6	13.7							

day 17 with 56,60,62,61,47,59,60 and 63 worms respectively indicating no loss of larvae. Hence, the dip in the curve obtained for the mean worm burden in males, during days 15 and 16 was most probably an artefact, due to the fact that no non-resistant individual was included in the groups killed on these days,(Fig.16). The worm burdens for male mice before day 13 were fairly consistent, with relatively small standard deviations. After day 15 they were extremely variable with very high standard deviations suggesting that some males resisted infection and rejected a proportion of their worms. Female mice were more consistent than males, there being no non-resistant individuals present. The highest worm burden recorded after day 15 was 35 and this was lower than any pre day 13 count.

DISCUSSION.

Philpot (1924) whilst studying the life-cycle of a number of Oxyurid nematodes, noted that in the case of A.tetraptera, there appeared to be fewer worms in the caecum 90 hours after infection than at earlier autopsy periods. This was interpreted by her as indicating that the emigration of larvae into the colon began about this time but Wells (1952) and Hsieh (1952), (both quoted by Chan (1955)) concluded that the emigration took place 24 hours after infection. Chan (1955) found that larvae were in the colon within 24 hours of infection, but that some still persisted in the caecum until the 96-th hour and worms could still occasionally be recovered from the caecum on day 28.

Chan's experiment indicated that the worms became established in the mid-colon and began to move towards the distal colon on day 4 before emigrating anteriorly towards the pro-

ximal colon. Thus although the majority completed this redistribution, some still persisted in the mid-colon throughout the experimental period.

Fisher (1958) could not confirm this emigration pattern and Anya (1966) reviewing all the major previous studies on the biology of A.tetraptera reinterpreted Chan's results as described in the introduction.

The present study differed from that of previous authors in a number of respects. The distribution of larvae in the colon was recorded far more precisely than in any other report, this being achieved either by the division of the colon into ten equal sections immediately after death or by ligaturing and freezing the colon immediately after death and subsequently dividing it into the ten regions for examination. This technique simplified the task of deciding whether or not a movement of the population of worms had taken place.

Chan found that for the first 3 days of infection the greatest number of larvae could be recovered from the mid-colon. He concluded that between days 4 and 6, more worms were found in the distal colon, than in the mid-colon, basing his conclusions on a consideration of the pooled totals of worms recovered from each region on the relevant day. The distribution in individual mice, however, showed that on day 4, two out of four, and on day 6 three out of five mice, had greater numbers of larvae in the mid-colon. It could be that by dividing the colon into three sections, Chan had bisected that region of the colon identified in the present study as the preferred site for the worms (approximately 60% along the length of the colon). Unlike Chan, Anya pooled all similar sections of the colon from the mice killed on a particular day, before examining them for worms. This procedure would

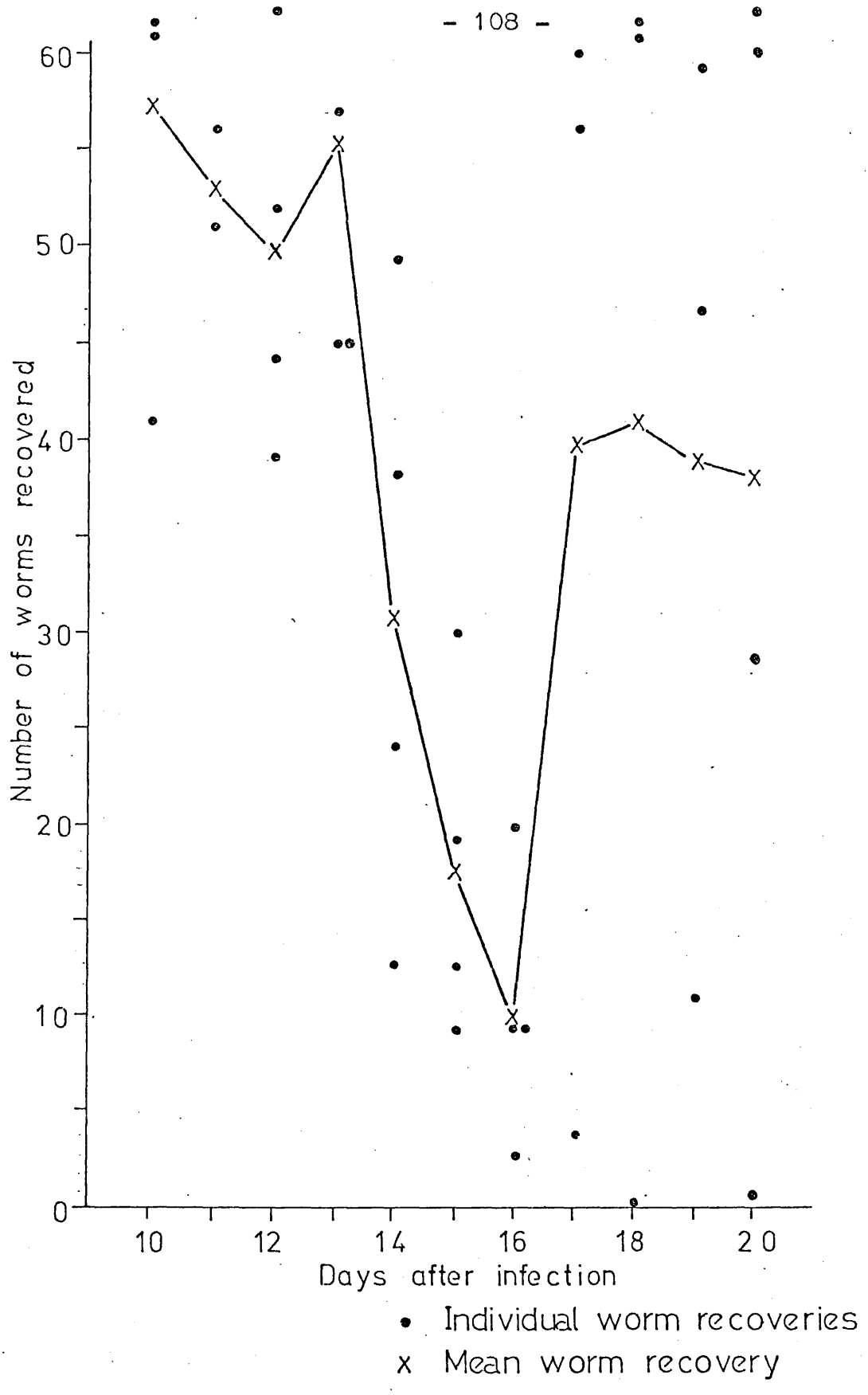


FIG. 16 EXPT. 4 Mean worm recovery from male mice infected with 500 eggs of A.tetraptera

TABLE 18 EXPT. 4 The distribution of the larvae of *A tetraptera* in the colon of male mice infected with 500 eggs.

Mean no.(±s.d.) of larvae recovered

Day killed	No. mice	M.w.r.	Regions of the colon C	1	2	3	4	5	6	7	8	9	10
10	4	57.5±11.2		0.3 (0.5)	22.8 (8.2)	33.0 (14.0)	0.8 (1.0)	0.3 (0.5)	0.5 (0.6)				
11	2	53.5±3.5		0.5 (0.7)	24.5 (6.4)	28.5 (3.5)							
12	4	49.5±10.5		10.0 (5.0)	27.5 (9.7)	9.0 (3.7)	0.8 (1.0)	0.3 (0.5)	0.3 (0.5)	1.8 (2.2)			
13	4	55.3±13.7		2.8 (1.5)	21.8 (13.4)	18.5 (9.4)	3.3 (2.6)	0.8 (1.5)	2.0 (3.4)	3.3 (3.6)	3.0 (2.6)		
14	4	31.0±15.8		1.5 (1.7)	13.0 (5.7)	4.0 (2.2)		1.5 (1.7)	4.8 (6.2)	1.3 (1.5)	0.8 (1.0)	2.5 (3.8)	1.8 (2.1)
15	4	17.8± 9.1		4.3 (4.2)	9.5 (9.8)	2.3 (3.9)	0.3 (0.5)	0.3 (0.5)		0.3 (0.5)	0.5 (0.6)	0.3 (0.5)	0.3 (0.5)
16	4	10.3± 7.1		1.0 (1.4)	4.3 (4.3)	1.0 (0.8)	0.3 (0.5)	0.8 (1.5)	0.8 (1.0)	1.0 (1.4)	0.8 (1.0)	0.5 (0.6)	
17	3	40.0±31.2		7.7 (12.4)	20.0 (16.0)	12.3 (19.7)							
18	3	41.0±35.5		6.0 (6.6)	28.0 (25.5)	7.0 (7.5)							
19	3	39.0±25.0		9.3 (7.6)	23.0 (13.0)	6.3 (5.7)	0.3 (0.6)						
20	4	38.0±29.3		7.5 (10.5)	24.5 (18.2)	6.0 (7.1)							

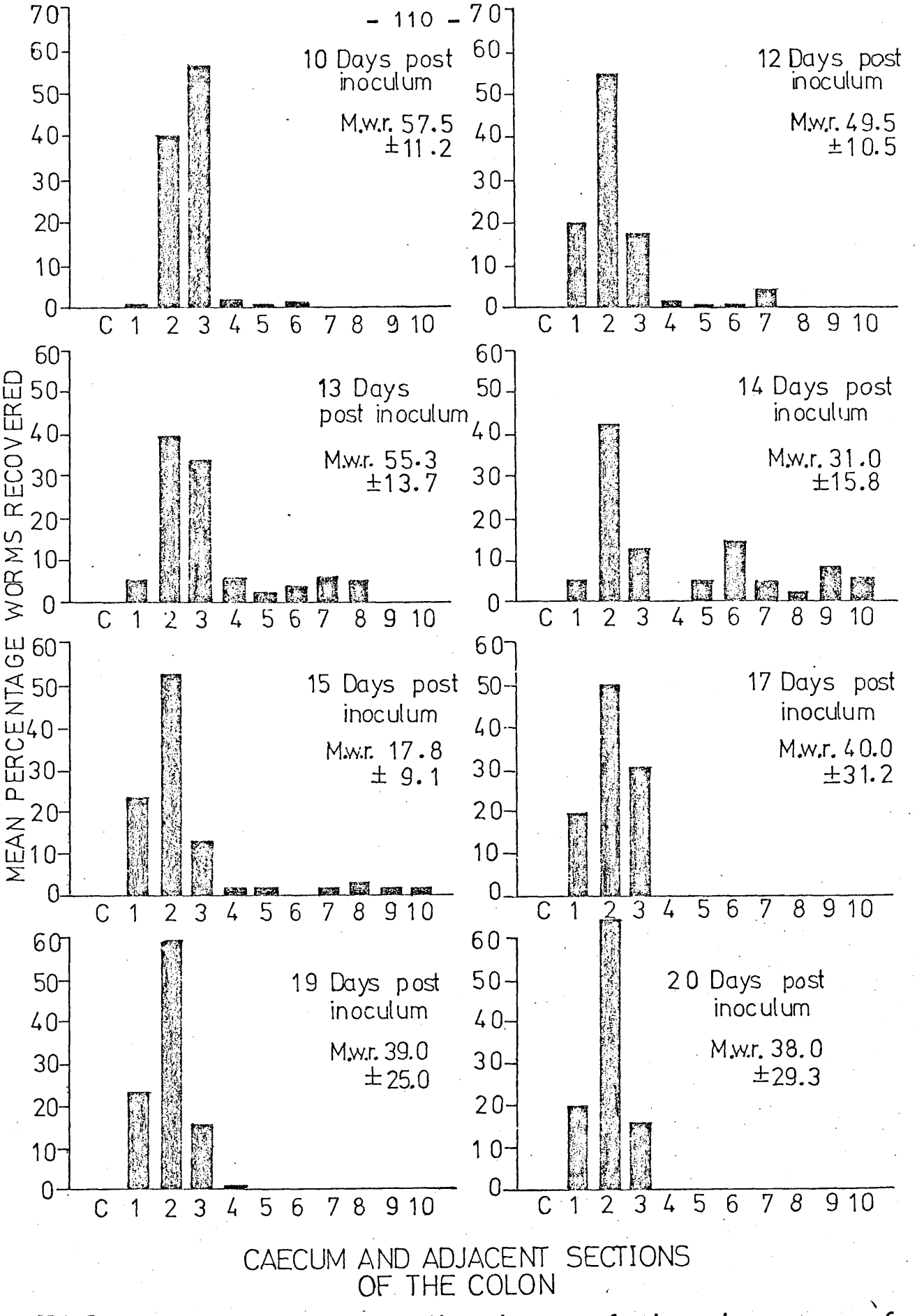


FIG. 17 EXPT. 4 The distribution of the larvae of A. tetraptera in the mouse (male) colon after infection with 500 eggs.

TABLE 19 EXPT. 4

The percentage distribution of the larvae of A. tetraptera in the colon of male mice infected with 500 eggs.

Day killed	Percentage of larvae recovered — Regions of the colon										
	C	1	2	3	4	5	6	7	8	9	10
10		0.5	39.7	57.4	1.4	0.5	0.9				
11		0.9	45.8	53.3							
12		20.2	55.6	18.2	1.6	0.6	0.6	3.6			
13		5.1	39.4	33.5	6.0	1.4	3.6	6.0	5.4		
14		4.8	41.9	12.9	0	4.8	15.5	4.2	2.6	8.1	5.8
15		24.2	53.4	12.9	1.7	1.7		1.7	2.8	1.7	1.7
16		9.7	41.7	9.7	2.9	7.8	7.8	9.7	7.8	4.9	
17		19.3	50.0	30.8							
18		14.6	68.3	17.1							
19		23.8	59.0	16.2	0.8						
20		19.7	64.5	15.8							

have disguised any individual variation between the mice and it is possible that Anya's technique, resulted in his failure to detect any local concentration. The present work has shown that when the colon was divided into ten equal sections, during the first week of infection, the majority of larvae could be recovered from the mid-colon. In some mice concentration appeared to be more pronounced than in others, but the region of maximum occurrence corresponded normally to sections five, six or seven. In light infections, consisting of no more than 60 or so larvae, most of the worms could be found in two adjoining sections, but a wider distribution (as many as four sections) prevailed in heavier infections where the mean worm burden was of the order of 150 worms.

Larvae were very rarely found in the caecum in the experiments reported in this chapter. It is possible that, because of their small size, some worms were overlooked in the comparatively large volume of caecal contents, but this is thought to be unlikely, since particular care was taken to ensure that all regions of the gut received equal attention. What ever the reason for the difference between the results of this work and that of previous authors (Philpot,1924; Hsieh,1953; Chan, 1955; Anya,1966 b) it is thought that worms remaining in the caecum after the first day of infection were unimportant to the survival of A.tetraptera. Anya suggested that these worms were lost with the hosts faeces and this seems to be the most likely course of events. In experiment three the mean worm burden in mice killed between days 1 to 5 was 170.9 ± 36.8, whereas for mice killed on days 7 to 10 it was 280.0 ± 65.8. It is suggested that this increase in number can be explained by the comparative difficulty in recovering all the second stage larvae, rather than by the survival of caecal worms.

The evidence presented in this chapter confirms Chan's observations on an emigration in the life cycle of A.tetraptera. The existence of a small region of the gut, which the larvae must reach shortly after infection in order to survive, must indicate that the worms are capable of detecting differences in the intestinal environment which trigger behavioural mechanisms directed towards ensuring the establishment of the larvae in the correct region and their subsequent development.

There have been no previous reports in the Literature concerning the movement of the larvae during the third week of infection. Chan's results showed that on days 12 and 14 there were more larvae in the mid colon and in the distal colon than on days 9 and 10 and also during the third and fourth weeks of the infection. It is tempting to suggest that this could indicate a movement of some of the larvae in the anal direction, but the figures for total worm recoveries, given by Chan, show no evidence of a loss of worms taking place at this time. Chan did not comment on this aspect of his results indicating that he must have considered the observation unimportant.

Stahl (1962) noted that female mice lose approximately 50% of their worm burden in the third week of infection. He interpreted this, however, in terms of a deleterious effect of oestrogen on the survival of larvae in an environment containing the hormone (Chapter six.).

The work reported in this chapter established conclusively that for CFLP mice, a redistribution of worms took place in most female mice and in approximately 50% of males, during the third week of infection. This change in distribution was associated with a movement of a proportion of the worms towards the anus where presumably the larvae were lost with

the host faeces. The residual worm burden (i.e.worms remaining in the proximal colon after the termination of the events of the third week) appeared to remain until at least the fifth week of infection.

It is suggested here, that this expulsion is immunologically mediated. Experiments reported in subsequent chapters attempt to elucidate the mechanism by which this is achieved and to discover how this aspect of the host-parasite relationship of A.tetraptera fits into the general picture obtained from similar studies on the biology of T.spiralis, N.dubius, N.brasiliensis and T.muris.

CHAPTER FIVE

Studies on the growth, egg production and the longevity of *Aspiculuris tetraptera* in CFLP mice; a histological examination of the infected colon.

INTRODUCTION

Anya (1966 b) studied the growth and the morphological development of *A.tetraptera* in laboratory mice. In the present study there was no specific experiment to confirm Anya's data but the opportunity arose on many separate occasions to compare the size of larvae of various ages and these pooled results will be presented. The onset of patency was also examined, together with the longevity of the parasite, the duration and the pattern of egg production.

Since differences were found between the results of experiments on the life cycle of *A.tetraptera* in CFLP mice and the results reported by previous authors (Chapter four), it was important to know the position and the orientation of the worms in the intestine at times during infection. In particular it was essential to confirm or disprove the entry of the larvae into the Crypts of Lieberkuhn and to determine the extent of host damage during this phase of the life-cycle. The intimacy of host-parasite contact would be important in determining the initiation of the antigenic stimulus, responsible for the host reaction ultimately leading to worm expulsion.

RESULTS.

A histological examination of the infected colon.

Experiment 5 - Twenty eight, four week old, female CFLP

mice were infected with 500 eggs and four were killed on each of the following occasions: 24, 48, 96 and 108 hours, 7, 10 and 14 days after infection. In addition six male and six female TFI mice, naturally infected with A.tetraptera were killed to examine a heavily infected colon, containing mature worms. The histological procedures adopted, have been fully described in Chapter three.

Examination of the histological material showed that the larvae were found in the lumen of the Crypts of Lieberkuhn for at least the first four days of the infection, sometimes persisting, in this location until day seven. (Fig.29). The anterior end of the larvae penetrated deeper and by 96 hours after infection could usually be seen at the base of the crypt, where the epithelial cells were compressed and flattened, indicating that the activity of the larvae in this situation may have been causing some host damage. As early as 48 hours after infection, crypts containing larvae appeared to possess epithelial and goblet cells which were smaller and contained less material than those in the non-infected neighbouring crypts (Fig.18,19,20 and 21). It is possible that in forcing their entry into the crypts the worms damaged the crypt walls. One such example is shown in Fig.22 and 23, where the anterior end of the worm has clearly penetrated into the lamina propria. The growth of the larvae in the crypts may also have contributed to the observed changes in epithelial and goblet cells in the crypt walls adjacent to the worms. There was,however, no substantial damage and larvae were not seen completely in the lamina propria. As described in the previous chapter, all the worms examined between days 0 and 7 were in the mid colon.

Seven days after infection the worms were in the lumen of the mid colon, (Figs 26 and 27) but some, however, still persisted in the crypts (Fig.29). Ten days after infection the larvae were in the anterior colon situated close to the epithelial cells, usually deep in the folds of the colonic rugae. By the fifteenth day of a primary infection the larvae were being rejected and sections of the rectum showed worms which were passing out of the host. Some of these worms are shown in Figs 30-33, where it can be seen that the gut cells have changed in appearance to a characteristically speckled or granular form, an observation recorded only at this time. In experiments where the colon was opened immediately after the death of the host, these worms were noted to be alive and capable of movement, implying that these were not post mortem changes.

Sections of the heavily infected colon, containing mature worms showed that superficial damage to the epithelium may occur but that it is relatively rare and presumably only results in overinfected hosts. Single worms located between the colonic rugae were in very close contact with the host epithelial cells (Figs 34 and 38), frequently leaving mirror images in the cell layers bordering on the parasite. The cephalic alae, in particular, left very distinct impressions in the cell layers, compressing the relevant cells and almost penetrating into the lamina propria (Fig.34). The anterior end of some mature worms may also have been responsible for damage, as can be seen in Figs.35 and 36. The high power photographs (Figs 34,37 and 38) of mature worms show the nature of the gut cells, which are here quite distinct from the appearance observed on day 15.

TWENTY-FOUR TO FORTY EIGHT HOURS AFTER INFECTION

Fig. 18.

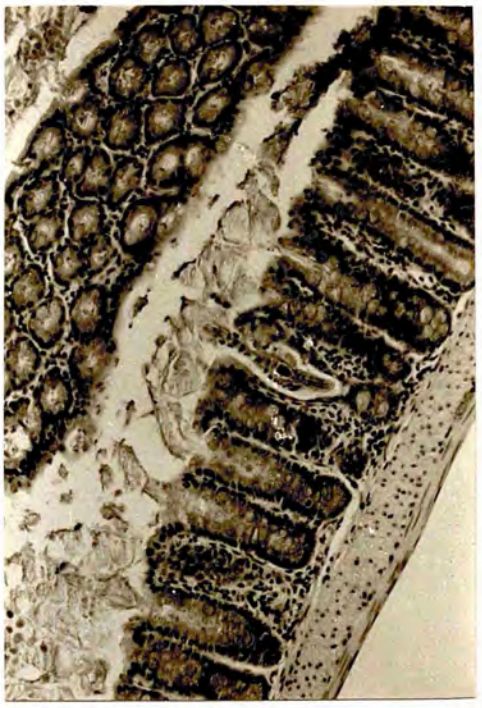
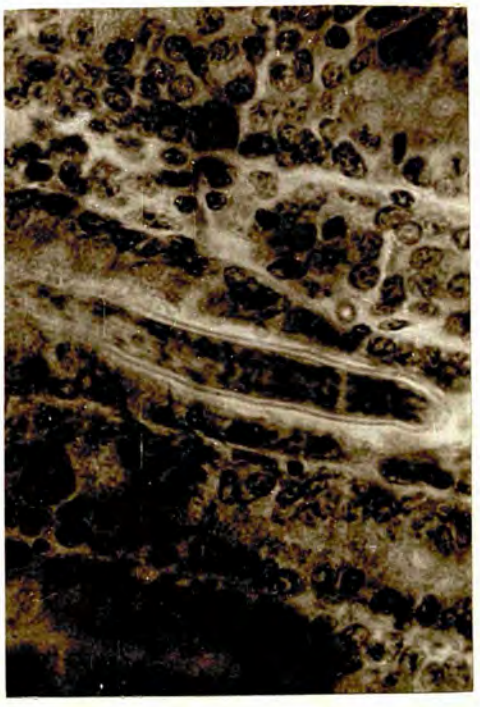


Fig. 19.

Fig. 20.

Fig. 21.



NINETY-SIX HOURS AFTER INFECTION.

Fig. 22.

Fig. 23.

Fig. 24.

Fig. 25.

Twenty-four to forty-eight hours after infection.

Fig. 18 - A 24 hour larva entering a Crypt of Lieberkuhn.

Fig. 19 - A transverse section of a 48-hour larva in the lumen of Crypt of Lieberkuhn. The large mucus-secreting goblet cells, seen in neighbouring crypts, appear to be smaller in the infected crypt.

Figs. 20 and 21 - A 48 hour larva deep in the Crypt of Lieberkuhn. The high power photograph clearly shows the larval cuticle in close contact with the external lumen facing border of the crypt goblet cells and epithelial cells.

Ninety-six hours after infection.

Figs. 22 and 23 - Two successive sections showing a larva pushing its anterior end against the walls of the crypt. In Fig.23 the anterior end has penetrated into the lamina propria.

Figs.24 and 25 - Sections showing the anterior end of two larvae which have entered deep into the crypt. In both photographs the epithelial cells and the goblet cells have been compressed compared to neighbouring crypts.

Seven days after infection.

Fig. 26 - A high power photograph of a 7 day larva showing the usual appearance of the gut cells.

Fig. 27 - Seven day larvae in the mid colon moving out of the crypts into the gut lumen, before the anterior emigration.

SEVEN DAYS AFTER INFECTION.

Fig. 26.

Fig. 27.

Fig. 28.

Fig. 29.

FIFTEEN DAYS AFTER INFECTION.

Fig. 30.

Fig. 31.

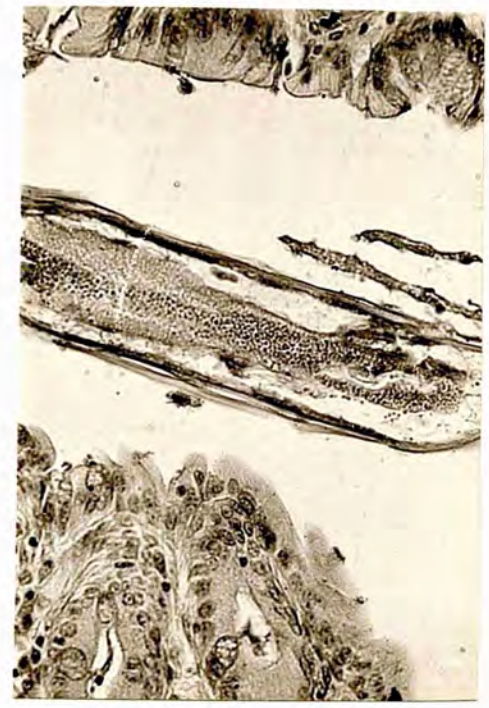


Fig. 32.

Fig. 33.

Fig. 28 - A 7 day larva lying in one of the colonic rugae in the mid colon. This worm has penetrated below the surface layer of epithelial cells and the cuticle is in direct contact with the lamina propria.

Fig. 29 - A 7 day larva still in the lumen of a Crypt of Lieberkuhn.

Fourteen days after infection.

Fig. 30 - Fourteen day larvae in the rectum, after elimination from the preferred site in the anterior colon during the response to a primary infection.

Figs.31,32 and 33 - High power photographs of 14-day larvae in the rectum. All the worms show the characteristic, granular speckled appearance of the gut cells.

Mature worms in the anterior colon

Fig.34 - Transverse section of the anterior end of a mature worm lying between two colonic rugae. The cephalic alae have left a clear imprint on the epithelial cells of the colon. In places the presence of the worm has resulted in cells becoming compressed.

Figs.35 and 36 - Both photographs show the anterior end of mature worms apparently probing the epithelium of the colon.

Fig. 37 - A transverse section of a heavily infected colon. The worms are crowding in the gut lumen and it is conceivable that this situation leads to host damage.

Fig. 38 - Three worms situated between two colonic rugae. An imprint of the two larger specimens is seen in the lower edge of the top layer of host tissue. The pointed alae have left a triangular impression in these cells.

MATURE WORMS IN THE ANTERIOR COLON.

Fig. 34.

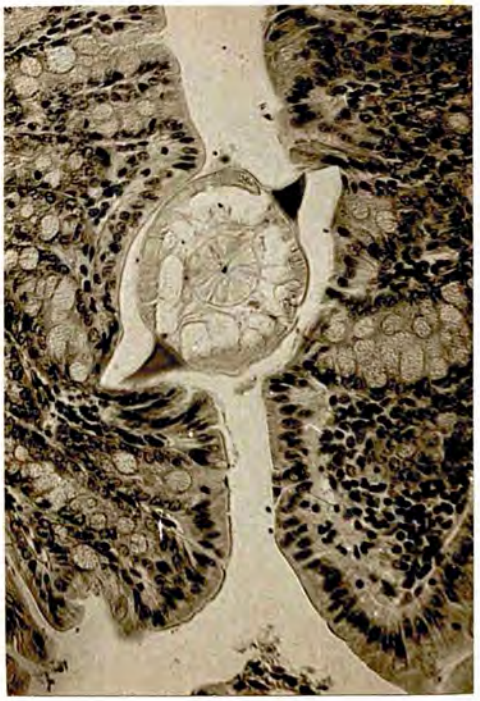


Fig. 35.

Fig. 36.

Fig. 37.

MATURE WORMS IN THE ANTERIOR COLON.

Fig. 38.

TABLE 20

EXPT. 6

THE LONGEVITY AND SEX RATIO OF *ASPICULURIS TETRAPTERA* IN MALE CFLP MICE INFECTED WITH 500 EGGS

Day killed	No.mice	Worms recovered			
		mean	±	s.d.	Percentage female worms
11	4	43.3		19.5	
20	4	28.3		23.1	
30	6	18.5		24.3	
40	5	22.6		35.3	71.6
50	4	21.3		23.2	73.0
60	5	21.0		9.1	71.4
68	4	9.0		8.8	77.7
80	5	19.6		12.7	67.3
110	5	17.6		10.9	82.9
135	4	4.3		5.0	94.1
170	4	8.3		7.9	93.9
179	8	9.0		7.7	94.4

The longevity and the pattern of egg production of A.tetraptera.

Experiment 6 - A single experiment was carried out in which 59, 26 day old male CFLP mice were infected with 500 eggs of A.tetraptera. Mice were killed in small groups at various times after infection, when the total number of worms recovered and the sex of the parasites was recorded (Table 20). Egg counts were carried out at the times indicated in Table 21.

Rejection of worms occured as usual between days 11 and 20, when the mean worm recovery was reduced from 43.3 ± 19.5 to 28.3 ± 23.1. There was thereafter a slight further loss and then a relatively stable residual worm burden persisting until day 110. The low worm count on day 68 (9.0± 8.8) was exceptional. The worm recoveries for autopsies after day 110 cannot be definitely assigned to the original infection since by this time several larvae were recovered with the mature worms, indicating that reinfection was taking place. The sex ratio, expressed as the percentage of female worms, was 71.6% on day 40 and rose steadily to over 80%, by day 110 and 90% by day 135 suggesting that in the residual worm burden, female worms lived longer.

Egg production began on day 26 rising to a peak on day 31 and then to a second higher peak on day 41. Thereafter egg production was more or less consistant for the duration of the experiment.

The growth of A.tetraptera in CFLP mice.

The larvae of A.tetraptera were measured on various occasions at different times after infection, the pooled results being recorded in Table 22. All the measurements were carried out using camera lucida tracings read with a calibrated map reader. Individual autopsy periods include a list

TABLE 21 THE MEAN EGG COUNT PER GM. FAECES OF TEN
EXPT. 6 MALE CFLP MICE INFECTED WITH 500 EGGS OF
ASPICULURIS TETRAPTERA

Day after infection	Egg Count	Day after infection	Egg count
23	0	38	711.1
24	0	39	1066.7
25	0	41	1657.0
26	66.7	43	1129.0
27	226.7	50	1040.0
28	369.0	57	951.0
29	351.0	64	889.0
30	366.7	71	702.0
31	618.0	78	538.0
32	569.0	85	662.0
33	484.4	92	520.0
34	418.0	99	982.0
35	289.0	106	1298.0
36	1000.0	114	1143.0
37	564.4	120	1115.0
		135	1182.0

of measurements, each one from a separate experiment, in which the mean ± s.d was calculated for all the worms studied. All the infections refer to CFLP and CFLPb strain mice, normally harbouring between 50 and 500 larvae. Only worms from control, untreated mice were considered for this table.

DISCUSSION.

Mya (1955) and Fisher (1958) recorded in their theses that the larvae of A.tetraptera enter the Crypts of Lieberkuhn soon after infection and these observations were confirmed by Anya (1966 b) but to-date no photographs have been published to illustrate these findings. The results recorded in this chapter confirm and expand the observations made by the previous authors. Thus here the larvae were recorded in the mid colon only during the first week of infection and it was in this location that the crypts were occupied. Evidence of superficial damage to host tissue was presented as manifest by the compressed epithelial cells adjacent to larvae and perhaps local distortion of cell function, such as a lowered mucus output by goblet cells which were smaller in infected crypts.

Neither Fisher (1958) nor Anya (1966 b) could confirm the existence of any local concentration of larvae in the colon (Chan,1955), and the possible explanation has been discussed in the preceeding chapter. In the present work, both the histological observations and the studies on distribution (Chapter four) agree that the larvae are concentrated in the mid colon in the crypts for up to a week after infection. No signs of an obvious host response were detected during this time but it is conceivable that the initiation of an immune response could have been triggered by the close

contact between the larval cuticle and the host epithelium in the crypts. Seven days after infection the larvae left the crypts, some perhaps earlier and collected in the gut lumen before the anterior emigration.

The expulsion of the larvae from the mouse colon during the third week of infection was described in Chapter four, this being amply supported by histological sections of the rectum on day 15. Larvae were found in the lumen of the rectum at this time and it was discovered that the gut cells of these worms were unusual in that under closer observation the cells were granular and speckled in appearance. Since no ultra structural or cytochemical studies were conducted, it was difficult to compare these observations to others in the Literature. Pertinent, however, is the work of Taliaferro and Sarles (1939) and Ogilvie and Hockley (1968) who recorded the changes taking place in Nippostrongylus brasiliensis immediately prior to expulsion during a primary infection. The adult N.brasiliensis showed vacuoles and dilations in the gut cells and a degeneration of the reproductive tract which has since been attributed to the action of host antibodies on the worms (Jones and Ogilvie 1971) although the mechanism may be indirect, the changes in the parasite arising from malnutrition, consequent to damage to the digestive enzymes. Lee (1969,1971) who made detailed ultrastructural observations on the changes brought about by immunity, suggested that the increased prevalence of lipid droplets in the worm tissue indicated a change in the respiratory metabolic pathways, following the movement of the worms away from the epithelium into the anaerobic environment in the gut lumen, this movement resulting from the action of immunity. Harkness, Smith and Bland (1973) recorded similar changes in 11-13 week old

Haemonchus placei in calves, although it was not possible to prove that the observed changes were undoubtedly attributable to the host response. Of the observations recorded above, the changes in lipid metabolism perhaps, offer the most promising explanation which can be advanced to explain the granular appearance of the gut cells in larval A.tetraptera. These worms are doubtless, being subjected to severe environmental alterations, since their passage down the colon involves a change from the semi liquid gut contents in the anterior colon to the dry fecal pellets in the rectum. Further, there must also be alterations in the oxygen availability since the rectum is open to the external environment, whilst the anterior colon is deep inside the body. It is not inconceivable that an oxygen gradient exists in the colon, influenced by factors such as blood vessels and the proximity to the anal sphincter. It is also possible that malnutrition, as in the case of N.brasiliensis, was instrumental in causing the changes. A further possibility is the coincidence during this time of the final larval ecdysis (Anya 1966 b). It may be that changes arising from the moulting procedure caused the granular appearance of the gut cells. Finally it must be concluded that whilst a definite explanation must await the results of precise ultrastructural and cytochemical studies, the possibility remains, that any one or perhaps several of the processes outlined above contributed to induce the physical or biochemical alterations which gave rise to the unusual appearance of 15 day rejected larvae.

In general the outline of worm growth recorded in Table 22 agrees with that of Anya (1966 b). Twelve hours after infection the four larvae measured 0.161 mm whereas Anya gave 0.120 mm but this difference was less at 24 hours

when the three separate experiments gave a range of 0.165 ± 0.009 to 0.185 ± 0.036 mm (Anya -0.150 mm). During the next two days growth was favourably comparable to Anya's results, maybe a little faster. Thus at 48-96 hrs Anya recorded measurements of 0.198mm to 0.231 mm, which is less than the 0.370 mm and 0.314 mm recorded for 96 hr larvae in Table 22. Although it was not possible to decide exactly, when the second moult took place, examination of the individual lengths of larvae from the results recorded in Table 22, suggest that there was a sudden change in length on or about the 72-nd hour after infection. If this was the second moult it occurred about 36 hours earlier than recorded by Anya. There was agreement that the third ecdysis occurred on or just before day 7 when a sudden increase in larval size was recorded. Similarly the final moult to the fifth instar occurred at the begining of the third week, as recorded by Anya.

Eggs were first recovered from the faeces of infected mice on day 26, two days later than recorded by Anya (1966 b) and Fisher (1958), but although this was the time to patency, maximum egg production was not achieved until two weeks later suggesting that either the initiation of the process was not simultaneous in all female worms or that growth continued after day 26 and was accompanied by an increase in fecundity.

Experiment 6 recorded the duration of worm survival as in excess of 110 days, which is in agreement with the results recorded in Chapter 13. Fisher (1958), however, concluded that male worms were rare by the 20-th.day of infection, whereas here some males lived for over 100 days. Further, Fisher stated that on the average females lived only 30-35 days with possibly some survival to 40 days. Fisher's results are clearly incompatible with the present findings and it can

TABLE 22 THE GROWTH OF ASPICULURIS TETRAPTERA IN CFLP AND CFLPb STRAIN LABORATORY MICE. (Pooled results from various experiments.)

Time after infection	No.larvae measures	Mean length (mm)	±	s.d
12 hrs.	4	0.161		0.006
24 hrs.	37	0.185		0.036
	14	0.170		0.005
	16	0.165		0.009
36 hrs.	44	0.192		0.009
48 hrs.	5	0.213		0.014
60 hrs.	34	0.228		0.017
72 hrs.	59	0.240		0.024
	48	0.318		0.037
	21	0.291		0.023
84 hrs.	21	0.303		0.018
4 days	39	0.370		0.024
	4	0.314		0.039
5 days	30	0.367		0.033
7 days	20	0.580		0.100
	19	0.767		0.045
8 days	59	0.742		0.035
9 days	54	0.750		0.064
10 days	32	1.197		0.103
	33	1.010		0.071
	31	1.272		0.084
	55	0.889		0.098
	79	0.792		0.094
12 days	53	0.835		0.099
	42	1.207		0.093
	45	1.132		0.084
14 days	36	1.389		0.150
	40	1.468		0.080
16 days	54	1.601		0.131
17 days	17	1.224		0.096
21 days	23 (♀)	2.633		0.496
	9 (♂)	1.904		0.386
28 days	14 (♀)	3.936		0.205
	3 (♂)	2.651		0.088

only be proposed that the biology of A.tetraptera in Fishers' system was fundamentally different from that recorded in this thesis.

CHAPTER SIX

The influence of the sex of the host on infection with *Aspiculuris tetraptera*

INTRODUCTION.

There are many factors which determine whether an animal can be infected by a particular parasite. It is important, for instance, that the parasite be adapted to survive and to exploit the physiological environment of the host and in this context, the hormonal status of the host is of particular interest. Sex hormones have frequently been implicated as influencing the course of parasitic infections (Haley,1958; Mathies,1959 b;Dobson,1966 a,b; Solomon,1966) and it has often been found that the female host is more resistant (Haley,1958; Mathies,1959 b; Solomon,1966,1969;).

Mathies,(1959 b) showed that male mice were far more susceptible to infection with *A.tetraptera* than female mice. He examined this further (Mathies,1959 b.) by comparing the levels of infection in both sexes under a variety of experimental conditions, which included gonadectomy and the administration of testosterone or oestrogen. He found that gonadectomy lowered the level of infection in both sexes, oestrogen being also effective in reducing susceptibility in males.

Stahl (1966 b) while studying the effect of concurrent helminth infection on *A.tetraptera* in mice found that in control groups autopsied on day 11, there was no difference in worm recovery. He realized the discrepancy between his own result and Mathies report and he reconciled the findings

in both papers (Stahl,1962) by demonstrating that whereas infection levels stay almost constant in male animals over a period of five weeks post infection, they are appreciably reduced in female mice by the fourth and fifth weeks of infection.

In a previous chapter (Chapter four) it was shown that in certain circumstances, both male and female mice rejected the worm burden during the third week of infection. Infections in male mice, however, were extremely variable and the results were complicated by the presence of non resistant individuals. There was consequently, some doubt as to whether Mathies interpreted his results correctly. He seemed unaware of the possibility that his results may have been due to differences in resistance between the sexes rather that in susceptibility. The experiments described in this chapter were carried out in an attempt to clarify some aspects of this situation.

RESULTS

The expulsion of the larvae of A.tetraptera in male and female mice.

Experiment 7 - The strain of mice used was a first cross between Ash females and CFLP males. Mice were given an oral dose of piperazine citrate (500 mg/kgm) on days 18,21,23 and 25 after birth, in order to prevent the establishment of a natural infection with A.tetraptera and Syphacia obvelata, which were present in the animal room at this time.

Twenty seven mice were divided into four groups consisting of 6 and 8 male mice (of which 1 died before day + 11) and 6 and 7 female mice. The infecting dose was 600 embryonated eggs per mouse. One group of each sex was killed on day 11 and the remainder on day 28. The results are presented

in Table 23.

There was no statistically valid difference in worm burden between the two groups on day 11, so clearly, susceptibility, as assessed by 11 day worm burdens was equivalent in both sexes. Sex resistance was, however, very obvious on day 28. Female mice lost approximately 89% of their worms during the third and fourth week, whilst the male counts were down by no more than 20%, a much weaker response. However, this figure (20%) is not truly representative of the group since 3/8 mice showed a stronger response whilst the remainder seemed to have suffered no loss at all.

The effect of gonadectomy on the expulsion of larvae from male mice.

Experiment 8 a - Thirty-one CFLPb mice were used in this experiment. Fifteen animals were gonadectomized 29-35 days after birth. They were given five days to recover before being infected, together with 16 control mice, with 1,000 eggs of *A.tetraptera*. Six control mice and nine gonadectomized mice were killed on day 9, but because the mice showed such low worm recoveries (probably due to a low infectivity in the inoculum) a second dose of a further 1,000 eggs was given on day 14.

The experiment was terminated on day 40. The results (Table 24) show that only 4 of the 20 mice killed on day 40 were infected and that these four were all in the control group. The difference between the means of the groups killed on both occasions was not statistically significant ($0.2 > P > 0.1$) but whereas it is uncertain whether there was a valid difference between the 11 day means and the 40 day mean in the control groups (Students t-test P=N.S, Wilcoxon test $P < 0.005$)

TABLE 23 EXPT. 7 Expulsion of the larvae of A.tetraptera in male and female mice.

Group	Number of mice	Day killed	Mean worm recovery	± s.d.
A (Male mice)	5	11	121.6	16.4
B (Female mice)	6	11	111.3	42.8
C (Male mice)	8	28	97.6	54.3
D (Female mice)	7	28	12.1	13.6

Analysis of results by Student's t-test and the Wilcoxon test

Groups compared	Probability	
A X B	N.S.	N.S.
C X D	P<0.01	P=0.001
A X C	N.S.	N.S.
B X D	P<0.001	P=0.001

the gonadectomized group lost all the worms by day 40 ($P < 0.001$).

The worm recovery in individual mice was extremely variable, giving rise to very high standard deviations and thus complicating the statistical analysis. No reliable conclusion was arrived at, but the results suggested that perhaps orchidectomy increased the ability of these mice to respond to and reject A.tetraptera. In order to further clarify this situation, the experiment was repeated.

The effect of gonadectomy on the expulsion of the larvae from male mice.

Experiment 8 b - Fourteen 2 month old CFLP mice were gonadectomized and infected together with 15 non-treated controls, two days after the operation. The infection consisted of 750 eggs in each of two doses given 20 hours apart. Four gonadectomized mice and five control mice were killed on day 12. The remainder were killed four weeks after infection.

As in the previous experiment there was no difference between the means of the two groups (Table 25) on day 12 ($0.7 > P > 0.6$). Susceptibility to infection, thus did not seem to be affected by orchidectomy, as postulated by Mathies (1959 b). The 28 day groups, however, showed that the gonadectomized mice were more efficient in eliminating the parasite. Not only did the mice lose worms ($P < 0.001$) but the residual worm burdens were lower than in control animals ($0.05 > P > 0.02$).

Comparison of the weight of the spleen, the testis and the terminal mesenteric lymph node in resistant and non-resistant mice.

Experiment 9 - 36 male CFLPb mice were infected with 1,250

TABLE 24 The effect of gonadectomy in male mice on the expulsion
EXPT. 8a of the larvae of A.tetraptera.

Group	Number of mice	Day killed	Mean worm recovery	± s.d.
A (Control)	6	9	98.5	111.8
B (Gonadecto - mised)	5	9	117.8	45.5
C (Control)	10	40	19.0	43.2
D (Gonadecto- mised)	10	40	0	0

Analysis of results by Students t test and the Wilcoxon test

Groups compared	Probability	
A X B	0.8 > P > 0.7	N.S.
C X D	0.2 > P > 0.1	P < 0.1
A X C	0.2 > P > 0.1	P < 0.005
B X D	P < 0.001	P < 0.001

eggs of A.tetraptera and with a further 1,500 eggs on day 5 (because the original inoculum was discovered to have a very low infectivity). The mice were kept in three groups of twelve. On day 10, seven mice were killed to assess the susceptibility of the group, the remainder were killed on day 31. All the mice were weighed and the weight of the testis, the spleen and the terminal mesenteric lymph node was also determined. The results are shown in Table 26.

If, as the previous results suggested, the level of sex hormone is an important factor in determining the effectivity of an animals resistance to infection, the size of the gonads should give some indication of this ability if it is related to the level of sex hormone secretion. Individuals which are non resistant should therefore be characterized by high hormone levels and correspondingly larger gonads, than in resistant mice. In experiment 9 an attempt was made to relate the size of the male gonads (as a percentage of body wt.) to the ability of resisting infections.

The experiment, however, was complicated by one unforseen circumstance, namely that of the thirty mice comprising the group killed on day 31, only one proved to be non-resistant. It was, therefore, impossible to make a statistical analysis of this result and consequently the hypothesis has not been adequately tested.

The effect of incubating mature A.tetraptera in an invitro system containing Hanks' saline and oestrogen.

Experiment 10 - Oestrogen is not a water soluble compound and it is extremely difficult to find a form which might be directly presentable to worms kept *in vitro* in Hanks's saline. A very crude experiment was attempted in an effort to determine

TABLE 25 The effect of gonadectomy in male mice on the expulsion
EXPT. 8b of the larvae of A.tetraptera.

Group	Number of mice	Day killed	Mean worm recovery	± s.d.
A (Control)	5	12	194.2	84.9
B (Gonadecto - mised)	4	12	171.8	61.9
C (Control)	10	28	79.8	80.8
D (Gonadecto- mised)	10	28	12.8	20.6

Analysis of results by Student's t-test and the Wilcoxon test

Groups compared	Probability	
A X B	0.7 > P > 0.6	N. S.
C X D	0.05 > P > 0.02	P = 0.01
A X C	0.05 > P > 0.02	P < 0.025
B X D	P < 0.001	P = 0.001

TABLE 26 EXPT. 9 A comparison of the weight of the testis, spleen and terminal mesenteric lymph node in resistant and non-resistant male mice.

	Resistant mice		Non-resistant mice	
	Mean	± s.d.	Mean	± s.d.
No mice	28		1	
Worm recovery	4.3	9.3	142	
Body weight (grams)	27.8	4.7	20.9	
Testis " (grams)	0.201	0.027	0.181	
Testis wt. / Body wt. %	0.732	0.09	0.867	
Spleen wt. (grams)	0.107	0.021	0.06	
Spleen wt. / Body wt. %	0.39	0.07	0.287	
T.m.l.node wt (grams)	0.0109	0.0071	0.0115	
T.m.l.node wt / Body wt. %	0.039	0.0071	0.0551	

whether oestrogen would have a rapid effect on the metabolism of the worms leading to a reduced survival in the abnormal environment.

Glass petri-dishes, containing 10 ml., of Hanks's saline and a total concentration of oestrogen ranging from 1.5×10^{6} nanograms/ml to 1.5×10^{-4} nanograms/ml., were incubated at 36-37°C. Fourteen to seventy-one mature worms of both sexes were placed in each petri-dish together with an equal number of controls in petri-dishes containing Hanks's but no oestrogen. The number of worms surviving on each day was recorded.

It can be seen that no difference between the two groups was demonstrable. There was no relationship between the concentration of oestrogen and the survival of worms. The most rapid death occurred on day 3, so that by day 4 all the worms were dead, as judged by their ability to respond to tactile stimuli.

The presence of oestrogen in the Hanks's precluded the changing of the saline every 12 or so hours, which would have been necessary to achieve a prolonged survival of the worms. Presumably, after 3-4 days incubation the concentrations of respiratory gases, metabolically vital compounds and metabolic waste products, were such as to no longer sustain the survival of the worms. A negative result is of little significance in an experiment of this type since there are so many other variables which might have affected the result. If Mathies's hypothesis is to be proved incorrect it must be shown that neither oestrogen nor any of its metabolic precursors or metabolites affect the survival of A.tetraptera and that this is true also of all the larval and developmental stages of the parasite. It could of course be that a particular

TABLE 27 EXPT. 10 The effect of incubating mature A.tetraptera in an invitro system containing Hank's saline and oestrogen.

Concentration of oestrogen	Number of worms alive Day 0	Percentage surviving to day 1	2	3
Control	50	100	96.0	0
Control	40	100	92.5	0
Control	37	100	100.0	0
Control	23	100	95.6	0
Control	41	100	92.6	0
1.5×10^6 nanograms/ml.	51	100	96.1	0
1.5×10^5 "	38	100	94.7	0
1.5×10^4 "	68	100	84.2	0
1.5×10^3 "	71	100	90.1	0
1.5×10^2 "	40	100	100.0	0
1.5×10 "	24	100	91.6	0
1.5 "	27	100	92.0	0
1.5×10^{-1} "	25	100	91.3	0
1.5×10^{-2} "	32	100	96.7	0
1.5×10^{-3} "	33	100	97.0	0
1.5×10^{-4} "	52	100	96.1	0

oestrogen compound affects only one stage in the life-history of A.tetraptera and if that is so then Mathies' hypothesis holds true.

DISCUSSION.

Sex differences in the responses of animal hosts to infection by nematode parasites, are well documented (Ackert and Dewhirst,1950; Haley,1958; Mathies,1959 b; Stahl,1962; Neafie and Haley,1962; Dobson,1961 a, 1961 b, 1964, 1965, 1966 a, 1966 b; Solomon,1966, 1969.). Most authors agree that the steroid sex hormones may be very important, in terms of susceptibility or immunologically mediated resistance, in determining the suitability of the host for infection by a parasite. This hypothesis is reinforced by the observation that the sex differences in the response do not appear until after maturity of the host when the secretion of sex hormones has reached the elevated adult level. How sex hormones manage to exert such a major disturbance on the resistance mechanism of the host is at present still unknown but several hypotheses have been proposed, and these will be discussed below.

Mathies suggested, and Stahl supported, the hypothesis that oestrogen has a direct detrimental effect on the metabolism of the gut lumen dwelling parasite. Mathies (1959 b) found that the gonadectomy of male and female mice led to reduced worm levels, as assessed by autopsy 20 days post infection. Since there is no sex difference in the number of larvae during the first eleven days (Stahl,1962) of infection, it could be concluded that the gonadectomised mice rejected larger numbers of worms than the controls inbetween day 11 and 20. A further possibility however, is that gonadectomy per se reduced the susceptibility of the mice to infection,

But unfortunately, Mathies's experiments had no controls to eliminate this possibility. It can be seen that the results shown in Table 23 Expt.7 are in agreement with Stahl's (1962) report and with the conclusions reached in Chapt.four, confirming the rejection of worms by female mice during the third week of infection.

Experiments 8 a and 8 b were carried out to amplify further these observations in an attempt to reconcile Mathies's results with the process of worm rejection.

Clearly gonadectomy had no effect on the susceptibility of mice, but it increased the ability of the male mice to expel worms. Two of the experiments (Expt.8 a and Expt.9) were complicated by the presence of an unusually large percentage of resistant animals (a complication probably arising from the breeding of mice in small colonies), but the results tended to support the concept that gonadectomy in male mice leads to more efficient expulsion of A.tetraptera.

A re-examination of Mathies's data (1959 b) suggested that his results may have been misinterpreted. Firstly, it can be seen that the administration of testosterone to male mice did not affect the worm burden. If it is assumed that all his male mice were non-resistant (and this is strongly suggested by the low standard deviation in the groups) this is not a surprising discovery, since the number of worms established in controls and testosterone-treated animals probably represented the maximum infectivity for the inoculum in the particular age and strain of mice used. It can be seen, however, that oestrogen reduced the worm burden in male mice but instead of adopting Mathies's "direct influence" explanation it is suggested that the reduction of worms was caused by the stronger immunologically-mediated response in these animals,

since oestrogen would be expected to lower the plasma testosterone level, presumably by a feedback inhibition acting on the secretion of the gonadotrophic hormones, follicle stimulating hormone and luteinizing hormone (Coppage and Cooner, 1965; Ganong,1967.). If Mathies's hypothesis is valid, then presumably it would follow that greater quantities of oestrogen would result in fewer worms, but his results showed that the treatment of female mice with oestrogen did not lower the residual worm burden, implying that in female mice the mechanism of worm expulsion was already working at maximum efficiency and could not therefore, be further increased. Presumably the administration of oestrogen would have increased the levels of hormone available to the worms in the gut and should have had a more potent antimetabolic activity, thereby reducing the residual worm burden even further.

Although Mathies's hypothesis is aimed at explaining a specific situation it is clear that this interpretation can not be applied to the many other aspects of sex dependant differences in response to infection, recorded in the Literature. It is difficult to conceive how the many different infecting agents can all have a similar susceptibility to the presence of oestrogen.

Human infections, it seems, are more prevalent in the male than in the female (Washburn et al 1965). Michaels et al (1971) found that in 120 hospitalized infants aged 6-14 months, there was a greater antibody response in females than in males, Similarly, in a field trial involving 15,000 children, girls were found to have mounted a greater antibody response to rubella vaccine (Michaels et al 1971). Other studies have shown that IgM levels may be higher in adolescent and mature females (Butterworth et al 1967;

Stoop et al 1969) and that higher levels of IgA polioantibody can be found in female patients in response to immunization (Ainbender et al 1968).

One explanation which has been proposed to explain this situation is based on genetic arguments (Rhodes et al 1969; Washburn et al 1965; Schlegel and Bellanti,1969;). Washburn et al (1965) proposed that the antibody response is at least partially regulated by genes on the X-chromosome and since inactivation of one of the X-chromosomes is random among the different cells, it is argued that the resultant mozaicism would give the female a greater genetic diversity and lead to an increased heterogeneity in antibody synthesis. Evidence to support this hypothesis was produced by Rhodes et al (1969) who showed that serum levels of IgM were highest in women with an additional X-chromosome (XXX), intermediate in normal women (XX) and least in males (XY). In addition, it is noted that many immunodeficient diseases have an X-linked mode of transmission, including X-linked thymic alymphoplasia and congenital agammaglobulinaemia (Schlegel and Bellanti,1969).

The superiority of the female in mounting an immune response has also become evident from the many laboratory studies which have been conducted to test this hypothesis. Female mice developed a stronger and a longer lasting immune response to bovine serum albumin (BSA) than did males and it was suggested that in part the greater phagocytic activity of the reticulo endothelial system in females may explain this situation (Terres et al,1968). Reticuloendothelial activity, as estimated by carbon clearance time, is known to be stronger in young animals than in old and in

in adult female mice compared with males (Hartveit and Andersen,1969). Agglutinin titres in rats given multiple injections of Salmonella typhosa H antigen were significantly higher in female rats than in male rats (Weimer and Roberts, 1972). IgM and IgG antibody against sheep red blood cells (SRBC), as measured by plaque forming cells/spleen (PFC/ spleen) was higher in female Syrian hamsters than in males. The authors (Blazkovec et al 1973) concluded that the efficiency of antibody production by individual cells in young females was greater than that of males and that it was lower still in older hamsters. There was, however, no such difference in the hemolysin or the hemagglutinin response. Blazkovec et al (1973) listed four points of sexual dimorphism in the hamster, which they considered important in interpreting their results: a) Male hamsters are shorter and lighter than females. b) Splenic weight and hematopoietic activity is greater in the female (Ravines,1961). c) The male hamster has heavier adrenals. d) Male hamsters have a higher pituitary LH content.

Various authors have attempted to modify the hormonal status of their experimental animals by treatment with hormones, ablation of the primary sites of hormone secretion and by a combination of these techniques. Using these procedures it has been universally found that orchidectomy has immunopotentiating consequences (Graff et al 1969; Castro and Hamilton,1972; Blazkovec et al 1973; Castro,1974 b) and that it is rapidly followed by hyperplasia in the lymphoid tissues (Dougherty,1952; Castro,1974 a). Androgens when administered to orchidectomised animals counteracted the effects of orchidectomy, suggesting that the acceleration of allograft

rejection (for instance) in orchidectomised animals was achieved by the deprivation of testosterone (Castro,1974 a). Several authors have attributed the advantage in immunoreactivity of female mice, to the lack of male sex hormones, (Blazkovec,1973; Castro,1974). Allograft survival was prolonged in testosterone treated mice (Castro,1974 b), confirming that this sex hormone has, indeed, immunosuppressive properties (Kappas, Jones and Roitt,1963).

Ovariectomy (Oophorectomy) and treatment with oestrogenic hormones has been found to increase the immune response (Weinstein,1939; Graff et al 1969) but the latter treatment was only effective if large doses of the hormone were given. Other authors have found ovariectomy to be without effect. Thus ovariectomised hamsters did not have an altered response to SRBC (Blazkovec,1973) nor did ovariectomy accelerate the agglutinin response to Candida albicans vaccine (Rifkind and Frey,1972) although in both model systems, orchidectomised mice responded more rapidly than controls, comparably in fact to intact females.

Castro's work (1974 a, 1974 b) perhaps the most exact so far has shown that where orchidectomy accelerated an immune response, the effect could be reversed by the administration of such immunosuppressive procedure as thymectomy and A.L.S. treatment. These results indicated that the effects of orchidectomy were mediated through a T-cell dependant process. Castro demonstrated that only T-cell dependant responses such as allograft rejection, GVH response, response to oxazalone, response to SRBC'c were affected by orchidectomy. Essentially less T-cell dependant process (i.e.more dependant on B-cells)such as the manufacture of cytotoxic antibodies

to H-2, response to BSA and antipneumococcal polysaccharide antibody synthesis remained unaffected, indicating that orchidectomy in his system augumented CMI and that the B cell response was not directly involved.

In parasitological studies on sexual dimorphism in susceptibility to infection, few authors have considered the effects of the sex hormones on immune mechanisms involved in resistance to infection (Solomon,1969). Haley (1958,1966 a and 1966 b) found that in infections with N.brasiliensis there was only a pronounced sex difference in the abnormal host the hamster. There was no such difference in rats in his experiments, but recently Waddell et al (1971) have shown that female rats expel worms sooner than males and that this difference can be removed by castration of the male. This study, one of the few parasitological works in which sex difference is related to hormonal influence on immunological reactions involved in worm expulsion, has demonstrated results which are closely comparable to those reported in this Chapter. It is proposed that the weaker response in male mice to infection with A.tetraptera, is primarily consequent to the presence of male sex hormones which exert an inhibitory effect on the immune response. The finding that gonadectomy alleviated this inhibition is in agreement with the work of Castro (1974) and Waddell et al, (1971). Castro's (1974) conclusion that orchidectomy removes a restraint on the T-cell dependant response is in accordance with current understanding of the mechanisms involved in worm expulsion, which have also been attributed to a T-cell dependant process (Selby and Wakelin, 1973; Ogilvie and Jones,1973). These findings suggest that the mechanism by which testosterone confers immunosuppression

to the host is basically different from that involved in cortisone induced immunosuppression, where it is thought that the B-cell component is the susceptible step in the immune response (Chapter nine). Presumably, in parasitic infections where sex resistance is manifest by a greater susceptibility of the male in the early stages of infection, (e.g. in abnormal host- Solomon,1966; Dobson,1961 b, 1966 b.) this is brought about by the antiinflammatory effects of testosterone, or by hormone induced changes in the physiology of the skin (Lewert,1958; Solomon,1969) in addition to the immunosuppressive properties outlined above.

CHAPTER SEVEN

The development of *Aspiculuris tetraptera* in hosts other than the laboratory mouse.

INTRODUCTION

Aspiculuris tetraptera has been reported from a number of hosts (Listed in Chapter two) other than the laboratory mouse. The majority of these reports were of natural infections, occuring in wild populations of small mammals. Only one previous worker, Mathies(1959 a) studied experimentally induced infections in foreign hosts.He found that the laboratory mouse harboured greater worm burdens (182.0) from an infection of 500 eggs, than the albino laboratory rat, *Rattus norvegicus albinus* (9.1), deer mice, *Peromyscus maniculatus bairdii* (0), and Mongolian gerbils *Meriones unguiculatus* (2.3). The first three of these species have previously been reported as hosts for the parasite. (Chapter two).

In the present study, laboratory Hooded Wistar Rats, Field mice (*Apodemus sylvaticus*) Bank voles (*Clethrionomys glareolus*) and Field voles (*Microtus agrestis*)were experimentally infected. Of the above species, only *M.agrestis* has never been reported as a host for the parasite, but it was included in the study since there was a population of these voles living in the trapping area.

The aim of the study was to determine whether Hooded Wistar Rat, Field mice and the two species of voles could act as hosts for *A.tetraptera* and if so, to ascertain those aspects of the host-parasite relationship which distinguish an infection in the abnormal host from the normal course of events in the more usual host, the laboratory mouse. It was of

particular interest to discover whether A.tetraptera would show the site specificity and a migration pattern identical to that already described for infections in the laboratory mouse.

RESULTS.

Infections in the Rat.

Experiments 11 and 12 - In the first experiment six Hooded Wistar Rats (one male and five female) and six male CFLPb mice were infected with 1,000 eggs. One animal from each group was killed on the days shown in Table 28. The larvae were recovered by incubation in Hanks's saline and were then cooled overnight at 4°C before being measured by camera lucida (normally completed within 24 hrs of removal from the host.) Three criteria were used in order to assess the suitability of the rat as a host for A.tetraptera : 1/ The number of worms present. 2/ The distribution of worms in the colon. 3/ The size of the worms recovered.

In the second experiment, mature worms, recovered from naturally infected, cortisone-treated, male TFI mice, were transplanted directly into the caecum of four (one male and three female) Hooded Wistar Rats and four female CFLPb mice. One animal from each group was killed on days two and three and the remainder were killed on day five. The worms were recovered, as in the previous experiment, by incubation in Hanks's.

The results of these experiments are presented in Tables 28,29 and 30 and in Fig.39. Three days after infection 173 larvae were recovered from the mouse, but only 65 from the rat in spite of the rat being infected with 2,000 eggs. The rat-worms were smaller ($P < 0.001$) than the mouse

worms and were found slightly distal to the preferred site in the mouse.

The remaining rats had considerably smaller worm burdens (The highest being 11) than the mice. The difference in size of recovered larvae was not significant on day 5, but a statistically valid difference was revealed on day 9($P<0.001$). No larvae were found in the rat killed on day 12 or in the one killed on day 17.

Whereas some larvae persisted for as long as 9 days, transplanted mature worms were less successful, only 29.8% of the worms surviving until day 2 (compared to 92.5% in the mouse) and 4.1% until day 3 (mouse - 95.0%). Both rats killed on day 5 were not infected (mouse - 92.5%, 100%). The distribution of the worms recovered on day 2 was similar to the normal pattern in mice. Thus of the recovered worms 78.6% were in section 3 and this is the normal preferred site for mature worms in mice (Chapter four).

Infections in Apodemus sylvaticus.

Experiment 13. - The field mice used in this experiment were caught in Longworth Traps at Trent Park (Chapter two). Within twenty four hours of capture they were given piperazine citrate while under a light ether anaesthesia. Seven *A.sylvaticus* (4 males and three females) and eight male CFLPb mice were given 1,000 embryonated eggs of *A.tetraptera*. The mice were killed on the days shown in Table 31 and the larvae were recovered by incubation in Hanks's saline. They were then kept at 4°C., overnight and were measured by camera lucida within twenty-four hours of recovery.

The results are shown in Tables 31 and 32 and in Fig.40.

Clearly *A.tetraptera* was not able to become established

TABLE 28 EXPT. 11 The recovery of the larvae of A.tetraptera from R.norvegicus and CFLP b strain laboratory mice after infection with 1,000 eggs.

Days post infection.	Number of larvae recovered.	
	R.norvegicus	M.musculus.
3	65	173
4	3	137
5	8	202
9	11	357
12	0	83
17	0	24

TABLE 29 EXPT. 11 The growth of the larvae of A. tetraptera in R. norvegicus and CFLP b strain laboratory mice after infection with 1,000 eggs.

Days post infection	The length of worms recovered (length in µm)					
	R. norvegicus			M. musculus		
	No. larvae measured.	Mean	± s.d.	No. larvae measured.	Mean	± s.d.
3	39	218.3[1]	32.6	59	239.9[1]	23.6
5	8	346.9[2]	38.9	30	367.1[2]	32.6
9	9	582.0[3]	47.8	54	750.0[3]	64.3
12				53	835.5	99.3

Statistical analysis of results by t-test

1 P < 0.001 2 P = N.S.

3 P < 0.001

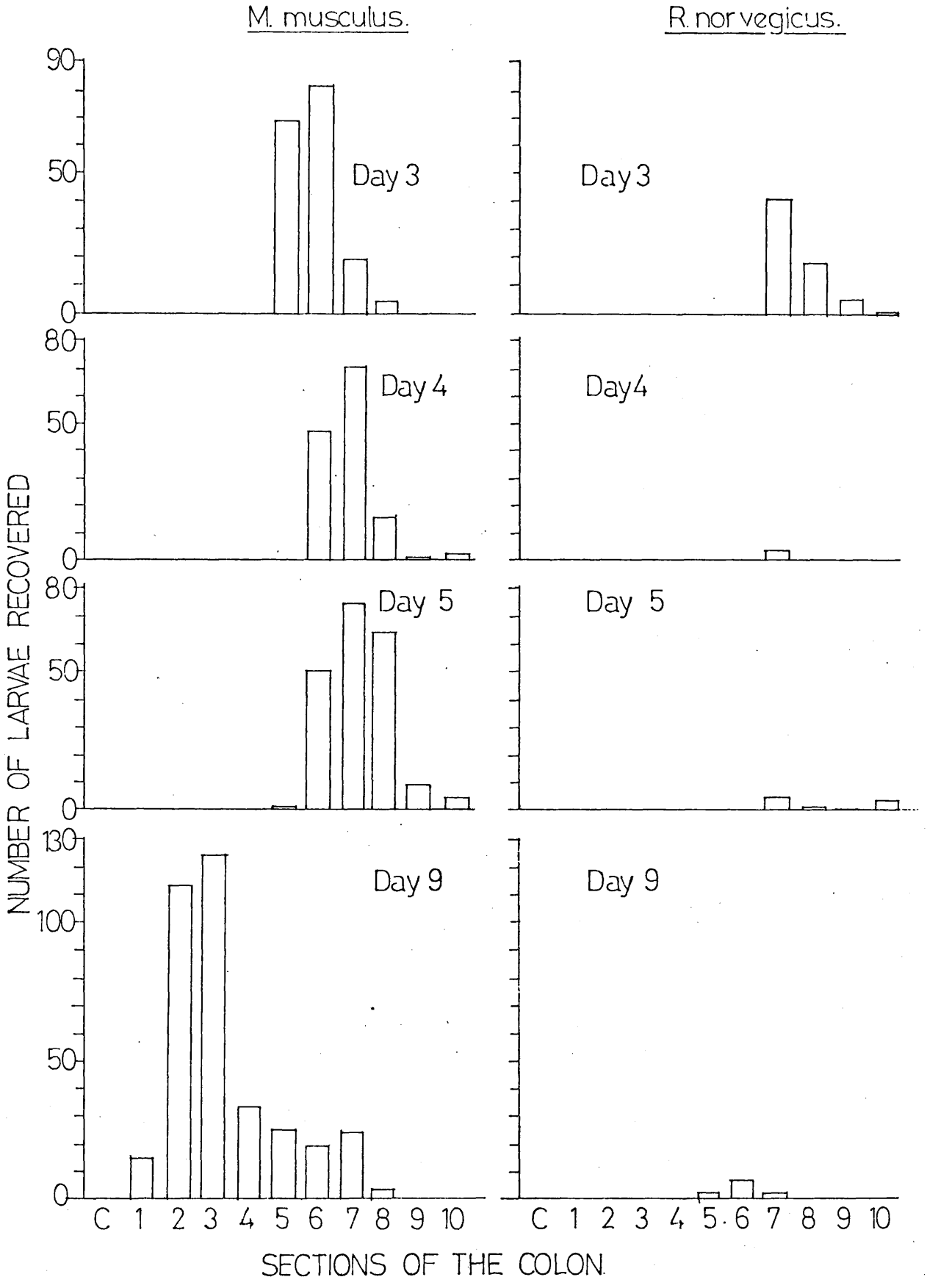


FIG 39 EXPT. 11 The distribution of the larvae of A.tetraptera in the colon of R.norvegicus and CFLP b strain laboratory mice after infection with 1,000 eggs.

TABLE 30 The recovery of worms from recipient *R.norvegicus* and CFLP b strain laboratory mice given approximately 40 mature worms by transplantation.

EXPT. 12

Days post infection.	Number of worms recovered.					
	R.norvegicus.			M.musculus.		
	Nos. of worms given.	Nos. of worms recovered.	% worm recovery.	Nos. of worms given.	Nos. of worms recovered.	% worm recovery.
2	47	14	29.8	40	37	92.5
3	49	2	4.1	40	38	95.0
5	46	0	0	40	37	92.5
5	45	0	0	44	44	100.0

as well in A.sylvaticus as it was in mice. Twenty-four hours after infection only 25 larvae were recovered whereas the control mouse had 218. On day 3 much the same picture emerged but by day 4 even this small worm burden was reduced. The growth of worms proceeded at a slower rate in A.sylvaticus, a statistically significant difference being obtained on day 3 ($P < 0.01$) and on day 4 ($P < 0.01$). The distribution of larvae showed that they became established in a region of the colon which would correspond to the preferred site in mice. The larvae, however, were more widely distributed and whereas on day 1, they were confined to 4 sections in the mouse (Sections 4-7), they were spread over six sections in A.sylvaticus (Sections 5-10). Not only was there a maximum number in the usual region (Section 5-7) but a second peak was observed in section 10, indicating that larvae were being lost from the first days of the infection. This was confirmed by the lower worm burden on day 4 and the absence of worms on days 8,10,14 and 16.

Infections in voles.

Experiment 14 - Whilst trapping A.sylvaticus in Trent Park, the opportunity to trap more than just this one species of small mammal presented itself. Clethrionomys glareolus and Microtus agrestis were found to enter the traps and were consequently recovered at inspection. Voles appeared to be more susceptible to cold than field mice and a number did not survive a night in the traps. Only two specimens from each species were available for study and these, together with two male CFLPb mice were infected with 1,000 eggs of A.tetraptera. M.agrestis did not become infected, as judged by the two animals killed on day 3 and 10 respectively.

TABLE 31 EXPT. 13 The recovery of the larvae of A.tetraptera from A.sylvaticus and CFLP b strain laboratory mice after infection with 1,000 eggs.

Days post infection.	Number of larvae recovered	
	A.sylvaticus	M.musculus.
1	25	218
3	32	187
4	9	113
8	0	305
10	0	254
14	0	316
16	0	227

TABLE 32 The growth of the larvae of A.tetraptera in A.sylvaticus and
EXPT. 13 CFLP b strain laboratory mice after infection with 1,000 eggs.

Days post infection.	The length of worms recovered (length in µm.)					
	A.sylvaticus			M.musculus		
	Nos. larvae measured.	Mean	± s.d.	Nos. larvae measured.	Mean	± s.d.
1	19	173.7[1]	31.7	37	185.8[1]	35.6
3	31	295.2[2]	25.3	48	318.2[2]	37.1
4	9	344.4[3]	24.3	39	370.5[3]	24.3
8				59	742.0	34.6

Statistical analysis of results by Student's 't'-test

1 – $P > 0.1$
2 – $P > 0.01$
3 – $P > 0.01$

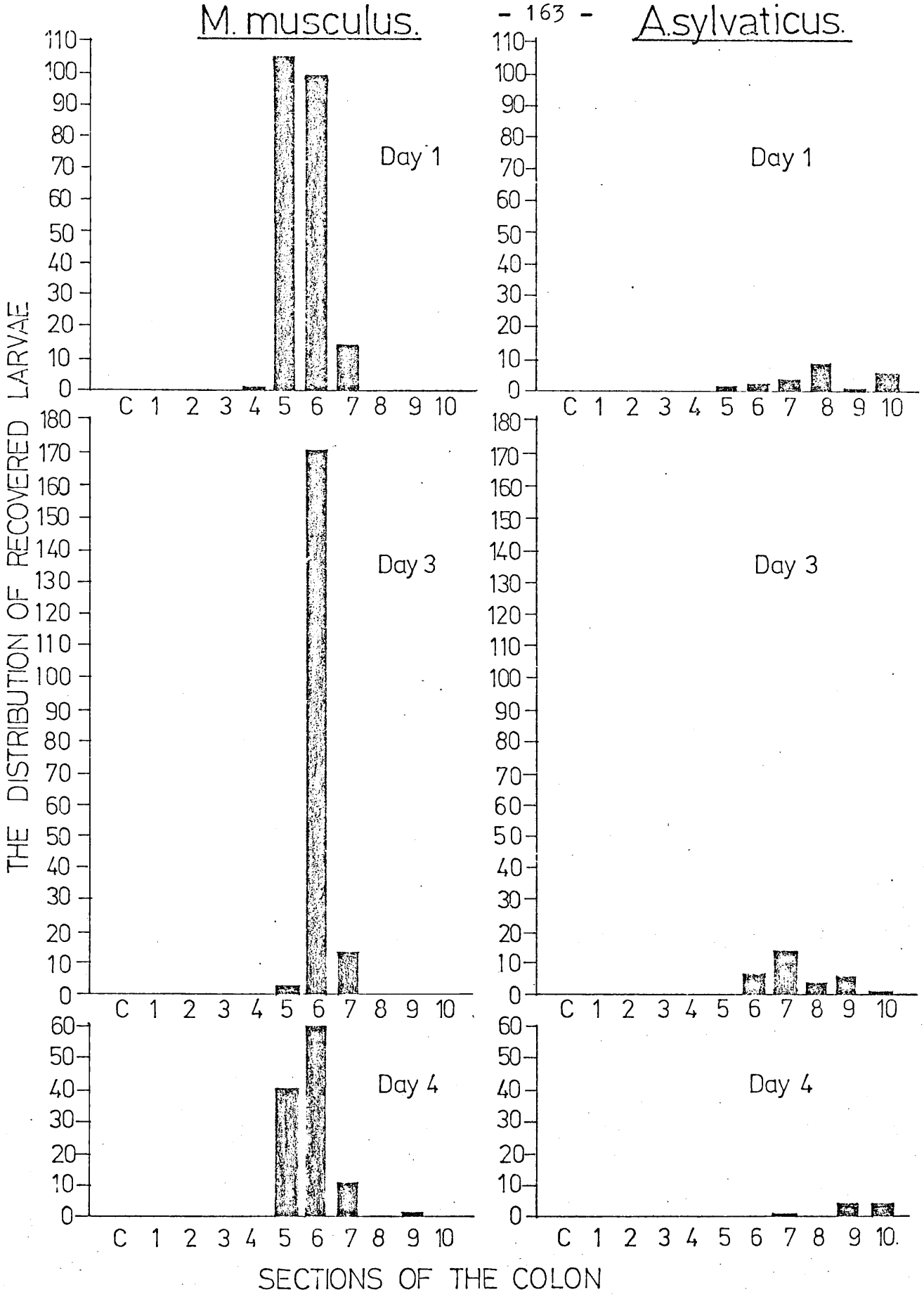


FIG 40 The distribution of the larvae of A.tetraptera in the colon of A.sylvaticus and CFLP b strain laboratory mice after infection with 1,000 eggs.

EXPT. 13

The one C.glareolus killed on day 3 was infected with 15 larvae and 3 mature worms. The larvae were in approximately the correct stage of development to have originated from the experimentally given infection, but the mature worms could only have been a natural infection. The remaining vole, killed on day 10 was not infected, but another vole (C.glareolus), not part of the present experiment, was autopsied after capture and was also found to be infected with five mature worms.

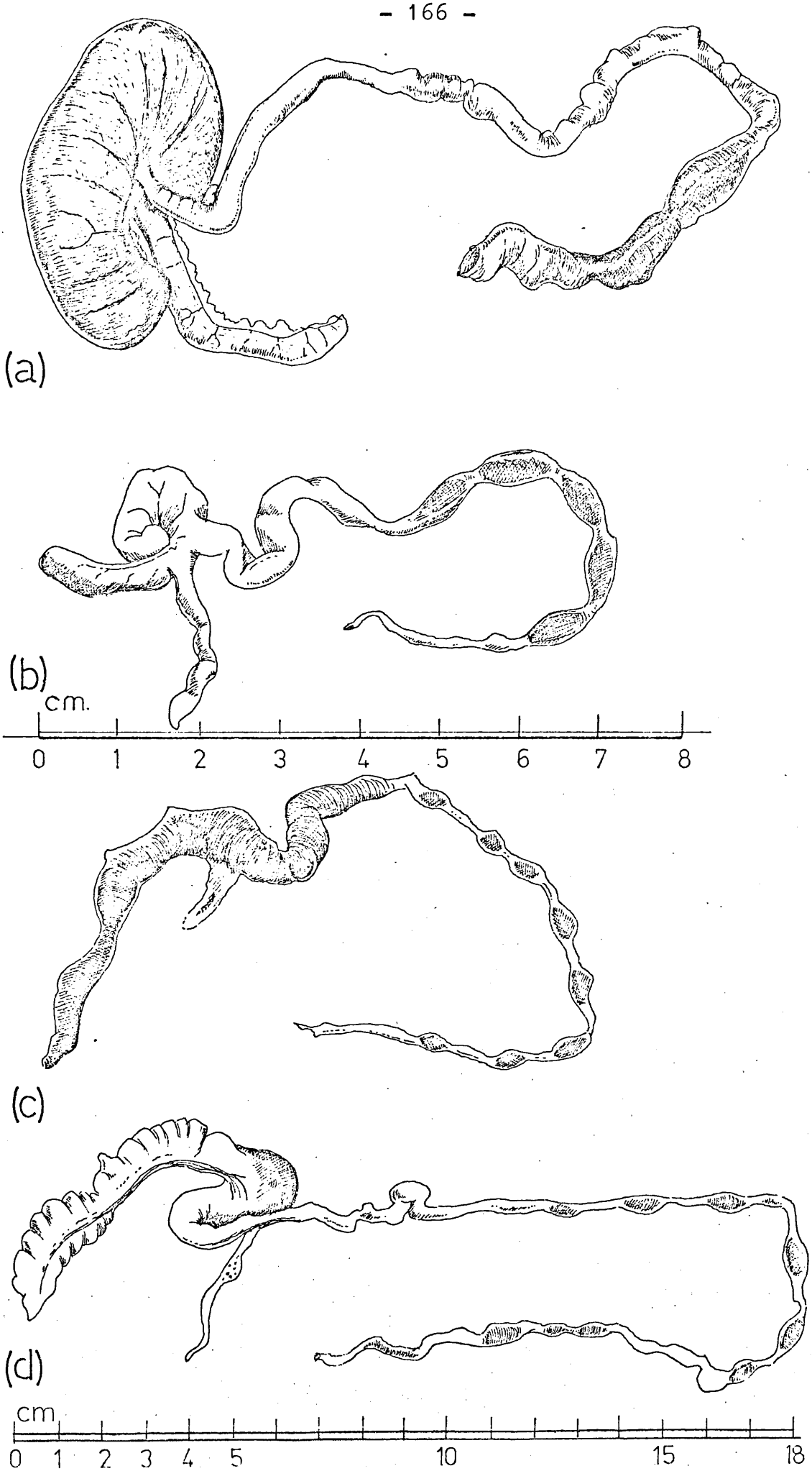
The larvae recovered from C.glareolus killed on day 3 had a maximum concentration in section 9, which was near the anus and more distal than the preferred site in mice. It could be, however, that the mature worms had already stimulated a degree of immunity in the animal and that the worms were being expelled by a secondary immune reaction, rather than by the unsuitable environment of the atypical host.

The effect of hydrocortisone on the survival of A.tetraptera in A.sylvaticus.

Experiment 15 - Eleven A.sylvaticus (Seven males and four females) and ten male CFLPb mice were arranged into four groups, as shown in Table 33, in which six A.sylvaticus (Three males and three females) and six CFLPb mice were treated with hydrocortisone acetate on days -2 and +1. All the animals were given 1,000 eggs of A.tetraptera on day 0 and were then killed on day 10.

As in the previous experiment (Expt.13) A.tetraptera larvae did not survive for long in control A.sylvaticus, male or female mice. Worms were, however, recovered from the anterior colon in hydrocortisone treated A.sylvaticus, indicating that the anterior emigration was accomplished by

Fig. 41. Sketch of the external morphology of the caecum and the colon in the rat (a)- CFLPb mice, (b)- *Apodemus sylvaticus*, (c)- and *Clethrionomys glareolus*, (d)- to show the relative size and the length of the intestine. *A.tetraptera* became established to a lesser or to a greater extent in the colon of all four species.


(a)
(b)
cm.
0 1 2 3 4 5 6 7 8
(c)
(d)
cm
0 1 2 3 4 5 10 15 18

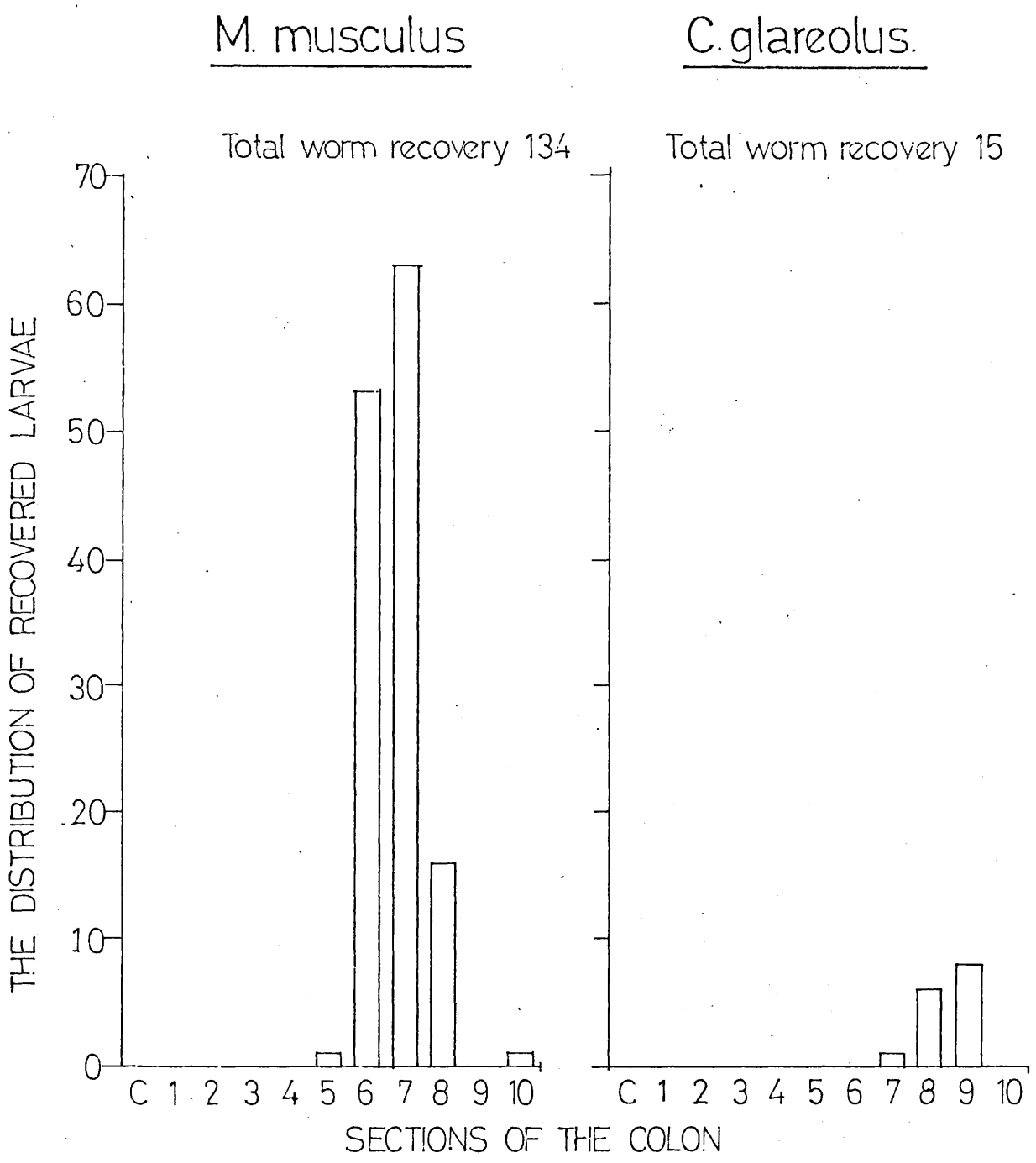


FIG. 42 The distribution of the larvae of A. tetraptera in the colon of
EXPT. 14 Clethrionomys glareolus and CFLP b strain laboratory mice after infection with 1,000 eggs.

these larvae and more over they were found to have grown as well as in hydrocortisone treated CFLPb mice (P= N.S.). Nevertheless there were strikingly fewer larvae in the treated abnormal host ($P < 0.001$) than in similarly treated CFLPb mice. Hydrocortisone was also found to have increased the mean worm recovery from CFLPb mice ($P < 0.01$) and to have increased the growth of these worms ($P < 0.001$).

DISCUSSION.

The concept of host-specificity with reference to parasitology has been amply reviewed in the past (Cameron,1964; Croll,1966; Baer,1971) and it is not proposed here to expand on these views. Rather, only those aspects of host-specificity having a direct relevance to the experimental work, will be discussed.

With the exception of M.agrestis, the three remaining abnormal hosts of A.tetraptera were found to be susceptible to infection. Thus in all three species an initial level of infection was established and further, these worms showed signs of a site specificity similar to that in CFLP mice (Chapter four). In all the animals autopsied, the worms were recovered from the mid colon or even distal to it, there being no larvae in the anterior colon. The site specificity observed in abnormal hosts differed from that in the mouse , there being a greater range of sections from which the parasite could be recovered. In the field mouse, the small population of established worms appeared to be gradually displaced distally, few larvae remaining in the colon after day four. The larvae were more successful in the rat, where the worms recovered on day 9 were anterior to the initial establishment site, suggesting that an emigration may have been

TABLE 33 EXPT. 15 The effect of hydrocortisone on the recovery of A.tetraptera from Apodemus sylvaticus.

Group	No. mice.	Mean worm recovery.	±s.d.
A (CFLP b strain control.)	4	73·8[1,2]	64·4
B (CFLP b-hydro cortisone treated.)	6	239·5[1,3]	75·1
C (A.sylvaticus control.)	5	0	0
D (A.sylvaticus hydrocortisone treated.)	6	12·8[2,3]	8·4

TABLE 34 EXPT. 15 The effect of hydrocortisone on the growth of A.tetraptera in A.sylvaticus.

Group	No. worms measured.	Mean length MM.	±s.d.
A	79	0·792[4,5]	0·094
B	77	1·034[4,6]	0·091
C	0	-	-
D	57	1·047[5,6]	0·095

Statistical analysis of results by Student's t-test and Wilcoxon test.

1	P < 0·01	P=0·01
2	P = N.S	P=0·01
3	P < 0·001	P<0·005
4	P < 0·001	-
5	P < 0·001	-
6	P = N.S.	-

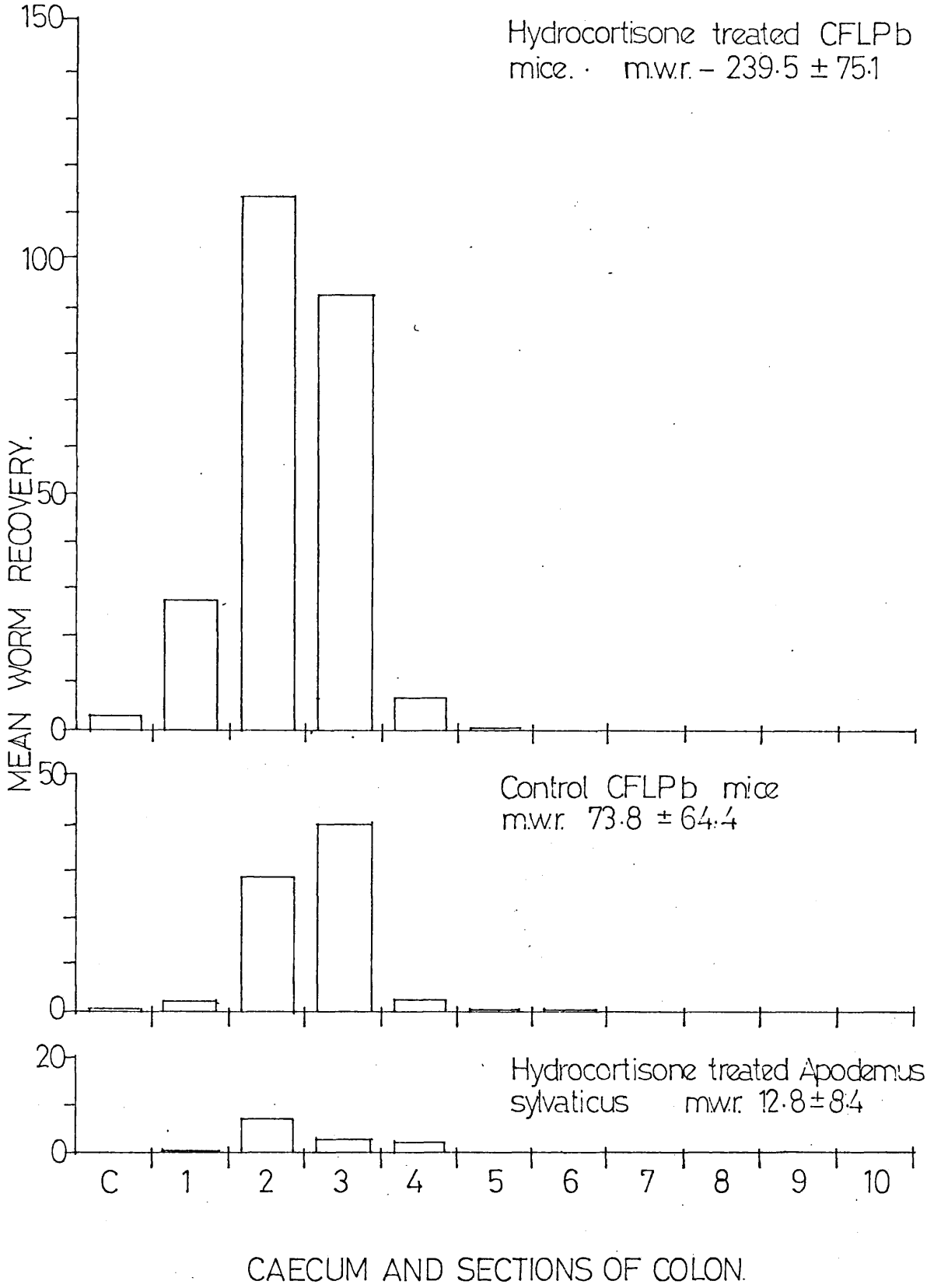


FIG. 43 The effect of hydrocortisone on the recovery and the distribution of A.tetraptera
EXPT. 15 in Apodemus sylvaticus and CFLP b laboratory mice.

undertaken in this host, but it lagged behind events in CFLPb mice where the emigration was already completed at this time. In neither the rat nor in A.sylvaticus did A.tetraptera grow as quickly as in CFLPb mice.

There could be several possibilities to explain the poor survival of larvae in the two abnormal hosts, studied in greater detail. Firstly, it may be argued that the lack of a suitable environment and the absence of the normal hatching stimulus prevented most of the larvae from becoming established in the preferred site. The existence of a very precise establishment site in the mouse suggests that extensive information must be available to the larvae in their normal host, by means of which they become orientated after hatching in the gut and are able to detect the location of the site. Location specifity is known in other nematodes, for instance the fourth stage larvae of Nippostrongylus brasiliensis are able to locate the duodenum even if inoculated, distal to this site, into the posterior ileum (Alphey,1970). Presumably even slightly modified information would hinder the establishment of some larvae and hence the lower susceptibility in the older mouse (Stahl,1959,1962; Mathies,1959 a) and in the abnormal host. Perhaps there are specific physiological conditions in the gut of some animals which are in themselves detrimental to the survival of the parasite. (e.g.faster rate of passage of gut contents.)

The loss of the larvae which have successfully established is, however, more difficult to explain. Firstly, it is possible that the conditions in the gut were such as to support the existence of the parasite for the observed time, but not such as to offer the worms food, thus leaving the

larvae to survive on stored nutrients until the supply was exhausted. The free-living infective larvae of several parasitic nematodes are known to rely on stored fats as an energy reserve (Lee,1965). Anya (1966 b) could not detect a mouth opening in 36 hour larvae and could only identify the mouth in third stage larvae recovered after 120 hours of infection. These observations make it feasible to suppose that there is no feeding during the crypt phase and consequently the larvae are lost only on the completion of this stage. On the other hand, experiments were conducted to determine whether the crypt phase larvae could survive in vitro in Hanks's saline at 37°C, for as long as the mature worms. Mature A.tetraptera survived for up to four days under these circumstances and yet it was not found possible to keep larvae alive for more than twenty four hours. Supporting the suggestion that these larvae were deriving nutrient from the host was the observation that growth proceeded from approximately 0.161 mm (Chapter five) on hatching to 0.582 mm in the rat on day 9 and 0.344 mm on day 4 in A.sylvaticus. In both instances this represented more than a two fold increase in length, perhaps a somewhat difficult task to accomplish on stored nutrients. Further to this, was the observation that A.tetraptera grew to 1.047 ± 0.009 mm and survived for ten days in hydrocortisone treated A.sylvaticus.

Secondly, it can be proposed that only the third stage larvae, on completing the crypt phase, were sensitive to the harmful factors in the environment in the abnormal host. If this is the case, then it must be pointed out that these same factors must have been abolished in hydrocortisone treated animals.

Thirdly, it must be considered, that a host response, functioning earlier in the abnormal host than in the mouse, caused the loss of larvae. Nematospiroides dubius, for instance when given to rats will establish in numbers comparable to those found in mice, but the worms become entrapped in a host response which destroys the larvae before they can return to the gut lumen, as in the mouse (Cross and Duffy, 1963). Evidence for this explanation is also forthcoming from the findings on the effect of hydrocortisone. It is unfortunate that an experiment was not conducted for a longer period to determine whether the parasite could achieve patency under these circumstances, but it was nevertheless found that in hydrocortisone treated field mice the anterior emigration was completed by the few larvae which became established and the worms survived for the ten days duration of the experiment.

Hydrocortisone is known to have a profound effect on the host response to infection, mainly reflected in its anti-inflammatory properties and its immunosuppressive capacity (Chapter nine) and moreover it has been found to greatly prolong the survival of parasites in abnormal hosts. The destruction of N.dubius by the host response of the rat was entirely prevented by hydrocortisone and the development of larvae in treated rats proceeded as in control mice with only a few minor differences, (Cross,1960). The drug had a similar effect on infections with this parasite in hamsters, another abnormal host in which development is usually quickly terminated by a host response, (Cross and Duffy,1963). Cross and Duffy (1963) reported that N.dubius developed normally in neonatal rats, the significance of this being that neonatal

rats are known to have an incomplete ability to resist parasitic infection, (Jarrett et al 1966,1968; Kassai and Aitken,1967; Ogilvie and Jones,1967.). Other examples of studies in which hydrocortisone extended the survival of a parasite in an abnormal host, include Strongyloides ratti in mice (Roman 1956).Trichinella spiralis in Chinese hamsters (Ritterson 1959), Litomosoides carinii in the white rat (Briggs 1959) and N.brasiliensis in the guinea pig (Parker, 1961). Of these model systems studied, the N.brasiliensis system has been examined in somewhat greater detail and it has been found that the first obstacle encountered by the larvae was skin penetration, since it was at this stage that the inflammatory response was stimulated (Lindquist,1950) in the guinea-pig. In hamsters, it was found that the larvae which accomplished the lung migration, developed no further, remaining as third stage larvae in the lungs for a long time (Haley,1966 a, Ogilvie and Jones,1971). Serial passage of the parasite in hamsters, however, improved the proportion of larvae which developed further (Haley,1966 a and b).Host-specificity of N.brasiliensis is clearly dependant in the first instance on the parasite being able to penetrate the host skin and then to perceive the environmental information which will allow it to eventually arrive in the intestine via the lung emigration. If the parasite is to become established in the abnormal host, then in addition, both of these processes must be achieved without the parasite succumbing to the innate, non-specific, host response which it must inevitably stimulate. In the case of N.dubius, the parasite has to penetrate the gut mucosa and then to undergo development to the fourth stage before returning to the gut lumen,

this particular phase being subject to harassment by innate immunity, (Cross,1960).It is thought that cortisone increases the host susceptibility by its suppressive effect on these innate mechanisms. Thus Lewert (1958) found a correlation between the relative susceptibility and resistance to infection, and "the physiological state of organization of integumentary structures", (Quoted by Solomon,1969). Solomon was in agreement with this interpretation, suggesting that testosterone may be acting similarly, i.e. by altering the skin structure, allowing a faster skin emigration by the larvae. The antiinflammatory properties of hydrocortisone are undoubtedly responsible for the prolonged survival of N.dubius larvae in rats and hamsters, (Cross and Duffy,1963) and N.brasiliensis in the guinea pig, (Parker,1961), once the initial penetration phase has been completed. It is pertinent to note here that Ogilvie and Jones (1971) found that when mature N.brasiliensis were surgically transferred to the intestine of rats and mice, they became equally well established in the normal and the abnormal hosts.

Unfortunately, no histological studies were conducted on A.tetraptera in the abnormal hosts, such a study being particularly necessary at this stage. Although consequently, it is not known whether A.tetraptera penetrated host tissue in the abnormal host, it is thought that the effect of hydrocortisone on infections in A.sylvaticus was different from that reported for N.brasiliensis and N.dubius. Hydrocortisone was shown to increase worm recovery from CFLPb mice on day 10, suggesting that the complete loss of larvae from A.sylvaticus was achieved by the same mechanism which effected partial loss of larvae from CFLPb mice after the crypt phase of infection,

(LP-2, Chapter seventeen). A host response may cause the normal preferred site of the parasite to become unsuitable for further occupation, thus initiating a redistribution of the parasite (Crompton,1973). N.brasiliensis (Brambell,1965), T.colubriformis (Connan,1966) and N.dubius (Panter,1969) undergo such a change, revealing, after the events of immunity, a different distribution pattern in the host intestine, from that observed in the non-immune host. It is possible that a local inflammatory response in the mid colon brought about an adverse situation, from which the third stage larvae escaped by an anterior emigration. Such a response might be expected to occur earlier in the abnormal host, perhaps forcing the larvae into the gut lumen, before they are sufficiently developed and prepared for such an emigration, thus causing the loss of all the worms in this situation. Hydrocortisone would abrogate the host response, permitting the development of the larvae to continue normally, and allowing the emigration to occur when the larvae were capable of undertaking it.

In the rat, it is suggested, that perhaps events proceeded in a slightly different way. The survival of the larvae in untreated rats for nine days implies that the worms were not forced into an early emigration, but the observation that such an emigration was attempted suggests that the worms were unable to complete it, most probably because of an inability to maintain a position in the lumen of the rat intestine.

In conclusion, then it is proposed that there may be two different, though not unrelated reasons for the eventual disappearance of the few established larvae in the abnormal

host. The abnormal hosts studied, here, have given important results, presenting an opportunity for future workers to gain even more information about the mechanisms underlying host-specificity and the host-parasite relationship in the abnormal host.

CHAPTER EIGHT

Acquired immunity to infection with *Aspiculuris tetraptera*.

INTRODUCTION

Immunological responses towards foreign antigenic material (derived either from natural disease causing agents or from experimentally introduced non-living substances) are characterized by the ability of the host, having once experienced the antigen to respond, when challenged a second time by the same antigen, with greater speed and efficiency than in the first instance (Humphrey and White,1971). The manifestation of a secondary response thus indicates that the original response was immunologically mediated since innate immune factors involved in "natural resistance" depend on prolonged stimulation before achieving sufficient enhancement to act in the capacity of acquired resistance. It is conceivable too that a period of non-stimulation would reduce the efficiency of these mechanisms to their former level.

Helminth infections in which an immunological spontaneous self-cure response leads to the removal of a large part of the worm burden from the intestine, usually during the 2-nd or 3-rd week of a primary infection, are also characterized by a more rapid expulsion of challenge infections. The rapidity of the onset of a secondary response after a challenge infection varies, however, in the different host-parasite models studied. Thus in *N.brasiliensis* infections, the challenge is eliminated four or five days earlier than the primary infection (Africa,1931; Chandler,1932; Sarles and Taliaferro,1936; Ogilvie,1965; Mulligan *et al*

1965; Jarret et al 1968; Neilson,1969.) Trichuris muris larvae on the other hand are rejected within two to three days of the administration of the challlenge dose, 2 weeks earlier than the normal primary response (Wakelin,1969). Trichinella spiralis shows a marked earlier response only in low level infections, the usual acceleration of expulsion from immune mice being of the order of 2 - 4 days. (Denham,1968).

Challenge infections with Aspiculuris tetraptera become weakly established, compared to controls, provided that the original infection is still present (Stahl,1966 a). The greater the interval between primary infection and challenge, the greater the reduction in the challenge larvae. These results suggested that acquired immune phenomena may be important in this host-parasite system, but Stahl preferred to interpret his results in terms of natural resistance, based on the protective function of intestinal mucus. Stahl's work was incomplete and consequently his conclusions are questionable. The work described in this chapter was undertaken to expand his observations.

RESULTS.

The effect of a challenge infection in resistant and non resistant male mice

Experiment 16 - 42 male CFLPa mice were arranged in two control groups (8 and 10 mice) and one experimental group (25 mice) from which one mouse died. The first control and the experimental group were infected (when approximately 1 month old) with 500 eggs of A.tetraptera. The control group was killed on day 11 to determine the progress of the infection. The remainder of the mice were given a dose of

piperazine citrate between days 28 and 34. The worms were recovered from the faeces after prompt removal from the cages, 2-24 hrs after drug treatment (Chapter three). During this treatment the mice were kept in single cages containing no sawdust or food. A mouse was designated either as resistant or non resistant on the basis of the number of worms found. The mice were then pooled in two groups containing 11 and 13 animals respectively. Thirty-six days after the original infection, the 24 experimental mice together with the 10 non-infected control mice were given 750 eggs of A.tetraptera. The control group was killed 10 days after challenge (day 46) whilst the experimental groups were killed on day 28 (day 64) post challenge.

The results show (Table 35.) that the experimental group was easily segregated into mice with low and high worm burdens. The surprising conclusion, however, is that both groups responded identically to the challenge infection. It is conceivable that some non resistant individuals may have acquired the ability to respond after exposure to the initial infection or by virtue of their age, but it is more difficult to explain the lack of response to the challenge infection, among some mice designated as resistant. This result, although somewhat a setback to the arguments presented later in this chapter, must be accepted as such and perhaps interpreted as an inexplicable experimental error. It might be that in spite of the consistency of the control group, the experimental animals received atypically low egg doses, thus concealing their non-resistant nature when examined after drug treatment.

Super-infection in male mice.

Experiment 17 - In this experiment the fate of challenge infection, given four days after the primary infection was investigated. Sixty male CFLP (4 wks old) mice were assigned to six groups of 10 animals. Four groups were infected with 500 eggs of A.tetraptera on day 0. Four days later, two of these groups, together with the previously non-infected groups received the challenge dose of a further 500 eggs. One control group to the first infection, one challenge control group and one group receiving both infections were killed on day 10. The remaining three groups were killed on day 28.

The results (Table 36 and 37) showed that there was no statistical difference between the means of any of the immunized and challenged groups and the corresponding control animals. The distribution of worms in the colon indicated that the larvae of the challenge infection may have become displaced distally by the previous infection. This, however, is not statistically significant and it was also noted that the primary infection in these mice was established further along the colon than usual. It is possible that the presence of the immunising infection in the normal preferred site (section 5,6 and 7) on day4 may have resulted in the establishment of the challenge infection in the more distal sections of the colon. Larvae from the immunising infection would have begun their anterior emigration three days after challenge, thus leaving their position vacant to the three day larvae of the challenge. These latter worms, however, would normally still be inside the Crypts of Lieberkuhn, only to emerge a day or so later. Since no mice were

TABLE 35 EXPT. 16 The mean worm recovery from mice infected with 500 eggs of A.tetraptera and challenged on day 36 with a further 750 eggs.

Group	Number of mice	Mean worm recovery	± s.d.
A (Immunising infection control day - 11)	8	102.9	18.6
B (Resistant 5th week m.w.r.)	11	19.9	16.2
C (Non-resistant 5th week m.w.r.)	13	85.9	23.8
D (Challenge control day - 10)	10	166.1	94.6
B (28 Days post challenge)	11	73.0	67.2
C (28 Days post challenge)	13	76.1	80.5

TABLE 36 EXPT. 17 The mean worm recovery from mice infected with 500 eggs of A.tetraptera and challenged on day 4 with a further 500 eggs.

Group	Days post infection	Mean worm recovery	± s.d.
A (Immunising infection control)	10	161.9	64.1
B (Challenge infection control)	10	129.0	37.3
C Immunising } Infctn.	10	167.6	54.6
C Challenge } Infctn.		127.4	29.4
D (Immunising infection control)	28	67.2	60.2
E (Challenge infection control)	28	67.2	49.7
F (Immunising and challenge infections)	28	110.3	91.9

TABLE 37 The distribution of the larvae of A.tetraptera in the mouse colon after a challenge infection on day 4.
EXPT. 17

Group	Number of mice	Mean worm burden	Percentage of larvae recovered Regions of the colon										
			C	1	2	3	4	5	6	7	8	9	10
A (Immunising infection control)	10	161.9 ± 64.1	3.4	4.9	49.0	37.4	4.1	0.9	0.3				
C (Immunising infection)	10	167.6 ± 54.6	1.1	1.3	12.8	46.4	32.6	4.7	0.8		0.2		
C (Challenge infection)	"	127.4 ± 29.4					0.2	4.4	31.2	41.8	18.3	3.8	0.6
B (Challenge infection control)	9	129.0 ± 37.3				0.4	10.2	16.7	55.6	15.2	1.2	0.6	

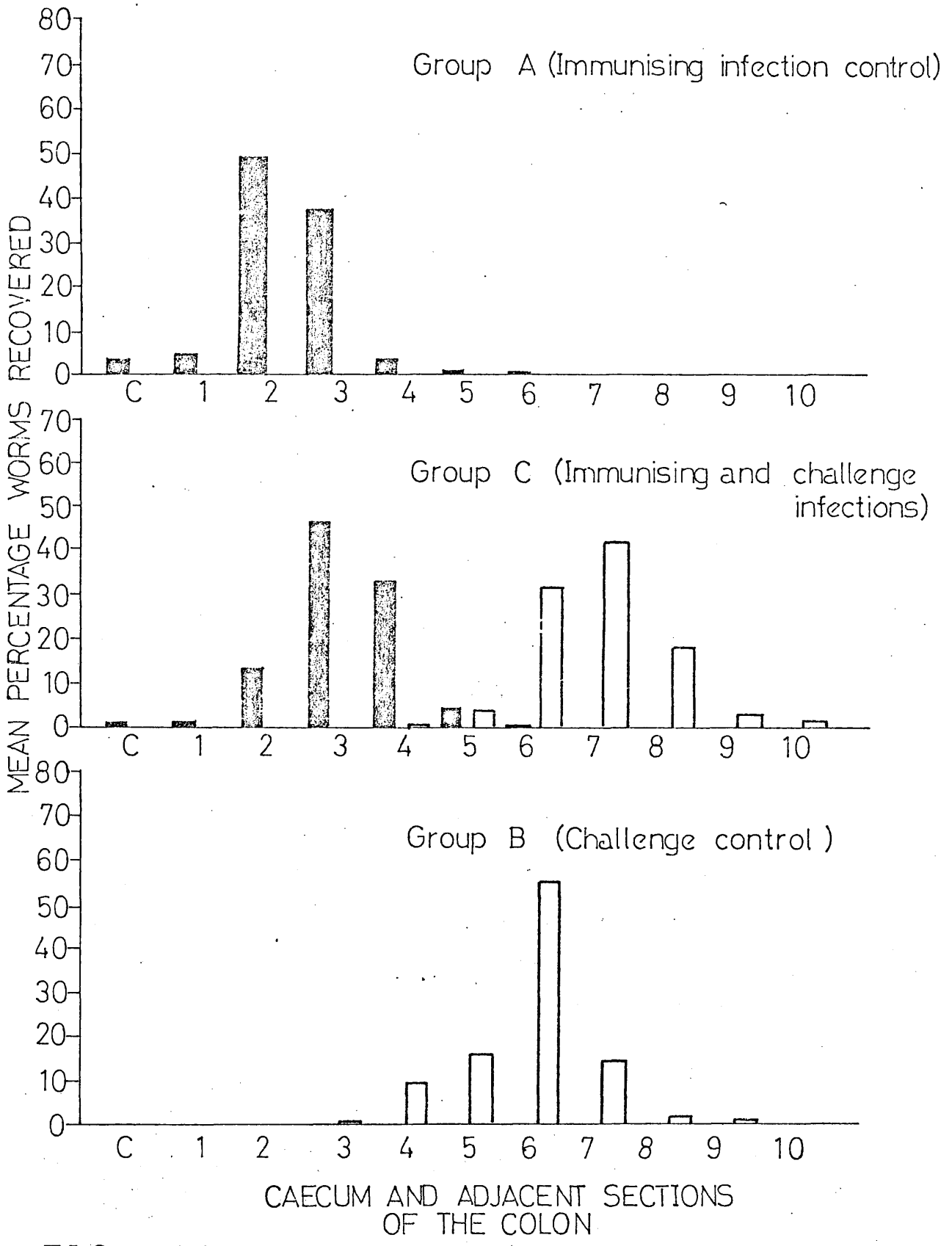


FIG. 44 EXPT. 17 The distribution of the larvae of A tetraptera in the mouse colon after a challenge on day 4.

examined during this time it can only remain to speculation whether the challenge larvae moved into the normal site after the first infection larvae moved anteriorly or whether the two groups co-existed in the same site from the time of the challenge.

The mice killed on day 28 showed that in spite of the almost total elimination of larvae from some mice and the apparent complete lack of response in others, the experimental group had a mean worm burden very closely correlating with the sum of the means of both control groups. The individual worm recoveries showed that there was a complete spectrum of worm burdens ranging from 0 - 189 in the control group and 0 - 275 in the experimental group. Since the 10 day controls indicated a relatively consistant 'take' in all the mice, it can be suggested that the low worm burden in the experimental group indicated mice which had resisted both infections with a degree of success. The intermediate worm burden must be assigned to mice lacking the full response and the heavy worm burden to mice totally incapable of resistance.

Because of the unsatisfactory results obtained in these two experiments using male mice, further work, examining acquired immunity was carried out with female mice only.

The effect of two immunising infections in female mice. (Table 38)

Experiment 18 - In this experiment, the immunising effect of two infections was assessed by comparing the survival of worms from a challenge infection given simultaneously to control mice and to mice subjected to the immunising regimen.

Thirty-nine, 30 day old female, CFLP mice were separated into four groups (9,10,10, and 10 mice respectively).

The first immunising infection (600 eggs) was given on day 0 to the first three (A,B,D) groups only. One of these groups (Group D/9 mice) was killed on day 10. Group A was given piperazine citrate and then both groups A and B were given a second infection 750 eggs (on day 15). On day 34, groups A B and C were given piperazine citrate and two days later (day 36) they were challenged with 1,200 eggs of A.tetraptera. All three groups were killed twelve days post challenge (day 48).

The control group D, (killed on day 10) showed that the first immunising infection was weakly established. There were no infectivity controls to the second immunising infection, but it was known to be more viable than the first egg suspension. Controls to the challenge infection (group C) had a mean worm recovery of 119.1 ± 65.4, which was fairly consistant, excluding two mice with low worm numbers. Both immunized groups resisted the challenge infection almost totally, showing only 3.4% and 7.1% of the control worm recoveries. The differences between the means of group A and C, and B and C were statistically significant. ($P < 0.001$ in both instances.)

The effect of a single immunising infection in female mice.

Experiment 19 - Forty-one female CFLP mice, approximately four weeks old at infection, were used in this experiment. Three groups (A,C, and D) were given an initial infection of 750 eggs. The immunising infection was examined on two occasions one before rejection and one following rejection to ensure that the mice used in this experiment behaved in the predicted way. One control group (group C) was killed on day 11 and the remaining group was killed on day 21.

TABLE 38 EXPT. 18 The mean worm recovery from mice, previously exposed to infection, after a challenge infection of 1200 eggs of A.tetraptera.

Group	Number of mice	Mean worm recovery	± s.d.
A (Two immunising infections)	10	3.9	8.6
B (Two superimposed infections)	10	8.2	16.1
C (Challenge control)	10	119.1	65.4
D (First immunising infection control day - 10)	9	16.2	15.5

Analysis of results by Student's t-test

Groups compared	Probability
A x B	Non significant
A x C	< 0.001
B x C	< 0.001

TABLE 39 EXPT. 19 The mean worm recovery from mice previously exposed to infection, after a challenge infection of 1200 eggs of A.tetraptera.

Group	Number of mice	Mean worm recovery ± s.d.
A (Challenge control)	10	108.2 124.1
B (One immunising infection)	10	0.7 1.0
C (Immunising infection control - day - 11)	8	137.3 64.1
D (Immunising infection control - day - 21)	11	7.5 13.9

Analysis of results by Student's t-test

Groups compared	Probability
A x B	0.02>P>0.01
C x D	<0.001

TABLE 40 EXPT. 20 The mean worm recovery from mice, previously exposed to infection, after a challenge infection of 1000 eggs of A.tetraptera.

Group	Number of mice	Mean worm recovery	± s.d.
A (Crypt infections only)	10	10.1	17.6
B (Challenge control)	12	273.4	254.3
C (Two infections only)	9	10.9	25.2
D (First infection only)	10	57.6	82.0

Analysis of results by Student's t-test and the Wilcoxon test

Groups compared	Probability	
A x B	<0.01	<0.001
B x C	<0.01	<0.001
B x D	<0.02	<0.001
D x C	N.S.	<0.001

Groups A and B were given a single dose of piperazine citrate on day 19 and then both groups were challenged on day 21 with 1,200 eggs. Twelve days after challenge both groups were killed in order that their worm burden could be assessed. (Table 39)

The primary infection was rejected in the normal way (Chapter four.). The initial level of 137 ± 64.1 worms on day 11 was significantly reduced by day 21 (7.5 ± 13.9 worms /$P < 0.001$/). A nineteen day exposure of mice to this single infection was sufficient to cause an almost complete state of immunity ($0.02 > P > 0.01$). The result was complicated by an extremely variable control group, but its significance, is none the less appreciable.

Immunization by the Intra-Crypt larval phase of the infection.

Experiment 20 - Forty-one, four week old female CFLP mice were arranged in four groups. Group A was given three 6 day infections (500 eggs on each occasion.) The infections were individually terminated on day 6 by the administration of piperazine citrate. A day or more was allowed to elapse before the next dose of infective material was given. Group B, the challenge control group, was given the same piperazine citrate regimen as in group A, but was not infected. Group C was given 500 eggs on day 0 and a further dose of 1,000 eggs on day 9. Group D received only the single initial dose of 500 eggs on day 0. All the groups were given piperazine citrate on day 36 to remove any worms still present. Four days later (day 40) groups A,B,C and D were given 1,000 eggs and then they were killed on day 51, eleven days post challenge. (Table 40)

Three mice in the control group failed to develop a

reasonable level of infection following the challenge with 1,000 eggs of A.tetraptera. None the less, the mean worm burden in this group was greater than in any of the other three groups ($P < 0.001$). All three immunising regimens were therefore, successful in protecting mice from the challenge infection. Groups A and C showed the stronger response, group D being less resistant to re-infection.

Expulsion of challenge infection larvae.

Experiments 21, 22, 23 and 24 - In the first of these four experiments, 4 of 9, 27 day old female CFLPb mice were given a single immunising infection on day 0. Piperazine citrate was given on day 25 and then both groups were challenged with further 1,000 eggs on day 29. The times when mice were killed and the resulting worm recoveries are recorded in Table 41 and Fig.45.

Experiment 22 was similar to Experiment 21, except that the mice used were 35 days old on day 0. The immunising infection (1,250 eggs) was followed on day 40 by the administration of a further 1,000 eggs before the termination of both infections on day 82 by piperazine citrate. The challenge infection (1,000 eggs) was given to the experimental group (5 mice) immunised by the above regimen and also to a group of five, non-infected control mice.

Experiment 23 consisted of a further group of ten female CFLPb mice, of which five were immunised with a single 42 day infection (1,000 eggs), terminated in the usual way before challenge (1,000 eggs) on day 46.

Experiment 24 - Eleven of twenty two female CFLPb mice were given three infections (mice 35 days old) consisting of 500 eggs of A.tetraptera on each occasion, The days on which the infection was given were 0, + 18 and + 39. The

parasites were cleared on day 52 and the group of mice together with the uninfected 11 controls, was challenged with 1,500 eggs.

In spite of the variation between individual worm burdens it was shown by the last four experiments that the rejection of larvae from immune mice was achieved during the first week of infection, usually before day 6. A surprising feature of these experiments was the loss of larvae from control mice. Most of the animals used in the experiments were over 60 days old, some over 100 days when the challenge infection was administered. It was shown by Mathies (1959 a) and Stahl (1962) that mice exibit a marked age resistance, after 4 weeks of age, based both on a lower susceptibility to infection and a greater resistance towards the established infection. It is proposed that the loss of worms from control mice can be attributed to the age of these mice at infection. In spite of this setback, however, it was demonstrated that expulsion in immune mice occured earlier than in controls. In some experiments a difference between the two groups could be detected as early as on day 3 and day 4.

DISCUSSION.

The purpose of this study, as stated in the introduction, was to investigate the possibility that mice acquire immunity to A.tetraptera, following exposure to a primary infection. Only one other study on a similar aspect of the life history of A.tetraptera has been reported in the literature. Stahl (1966 a) rejected the possibility of an immune response, on the basis of rather weak arguments. The choice of the interpretation involving "natural resistance" in the

form of mucus secretion was arrived at when the author failed to detect the presence of either larval immobilization or agar-double diffusion precipitates using sera from infected animals. Chapter 12 describes experiments which showed that antibodies against A.tetraptera were present in the serum of immune mice.

The experiments reported here firmly establish that female mice are capable of developing an acquired resistance and that in some instances an almost complete immunity is achieved after a single immunizing infection. The results from studies involving male mice proved difficult to interpret and it must be concluded that no obvious signs of acquired immunity were discovered. The experimental plans were not as effective in evoking a detectable acquired immunity as in the later experiments with female animals.

A single 19 day infection (137.3 ± 64.1 larvae) was capable of protecting female mice from a challenge infection in which the mean worm recovery for control mice was 108.2 ± 124.1 larvae (Expt.19.). Furthermore a thirty six day infection in which the mean worm recovery was 24.5 ± 14.9 larvae, protected mice from a challenge with 1,000 eggs (Expt.20.). The control mice had 273.4 ± 254.3 larva whereas the experimental group had only 57.6 ± 82.0 larvae. These results, then indicate that a single or a multiple infection produces a detectably stronger secondary response which is effective in eliminating the greater part of the worm burden before days 10-12. As Stahl (1966 a) pointed out, the larvae of A.tetraptera enter the Crypts of Lieberkuhn in the mid colon, soon after infection. There is ample opportunity in this situation for a fairly close host-parasite contact and

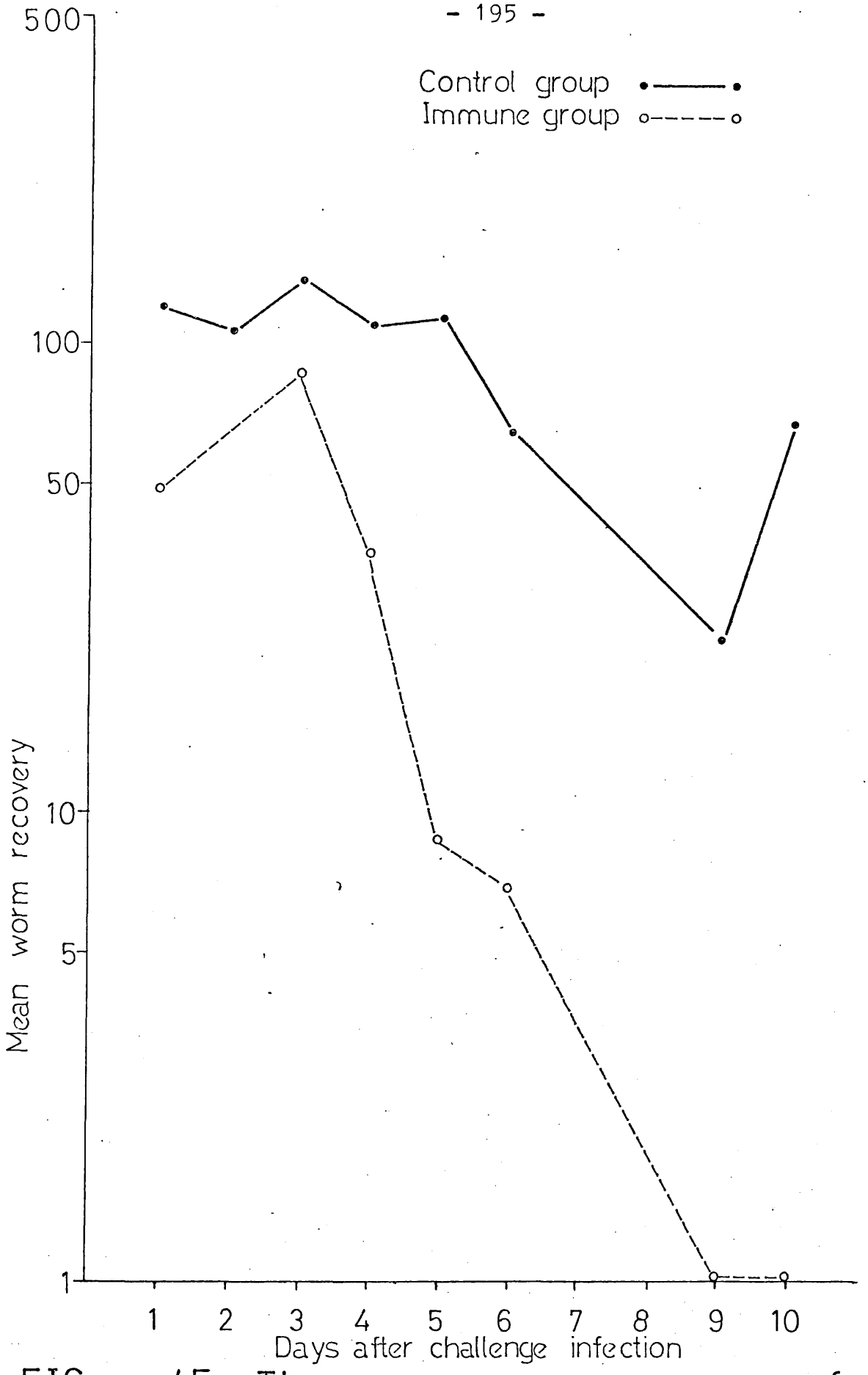


FIG. 45 The mean worm recovery from previously infected and from control mice after a challenge infection with A.tetraptera.

EXPTS. 21-24

(Pooled results)

TABLE 41 EXPTS. 21-24 Individual worm recovery from previously infected and from control mice after a challenge infection with *A. tetraptera.*

Days post infection	Expt.24 C.	Expt.24 I.	Expt.21 C.	Expt.21 I.	Expt.22 C.	Expt.22 I.	Expt.23 C.	Expt.23 I.
1			119	48				
2			107					
3	89 166	14 113			125	2	167	220
4					145	23	74	49
5	21 58 182	0 1 18			236	15	65	10
6	2 79	0 4	52 52 98	0 0 2	33	41	139	2
7								
8								
9	0 47	0 0						
10	0 0	0 0			273	0	0	1

it is possible that the antigenic stimulus, which elicits the immune response is generated at this time. The third stage larvae emigrate anteriorly on day 7 (Chapter four) and thereafter the worms become almost entirely lumen dwellers. Histological examinations (Chapter five) showed that even in the anterior colon, there is some degree of host-parasite contact. The worms are frequently found adhering closely to the mucosa and imprints in the surface of the gut tissue are observable in sections of the infected colon, indicating that when occupied, there was close contact between parasite and host in the particular site. It is, therefore, still conceivable for the antigenic stimulus, responsible for worm expulsion, to be delivered after the anterior emigration on day seven. It has been shown that nematode parasites can be expelled from the intestine, within a week of transplantation to rats with no previous experience of infection.(Ogilvie,1971).

In experiment 20, the antigenicity of the crypt dwelling larval phase was investigated. Care was taken to terminate each of the three infections before the emigration. It was shown, using the experimental plan outlined before, that mice were strongly resistant to the challenge infection, when exposed to pre six day larvae only. Assuming, therefore, that the piperazine citrate was effective in terminating each of the immunizing infections at the appropriate time, the result can be ascribed to the antigenicity of the pre 6 day larvae and it can be concluded that the crypt phase is effective in delivering a stimulus capable of inducing a state of immunity in mice exposed to infection in this way. The last four experiments described in this chapter were concerned with establishing the time at which the secondary response takes effect.

In spite of the complication resulting from the use of older mice (a necessity arising from the fact that immunization takes time) it was concluded that a large proportion of the larvae developing in previously infected mice, was lost during the first week of infection, during the crypt phase or directly after it. The reaction of the host to the antigenicity of the first and second stage larvae entering the Crypts of Lieberkuhn during a challenge infection was typically stronger and took effect before the larvae had time to emigrate anteriorly. This, in itself, suggested that the emigration may be not only a move to a suitable environment, but that it may also be an escape mechanism, protecting the larvae from a local host response engendered by the closeness of the host-parasite contact during the crypt phase. If this is so then it would not be unreasonable to suggest that the response to a primary infection in older mice, may be stronger and that in these animals the main part of the infection is lost during the crypt phase in the first week as in challenge infections, rather than in the second week as in primary infections in younger mice. Worms which may have avoided the early response might then still be expelled during the second week of infection in the normal way, to account for the low residual worm burden in old mice.

In conclusion, the results presented in this chapter established that mice can acquire resistance to infection with A.tetraptera and they add strength to the hypothesis (suggested earlier, in Chapter four) that the primary expulsion of A.tetraptera is achieved by immunologically mediated processes.

CHAPTER NINE

Attempts to alter immunity

I - The effect of hydrocortisone acetate.

INTRODUCTION.

Since the early days of immunology it has become apparent that the immune response is a complex, multi-component process and that as such, the integrity of this process can be interfered with, by treatment of the host in specific ways designed to modify the functioning of one or more components in the response. The immune response can be suppressed in two basic ways; namely by specific inhibition, such as immunological paralysis and by non-specific inhibition which can be achieved by such procedures as X-irradiation, neonatal thymectomy, A.L.S. therapy, thoracic duct drainage and treatment with immunosuppressive drugs (Humphrey and White,1971).

The understanding of the action of an immunosuppressive drug in a specific experimental situation is consequent to a consideration of all the possible components in the immune response which may have been affected by the agent. Thus there are several classes of such drugs, each achieving its effect by immobilising one or more stages in the immune response. In general, all the immunosuppressive drugs have some antimitotic activity, since most were discovered after their effects on neoplasms were detected. The radiomimetic alkylating agents, such as nitrogen mustards and cyclophosphomide are believed to exert their activity at the D.N.A. level, thus interfering with cell replication. The antimetabolic drugs, such as the folic acid antagonists (Chapter 10), which inhibit vital enzymes required in cell metabolism and the purine analogues, such as 6-mercaptopurine (6 MP) which block

protein synthesis, prevent cells of the lymphoid component from undergoing changes following antigenic stimulation (Schwartz,1965).

The glucocorticosteroid drugs in particular have been extremely useful in clinical practise where they have been used to alleviate the symptoms of rheumatoid arthritis (Hench et al,1949) and to treat other inflammatory states (Germuth,1956). Corticosteroid drugs suppress antibody production and the homograft rejection (Elliott and Sinclair, 1968; Gabrielsen and Good,1966; Dukor and Dietrich,1968)and inhibit the development of inflammation (Germuth,1956), processes which are complex and in which the effects of cortisone result from its effect on specific components of each process. Such properties as the reduction of the immigration of leukocytes into sites of inflammation and depletion of peripheral lymphocytes and of lymphoid tissues (Germuth,1956; Cohen,1971) must all contribute in part to the overall effect.

It has also been shown that the development of immunity to parasitic infections can be retarded and/or abolished by cortisone treatment. A number of host-nematode parasite situations have been examined and it is clear that the effects of the drug are not identical in each case. Thus, whereas rats appear to recover their ability to expel infections of Nippostrongylus brasiliensis, when cortisone treatment has been terminated (Ogilvie,1965 b), Trichuris muris will survive to patency and longer in most mice given cortisone during the first two weeks of infection (Wakelin,1970, Wakelin and Selby,1974), these mice becoming thereafter non-resistant to challenge infections, whereas controls, infected for the first time, eliminate the worms during the third

week. Trichinella spiralis is expelled more slowly from cortisone-treated mice than from untreated controls, (Coker, 1955; Briggs,1964) suggesting that cortisone is only partially successful in depressing worm elimination in this host-parasite system.

Mathies (1962) studied the effect of cortisone on the host-parasite relationship of A.tetraptera. He found that cortisone treatment of infected mice resulted in higher worm burdens at autopsy on day 12. A difference between the susceptibility of male and female mice was noted but it was not altered by drug treatment. In spite of the heavy doses used (75 mg/kg body wt) older animals (6-7 wks) were less affected by the drug.

Preliminary experiments showed that some of Mathies's results were repeatable, but important differences between his results and those presented here necessitated a reappraisal of this aspect of the host parasite relationship of A.tetraptera.

RESULTS.

Suppression of expulsion in male mice.

Experiment 25 - Forty four, male, CFLP mice (approx-4 wks old) were arranged into four groups, two control groups (A and C) containing 10 mice each and two experimental groups (B and D) with 12 mice in each group. Hydrocortisone acetate, given by subcutaneous injection, was administered on days -4, -2, 0, +2, +6, +13, +20, +27 at a dose of 2.5 mg. On day 6 the dose was halved and then the previous concentration of 2.5 mg., was again used on days 13, 20 and 27. All the mice were given 650 eggs of A.tetraptera on day 0. One control group and one experimental group were killed on day 10 and the remaining ani-

mals were killed on day 28.

The results are presented in Table 42. There was no significant difference between the means of the worms recovered from control and cortisone treated mice on day 10. Only three of the remaining 12 cortisone treated mice survived to day 28 and the worm recoveries from these mice suggested that there had been no loss of worms. The control mice were inconsistent in their response. Five mice had very low worm recoveries, suggesting that they were resistant, whereas the remaining four had a mean worm recovery of 170 ± 16.9 which is comparable to the 10 day means in both groups.

Comparison of the effect of hydrocortisone in male and female mice.

Experiment 26 - It was shown earlier (Chapter six) that female mice made a stronger response to infection than did males and that they were more uniform in their ability to reject the worms. The effect of hydrocortisone on this response was investigated in this experiment.

One hundred, 4 week old CFLP mice (50 males and 50 females) were arranged into single sex groups of ten. Twenty mice of each sex were used as controls, half of each group being killed on day 10 and the remainder on day 28. Ten male and ten female mice were given hydrocortisone (1.25 mg/dose) on days 7, 10 and 12 and they were killed on day 28. The remaining twenty male and twenty female mice were treated with hydrocortisone on days -1, +1, +3, +5 and +7, and then half of each group was killed on day 10 and the remainder on day 28. The infective dose was 500 eggs of A.tetraptera given on day 0.

The results of this experiment are in agreement with

TABLE 42 EXPT 25 The effect of hydrocortisone on the loss of the larvae of A.tetraptera in male mice

Group	Number of mice	Mean worm recovery	± s.d.
A (Control day 10)	10	190.9	49.2
B (Cortisone day 10)	12	214.8	46.0
C (Control day 28)	9	91.0	76.9
D (Cortisone day 28)	3	189.3	57.0

Analysis of results by Students t-test and the Wilcoxon test

Groups compared	Probability	
A x B	N.S.	N.S.
B X D	N.S.	N.S.
C X D	$P<0.05$	N.S.

TABLE 43 EXPT. 26 Comparison of the effect of hydrocortisone in male and female mice.

Group		Mean worm recovery	
		Killed day 10	Killed day 28
Male mice	Control	161.9[1,4] ± 64.1	67.2[1,2,3] ± 60.2
Female mice	Control	159.1[5,8] ± 69.8	27.6[5,6,7] ± 21.8
Male mice	Hydrocortisone given on days -1,+1,+3,+5,+7	237.6[4] ± 63.0	232.4[2] ± 74.1
Female mice	Hydrocortisone given on days -1,+1,+3,+5,+7	208.5[8] ± 62.7	219.7[6] ± 62.9
Male mice	Hydrocortisone given on days +7,+10,+12		160.2[3] ± 71.0
Female mice	Hydrocortisone given on days +7,+10,+12		148.0[7] ± 46.5

Statistical analysis of results

1 = $P < 0.01$ 2 = $P < 0.001$ 3 = $P < 0.01$ 4 = $P < 0.02$
5 = $P < 0.001$ 6 = $P < 0.001$ 7 = $P < 0.001$ 8 = $P > 0.1$

previous experiments (Chapter six), in that there was no sex difference between the control groups on day 10, and the rejection of larvae was more consistent in female mice, which harboured fewer worms on day 28.

The hydrocortisone treated group killed on day 10 showed a higher worm burden than the controls indicating that the drug had an early effect, prior to day 10. There was no loss of larvae from this group between day 10 and day 28, confirming that hydrocortisone was capable of completely suppressing the expulsion of larvae which occured in the control groups. The groups receiving the hydrocortisone on days 7,10 and 12 had a similar level of infection on day 28, to the 10 day control group. Since the first injection of hydrocortisone was not given until the end of the first week of infection, the drug could not have influenced the initial establishment of the infection. The worms harboured by these mice on day 7 must have been fairly close to the levels in control mice on day 10. No loss of worms was incurred and consequently the 28 day mean worm recovery is comparable to that found on day 10 in untreated mice.

The effect of three injections of hydrocortisone administered at different times during infection.

Experiment 27 - This experiment consisted of ten groups of 10-12, 33 day-old, female mice. The mice received three doses, a total of 3.75 mg., of hydrocortisone acetate, spread over a period of five days. The days of treatment of individual groups, the number of animals in each group at the begining and at autopsy and the day on which each group was killed are all recorded in Table 44. The results of this

TABLE 44 THE EXPERIMENTAL TREATMENT OF

EXPT. 27 FEMALE MICE WITH HYDROCORTISONE

Group	No.mice in group Day -7	Day killed	Cortisone given on days	Day killed
A	10	8	-7 -5 -3	28
B	12	12	-5 -3 -1	28
C	12	10	-3 -1 +1	28
D	12	12	+1 +3 +5	28
E	12	12	+5 +7 +9	28
F	11	11	+9 +11 +13	28
G	11	10	+13 +16 +17	28
H	10	10	+17 +19 +21	28
I	10	10	None	10
J	10	10	None	28

TABLE 45 EXPT. 27 The effect of three doses of hydrocortisone administered at different times during a primary infection.

Group (Days of hydrocortisone treatment)	Number of mice	Mean worm recovery ± s.d.	
A (-7,-5,-3)	8	129.6	37.6
B (-5,-3,-1)	12	123.8	55.2
C (-3,-1,+1)	10	146.4	90.2
D (+1,+3,+5)	12	155.4[2]	39.1
E (+5,+7,+9)	12	145.9[3]	28.9
F (+9,+11,+13)	11	109.1	41.5
G (+13,+16,+17)	10	86.1	21.5
H (+17,+19,+21)	10	38.7	42.8
I (Control day 10)	10	104.4[1,2,3]	50.2
J (Control day 28)	10	22.6[1]	38.1

Statistical analysis of results

1 = $P < 0.001$ 2 = $P < 0.02$ 3 = $P < 0.05$

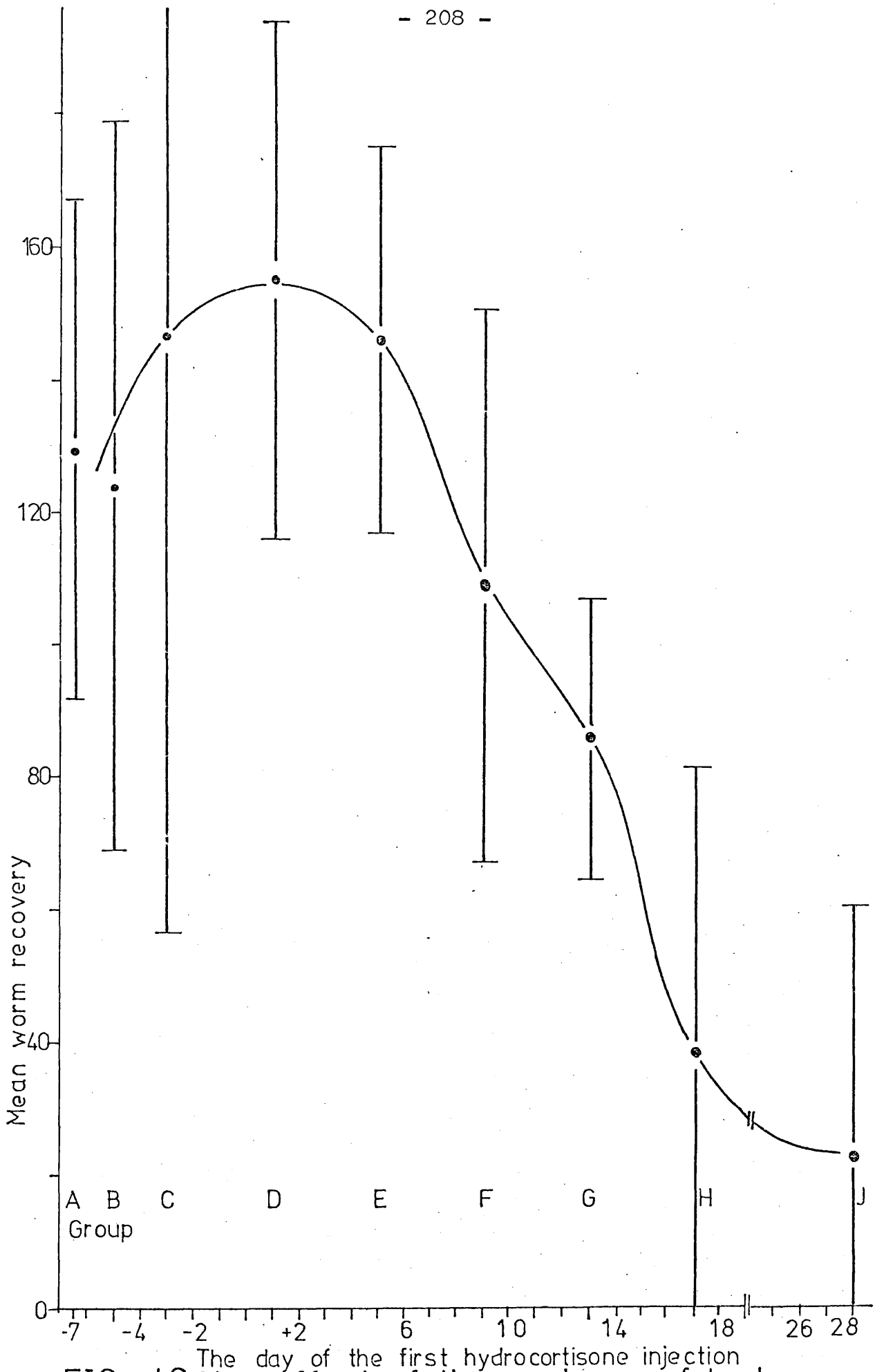


FIG. 46 The effect of three doses of hydrocor-
EXPT. 27 tisone at different times during infection.

experiment are presented in Table 45 and in Fig.46.

When hydrocortisone acetate was given on days -7, -5 and -3 it suppressed the expulsion of worms and this group had significantly more worms than the control group on day 28. The heaviest worm recoveries were recorded in group D which received hydrocortisone on days 1, 3 and 5. Group C and E had similar worm burdens. Group F had virtually the same number of worms as group I, the 10 day control group. Group G and H had intermediate worm recoveries between all the earlier groups and group J the 28 day control group.

The percentage of female worms in the worm burden of the different groups was fairly consistant (58-61%). It is suggested that this is the normal sex ratio for A.tetraptera. By the completion of worm expulsion the sex ratio had altered to 48.6% in the control group.

The duration of the effect of hydrocortisone acetate.

Experiment 28 a - Fifty-four female mice were separated into two groups, (30 and 24 mice respectively) and infected with 750 eggs of A.tetraptera. The first group was treated with hydrocortisone on days 8,10 and 12 only; these days being chosen because they were immediately prior to worm expulsion and consequently the effect of hydrocortisone given during this time was more easily explained in terms of an immunosuppressive action than the effect in mice treated more than ten days before expulsion. The second group received in addition a further dose on days 28, 35, 42, 49 and 58. The standard dose of hydrocortisone was 1.25 mg. Mice from both groups were killed and autopsied as shown in Fig.47.

The infection was extremely variable in both groups. In the group treated with hydrocortisone throughout the

experimental period, four of the 14 animals (28%) killed between day 35 and 52 contained more than 100 worms. The percentage of animals infected with more than 100 worms in the other group (between day 11 and 52) was also 28% (6 out of 21). Using this item (Percentage of mice with more than 100 worms) as a criterion of the duration of the action of hydrocortisone, it was found that by day 60, the worm burdens were appreciably lower in both groups. Thus of 19 animals only 1 had a high worm burden.

Furthermore it was noticed that the colons of a number of the animals killed on day 60 were swollen and large, as if heavily infected, but actually contained few worms, indicating that some loss must have occured recently.

The duration of the effect of hydrocortisone acetate. Experiment 28 b - Sixty-eight, 5 week old female mice were infected with 750 eggs of *A.tetraptera* and then they were injected with hydrocortisone (1.25 mg/dose) on days 5, 7 and 10, slightly earlier than in the previous experiment in order to eliminate the variation in worm burden caused by the loss of worms just after the crypt phase on days 6-8. Twenty five of these mice were given a further dose of the drug on days 17, 24, 32 and 40. Ten nontreated mice were killed on day 21 to give some indication of normal rejection. The times at which mice were killed and the worm recoveries are shown in Fig.48.

The mice used in this experiment were almost a week older than those used in Expt.28 a. Furthermore, cortisone was given on days 5, 7 and 10 instead of on 8, 10 and 12. The earlier administration of hydrocortisone may have contributed to the more uniform "take" of the infection. (Con-

sider the results of Expt.27).

Although on day 21 all three mice had a much higher worm burden than the controls, by day 32 there was some evidence of a lowered worm recovery in three of the seven mice given the short cortisone treatment and two of the six mice given the continuous treatment. By day 40 there appears to have been a greater loss of worms in both groups, especially the continuously treated group. There is very little change between day 40 and 50 except for a few mice with greater worm recoveries in the continuously treated group. The reason for this is obscure, possibly explicable by the selection of mice during the allocation of animals to the two groups.

It is clear, however, that although the majority of mice, treated with hydrocortisone, either for a limited period or continuously, had far greater worm burdens on day 21 than the control animals, by the fourth week of the infection, both groups showed some degree of worm loss. Certainly by day 40 more than half of the animals had fewer worms than on day 21 and it is possible that worm senescence was responsible for this. (Chapter thirteen).

DISCUSSION.

The experiments reported here confirm the observation made by Mathies, that cortisone is effective in raising the worm burden of mice, when administered from the time of infection. The first two experiments showed quite conclusively that more worms survived to day 28 in male and female treated mice than in control mice. When hydrocortisone was given before the day of infection, then, except in experiment one, higher worm recoveries were observed on day 10 and this indicated that either the drug increased the susceptibility

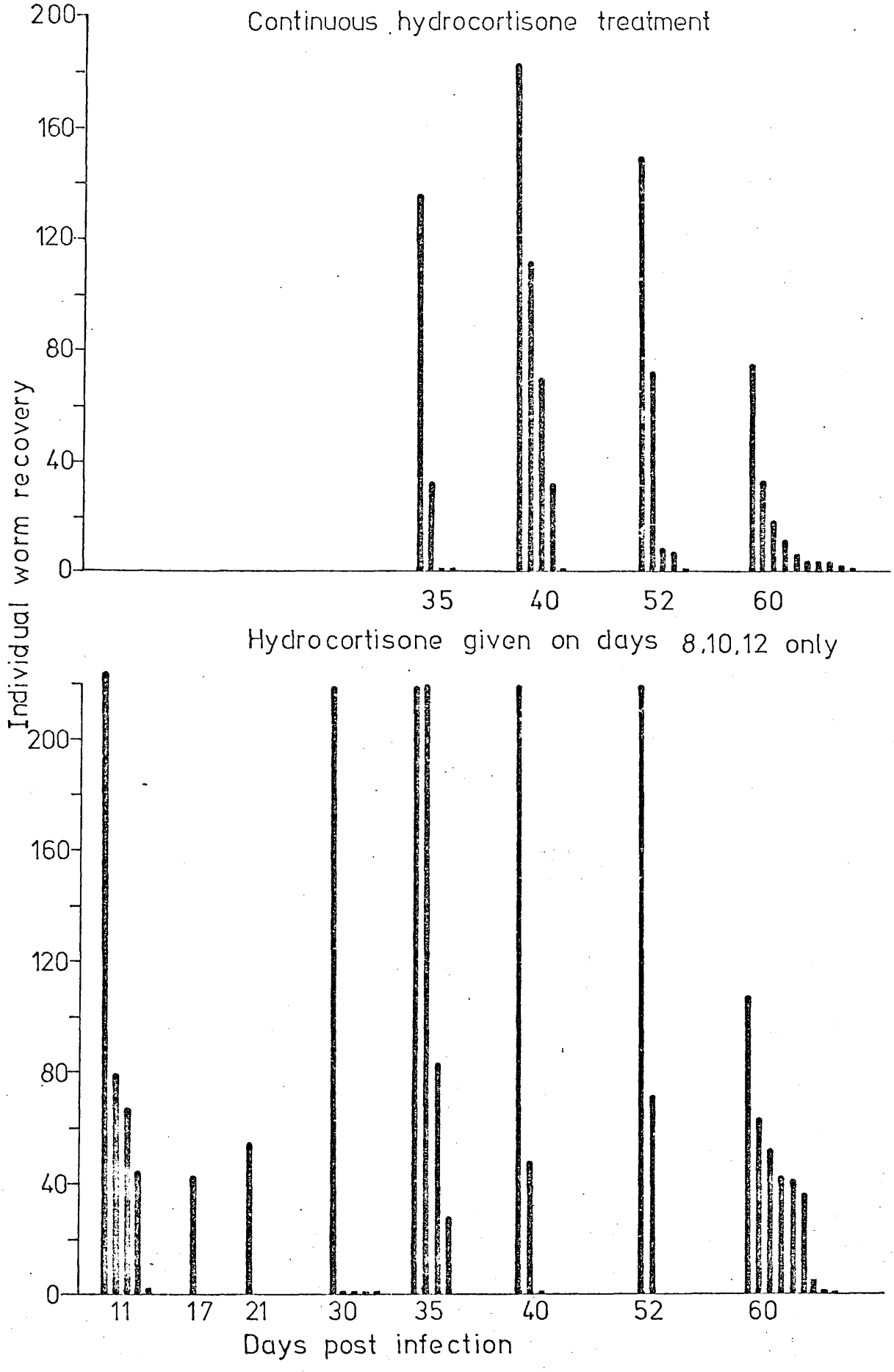


FIG. 47 The duration of the effect of
EXPT. 28a hydrocortisone acetate.

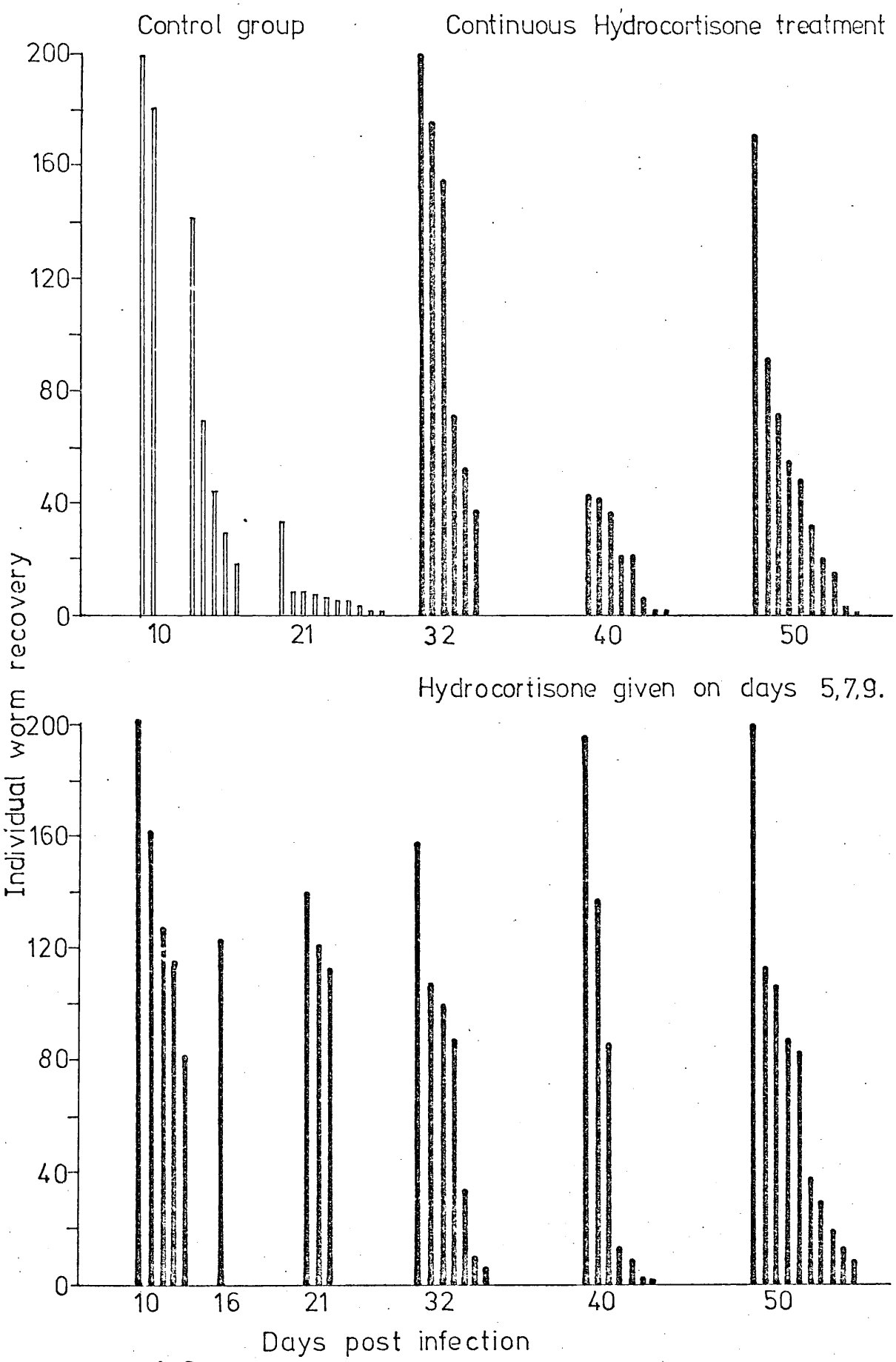


FIG. 48 The duration of the effect of
EXPT. 28b hydrocortisone acetate.

of the mice to infection or that some loss of larvae occurred in the control mice during the first week of infection. Hydrocortisone administration between days 7 and 12 ensured the survival of the worms present to day 28 and resulted in worm recoveries at autopsy comparable to those in mice killed on day 10.

The following conclusions were arrived at in experiments 25 - 28 :

a) The effects of hydrocortisone lasted at least two weeks and probably longer. Mice given the last dose of the drug on day -3 (Group A) did not eliminate their worm burden, harbouring large numbers of worms 28 days after infection.

It is possible that some mice in groups A and B were already recovering from the effects of hydrocortisone treatment, during the third week of infection, since these mice had slightly fewer worms than the mice in group D.

Continuous treatment with hydrocortisone, however, did not seem to markedly extend the survival of worms, over the worms in mice given the restricted treatment, suggesting that perhaps there may be a non-immunological explanation for the eventual loss of worms. It is possible that senility was an important factor here. Fisher (1958) found that female worms did not live longer than 40 days, but in Chapter five it was shown that worms of both sexes persisted over 100 days after infection and in Chapter thirteen this was amply confirmed by the survival of 31.9 ± 16.5% of transplanted 14-day larvae up to day 85. It does not seem likely that the signs of worm loss observed in hydrocortisone treated mice, can be assigned to the same cause as the worm loss in mice receiving transplanted larvae, where the time from transplantation

for the transferred worm burden to be reduced by 50% was calculated as 64.8 days. Taking into account the fact that these worms were already 14 days old, this gives a figure representing the age of worms at this stage as 78.8 days. One difference between these experiments which might conceivably explain this inconsistency, if both results are due to senility, is the difference in the worm burden since mice receiving transplanted larvae never received more than 50, 14-day larvae whereas the mice in Expts.28 a and 28 b, had much higher initial levels of infection. Consequently it is not possible at this stage to differentiate between the two possibilities, senility or host response, in accounting for the observations reported in this chapter.

b) Experiments 25 and 26 suggested that hydrocortisone increased the susceptibility of mice to infection with A.tetraptera. Examination of the data (Expt.27) showed that Groups D and E had as many worms as group C, although these groups had their first injection of hydrocortisone on day +1 and +5 respectively, and could not therefore have had a hydrocortisone elevated susceptibility to infection on day 0. Both of these groups had more worms on day 28 than Group I-the 10 day control group.

The results, therefore, indicated that there was a period between day 5 and day 10 of the infection, during which a small proportion of the worms was lost. This would have coincided with the anterior emigration by the worms in the colon (Expts. 1-3) and it was not improbable that there may have been some loss of worms during this time. A small loss could easily have been overlooked in previous experiments where the pre 10 day worm burden was no more than about 50 ± 10 worms.

c) The expulsion proper, occurred after day 10 and administration of hydrocortisone as late as on day 13 was effective in suppressing a large proportion of the expulsion. By day 17, most worms were expelled and administration of hydrocortisone was no longer effective in preventing stunting and damage to the worms. A large proportion of female worms recovered from group H, had none or only a few eggs, some worms being very small and immature, whereas worms from mice in Groups A to F were larger and contained large numbers of eggs. The expulsion of worms continued for longer than seventeen days, especially in heavily infected mice.

d) Examination of the sex-ratio of worms in mice where there was no expulsion, showed that about 58-62% of the worms were females. During the course of expulsion this sex-ratio was altered, more female worms being expelled overall. The change in the ratio did not appear until after day 17, because the presence of only a few mice with non-completed expulsion ensured that the ratio was not substantially altered from that in mice in which expulsion was completely suppressed.

In Experiment 27 the drop in percentage of female worms was from 61.24% (Group H) to 48.67% (Group I). Similarly in Experiment 25 the percentage of female worms fell from 62% in hydrocortisone treated mice to 46.6% in the 28 day untreated controls.

It is clear then, that hydrocortisone had a profound effect on the host-parasite relationship of A.tetraptera. There are several ways in which this effect could have been brought about and these will be discussed here. It has been suggested that cortisone depresses the host defence mechanism by its antiinflammatory effect, its immunosuppressive effect

and by alteration to the reticulo-endothelial system (Robinson,1960), but in bringing about these phenomena, cortisone acts on specific steps in each process. In the first place, cortisone has a rapid vasoconstrictive effect on the blood vessels in inflamed areas, a response which immediately restricts the number of polymorphs, macrophages and other cells which can leave the blood stream for the inflamed site (Ashton and Cook,1952). It has been found that polymorphonuclear leucocytes although more plentiful in cortisone treated animals, do not accumulate at the site of inflammation as quickly as in control animals (Germuth,1956), and although some workers have suggested that the phagocytic activity of these cells may be altered, the evidence for there being no effect is equally convincing (Germuth et al,1952). Cortisone treatment is rapidly followed by a pronounced oesinopenia, lymphocytopenia and a diminution of lymphocytes in lymphoid tissues such as the thymus, spleen and the lymph nodes (Germuth,1956; Cohen,1971). The accumulation of macrophages in inflamed areas appears also to be less efficient, but it has been proposed that this is an indirect effect caused by the destruction of committed lymphocytes which are needed at the inflamed site to mediate in the events leading to the accumulation of monocyte derived macrophages (Worth,1971). In general the reticulo-endothelial system does not seem to be greatly affected by cortisone (Cope,1964).There is, however, ample evidence to show that cortisone inhibits the formation of antibody and it is generally recognised that the cortisone sensitive step in the induction of the humoral response is the antibody forming, short lived marrow derived B-lymphocyte, but the drug must act before exposure of these

cells to antigenic stimulation, since committed cells are cortisone resistant (Cohen,1971). Cortisone suppresses cell-mediated immunity (CMI), this being reflected in its capacity to abolish hypersensitivity reactions, homograft rejection and graft versus host responses (G.V.H.)(Cohen,1971), processes which are dependant on the mediation of the thymus derived long-lived lymphocyte. The immunosuppressive effect, however, can not be achieved by the destruction of this cell-type since it is known that long lived lymphocytes are cortisone resistant and are capable of mounting the GVH response on transfer of the cortisone treated thymus and spleen to recipient mice (Cohen et al,1970; Cohen and Claman,1971). Cortisone in CMI is thought to exert its effect by the inhibition of the non-specific cellular elements which are mobilized in response to infection (Cohen,1971) and are mediated by the T-lymphocyte. Recent results by North(1972),however, have suggested that a short-lived T-cell which is cortisone sensitive may also be involved.

In parasitic infections, it has been found that cortisone will enhance the survival of helminth parasites to maturity (Wakelin,1970) or prolong the infection after maturity has been attained (Ogilvie,1965 b). These findings are thought to be achieved by the immunosuppressive effects of the drug since similar results can be obtained by the use of procedures such as A.L.S. treatment, irradiation, neonatal thymectomy and thoracic duct drainage (Ogilvie and Jones,1967;Rethy and Toth,1968; Dineen and Adams,1971; Hopkins et al,1972; Di Netta, Katz, and Campbell,1972;). The effect of cortisone in prolonging infection with T.muris, N.brasiliensis, and T.spiralis is therefore almost certainly

immunologically mediated and it is not unreasonable to suggest that this is also the case in infections of mice with A.tetraptera.

It cannot be stated, however, whether the enhancement of worm recoveries on day 10 by the administration of cortisone during the first week of infection is also an immunosuppressive effect, although worm expulsion can follow as early as day 7 after infection in other systems (e.g. N.brasiliensis in the rat, Ogilvie and Jones,1971) and there is sufficient host-parasite contact in A.tetraptera in the mouse during the first week to generate such a response. There is evidence, also, that the earlier response is stronger in the abnormal host (Chapter seven), where it can be suppressed by hydrocortisone, suggesting that perhaps a non specific inflammatory response may be involved. On the other hand, gluco-corticosteroid drugs are known to affect many physiological processes, such as gastric and pepsin secretion resistance of the mucosa to gastric secretions, absorption of water insoluble fats, excretion of water, as well as effects on the metabolism of fats, proteins carbohydrates and water (Ganong,1967). It is possible that some of these effects contributed to the abnormal appearance of the gut tissue in hydrocortisone treated mice and it may be that under these circumstances the anterior emigration was more efficient, the normal loss of larvae being abolished by the abnormal environment.

The results obtained from Experiments 26 and 27 leave one very interesting question unanswered; the reason for the long-term survival of worms in mice given only a short spell of cortisone treatment in the first week of infection. In other host-parasite systems e.g. T.spiralis (Coker,1955),

N.brasiliensis (Ogilvie,1965 b), and H.diminuta (Hopkins et al, 1972) involving the mouse, the termination of cortisone treatment is followed a week later by a resumption of the normal events leading to a spontaneous self-cure response. The exceptions to this rule are T.muris (Wakelin,1970) and Taeniae taeniaeformis (Olivier,1962). Three possible explanations can be proposed to explain this situation.

It could be that the host response is permanently altered in that a state of immunological unresponsiveness to the parasite may have been induced. Wakelin and Selby (1974) showed that when cortisone was given early in T.muris infection, the expulsion of worms, which normally takes place during the third week, did not occur and the parasite lived for 7-10 weeks after reaching maturity. This situation was compared by the authors to drug-induced immunological tolerance (Many and Schwartz,1970) and it was found to be similar in that the state could only be induced by treatment with cortisone during the period of initial antigenic stimulation, it was dependant on the persistance of antigenic stimulation until after the period of immunosuppression thereafter mice remaining tolerant for up to 7 weeks without further antigenic stimulation, and it was a specific tolerance since treated mice responded normally to concurrent stimulation with sheep red blood cells (unless the day of administration of SRBC's coincided with cortisone treatment). It was suggested that the tolerant state may have been brought about by the action of cortisone on the B-cell component of the response, the lack of which would prevent the T-cells from effecting expulsion, since immunity to T.muris is known to be T-cell dependant (Selby and Wakelin,1973).

It is possible that in part at least, a similar mechanism may be operating in A.tetraptera infections in mice. The antigenic stimulus is thought to be initiated during the first week of infection, during the crypt phase since repeated six day infections stimulate a high level of immunity (Chapter eight). The coincidence of hydrocortisone treatment and antigenic stimulation in the first week of infection, might be expected to result in a similar situation as in T.muris, but whether the longterm survival of worms, in mice treated with cortisone in the first week is truely a comparable situation, is not known at present and must await the result of further experiments.

Secondly it is conceivable that only the larvae of the parasite are immunogenic. Thus the immune response may have been hindered during larval development by cortisone, until a nonimmunogenic stage was reached by the worms, after which they could no longer be expelled. It is thought that this is an unlikely situation since the mature worms are known to be immunogenic (Chapter thirteen).

Lastly, it must be considered that cortisone may have been protecting the larvae from a host response until a nonsusceptible stage in development was attained by the parasite. That this could be so, is shown by the fact that mature worms (and 5-th stage larvae) although stimulating immunity against challenge infections, were themselves unaffected by it (Chapter thirteen). Thus it is suggested that the capacity of hydrocortisone to suppress expulsion when administered in the second week of infection was the result of its immunosuppressive effect on the host response at this time, protecting the larvae until they were no longer susceptible to immunity.

Such an immunosuppressive effect may be exerted by the inhibition of antibody production (Precipitating antibodies are rare in hydrocortisone treated mice - Chapter twelve), the T-lymphocyte population of committed cells or by the inhibitory effect on non specific cellular events which are mediated by specifically committed cells.

In summary then, it is suggested that the results of the experiments reported in this chapter are compatible with current work on immunological phenomena underlying worm expulsion. It is proposed that the survival of worms in mice given hydrocortisone during the first two weeks of infection is explicable by considering four different mechanisms. The survival of worms in mice given cortisone before infection and in the first week is explicable, it is proposed, by the induction of immunological unresponsiveness to the functional antigens of A.tetraptera. Suppression of the loss of larvae during the anterior emigration may have been achieved by the antiinflammatory effect of hydrocortisone on the non-specific cell response. The survival of worms in mice treated in the second week may have been brought about by the immunosuppressive properties of the drug. The long term survival of worms in all these mice was undoubtedly associated with the finding that after the fourth ecdysis A.tetraptera was no longer as susceptible to immunity as in the younger stages of development.

CHAPTER TEN

Attempts to alter immunity

II - The effect of indomethacin, sodium methotrexate, Freund's complete adjuvant and heterologous antilymphocytic serum on the expulsion of Aspiculuris tetraptera from female mice.

INTRODUCTION

Hydrocortisone was found to be extremely effective in suppressing the expulsion of the larvae of A.tetraptera from mice. This chapter gives the results of experiments in which mice were treated with two other known immuno-suppressants, sodium methotrexate (MTX) and horse anti-mouse lymphocyte serum (A.L.S.).The effect of Freund's complete adjuvant (F.C.A.) and the antiinflammatory drug indomethacin was also investigated.

RESULTS.

The effect of Indomethacin.

Experiment 29 a - Sixteen control mice and nine experimental mice were infected with 500 eggs when 40 days old; all the mice were CFLPb males. Four mice from the control group were killed on day 10 (Group A), and the remaining animals (Groups B and C) were killed thirty four days after infection. The experimental group (Group C) received 0.1 mg of indomethacin in 0.2 ml. of milk every two days until day 34. On two occasions the interval between the doses was a day longer (i.e. days 9-12 and days 18-21).

In spite of the low "take", there was no difference between the groups killed on day 34. The Wilcoxon test gave P as < 0.005 for the comparison between groups B and C, but since expulsion clearly took place in both groups, indomethacin can not have had a major effect here.

The effect of Indomethacin.

Experiment 29 b - Eighteen male CFLPb mice were infected with 750 eggs of A.tetraptera on day 0. Six mice, the experimental group, were given 0.1 mg of indomethacin in 0.2 ml. of milk (as previously) on days 10,12,14 and 18. Four control mice were killed on day 10. The remaining animals were killed on days 20,21 and 24 as shown in Table 47.

A significant difference was detected between indomethacin treated and control animals in this experiment (Student's t-test $P < 0.01$, Wilcoxon test $P < 0.001$) but nevertheless, expulsion occurred in both groups. Since the autopsies were carried out on day 20-24, the indication is that indomethacin temporarily delayed rejection but did not prevent it entirely, as did hydrocortisone.

The effect of sodium methotrexate.

Experiment 30 - Nineteen control mice and eleven experimental mice were infected with 750 eggs of A.tetraptera when approximately four weeks of age. Eight control animals killed on day 11 (Group A) gave a mean worm recovery of 136.9 ± 64.1. The remaining eleven mice were killed on day 21 (Group B), when the mean worm recovery was 7.5 ± 13.9 worms.
MTX was given intra muscularly every two days, alternatively into the left and the right hind limb. The dose was 0.05 ml. of a 2.5 mg/ml. concentration. Treated animals were killed on day 21 (Group C), when the mean worm recovery of 140.4 ± 110.2 was statistically different from the control group ($P < 0.001$). The expulsion of larvae occurred in the usual way in control mice ($P < 0.001$) but not in treated mice ($P < 0.6$).
Recovered worms were left in Hanks's saline overnight

at 4°C and were measured by camera lucida on the following day. Not more than ten worms were taken from any one mouse. Seventeen larvae, measured from the control group(2.38±0.47mm) were longer than the twenty nine larvae measured from the MTX treated group (1.67±0.48 mm). This difference was statistically valid ($P < 0.001$).

The effect of indomethacin and of sodium methotrexate.

Experiment 31 - Sixty-four, CFLP female mice, 33 days old were infected with 650 eggs of *A.tetraptera*. Eighteen mice (Groups C and D) were given indomethacin, (0.1 mg in 0.2 ml milk) every two days until day 21. Groups E and F, again twenty mice, were injected intramuscularly alternatively into the right or left leg, (every two days) with 0.05 ml of a 2.5 mg/ml concentration of MTX. The days on which mice were killed are shown in Table 50.

The inoculum used in this experiment was tested on young mice and was found to be infective. The infection became well established in the mice and worm recoveries during the first week of the infection were quite consistant. During the second week, however, some mice began to loose larvae, almost a week earlier than usual. On day 10, seven of the sixteen control mice had fewer than 50 larvae still remaining. By day 21, almost the entire infection had been eliminated in most mice. The greatest worm burden was 33 worms. The nine indomethacin treated mice, killed on days 10-11 had a slightly lower mean recovery than controls. Five of these mice had a low worm count. This was reduced to 46.3 ± 48.9 larvae by day 21 when three of the ten mice still had quite high levels of infection. Four of the ten methotrexate mice had high worm

TABLE 46 The effect of indomethacin on the survival of *A.tetraptera* in
EXPT. 29a male mice

Group	No. of mice	Day killed	Mean worm recovery	± s.d.
A (Control)	4	10	49.3[1]	10.4
B (Control)	12	34	3.0[1,2]	6.6
C (Indomethacin)	9	34	7.5[2]	7.2

TABLE 47 The effect of indomethacin on the survival of *A.tetraptera* in
EXPT. 29b male mice.

A (Control)	4	10	169.5		84.7	
B (Control)	3	20	19.0		23.6	
C (Control)	3	21	7.0	8.8[3]	4.6	14.6
D (Control)	3	24	0.3		0.6	
E (Indomethacin)	2	20	67.0		29.7	
F (Indomethacin)	2	21	62.5	65.7[3]	62.9	31.2
G (Indomethacin)	2	24	67.5		2.1	

Statistical analysis of results by Student's t-test and Wilcoxon test

1	$P < 0.001$	$P < 0.001$
2	P = N.S.	$P < 0.005$
3	$P < 0.01$	$P < 0.001$

TABLE 48 The effect of sodium metho - trexate on the survival of
EXPT. 30 A.tetraptera in female mice.

Group	No. of mice	Day killed	Mean worm recovery	± s.d.
A (Control)	8	11	136.9[1,3]	64.1
B (Control)	11	21	7.5[1,2]	13.9
C (Methotrexate)	11	21	140.4[2,3]	110.2

TABLE 49 The effect of sodium metho-
EXPT. 30 trexate on the growth of A.tetraptera in female mice.

Group	No. of larvae measured	Mean length (mm)	± s.d.
B (Control)	17	2.28[4]	0.47
C (Methotrexate)	29	1.67[4]	0.48

Statistical analysis of results by Student's t-test and the Wilcoxon test

1	$P < 0.001$	$P < 0.001$
2	$P < 0.001$	$P < 0.001$
3	P = N.S.	P = N.S.
4	$P < 0.001$	

TABLE 50 EXPT. 31 The effect of indomethacin and sodium methotrexate on the survival of A.tetraptera in female mice.

Group	Nos. of mice.	Day killed	Mean worm recovery	± s.d.
A (control)	16	10-11	87·9[1,4,5]	66·0
B (control)	10	21	7·5[1,6,7]	9·4
C (Indocid)	9	10-11	79·9[2,4]	75·8
D (Indocid)	9	21	46·3[2,6]	48·9
E (Methotrexate)	10	10-11	65·7[3,5]	58·9
F (Methotrexate)	10	21	32·1[3,7]	48·1

Statistical analysis of results by Student's t-test and the Wilcoxon test.

1	$P < 0.001$
2	P = N.S
3	P = N.S
4	P = N.S
5	P = N.S
6	$0.05 > P > 0.02$
7	P = N.S

counts on day ten and eleven, but there was a loss of worms by day 21 when only three mice had greater worm burdens than controls.

The effect of A.L.S. and Freund's complete adjuvant.

Experiment 32 - This experiment was run concurrently with experiment 27 Chapter nine. The control group was the same as in that particular experiment and the mice were of the same origin. (33 day old, CFLP females). The mice were infected with 500 eggs and the control group (A) was killed on day ten. The second control group (B) was killed on day 28 together with groups C,D,E and F. Group C was given 0.5ml i.p. of ALS every four days starting on day -5. Groups D and E received 0.3 ml of FCA intra-peritoneally on day 0, and then a further identical dose was given to group E on day 10. A hydrocortisone treated group was included as a control of total inhibition of worm rejection, such as was demonstrated in Chapter nine. Group F received 1.25 mg of hydrocortisone acetate on days 9,11 and 13. The results are shown in Table 51.

DISCUSSION.

None of the four agents tested in these experiments was as effective in suppressing worm rejection as was hydrocortisone. The only drug which successfully inhibited worm expulsion was MTX.

Methotrexate (N-{P -/2,4-diaminopteridin-6 ylmethyl/ methylamino} benzoyl) - L - glutamic acid is a cytotoxic antimetabolite, whose principal mode of action is the competitive inhibition of dihydrofolic reductase, an enzyme essen-

tial in the metabolism of folic acid (OBrien,1962). This compound and its derivatives is vital in the biosynthesis of purines, pyrimidines and amino acids essential to the process of cell replication (Schwartz,1965). MTX consequently interferes with the reproduction of malignant cells and to a lesser extent with normal, slower growing cells. Particularly susceptible to MTX are neutrophils, leucocytes, platelets, bone marrow cells and the epithelial cells of the mouth and the gastrointestinal tract, the inhibition of which may cause hemorrhagic enteritis (Burchenal,1955;British Paharmaceutical Codex,1973).

MTX has a profound effect on antibody synthesis (Friedman et al, 1961; British Pharmaceutical Codex,1973) due mainly to its action in inhibiting the development of plasma cells and immunologically committed cells from the hemocytoblasts, which themselves appear to remain unaffected (Schwartz,1965). The drug has been found, however, to depress not only the humoral immune response but also the cell-mediated immune reaction and this is reflected in such observations as the suppression of delayed hypersensitivity (Friedman et al,1961; Friedman,1964) and the prolongation of the survival of skin homografts (Hechtman et al,1962; Berenbaum,1963). In addition MTX treatment, together with the i.v.administration of transplantation antigens, results in an extended period of tolerance to skin homografts in mice (Medawar,1963). The induction of immunological unresponsiveness by MTX was first observed in mice infected with Lymphocytic choriomeningitis virus (Haas et al,1956, 1957) and was later shown to depend closely on the coincidence of the highest MTX concentration with the maximum virus titres in the mice (Hotchin,1962).

MTX has been used to suppress resistance to parasitic infections. Ritterson (1968) showed that Chinese hamsters, autopsied 30-35 days after infection with Trichinella spiralis developed more than 50 times as many larvae as controls, when treated with MTX. Hymenolepis microstoma, in another experiment with identically treated Chinese hamsters, yielded an average worm weight fourfold greater than from control animals (Ritterson,1971) but the result was complicated by possible toxic effects. Retardation of the growth of larval parasites in MTX treated hosts was observed by Wilson (1971) who found that the drug acted on the third and fourth stage larvae of Dictyocaulus viviparus in guinea-pigs, not only slowing development of the parasite, but also delaying the onset of worm expulsion and preventing the appearance of anaphylactic antibody in the serum of infected animals.

In the present work it was found that the larvae of A.tetraptera were smaller on day 21 in MTX treated animals than in controls and this could have been a direct consequence of the drugs presence in the worm's environment. Methotrexate has an effect on the gut epithelial cells, the resultant disorder possibly facilitating the passage of the drug into the worm's environment. In contrast, Hopkins et al, (1972) found that MTX did not affect the growth or the maturation of Hymenolepis diminuta in mice. Indeed, the worms grew better in treated mice than in controls, presumably because of the drugs immunosuppressive action, protecting the worms from the stunting effects of immunity. It could be that cestode parasites are not affected by MTX in the same way as nematodes.

Since the early attempts to produce specific antiserum

against lymphoid tissue, late in the nineteenth century, and in the early 1900's heterologous antilymphocyte serum has proved to be a powerful immunosuppressant of low-toxicity, (Lance et al,1973). Antilymphocyte serum (ALS) is prepared by immunising one species, e.g. horse or rabbit, with heterologous lymphocytes, e.g. mouse (Levey and Medawar,1966 a; Gray et al,1966), rat (Woodruff and Anderson,1964) pig (Lucke et al, 1968), dog (Iwasaki,et al,1967) and man (Monaco et al,1967).

It is generally agreed that the activity of ALS resides predominantly in the 7S IgG fraction of serum gamma globulin. (Woodruff et al,1967; James and Medawar,1967; Lance,1967), and its mode of action has been intensively studied both in vitro and in vivo (Taub,1970). The immunosuppressive activity of ALS is exemplified by five main observations in vitro, these being: agglutination of target lymphocytes, cytotoxicity (Lysis of target lymphocytes), inhibition of antibody synthesis, inhibition of the inhibitory effect exerted by lymphocytes on target cells, and stimulation of D.N.A. synthesis, (Taub,1970). Animals given ALS show lymphopenia as perhaps the first major symptom of treatment (Gray et al, 1966; Woodruff and Anderson,1963), but there is a lack of correlation between early lymphopenia and immunosuppression (Woodruff and Anderson,1963; Levey and Medawar,1966 a), although some relationship has been shown, when the extent of early lymphopenia and the later rise in short-lived lymphocytes was compared to immunosuppressive activity (Taub,1969). ALS has a profound cytotoxic effect in vivo. Changes in the thymus-dependant regions of lymph nodes and the spleen are characterised by an almost total absence of lymphocytes (Turk and Willoughby,1967),

TABLE 51 EXPT. 32 The effect of heterologous anti-lymphocyte serum, Freund's complete adjuvant and hydrocortisone acetate on the survival of A.tetraptera in female mice.

Group	No. of mice	Day killed	Mean worm recovery	± s.d.
A (Control)	10	10	104.4[1]	50.2
B (Control)	10	28	22.6[1,2,3,4,5]	38.1
C (A.L.S.)	8	28	10.4[2]	17.8
D (F.C.A. day 0)	10	28	23.6[3]	32.1
E (F.C.A. day 0,10)	7	28	20.3[4]	24.0
F (Hydrocortisone)	11	28	109.1[5]	41.5

Statistical analysis of results by Student's t-test and the Wilcoxon test

1	$P < 0.001$	$P < 0.001$
2	P = N.S.	P = N.S.
3	P = N.S.	P = N.S.
4	P = N.S.	P = N.S.
5	$P < 0.001$	$P < 0.001$

but the thymus, the germinal centres and the cortical areas of lymph nodes remain unaffected. Clearly ALS is a powerful inhibitor of cell-mediated immunity, whose principle target is the thymus-derived, long-lived lymphocyte. This is reflected in its suppression of primary transplantation immunity (Waksman et al,1961; Lance and Medawar,1968), the secondary response (Levey and Medawar,1966 a), the rejection of heterografts (Monaco et al,1966; Lance and Medawar, 1968) and the abolition of cutaneous signs of delayed hypersensitivity responses (Waksman et al,1961). ALS, in some instances, inhibits the humoral antibody response, but in general this is a much weaker effect than that on cell-mediated immunity (Taub,1970). Although ALS prevents the development of the immune response to antigen, it does not prevent the antibody response to its own constituent proteins. This is characterised not only by a rise in specific antibody but also in some hyperplasia of lymphoid tissue, thought to represent the development of immunity to the immunogenic proteins in ALS (Taub and Lance,1968; Taub,1970).

Surprisingly ALS had no effect on the development of immunity to A.tetraptera. ALS mice had a lower worm burden overall than the controls. There could be two possible explanations to account for this.

Firstly it may be that the regimen for administering ALS was incorrectly devised and thus the serum was present in effective concentrations at a time when it had a minimal effect on the development of the events which caused worm expulsion.

Secondly, it may be that ALS does not inactivate the processes responsible for worm expulsion, this being effected

by processes not dependant on the presence of the primary targets of ALS. For instance some humoral antibody responses are not affected by ALS, and this could be the basis of the effector mechanism.

The last alternative, however, would be inconsistant with other reports in the Literature on the effect of ALS during the development of immunity to helminth parasites in mice. Almost invariably, where the administration of ALS by one regimen has failed to suppress immunity, the experiment was adapted to coincide high concentrations of ALS with the maximal vulnerability of the developing response; during the period of antigenic stimulation. Thus for instance ALS can suppress immunity to T.muris (Wakelin and Selby,1974 in press) H.diminuta (Hopkins et al,1972), Trichinella spiralis (Dinetta, Katz,Campbell,1972) and Nippostrongylus brasiliensis (Kassai, Szepes, Rethy, Toth,1968) in mice. For various reasons the experiment could not be repeated, but not a great deal of significance is attached to the failure of ALS to suppress worm expulsion in this particular experiment.

FCA has been shown to delay the development of immunity to H.microstoma in hamsters (Ritterson,1971) and H.diminuta in mice (Goodall,1972). Ritterson attributed this immunosuppression to the granuloma which FCA induced in the abdominal cavity especially surrounding the spleen, suggesting that the participation of the spleen was of vital importance in the normal host response to the parasite. Recently, antigenic competition has been forwarded as an alternative hypothesis (Goodall,1973). Its effect in other parasite systems has not been extensively studied. FCA failed to suppress the expulsion of A.tetraptera larvae from mice in two groups, one of which

was treated on day 0 and the other on days 0 and 10. There was some evidence for suppression in one or two animals in both groups, but overall it can only be concluded that the administration was ineffective.

The antiinflammatory drug, indomethacin (1-P-chlorobenzoyl-5-methoxy-2-methylindol-3-ylacetic acid) is known to have in addition antipyretic and analgesic properties. When taken orally it is quickly absorbed and its action can be detected within 2 hours. Indomethacin has been used clinically to relieve the pain and swelling in gout and rheumatoid arthritis as well as in allied disorders such as osteoarthritis and ankylosing spondylitis (British Pharmaceutical Codex, 1973.). In the present study its antiinflammatory effect was the primary concern.

A consistant effect, comparable to that of hydrocortisone, could not be demonstrated in indomethacin treated mice. In experiment 29a, no effect was identified. Experiment 31 was not more informative, although experiment 29b demonstrated some delay in worm rejection. It does not, therefore, seem likely that indomethacin has a profound effect on the expulsion process, and it is probable that the partial effect was a result of delaying worm rejection by an anti-inflammatory action rather, than by total suppression of the process. The failure of indomethacin to prevent worm rejection entirely, suggests that the more successful results obtained using hydrocortisone were not consequent solely upon the antiinflammatory action of this drug but rather that complete suppression of worm expulsion was attributable to the immunosuppressive properties of hydrocortisone.

CHAPTER ELEVEN

Infection of neonatal and young mice; attempts to demonstrate passive transfer of immunity from mother to young; the effects of splenectomy and a quantification of the response of the colon to heavy infection with *Aspiculuris tetraptera*.

INTRODUCTION

Interest in the progress of infections in neonatal animals was aroused when it was discovered that young rats did not eliminate *Nippostrongylus brasiliensis* on day 13, when infected with sufficient larvae to evoke a normal primary spontaneous self-cure in adult rats (Jarrett,Jarrett and Urquhart,1966; Kassai and Aitken,1967; Jarrett,Jarrett and Urquhart,1968.) Immunological unresponsiveness of this type has also been described in sheep infected with *Haemonchus contortus*, where the period of tolerance to the parasite lasted for as long as six months (Manton *et al*, 1962; Urquhart *et al*, 1966; Lopez and Urquhart,1968). It is possible that this is an important factor in the survival of parasites in the wild, since if ecological circumstances were such as to permit the infection of immature animals in the immediate post-natal period then the induction of tolerance would be a reasonable possibility.

Neonatal infections, however, have not been succesful in all systems studied. For example, it was found that mice under fourteen days of age could not be infected with the eggs of *Trichuris muris* (Wakelin,1972). Because of the possible importance of neonatal infections to the survival

of parasites in wild host populations, an experiment was carried out to determine, the earliest age at which mice could be infected with A.tetraptera.

Another related aspect of immunity to helminth infections in young animals, is the converse situation where the development of parasites is prevented in young animals by the passive transfer of immunity, via the milk from the mother to the offspring. This was successfully demonstrated in Trichinella spiralis infections in mice, where it was found that although immunity could be demonstrated three weeks after birth in mice suckled on immune mothers the strongest immunity was passed to eight - ten day old baby mice (Duckett, Denham and Nelson,1972).

Similar observations had previously been made in the Nippostrongylus brasiliensis system (Jones and Ogilvie,1967) where it was shown that young rats born to immune mothers and suckled by them, had hemagglutinating antibody titres in the plasma comparable to those of the mothers (Greenberg, 1971).

A preliminary experiment is described in which young mice suckled by immune and control mothers, were infected with the eggs of A.tetraptera in an attempt to discover whether baby mice could be protected by the passive transfer of resistance from the mother. In addition two unrelated experiments are reported. In the first of these, the effect of splenectomy, on the expulsion of worms, was investigated and in the second experiment an attempt was made to quantify the response of the colon to the presence of a heavy infection with A.tetraptera.

RESULTS.

The infection of neonatal and young mice.

Experiment 33 - Female CFLPb strain mice were mated with male mice of the same strain, at various times during a three week period. Five female mice which became pregnant were isolated as soon as pregnancy was confirmed and their litters were adjusted to five babies each,within 24 hours of birth. On the day of infection (750 eggs) the litters were 5,10,14, 18 and 26 days old. All the baby mice were killed ten days after infection and the worms were recovered in the usual way (Table 52).

No worms were found in any of the mice in groups A and B (five and ten days old at infection, respectively). The youngest litter from which larvae were successfully recovered was group C, which was infected 14 days after birth. The three male mice yielded 10,18 and 21 larvae and the two females 0 and 74 (mean worm recovery 24.6 $\pm$ 28.8). The greatest number of parasites was found in the last two groups, C and D, infected on days 18 and 26 after birth respectively.

The transfer of immunity from mother to baby mice.

Experiment 34 - Fifteen female CFLPb mice (4-5 wks old) were given four immunising infections consisting of 500, 500, 1,000 and 1,000 eggs respectively. A period of 14-20 days separated consecutive infections. Together with fifteen control mice, (same age and strain) these animals were mated to male CFLPb mice, two days after the third challenge infection. Experimental and control groups were arranged from litters born within 24 hours of one another, ten groups A-J being successfully formed by this method. The litters and their mothers were infected with 750 - 1,000 eggs and the

TABLE 52 The recovery of the larvae of *A.tetraptera* from neonatal and
EXPT. 33 young mice infected with 700 eggs.

Group	Age at infection.	Number of mice		Worms recovered.	
		Day 0	Day 10	Mean	± s.d.
A	5	5	3	0	0
B	10	5	4	0	0
C	14	5	5	24.6	28.8
D	18	5	5	114.2	48.2
E	26	5	5	158.8	77.2

Statistical analysis of results by Student's 't'test & Wilcoxon test
Groups compared.

C X D	P < 0.01
C X E	P < 0.01
D X E	P – N.S.

groups were killed 7-11 days after infection, when the larvae were recovered and counted.

There was no indication of a passive transfer of immunity from mother to the babies in any of the ten groups studied. The age of the litters on the day of infection varied from sixteen to twenty one days, the youngest litters being infected within two days of the onset of susceptibility to infection. It was found that the mice in litters born on the same day, to different mothers, did not always grow at an equal rate, as judged by the size of the young and the acquisition of hair. This gave rise to a great deal of variation in susceptibility to infection, some mice still being insusceptible as late as on day eighteen after infection.

The effect of splenectomy.

Experiment 35 - The twelve experimental mice used in this experiment (4 wk old, female, CFLP strain mice) were splenectomised as described earlier in Chapter three. They and the ten control mice were given 1,000 eggs of *A.tetraptera* one day after splenectomy. Mice from both groups were killed on two occasions after infection; on day 10 (four mice from both groups) and on day 20 (the remainder) and the worms were recovered, counted and recorded.

The results, (Table 54) show that both groups were equally susceptible as indicated by the autopsies carried out on day 10. Both groups responded similarly to the infection, eliminating the greater part of the worm burden, and consequently there was no difference between the groups in the residual worm burden recorded on day 20.

TABLE 53 An attempt to detect the existence of maternal transfer of immunity
EXPT. 34 in mice immune to A.tetraptera.

Group	Age at infection in days.	Day killed	Mean worm recovery					
			Control mothers			Immune mothers		
			Nos. mice	Mean	± s.d.	Nos. mice	Mean	± s.d.
A	18	7	4	762.0	132.7	3	761.0	82.4
B	17	10	4	437.3	137.6	5	538.0	62.4
C	17	11	5	176.0	155.6	4	661.3	389.5
D	21	10	3	385.0	185.2	2	299.5	357.1
E	20	10	5	360.8	161.1	5	431.2	158.7
F	18	11	5	297.4	219.7	5	254.8	177.6
G	17	11	5	386.6	140.8	5	439.6	277.6
H	16	11	5	134.0	121.2	4	346.5	260.3
I	20	10	5	469.4	36.6	5	477.6	69.9
J	18	11	5	202.2	132.7	4	376.3	170.9

Statistical analysis of results
Student's t test — all results – N.S.
Wilcoxon test —

A quantification of the response of the colon to heavy infection

Experiment 36 - While autopsying naturally infected TFI mice and also hydrocortisone treated CFLP mice, it was noted that the colon in heavily infected animals was relatively large and much thickened in appearance. An attempt was made to quantify this observation.

Twenty 20-33 day old female CFLPb strain mice were weighed, divided into two groups, given 0.025 ml of hydrocortisone and then two days later one of these groups was infected with 1,500 eggs of A.tetraptera. Both groups were given a further dose (0.05 ml) of hydrocortisone on day 6 and the experimental group was further infected on day 7 (2,000 eggs) and on day 14 (1,000 eggs). Both groups were killed on day 35 and the mice were individually weighed immediately after death. The terminal mesenteric lymph node and the spleen were removed, blotted dry on tissue paper and weighed. The entire colon was removed, opened under tap water and emptied of its contents. It was quickly rinsed, dried three times on tissue paper and then weighed. The number of worms present, in the infected group, was recorded.

The weight of individual mice in both groups was extremely variable, but there was no statistical difference between the groups on either day -2 or day 35. There may be some significance in the fact that the infected group was lighter than the control group, the heaviest worm burdens being associated with the lightest mice. There was no control group to compare normal growth in the absence of hydrocortisone treatment but it was noted, however, that both groups of mice grew slower than if they had not been injected with the drug.

TABLE 54 The effect of splenectomy on the expulsion of the larvae of
EXPT. 35 A.tetraptera in female mice.

Group	No. mice	Mean worm recovery.	± s.d.
A (control day-10)	4	221.3	171.3
B (splenectomised day-10.)	4	95.3	69.7
C (control day-20)	6	22.7	33.4
D (splenectomised day-20.)	8	19.4	24.2

Statistical analysis of results by Student's t-test & Wilcoxon test.
Groups compared.

A X B	P N.S.
C X D	P N.S.
A X C	P < 0.05
B X D	0.1 > P > 0.05.

TABLE. 55 EXPT. 36 A comparison of the weight of the spleen, the terminal mesenteric lymph node, the colon and the total body weight in hydrocortisone treated, infected mice and in hydrocortisone treated controls.

Group	No. mice	Spleen wt. % / Body wt.	Tml node wt. % / Body wt.	Colon wt. % / Body wt.	Body wt.
		Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
A (Control day-2)	10				11.6[1,2] 3.7
B (Infected day -2)	10				12.5[1,3] 4.4
C (Control day 35)	10	0.45[7] 0.08	0.042[6] 0.016	1.69[5] 0.14	18.9[2,4] 5.9
D (Infected day 35)	9	0.42[7] 0.06	0.058[6] 0.036	2.86[5] 0.3	16.5[3,4] 7.2

Statistical analysis of results by Student's t-test and the Wilcoxon test.

1	P = N.S
2	0.01 > P > 0.001
3	0.2 > P > 0.1
4	P = N.S.
5	P < 0.001
6	P = N.S.
7	P = N.S.

When the weight of the colon (expressed as percentage of the body wt) was compared in the infected and the non-infected group, the difference was found to be statistically significant, ($P < 0.001$). There was no such difference, however, between the weight of the spleen or the weight of the terminal mesenteric lymph node in these mice.

DISCUSSION.

Whereas eight day old CFW mice could be infected with *Trichinella spiralis* (Duckett, *et al*, 1972), CFLP mice of the same age were refractory to infection with *Trichuris muris* (Wakelin, 1972) and *Aspiculuris tetraptera*. This finding, it is suggested, may be explained by the difference in the sites in the intestine, normally occupied by these parasites and/or the ability to survive in atypical environmental situations. Since, the initiation of hatching of *A. tetraptera* larvae *in vitro*, depends on the pH, the temperature and on an aerobic environment (Anya, 1966 c) it may be that the optimal conditions for the stimulation of hatching were not found in the intestine of mice under fourteen days of age. Thus either hatching was not triggered or if it did take place, then the larvae failed to survive in the preferred sites for the ten days between infection and death.

A comparison of the intestines in weanling and older mice shows immediate differences. Mice under 14 days of age feed almost exclusively on milk and it is presumably the products of this food and bile that give rise to the bright yellow-orange appearance of the gut contents. The caecum is very small and the colon does not contain distinct faecal droppings as in older mice. The gut of older animals is obviously generally larger, longer and has a wider lumen.

The gut contents are darker and contain numerous undigested elements such as sawdust particles, grains, husks etc. Presumably the typical micro flora is acquired when baby mice cease to depend solely on maternal milk and begin to forage and investigate their surroundings. Since this behaviour begins soon after the eyes open, towards the end of the second week after birth this could be associated with the initiation of susceptibility to infection at this time. Perhaps Trichinella spiralis larvae, which do not have to hatch are not as dependant on these factors and/or the differences in the small intestine of weanling and older mice may not be as marked as those in the caecum and the colon.

Maximum levels of protection to Trichinella spiralis were demonstrated in 8-10 day-old mice suckled by immune mothers and this protection was still strong in 13-16 day old mice (Duckett, et al,1972). As late as 25-28 days after birth, passive transfer of resistance was detectable, though 41-43 day old mice were not significantly protected. In order to ensure that infection took place, the youngest mice infected in experiment two were 16 days old, but in the ten groups studied, no immunity could be detected. The inoculum used to infect mice gave rise to heavy infections, but this is not altogether surprising since 21-day mice exhibit maximum susceptibility to infection (Stahl,1962) with A.tetraptera . Thus the conditions in the gut change dramatically during the third week, since on day 14 mice are only just susceptible to infection, but by the end of the third week peak susceptibility is reached, before the effects of age and sex resistance reduce susceptibility to lower levels in older mice. Nevertheless, the failure to demonstrate transfer of immunity can be accounted for in a number of possible ways.

Firstly it may be that the level of antibodies transferred was not sufficient to deter the establishment and the survival of the worms. There is also the possibility that the experiment failed purely on technical grounds, thus either the inoculum used was too large or the mothers were not made adequately immune. These two experiments warrant further attention since it would be interesting to discover the nature of insusceptibility to infection in weanling mice, and in particular it would be interesting to know whether spontaneous self-cure takes place in mice infected fourteen days after birth.

Two unrelated experiments were included in the second half of this chapter. In the first of these, the effects of splenectomy were investigated. Splenectomy has been shown to result in the continuation of peak parasitaemia in infections with malaria and other protozoan parasites (Taliaferro,1929). Although a weak response is initiated by splenectomised animals against intravenous challenge with antigen, the same animals respond normally when the antigen is given intradermally or intraperitoneally (Humphrey and White,1971). Splenectomy did not affect the expulsion of Nippostrongylus brasiliensis (Ogilvie and Jones,1967) when the operation was carried out before or during the initial infection or before challenge infection in immune rats, nor was the response to Trichuris muris in mice altered by this procedure (Selby and Wakelin, 1973). In contrast, spleen cells have been shown to transfer immunity to T.spiralis (Larsh et al,1969,1970), Trichostrongylus colubriformis (Dineen and Wagland,1966) and to N.brasiliensis (Ogilvie and Jones,1968; Kelly and Dineen,1972), but in these systems as in T.muris, lymph node cells can also more

effectively protect recipient mice.

Mathies (1962) in a report dealing with the effects of hydrocortisone on infection with A.tetraptera compared the m.w.r. in splenectomised male and female mice to that in control animals twenty days post inoculum. His results indicated that splenectomy did not alter the m.w.r. which is in contrast to the findings of Stahl (1959) who found that splenectomised mice harboured more worms than controls. In Mathies's experiments mice were infected on the day following splenectomy, whereas Stahl left a longer interval (Quoted by Mathies,1962) between splenectomy and infection. This inconsistency in experimental protocol was proposed by Mathies as a possible explanation of the different results. Experiment 35 in this study included two groups of splenectomised and two of control mice in order to distinguish between the effects on susceptibility to infection and those on resistance. The result confirms that if the spleen is involved it is not necessary and its absence does not abrogate the primary expulsion of A.tetraptera.

Experiment 36 was concerned with the effect of a heavy worm burden on the colon. It was shown that the colon of infected mice was heavier than that of control mice, and its appearance was different being wider, larger and with thicker internal transverse folds. In some ways these changes resembled those observed by Symons (1957) for N.brasiliensis infections in rats. Symons found that not only was the jejunum thickened, but that it was filled with a large amount of fluid. Both the weight of the jejunum and its diameter were twice that recorded for controls. Changes in the muscularis externa were attributed to hypertrophy and to hyperplasia. Although no histological observations were made here, Symons's description fits in well with the changes observed in the heavily

infected colon, the exception being that the lumen was not fluid filled. This, however, may not occur as markedly as in the jejunum since the colon is predominantly a water absorbing region, (Bockus,1966; Guyton,1967; Goligher,1967) condensing the fluid gut contents to relatively dry faeces. The thickening of the gut walls probably represents a non specific reaction to extensive mechanical stimulation and damage to the mucosa. Trichuris muris, for instance, produces similar changes in the host, and is known to penetrate the mucosa as a larva and later as a mature worm with the extended oesophageal region of the body (Wakelin,1973). The penetration of host tissues by A.tetraptera has also been suggested by some authors. Mya (1955), Fisher (1958) showed that the larvae enter the lumen of the Crypts of Lieberkuhn and Mullink, (1970), observed immature parasites in the lumen of the crypts and between the epithelium and the basement membrane in the proximal colon. A cellular reaction was stimulated by the worms which was described by Mullink as a hyperplasia and could equally well have brought about the increase in weight described in experiment 36. Mullink described the cellular changes as an infiltration of the mucosa with lymphocytes, plasma cells and also neutrophils, oesinophils and granulocytes. These observations, however, usually become manifest only in heavy infections which in CFLP strain mice are rare and experimentally difficult to bring about, without the use of immunosuppressants. This is in contrast to CFI mice which are frequently heavily infected, suggesting that there are fundamental differences in the normal response between this strain of mice and CFLP mice.

CHAPTER TWELVE

Serological studies.

INTRODUCTION

The invasion of a host by foreign matter, whether by living organisms or inanimate material (e.g. dust,pollen etc), invariably stimulates a host response which may involve the production of specific antibodies. Such antibodies combine with homologous antigen and either "inactivate" it, facilitate its destruction by complement (foreign cells), or promote its phagocytosis by polymorphonuclear leucocytes and macrophages. The presence of specific antibodies can be detected in various ways, among which the precipitin reaction is a widely used method.

The manifestation of a precipitin reaction by the serum of infected animals, however, does not necessarily indicate that all the types of antibody, produced in response to the antigen, are represented in the reaction, for it is known that certain antibodies do not precipitate homologous antigen (IgA). Precipitation, usually involving the participation of high molecular weight carbohydrate or protein antigens, will only take place in an environment with optimal electrolyte concentrations, containing the correct ratio of antigen to antibody. A convenient method of detecting these antibodies is double diffusion in two dimensions (Ouchterlony,1948) in agar gel, with the advantage of direct comparison between the test antibodies and antigens.

Although precipitating antibodies have been widely studied in parasitic infections, there has been little evidence to indicate an association with protection. Antibodies produced both by the immunization of the host with

parasite extracts (Kagan,1957; Soulsby,1957) and also by subjection to an infection with the parasite (Sarles and Taliaferro,1936; Soulsby and Stewart,1960) have been investigated and it has been possible to associate the pattern of precipitation lines with the stage of infection, (Soulsby and Stewart,1960), specific lines, resulting from a reaction with metabolic products, somatic antigens and exsheathing fluid.

Of the authors interested in A.tetraptera only Mathies and Stahl discussed the possibility of an antibody response. Mathies (1962) rejected the possibility that the increased susceptibility following cortisone treatment may have been caused by immunosuppression without sufficient evidence. Stahl (1966 a) reported that in studies on larval immobilization and agar gel double diffusion tests, using saline extract of adult and larval worms, no evidence of antibodies was detected. The present chapter will describe experiments in which precipitating antibodies were detected and studied in various ways.

RESULTS.

Precipitating antibodies in the serum of hyperimmune mice.

Experiment 37 - In the first experiment, four groups of ten mice, immunised by the protocol outlined in Table 56, were bled and the serum was tested against two antigen preparations. Groups A-C were female, 33 day old CFLP mice, whereas group D was 1 month younger. The results (Fig.49) are recorded on slide 28.

TABLE 56 The experimental protocol by which mice were immunised.
Expt. 37

	DAY OF EXPERIMENT					
	0	7	14	33	45	56
No.eggs given	500	500	500	500	600	
Group						
A	✓	–	–	✓	✓	All mice
B	✓	✓	✓	✓	✓	
C	–	–	–	✓	✓	killed
D	–	–	–	✓	✓	

Groups A and B both gave strong precipitates which were most probably composite in nature. Group C showed a weak line in slide 28 and group D a weaker one.

The detection of antibodies using larval antigen.

Experiment 38 - Larval antigen (Ag6) was tested against HIS (as above) and NIMS. A comparison was made with the mature worm antigen (Ag4 and Ag5). The results are recorded on slide 69.

Whereas both Ag4 and Ag5 gave distinct lines, Ag6, the larval antigen, gave a barely detectible tracing. Its presence, however, was emphasised by the curve in the Ag5 line towards the Ag6 well (slide 69). Ag4 did not cross react as clearly.

Precipitating antibodies in immuno incompetant mice.

The effect of hydrocortisone, A.L.S. and F.C.A. on the precipitating antibody response.

Experiments 39 and 40 - In Expt.27 (Chapter nine) the effect of hydrocortisone on A.tetraptera infection in mice was investigated. All the groups killed on day 28, were bled and the pooled blood from the mice in each group was examined for precipitating antibodies. Group J, the 28 day control group gave a strong, well defined line, as expected, but with the exception of Group C, no lines were made by any of the other, hydrocortisone-treated groups.

Experiment 32, (Chapter ten) studied the effect of ALS and FCA on infection with A.tetraptera. As in the previous experiment all the mice were killed on day 28, bled and the serum was tested. Group B, the control group gave a distinct line. A weaker line was seen for both FCA treated groups, but neither the hydrocortisone-treated group not the ALS-treated group gave signs of a precipitate.

Comparison of precipitating antibodies in the sera of naturally infected TFI mice and in hyper immune mice.

Experiment 41 - In this experiment two serum pools were used, obtained each from 10 TFI mice. Pool TFI.1 was derived from 8-wk old freshly purchased male mice. Pool TFI.2 was obtained from the remainder of the same batch of mice, killed 4 weeks later. Hyper immune serum (HIS) was from group A in the previous experiment. The serum of non-infected mice (NIMS) was included as a further control. The results are shown in Fig.51.

TFI.2 gave one clear but 1 weak line with possibly an additional line nearer to the serum well. TFI.1 produced an extremely weak line, sometimes impossible to detect and possibly a second weak line. NIMS gave no line whereas HIS made a very strong clear possibly composite precipitate.

Cross reaction between A.tetraptera, N.dubius and T.muris and the corresponding serum of mice exposed to infection with any one of these parasites.

Experiment 42 - The A.tetraptera antigen Ag4 and HIS were used as in the previous experiment. N.dubius antigen and the serum from mice given three infections with 500 larvae at 14 day intervals, bled 10 days after the last infection, were tested, as was the T.muris antigen (TmAg). There was no serum from T.muris infected mice. The results are recorded on slide 48 and 52, Table 57 gives a summary of these findings.

TABLE 57 Experiment 42. A summary of cross reactions between the sera of mice infected with A.tetraptera and N.dubius and the corresponding antigens with T.muris. (The numbers in brackets refer to the relevant slides. + denotes a single line and (+) a possible line.

Antigen	Serum	
	A.tetraptera	N.dubius.
A.tetraptera	+ (59)	+(++) (48)
	++ (52)	+ (50)
	++(+) (48)	
	++ (50)	
N.dubius	+ (59)	+++ (59)
	+(+) (52)	+++ (48)
	+ (50)	+++ (50)
	+ (48)	
T.muris	(+) (59)	+ (59)

T.muris gave a weak line with N.dubius anti-serum on only one of the three occasions on which it was tested. N.dubius antigen gave three lines with homologous antiserum, of which one was strongest. A.tetraptera gave only one well defined line with homologous antiserum, but sometimes a second weaker line was also visible. N.dubius antigen reacted with A.tetraptera antiserum with one faint line and possibly a second but the reverse situation only gave one line, except in slide 48 where 2 additional weak lines were observed.

Tests for cross-reactions with bacterial polysaccharide and caecal wall antigen.

Experiment 43 - HIS was tested against Ag5 (control,well 5, Fig.53) a caecal wall antigen (courtesy D.Wakelin,well 19, Fig 53) and a bacterial (pneumococcal) polysaccharide (SSS 3, well 20, Fig.53). Whereas the control well with Ag5, gave a strong reaction, the remaining two antigens failed to make

precipitates.

Electrophoretic studies.

Experiments 44 and 45 - Plasma proteins can be separated into components on the basis of their mobilities in an electric field, by the method of cellulose acetate electrophoresis. Changes, resulting from altered antibody levels, in the gamma globulin component can be conveniently studied by comparing the density of this band on the strip, before and during infection (Humphrey and White, 1970; Leland,1961). Because of the success in detecting precipitating antibodies to A.tetraptera, an attempt was made to study the gamma globulin fraction of mouse serum, before and at various times during infection. The methods used, were described in Chapter three. In both experiments, five mice were infected and five were left uninfected, each mouse being bled once a week from the tail. The plasma was isolated and an electrophoretic pattern was obtained.

Unfortunately, in spite of some quite reasonable tracings (Fig.58) it proved difficult to detect any changes in the gamma globulin, principally because of variation in the amount of each component in animals treated identically.

TABLE 58. Key to the numbers used in Figs. 49 to 58.

Number on photograph	Agent represented
1	A.tetraptera antigen 1 (Ag1)
4	A.tetraptera antigen 4 (Ag4)
5	A.tetraptera antigen 5 (Ag5)
6	A.tetraptera antigen 6 (Ag6) larval
7	Hyperimmune A.tetraptera antiserum (HIS)
8	Non infected mouse serum (NIMS)
9	TFI mice A.tetraptera antiserum 1
11	Experiment 37 Group A
12	Experiment 37 Group B
13	Experiment 37 Group C
14	Experiment 37 Group D
15	N.dubius antigen
17	N.dubius antiserum
19	Caecal wall antigen
20	SSS3-Bacterial (Pneumococcal) polysaccharide.

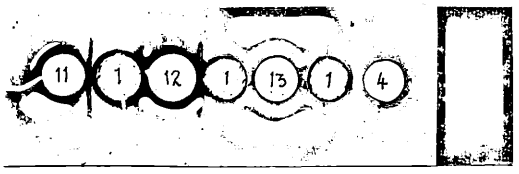


Fig. 49. Precipitating antibodies in the
Expt. 37. serum of hyperimmune mice.

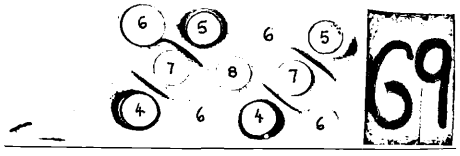


Fig. 50. Tests with larval
Expt. 38. antigen (Ag 6)

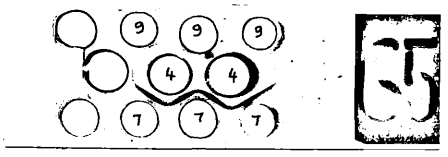
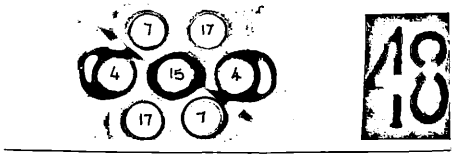


Fig. 51. Antibodies in
Expt. 41. TFI mice.



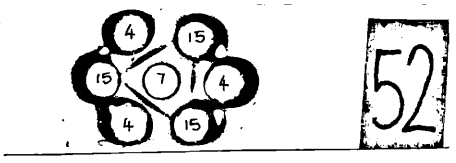


Fig. 52. Experiments to determine the extent of cross
Expt. 42. reactions between A.tetraptera antigen and
antigen derived from other murine nematode
parasites.

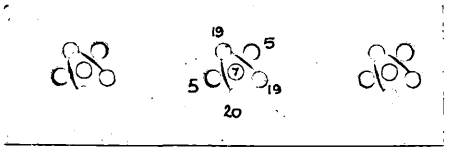


Fig. 53. Tests for cross reaction with bacterial poly-
Expt. 43. saccharide and caecal wall antigen.

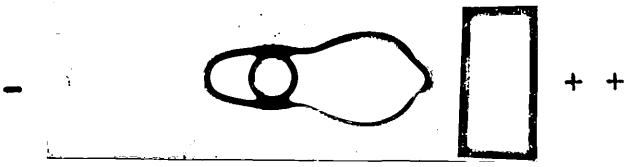


Fig. 54.

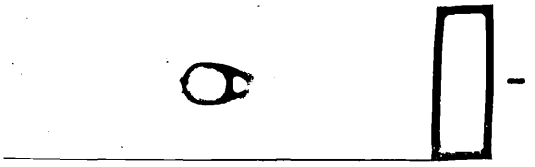


Fig.55.

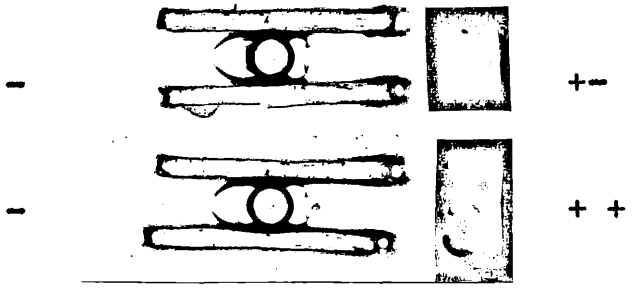


Fig. 56.

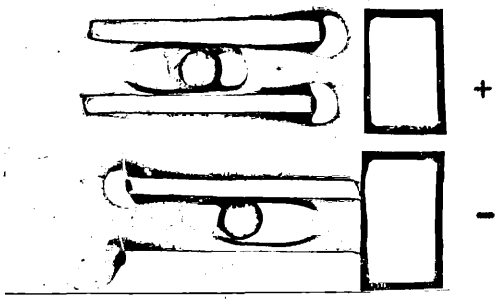
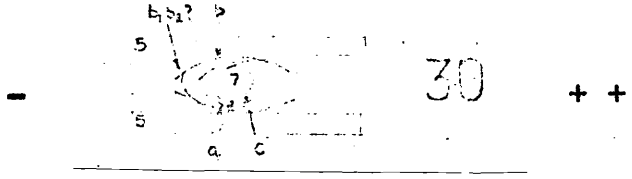


Fig.57.



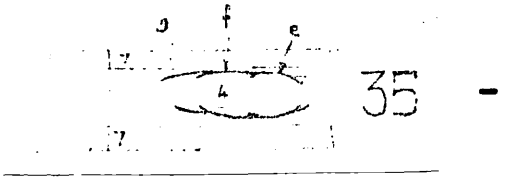


Fig. 54-57 Immunoelectrophoresis of hyperimmune
Expt. 46-49 serum and antigen.

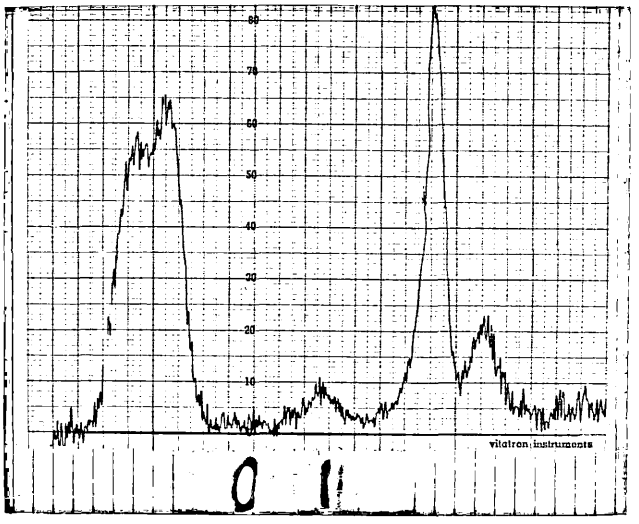


Fig.58. An example of typical densitometer trace
Expt.50. of an electrophoretic strip on which serum protein were subjected to a current of 1MA for 2 hours.

Immunoelectrophoretic studies.

A series of experiments was carried out in which sera from immune and hyperimmune mice were examined by immunoelectrophoresis.

Immunoelectrophoresis of immune and hyperimmune serum.

Experiments 46,47 and 48 - A number of different serum pools were examined, some taken from 28 day primary infection mice, others from mice given repeated infections with *A.tetraptera* as in Expt.37.

Most slides showed a distinct line, a, forming a semicircle on the negative side of the serum well and a second line, b, running in a long arc parallel to the antigen trough. This line bifurcated at the negative end into two components b_1 and b_2. A further line, c, was sometimes detected surrounding the serum well, especially in experiments with HIS. A typical example of electrophoresis of hyperimmune serum is seen in Fig.56.

Immunoelectrophoresis of antigen.

Experiment 49 - Experiments were carried out in which antigen was subjected to electrophoresis and then allowed to diffuse against HIS. The results are recorded in Fig.57.

The migration of antigen proteins was towards the anode, where two regions were identified. A lighter staining component moved faster than the second fraction which stained more intensely and remained near to the antigen well. The precipitation line, on further examination was divided into at least four separate components. Line e bifurcated into e_1 and e_2 at the negative end and line f, although continuous with line e, was in fact distinct, splitting into two lines f_1 and f_2 at the positive end. A further component, g, was seen in the positive end of the slide.

Separation of the components in HIS by Sephadex column chromatography.

Experiment 50 - Plasma proteins can be separated according to their molecular size by passage through a column loaded with standardised cross-linked dextrans. Larger molecules pass quickly through this system, whilst smaller ones are retained to lesser or a greater degree depending on size, (Lathe and Ruven,1956; Porath and Flodin,1959; Diamond and Denman,1973).

A sephadex G200 column was set up as explained in Chapter 3. HIS was passed through this column and the fractions constituting the first peak were pooled (Fraction 1). The first half of the second peak was called fraction 2 and the remainder was fraction 3. Each fraction was concentrated and then placed into an Ouchterlony plate, to be diffused against antigen. A precipitate was obtained with fraction 1 and 2, but there was no detectable reaction between fraction 3 and antigen.

Haemagglutination and Passive cutaneous anaphylaxis.

Experiments 51 and 52 - Neither of these two tests was successful in detecting antibodies in HIS and in primary infection serum.

Incubation of larval and mature A.tetraptera in immune and hyperimmune serum. Incubation products of A.tetraptera.

Experiments 53 and 54 - An experiment was set up in which larvae and mature worms were incubated in control, immune and hyperimmune serum at 37°C in Hanks's saline for 24 hours. Worms were examined at hourly intervals and then after overnight incubation, but worm survival was not affected by the presence of immune or hyperimmune serum. The worms did, how-

ever, live longer in Hanks's saline. No precipitates were observed in these experiments.

Worms were incubated in a small petri-dish in Hanks's saline for 24 hours. The saline was then tested against HIS in Ouchterlony plates. No precipitates were detected by this technique.

DISCUSSION.

The results of a preliminary study, defining some of the characteristics of antibodies present in the serum of infected mice, have been described. There can be no doubt, at this stage, that immune serum gave distinct precipitin lines in Ouchterlony plates, when tested against homologous antigen. At least two components were identified as constituting this reaction, a larger mobile fraction and a second weaker, sometimes imperceptible, less mobile line. The existence of a minimum of two components was confirmed by immunoelectrophoretic studies in which a fast fraction, forming a long arc (b) and a slow, relatively weak, component, situated usually in close proximity to the antibody well, were described. Whether these two components are identical, to the previous two, cannot be concluded for certain.

In order to obtain some more information, concerning the nature of lines a and b, a further experiment, not reported in the results section, was carried out, in which specific antimouse IgG was compared to antigen in its formation of a precipitate with immune serum after electrophoresis of the latter agent. This showed that line b was very similar, to the line created by anti IgG, indicating that they were probably identical.

The nature of line a and its component parts was more

difficult to ascertain, with the methods available to the author. It is significant, nevertheless, that this component was less mobile than line b and that a precipitate was obtained with the first peak on the sephadex column. Both of these observations support IgM as a possible candidate for line a. It is emphasized, however, that the studies carried out were elementary and the conclusions arrived at are merely speculative in nature, awaiting more precise examination of the system for the final verdict.

Studies on A.tetraptera antigen revealed that this also had at least four reacting fractions. It would be interesting to discover whether these antigens are related to the antigens which elicit the host response leading to worm rejection. A significant observation here was the fact that almost complete immunity could be stimulated by the crypt phase of the infection (Chapter eight, Expt.20) and by 7 day larvae (Chapter thirteen,Expt.58), whereas a larval antigen gave only a very weak precipitate. At least one of the precipitating antigens was found to react with N.dubius antiserum, not necessarily implying cross protection, since there is no substantial evidence to support the conjecture that cross reacting antigens are in any way, connected with functional antigens and immune protection (Michel,1968). That this is essentially so, was shown by Stewart (1950) who found that sheep may be made immune to either Hemonchus contortus or Trichostrongylus spp, and fully susceptible to the other, yet in either case they possess antibodies which will cross-react serologically with antigen prepared from either parasite. The origin of the A.tetraptera antigens was not studied. Somatic proteins, excretory products and exsheathing fluid (Soulsby,1963) may all

be implicated but the observation was made that incubation products did not produce reacting antigens, nor were precipitates seen in the oral, excretory or genital openings (Sarles, 1937,1938) of worms incubated in HIS. Larval antigens reacted weakly, the strongest lines were achieved using mature worm antigen, which would not include exsheathing fluid since the final moult occured on or about day 14. Thus it seems likely that somatic antigens are implicated in the precipitin reaction but it is difficult to conceive how such antigens become available to the host immune response, without a tissue penetration phase.

An important discovery was that of the weaker response made by TFI mice. These mice, when recently purchased were moderately infected with A.tetraptera, but the serum of these mice showed only weak lines, far less apparent than those observed for 28 day, primary infection serum from CFLP mice. It may be that the lack of a serological response in these animals was associated with their inability to reject worms. The synthesis of both precipitating and functional antibodies is an immune phenomenon and unless the two are identical TFI mice must have a central defect or inability to respond strongly to A.tetraptera infection. It is pertinent to note here that hydrocortisone abolished the precipitating antibody response in CFLP mice and also the elimination of worms. Conversely whereas ALS failed to suppress rejection, it too abolished the precipitating, antibody response, showing that worm rejection can still operate effectively in mice with reduced precipitating antibody levels.

On the other hand antibodies are known to play a vital role in worm rejection (Ogilvie and Jones,1973), and it has been shown that IgG1, and to a lesser extent IgG2 contain the

functional components responsible for worm damage in the N.brasiliensis system. It was suggested earlier that at least one component of the anti A.tetraptera response was IgG but it is too early yet to state whether it has a related function.

The results reported here are not altogether irreconcilable with those of previous authors. The explanation of their failure to detect anti A.tetraptera antibody could simply depend on the strain of mice used in their experiments, since as has been seen here, host strain characteristics may vary. In conclusion it can be said that CFLP mice have the ability to reject A.tetraptera larvae during a primary infection and also to make a precipitating antibody response to this parasite. The two phenomena are related in that they are both immune in nature, but functionally they may well be independant. The results recorded here amply justify further studies on this system since similar serological responses by non tissue penetrating parasites are rare.

CHAPTER THIRTEEN

The response of mice to transplanted larval and mature *Aspiculuris tetraptera*.

INTRODUCTION.

The response of outbred, wild animals of any species to a particular environmental irregularity or to a disease is seldom identical for all the individuals. This variability is inherent in all animal populations and it forms the basis of evolution as it is understood. For laboratory workers, variations in the responses of individual animals are a hardship which has to a certain extent been overcome by the use of laboratory strains.Even the use of specific strains, however, does not eliminate all the problems involved and for these cases inbred animals are available, as for instance in immunological studies, where extremely close similarity between the animals in one experimental group is a prerequisite to the successful elucidation of the problems under investigation.Inbred mice, however, have one important drawback and that is, they are relatively expensive.

In the experiments so far described in this thesis, a constant feature has been the large variation between the individuals in identically treated groups of animals. Inbred mice were not the answer to this problem, for the pre stated reason so an alternative solution was sought.

Various workers in the past have demonstrated that the surgical transplantation of nematode parasites to recipients is feasible and a useful tool in evaluating certain aspects of the host-parasite relationship, (Mulligan *et al*,1965; Ogilvie,1965 a; Barth *et al*,1966; Ogilvie and Hockley,1968). *N.brasiliensis* has been extensively studied in this way. Rats

have been exposed to truncated infections consisting solely of mature worms. In immunological studies this has eliminated the need to consider the role of the larvae in bringing about the observed phenomena and has given more consistent results (Ogilvie and Jones,1971). The surgical transfer of tapeworms has also given precise information on the host-parasite relationship (Braten and Hopkins,1969). The procedures used in this study have been precisely described in Chapter three. The final method was devised after much experimentation and the first three experiments will show how the method was evaluated before tackling more ambitious targets. Finally, the duration of infection with transferred larval and mature A.tetraptera was studied and the results are presented in this chapter.

RESULTS

The transfer of mature worms to the ileum and the colon in male mice.

Experiment 55 - Five, non-infected Porton strain male mice received about eighteen mature worms. In three of these mice the worms were transferred into the colon and in the others, into the ileum. All the mice were killed twenty four hours later and the intestines were examined for worms.

Table 56 shows that the survival of worms in the colon was not unreasonable. The ileal worms, however, did not survive as well.

Comparison of the survival of mature worms after transfer to the ileum, caecum and the colon.

Experiment 56 - Twelve Porton strain males were infected by transplanted mature worms. The four mice in group A had the worms transferred into the ileum, Group B - into the caecum and group C into the colon . All the mice were killed on day 2.

TABLE. 59 EXPT. 55 The survival of mature A.tetraptera after transfer to the ileum and the colon of recipient mice.

Mouse	No. worms transferred.	No. of worms recovered from sections of the intestine.				
		Ileum	Caecum	Colon		
				1-3	4-7	8-10
L_0R_0	18 (Ileum)	-	-	4	-	-
L_1R_0	2 (Colon)	-	-	2	-	-
L_0R_1	18 (Colon)	-	-	18	-	-
L_1R_1	18 (Colon)	-	-	17	-	-
L_2R_0	18 (Ileum)	-	-	-	-	-
Control	- -	-	-	-	-	-

EXPT. 56 The survival of mature A.tetraptera after transfer to the ileum, caecum and colon of recipient mice.

Group A.

L_1R_1	19 (Ileum)	-	-	8	-	-
L_0R_0	19 (Ileum)	2	-	9	-	-
L_1R_2	25 (Ileum)	-	-	24	-	-
L_2R_0	25 (Ileum)	4	-	3	-	-

Mean survival 57.9 %

Group B.

L_0R_1	20 (Caecum)	-	1	14	-	-
L_2R_0	25 (Caecum)	-	-	8	2	1
L_2R_1	20 (Caecum)	-	-	20	-	-
L_0R_2	25 (Caecum)	-	-	14	1	-

Mean survival 67.7 %

Group C.

L_1R_0	20 (Colon)	-	-	18	-	-
L_0R_2	25 (Colon)	-	-	19	2	-
L_1R_2	20 (Colon)	-	1	12	2	-
L_0R_0	19 (Colon)	-	-	7	-	-

Mean survival 72.6 %

The results in Table 56 show that a large proportion of the worms survived until day 2. The worms transferred into either the colon or the caecum were equally succesful in becoming established, indicating that the parasite can migrate to its preferred site when transferred to the caecum, (not normally a favoured region). Perhaps the more unusual conditions in the ileum prevented the worms transferred to the ileum from finding their way to the colon.

The transfer of mature worms to hydrocortisone treated recipients.

Experiment 57 - Six female CFLP mice were given 1.25 mg. of hydrocortisone acetate on day -2 and on day 0, in order to eliminate any inflammatory response, resulting from the transplantation (Chapter nine). It was thought best to study the survival of transferred worms in immunosuppressed recipients first before attempting the same in non-treated recipients. Each mouse received approximately thirty mature worms, (50% of both sexes) obtained from 16-wk old naturally infected, male TFI mice. A single mouse was killed on each of the occasions shown in Table 57 and the distribution of the recovered worms was recorded.

A few worms survived in the caecum until day 3. The majority, however, moved into the colon within two days of transfer. There was only minor loss of non-viable worms in this system. The highest recorded loss was on day 8 when the particular animal had 70% of the transferred worms still present.

The transfer of 7-10 day larvae to male and female mice.

Experiment 58 - Because the transfer operation is a complex procedure and thus time consuming, a maximum of twelve to fifteen mice only, could be operated on in any one day's work. Experiment 58 consisted of five individual experiments in each

TABLE 60 The survival of mature A.tetraptera after transplantation to
EXPT. 57 the caecum of hydrocortisone treated recipient mice.

Mouse	No. of worms transfered.		Day killed	No. of worms recovered from sections of the intestine										Total no. of worm recovd.	% recovery.
				Ileum		Caecum		Colon 1-3		Colon 4-7		Colon 8-10			
	MALE	FEMALE		MALE	FEM.	MALE	FEM.	MALE	FEM.	MALE	FEM	MALE	FEM		
$L_0 R_2$	17	15	12 hrs.	-	-	9	7	8	8	-	-	-	-	32	100·0
$L_2 R_0$	16	15	2 days.	2	-	4	1	10	13	-	-	-	-	30	96·8
$L_1 R_0$	15	15	3 "	-	-	1	2	10	10	-	-	-	-	23	76·7
$L_0 R_0$	15	15	4 "	-	-	-	-	13	12	-	1	-	-	26	86·7
$L_1 R_1$	15	14	6 "	-	-	-	2	15	10	-	-	-	-	27	93·1
$L_0 R_1$	15	15	8 "	-	-	-	-	9	12	-	-	-	-	21	70·0

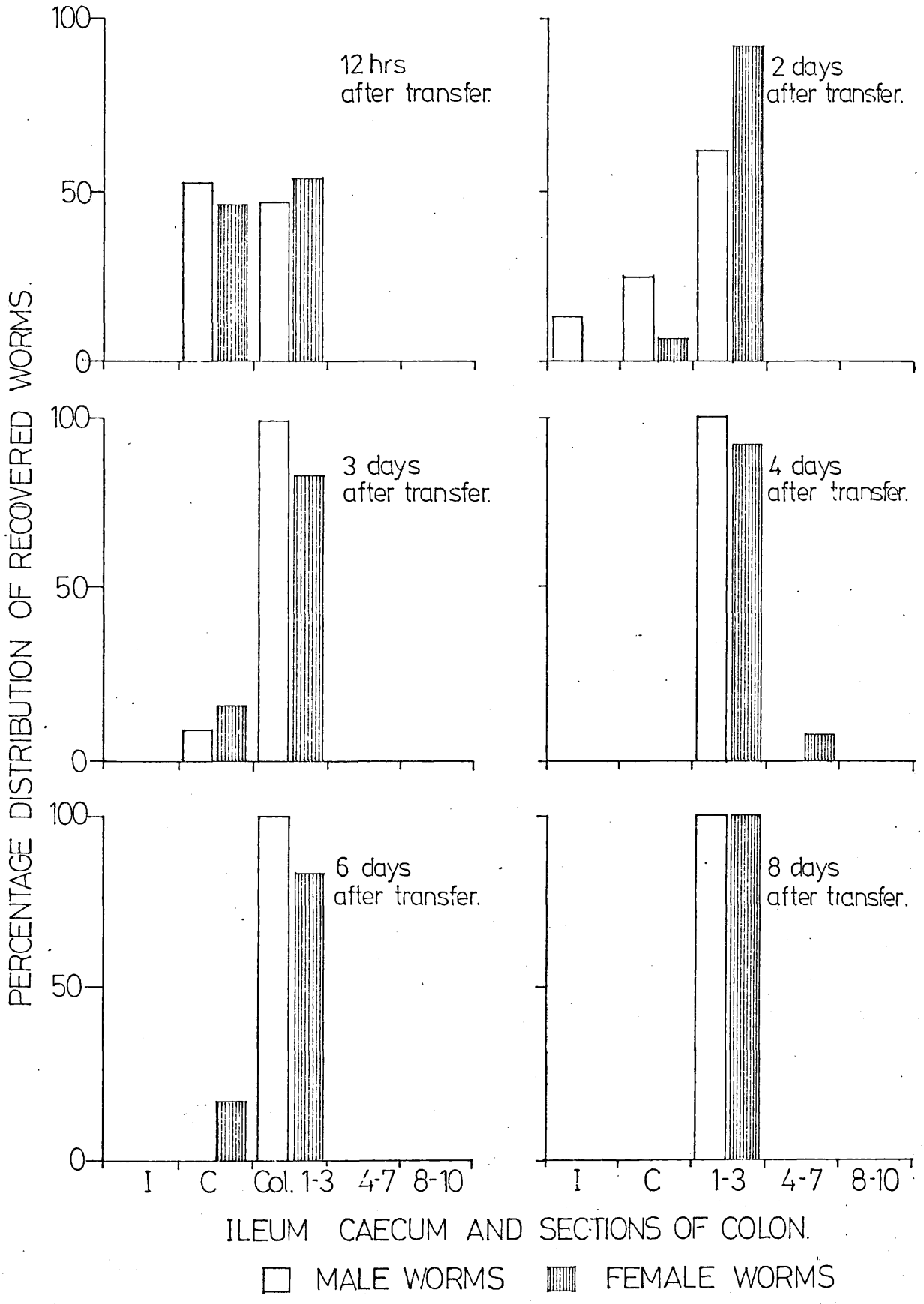


FIG. 59 The percentage distribution of A. tetraptera after transfer to
EXPT. 57 cortisone treated recipient mice.

in each of which 65-160, seven to ten day, larvae were transferred to CFLPb, 4-5 wk old mice. The larvae were obtained from heavily infected CFLPb males treated with 0.05 ml. of hydrocortisone on at least two separate occasions during the infection period. Recipient mice were killed at various times after the transfer and their worm burden was assessed in the normal way. The results are difficult to analyse because there were five groups and in each group mice were killed on as many as eight different occasions. It was observed that the worm numbers remained constant for about a week after infection. On days 6-7 a proportion of the worms were displaced posteriorly in the colon and this was followed by a loss of worms over the next two weeks. In order to assess this change statistically all the mice killed before day 7 were treated as one group in each of the five separate experiments. The mice killed on day 8 and later, formed the second group. Table 58 shows the means, standard deviations and the statistical analysis of the results when interpreted in this way. The worms recovered from a particular mouse were expressed as the percentage recovery of transferred worms. This facilitated comparison between the groups from the different experiments.

The second method used was to pool the percentage recoveries for a particular day from all 5 experiments and to calculate the means for groups killed between the following times, days 1-4, 5-7, 8-12, 13-16, 17-20 and 21+ (Table 59). Lastly all the individual percentage worm recoveries were plotted against time and the means (Table 59) were superimposed. (Fig.60).

Clearly there is a drop in worm numbers after day 7. In three of the five experiments a statistically significant difference was obtained. In experiment D a non-resistant mouse

TABLE 61 The recovery of 7-10 day larvae
EXPT. 58 after transfer to recipient mice.

Group	Mean percentage recovery					
	Mice killed on days 1-7			Mice killed on days 8+		
	No. mice.	Mean ±	s.d.	No. mice.	Mean ±	s.d.
A	4	87.8[1]	17.3	5	66.1[1]	17.2
B	5	92.0[2]	10.5	5	53.9[2]	33.4
C	4	83.7[3]	3.2	5	20.3[3]	24.3
D	5	81.8[4]	18.5	5	49.8[4]	35.9
E	5	85.7[5]	2.8	7	21.8[5]	21.4

TABLE 62 The recovery of 7-10 day larvae
EXPT. 58 (pooled results) after transfer to recipient mice.

Mice killed on days.	No. of mice.	Mean percentage worms recovered.	±	s.d.
1-4	7	83.0		12.4
5-7	16	87.7[6,7,8]		11.6
8-12	5	61.3		12.8
13-16	10	54.3[8]		34.5
17-20	5	20.3[6]		24.3
21+	7	21.8[7]		21.4

Statistical analysis of results by Student's t-test & Wilcoxon test.

1	P= N.S.	P=N.S.
2	P< 0.05	P< 0.01
3	P< 0.001	P< 0.01
4	P= N.S.	P= N.S.
5	P< 0.001	P< 0.005
6		P< 0.001
7		P< 0.001
8		P= 0.005

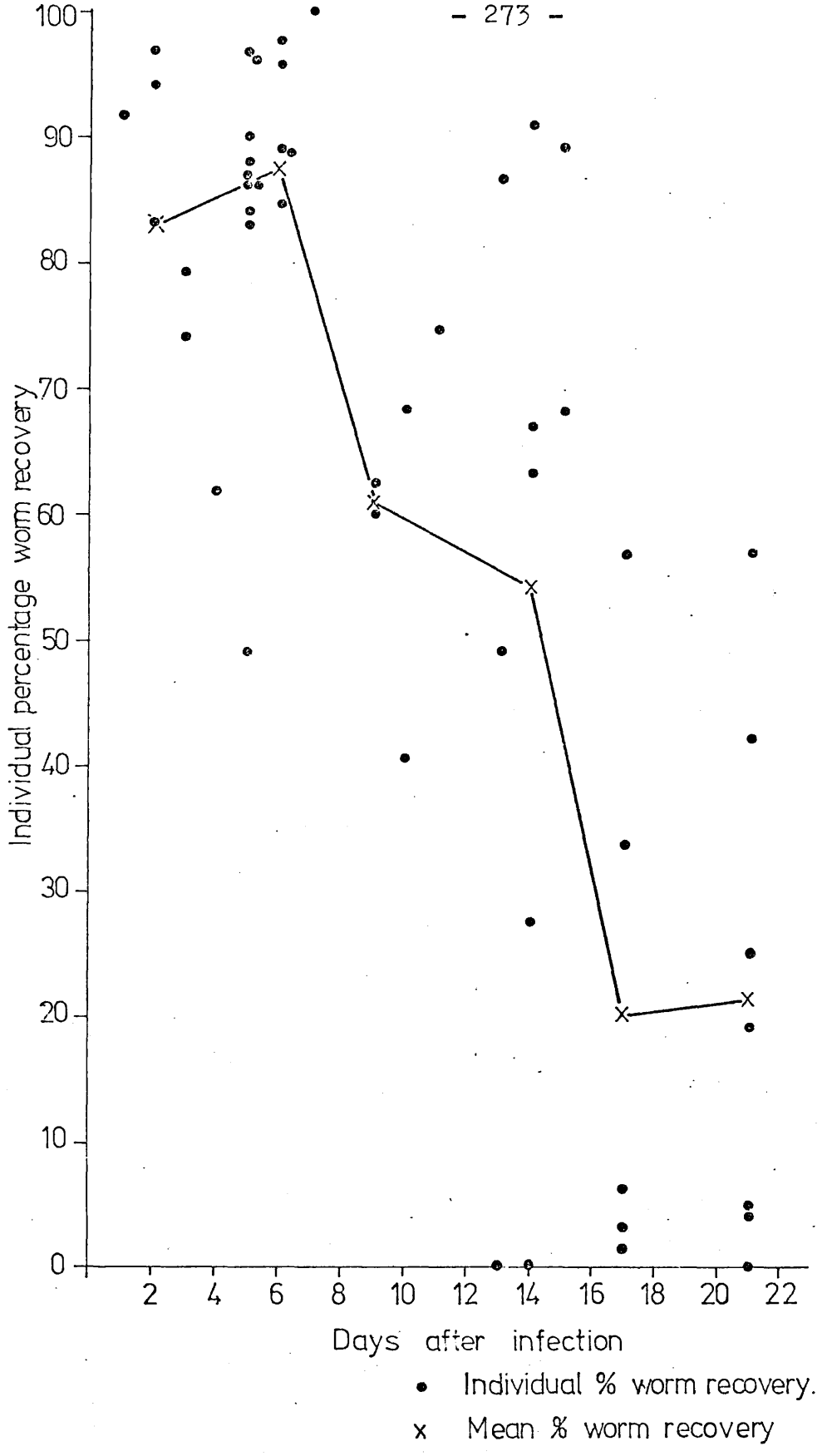


FIG. 60
EXPT. 58

Individual worm recoveries (pooled results) from recipient mice given 7-10 day larvae by transplantation.

killed on day 14 complicated the interpretation. The omission of this one mouse, however, gave P=0.05, which is the borderline of statistical significance. In experiment A, complete expulsion was achieved in a number of the day 8+ mice.

The transfer of mature worms to male and female recipients.

Experiment 59 - In this experiment, approximately forty worms (20 male and 20 female) were transferred to CFLPb and CFLP, male and female mice, with no previous experience of infection. Most of the recipients were 4-6 wks old on the day of the operation. Worms for transfer were obtained from TFI male mice 10-18 wks old. These animals were naturally infected with A.tetraptera and were injected once a week with 2.5 mg of hydrocortisone from the 10-th week of age.

This experiment was planned along lines similar to those of Expt.58. Thus there were seven separate experiments, Groups A-G, in which the worm transfer was carried out on seven separate occasions. The total percentage recoveries were pooled and then arranged into groups corresponding to the day on which the mice were killed. There were seven of these groups corresponding to mice killed on days 1-5, 6-10, 11-15, 16-20, 21-25, 26-30 and 31-35.

The data was analysed for correlation and the correlation coefficient was calculated as -0.45, which means that for sixty eight samples the relationship between the number of worms and the day of infection is highly significant ($P < 0.001$). Regression analysis shows that the data fit into a straight line graph represented by $Y=91.7 -1.1X$.

The transfer of fourteen day larvae to male recipient mice.

Experiment 60 - Fourteen day larvae obtained from groups

TABLE 63 EXPT. 59 The recovery of mature worms (pooled results) after transfer to recipient mice.

Mice killed on days.	Nos. of mice.	Mean percentage worm recovery.	± s.d.
1-5	25	89.0	14.5
6-10	11	80.5	18.3
11-15	7	84.7	12.2
16-20	13	66.4	34.4
21-25	2	61.3	1.8
26-30	6	82.2	18.0
31-35	4	32.2	30.1

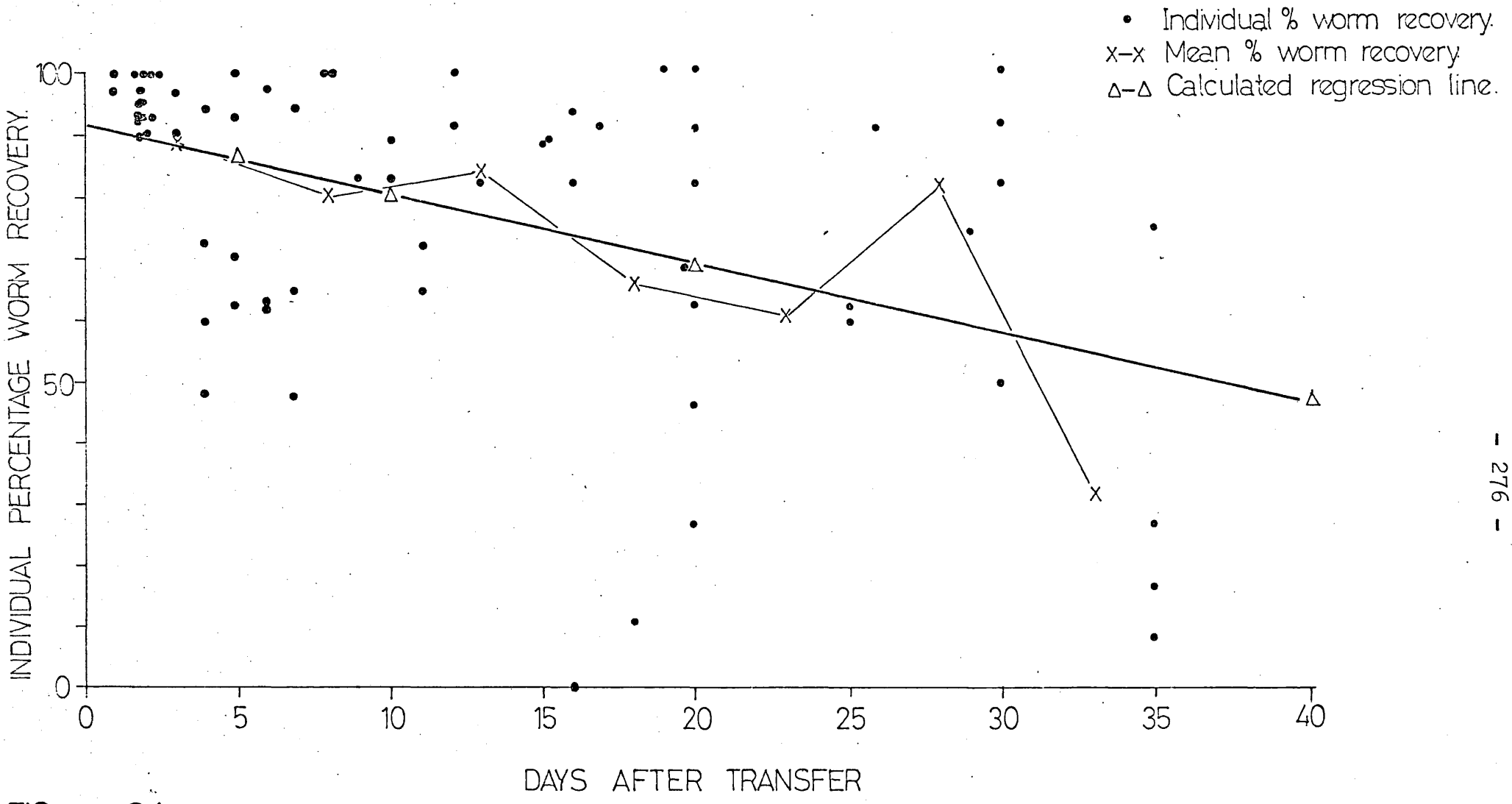


FIG. 61 Individual worm recoveries (pooled results) from recipient mice
EXPT. 59 given mature A.tetraptera by transplantation.

of infected, hydrocortisone treated male CFLPb strain donor mice. The recipient mice in this experiment were 4-6 wk old male mice of the same strain. As in the last two experiments, mice were killed at various times after infection, in each of the five groups, (Table 62). The results were interpreted either independantly for each group or they were pooled and analysed accordingly.

No significant loss of worms was detected before the third week of infection and thereafter the loss was small. The correlation coefficient calculated for the pooled results was - 0.9014, which is a highly significant result showing a negative correlation between the number of worms present on days after the transfer operation. The regression formula was calculated as $Y=93.8 - 0.66X$ which gives a time of 64.8 days for the transferred worm burden to be reduced by half. Experimentally this correlates well, since the autopsy worm recovery on day 68 was in fact 55.6%.

The survival of mature worms in immune recipients.

Experiments 61a and 61b - This experiment consisted of two smaller experimental groups. In the first experiment, fifteen male and fifteen female worms were transferred to each of eight immune mice. The three immunizing infections, consisting of 1,000, 1,500 and 1,500 eggs were given on days 0, 102, and 131 respectively and the worms were transferred on day 147. Mature A.tetraptera were obtained from hydrocortisone treated TFI, naturally infected mice. Three of these recipients, the control mice, were injected with hydrocortisone on days 0, 2 and 4. Both groups were killed on day 5.

In the second experiment the immunizing infections consisting of 750, 1,000 and 600 eggs given on days 0, 33 and 47 respectively were administered to six recipient mice. Twenty

TABLE 64 The recovery of fourteen-day larvae (pooled results) after transfer to recipient mice.
EXPT. 60

Mice killed on day	No. of mice.	Mean percentage worm recovery.	± s.d.
1-5	13	88.9	8.1
6-10	10	91.4	3.9
11-15	3	94.7	5.0
16-20	6	88.0	12.5
21-30	3	78.6	10.0
31-40	7	62.0	23.4
41-50	3	61.3	23.2
68	3	55.6	7.8
85	4	31.9	16.5
147	4	0	0

Table 65 The recovery of fourteen day larvae after
Expt. 60 transplantation to recipient mice.

Group	Day killed	Number of mice	Mean percentage worm recovery	± s.d.
A	1	1	98	
	2	1	96	
	5	3	95.3	2.3
	12	3	94.7	5.0
	19	3	94.0	5.3
B	2	1	90.0	
	3	1	83.3	
	4	1	84.0	
	6	3	90.7	5.0
	20	3	82.0	15.9
	49	3	61.3	23.2
C	1	1	95.9	
	2	1	88.0	
	5	3	78.5	6.4
	28	3	78.6	10.0
	68	3	55.6	7.8
D	6	4	91.5	5.0
	35	3	84.0	6.9
	85	4	31.9	16.5
E	6	3	92.0	2.0
	34	4	45.5	14.8
	147	4	0	0

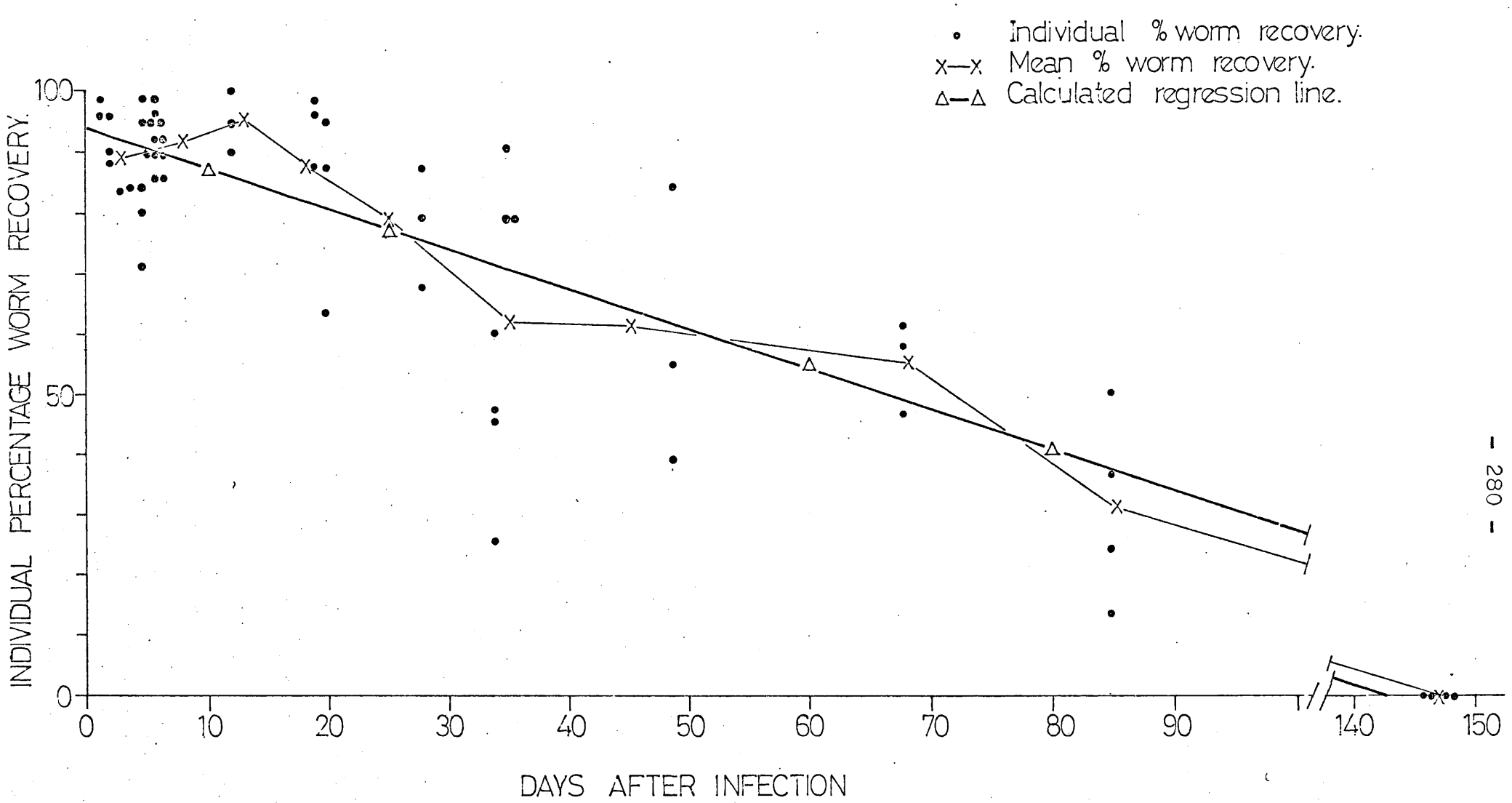


FIG. 62 Individual worm recoveries (pooled results) from recipient mice
EXPT. 60 given 14-day larvae by transplantation.

male and twenty female worms were then transferred on day 61. Two mice received hydrocortisone by the previous regimen and then both groups were killed on day 5.

When the results were analysed, independantly, neither the Student's t-test nor the Wilcoxon test gave a statistically significant result. A borderline significant difference was, however, obtained when the results of both experiments were pooled and compared by the Student's t-test. It is not at all surprising that the results proved difficult to interpret since the variation between individual mice was so great and the number of animals used, was so small. The pooled results gave the following recoveries : 78.0, 78.5, 87.0, 97.0 and 100% in hydrocortisone treated mice and : 0, 15, 47, 50, 53, 55, 90, 93 and 100% in non-treated mice. In spite of the non-significant result, there is some indication that the worms did not survive as well in immune recipient mice, but because the group included three mice with recoveries typical of the hydrocortisone treated group, the analysis of pooled results could not detect a valid difference between the two groups.

The stimulation of immunity by transferred 14-day larvae.

Experiment 62 - Fourteen day larvae were obtained from a single male CFLPb mouse, infected with 2,500 eggs and given hydrocortisone on days -1 and 3. The recipients were female CFLPb mice, about 6 weeks old on the day of the transfer. Approximately 50, 14-day larvae were transferred on day 0 to five mice. Seven mice were sham-operated and ten mice were included in an untreated control group. The transferred infection was terminated on day 28 with piperazine and the worms were counted as they were recovered with the faeces. The mean worm recovery was 34 ± 10.0. Four days later all three groups were challenged with 750 eggs and they were killed on day 10.

TABLE 66 EXPTS. 61a,b The effect of hydrocortisone on the survival of mature worms after transplantation to immune mice.

Group	No. mice	Percentage worm recovery.	± s.d.
Experiment 61,a			
A (Immune)	5	68.0[1]	25.0
B (Immune hydrocortisone treated)	3	87.5[1]	9.3
Experiment 61,b			
C (Immune)	4	40.8[2]	41.9
D (Immune hydrocortisone treated.)	2	89.0[2]	15.6
Pooled results Experiment			
Immune mice	9	55.9[3]	34.2
Immune hydrocortisone treated.	5	88.1[3]	10.2

Statistical analysis of results by Student's 't'-test and Wilcoxon test.

1	P = N.S.	P=N.S.
2	P = N.S.	P=N.S
3	P < 0.05	0.1 > P > 0.05

The larvae were recovered and the results are shown in Table 67.

The results were analysed by Student's t-test and by the Wilcoxon test and once again interpretation proved difficult. There was no difference between the control group and the sham-operated group, by either of the two tests. Student's t-test gave negative results for both of the two remaining comparisons, but the Wilcoxon test gave $P < 0.025$ for the comparison between the control group and the experimental group and $P=0.05$ (borderline of significance value) for the sham-operated group compared to the experimental group. The validity of the comparison is underlined when the actual worm recoveries are compared. Thus for the experimental group, the values are 0, 0, 0, 2 and 168, and for the sham-operated group 5, 9, 13, 22, 26, 118 and 222. The single high count (168) in the experimental group suffices to upset the variance value for this group and hence, the non-significant result. All things considered, however, it is probably valid to conclude that some degree of immunity has been demonstrated.

The stimulation of immunity by transferred mature worms.

Experiment 63 - Thirty to fifty mature worms obtained from hydrocortisone treated TFI male donor mice were transferred to eleven CFLPb male and female recipients. Five mice were sham-operated and four untreated control mice were included in the experiment. Three mice from the first group were killed on days 1-3 and the worm recoveries were 87.5%, 93.9% and 81.6%, indicating that the transferred worms were well established. Four weeks later, all the mice were given piperazine citrate and then, after a further four days, they were challenged with 1,000 eggs of A.tetraptera. All the mice were killed on the tenth day of the challenge infection and the

TABLE 67 The effect of transplanted mature *A.tetraptera* on a subsequent challenge with 1,000 eggs.
EXPTS. 62 & 63

Group	No. mice	Mean worm recovery.	± s.d.
Experiment 63			
A (control)	4	137.3 [2,3]	78.6
B (sham operated)	5	38.8 [1,3]	40.7
C (previously infected by transplanted mature worms.)	8	10.4 [1,2]	15.8
Experiment 62			
A (control)	10	97.7 [5,6]	94.7
B (sham operated)	7	59.3 [4,6]	81.7
C (previously infected by transplanted mature worms)	5	34.0 [4,5]	74.9

Statistical analysis of results by Student's 't'-test and Wilcoxon test

1	P = N.S.	P = 0.1
2	P < 0.02	P < 0.005
3	P = N.S.	P = 0.05
4	P = N.S.	P = 0.05
5	P = N.S.	0.025 > P > 0.01
6	P = N.S.	P = N.S.

results are recorded in Table 64.

The control group had more worms than either of the other two groups, P=0.05 (compared to the sham-operated group) and P< 0.005 (compared to the experimental group.). The difference between the sham and the experimental group was not, however, significant because of the unexpectedly low worm recoveries from the sham-operated group. The individual worm recoveries of 0, 0, 0, 0, 5, 7, 33 and 38, from the experimental group do not differ significantly from the sham-operated group where the values are 0, 9, 25, 62 and 98. Perhaps if more mice had been included in the second group, a more consistant difference might have been obtained. However, whereas 6/8 (75%) experimental mice have fewer than 10 worms, 2/5 (40%) of the sham-operated group fall into the same category; once again a not too convincing difference.

DISCUSSION.

The exploratory, preliminary experiments (Expts.55-57) showed that the procedure for transplantation was feasible and that worms transferred in this way would survive in recipient mice. It was demonstrated, using hydrocortisone to prevent the onset of immunity and to inhibit inflammation, that worms transferred to the caecum rapidly found their way back to the anterior colon. Further attempts suggested that hydrocortisone was unnecessary and that events proceeded similarly in "normal" mice. It was thus possible using this transfer system, as a convenient laboratory procedure, to begin the examination of the host parasite relationship in greater detail than would otherwise have been possible.

When 7-10 day larvae were transferred to recipient mice, they stimulated events, which ultimately led to the removal of about 75% of the worm burden. There was undoubtedly

inflammation in the caecum but rarely did this extend to the colon. The larvae did not appear to be obviously affected by it and they were lost irrespective of whether there was a reaction or not. Mice autopsied on days 6-8 in which larvae were observed along the length of the colon in the process of being lost, commonly showed no obvious inflammatory symptoms.

The loss of 7-10 day larvae occurred almost a week after transfer, i.e. when the larvae were about 14 days old and therefore moulting to the adult stage. It is at this time that they are usually rejected by mice infected by the normal route (oral) and it suggests that larvae of this age are capable of eliciting the phenomena which lead to their expulsion. The period of time required to stimulate rejection is similar to that of other parasites, e.g. transferred mature *N.brasiliensis* evoke immune expulsion after seven days of infection (Ogilvie and Jones,1971).

Mature worms, on the other hand were not expelled in the first three weeks of infection. The equation $Y=91.7-1.1X$ (as calculated for Expt.59) predicted that almost five weeks after infection (38 days) only 50% of the worm burden would be lost. This is a very slow rate of loss and could be attributed either to the development of senility in the worms or to a slow immunological response.

A similar result was obtained in Expt.60 where 14 day worms were transferred instead of mature worms. In fact the majority of 14-day larvae raised in immunosuppressed mice were already in the fifth instar, pre-adult stage. Since patency is reached on days 22-24, a further ten or so days is required by the larvae before they reach a comparable stage to the worms used in Expt.59. Unlike the 7-10 day larvae these worms were not rejected by a rapid spontaneous self-cure

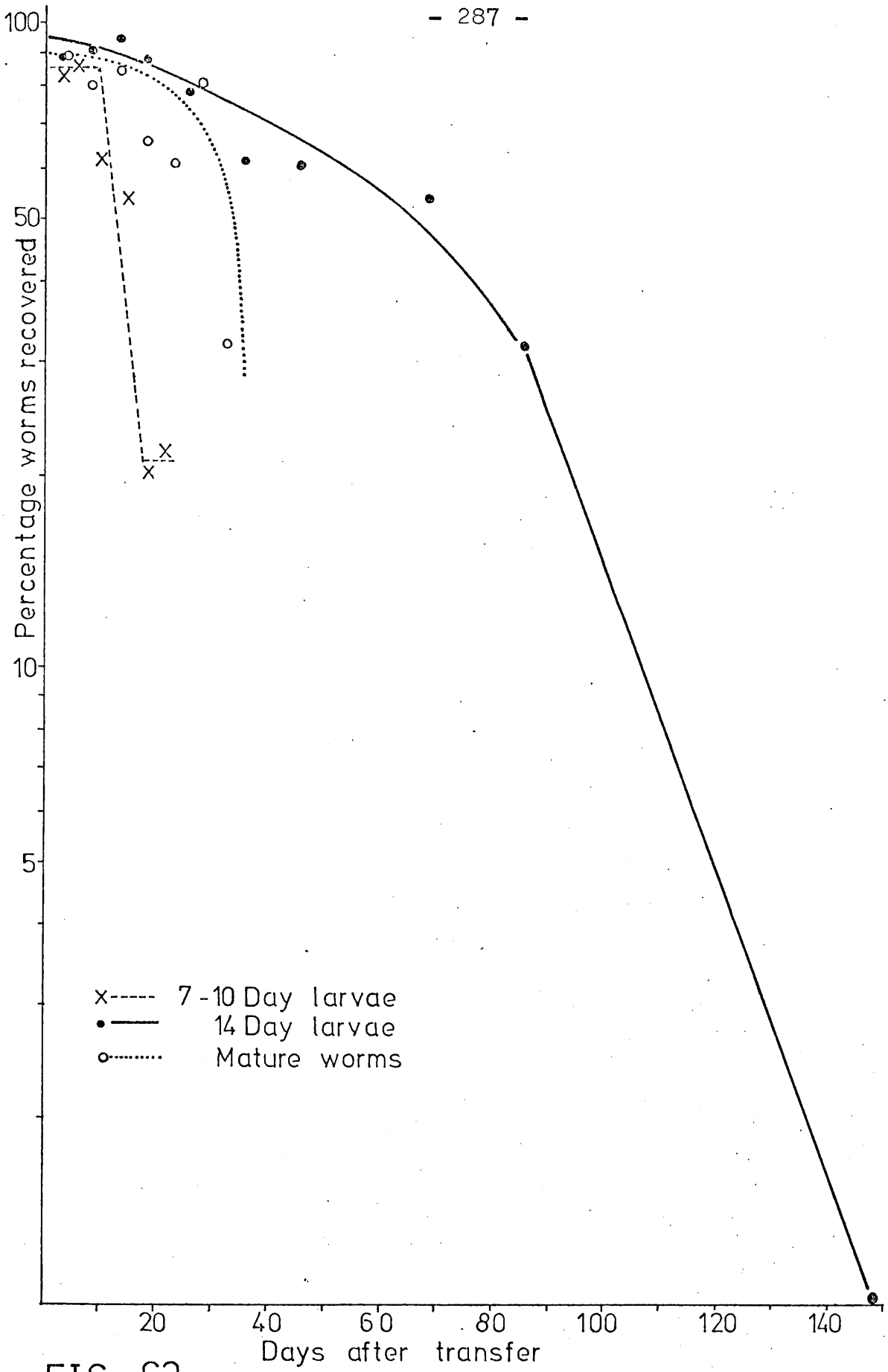


FIG. 63 The survivorship curves of 7 EXPTS. day larvae, 14 day larvae and 58, 59 & 60 mature worms in recipient mice.

response. One advantage of using 14-day larvae was the precise number of viable worms which could be transferred to recipient mice. The percentage of worms established was always greater than 80% and usually 90% or more. The standard deviations for groups killed within the first twenty days were comparatively small and indicated a uniform "take" in most cases. The recoveries over the next five months gave a consistant straight line graph represented by Y=93.8-0.66X, and the correlation of the relationship represented by this graph was highly significant (-0.9014). There was a long gap between the group killed on day 85 and that killed on day 147 and it could be that the loss of all the worms was accomplished well before day 147.

When the mean worm recoveries (Fig.63) were plotted logarithmically against an arithmetic time scale, it can be seen that the most important values were in fact those between days 85 and 147, for it was during this time that the rate of worm loss was accelerated. The convex curve indicated a low mortality before day 70 or so and then the death of the remainder of the population during a relatively short period. This is the usual picture for survivorship curves of known laboratory populations such as Drosophila, Hydra, the rotifer Floscularia conifera, the vole (Lelie and Ranson,1940) and also man. Fig.63 compares the results obtained in Experiments 58,59 and 60 on a logarithmic scale against the arithmetic time scale. Whereas the fourteen day larvae and the mature worms show a normal type of survivorship curve, the 7-10 day larvae clearly are lost by another means and it is proposed that the main factor involved, is the effect of immunity on these larvae.

An interesting fact arising from experiments 59 and 60

was the difference in the predicted time for the loss of 50% of the worms, between mature worms (38 days) and the 14-day larvae (64.8 days). However, the mature worms used in Experiment 60 were at least 28 days old and probably older; the addition of 14 days to the predicted value for mature worms would bring it to 52 days, much closer to the value for 14-day larvae. Furthermore this value was in approximate agreement with results of Experiment 6 in Chapter five and Experiment 28 in Chapter nine, for it was shown then that between days 50-60, there was a noticeable drop in worm recoveries from mice continually treated with hydrocortisone. It thus appears that the life-span of A.tetraptera lies somewhere between two and three months.

In conclusion, mice were capable of eliminating a large proportion of 7-10 day larvae soon after transfer, but they were not so successful when the transferred worms were in the fifth stage of larval development. This finding correlated well with some earlier results. In Experiment 27 Chapter nine it was shown that three doses of hydrocortisone given during the first week of infection were sufficient to ensure that most worms survived to patency, a result for which three possible explanations were suggested. The present findings are relevant to both of the last two possibilities, these being that mature worms were not rejected because they did not stimulate immunity or because unlike the larvae, they were resistant to the effects of immunity. However, if mature worms were capable of stimulating acquired immunity against larvae, as suggested by experiments 62 and 63, it seemed reasonable to propose that the explanation most consistent with the results was the third possibility. In other words, the mature worms were in some way adapted to withstand immunological attack, more effectively than

the larvae. Thus hydrocortisone was protecting the larvae from immunity until they no longer required assistance in overcoming the host response.

Here, then,is evidence from a number of sources indicating that worms in the fifth stage are capable of avoiding immune expulsion. This is not, however, a unique example. N.dubius can stimulate acquired immunity in mice after a single infection of 500 larvae (Bartlett and Ball,1972; Hosier and Feller,1973). Challenge infections do not become established as successfully as in control mice and yet the original immunizing infection persists for as long as eight or so months. This indicates that here too, is a parasite where immunity against subsequent reinfection is elicited by the larvae and possibly by the mature worms, but that the mature worms of the immunizing infection are not susceptible to its action since there is little worm loss after the larvae emerge into the intestine and become lumen dwelling adults.

When mature worms were transferred to immune mice, they were less successfull in becoming established, although because of the small number of animals used, the results were difficult to interpret. The numbers recovered from immune mice, however, were greater in terms of percentage recovery, than would normally be expected from immune mice, either challenged with eggs (Chapter eight) or with transferred 7-10 day larvae (Chapter fourteen)

CHAPTER FOURTEEN

The survival of "normal" and "damaged" worms in immune, non-immune and immunosuppressed recipient mice.

INTRODUCTION.

The immunological rejection of nematode parasites has received careful attention during the last few years, centred particularly around the precise mechanism of worm expulsion. The development of ideas and the recent experimental findings were reviewed by Ogilvie and Jones(1971) and more recently by Dineen,Ogilvie and Kelly (1973) and Ogilvie and Jones (1973). It has been shown that the process of rejection in N.brasiliensis infected rats is at least diphasic. During the course of a normal primary infection the first essential step is damage to adult worms (Ogilvie and Hockley,1968) by immunoglobulins stimulated by the presence of mature worms in the gut (from day 6 onwards). Ogilvie and Jones, defined damaged worms as:

> "Worms permanently damaged by immunity and found in the intestines of initially infected rats after the tenth day. For experimental purposes, worms are taken on day 14 (e.g. Edwards et al,1971) but this definition also includes the residual worm population found in adult rats as well as worms found in neonatal rats from approximately day 17 after infection."

The second phase is the actual rejection of worms which is thought by some workers to be mediated by sensitized lymphocytes (Keller and Keist,1972; Dineen, Ogilvie and Kelly, 1973), although the exact process by which this is achieved is not known. It has been postulated that lymphokines may be involved, exerting their damaging action on the metabolism of the parasite, (Dineen, Ogilvie and Kelly,1973). Other workers Urquhart et al,1965, Barth et al,1966, have implicated pharmacologically active amines in this context, but again

the evidence is still controversial (Wells,1962; Murray et al, 1971 a; Keller,1971; Kelly and Ogilvie,1973), and the mechanism awaits elucidation. There may be as yet undescribed factors involved.

Earlier in this thesis it was shown that A.tetraptera larvae were rejected by female mice during the third week of infection (Chapter four). Similar events occurred when 7-day larvae were transferred to mice with no previous experience of infection but the expulsion occurred seven days after transfer, by which time the larvae were 14 days old, (Chapter thirteen). Studies using hydrocortisone showed that worms were damaged before or during the actual rejection and that even in the presence of immunosuppressants the surviving worms remained small, stunted, delayed in development, and the females frequently had no eggs. (Chapter nine).

It was decided to use the transfer system to investigate the survival of larvae, taken a week prior to and immediately before rejection, after transplantation to recipient mice of varying immunological status. It must be explained here that "damaged" worms in the context of this chapter, are not synonymus with the damaged worms as defined by Ogilvie and Jones, (1971). There is, as yet, no histological evidence in A.tetraptera of the type of damage incurred by N.brasiliensis before expulsion, but the experiments described here will show that there may be some similarity between the ability of the "damaged" A.tetraptera and"damaged" N.brasiliensis,to survive in non-immune, immune and immunosuppressed hosts.

RESULTS.

The transfer of larvae to immune mice.

Experiments 64 and 65 - In the first of these two experiments, approximately 80 9-day larvae, obtained from hydro-

TABLE 68 EXPTS. 64 & 65 The survival of "normal" 8-9 day larvae after transplantation to immune and control recipient mice.

Group	No. mice.	Worms recovered on day 7			
		Mean worm recovery.	± s.d.	Percentage worm recovery.	± s.d.
Experiment. 64					
A (control mice)	4	76.0	11.2	86.2[1]	10.8
B (immune mice)	4	22.0	5.7	27.4[1]	5.7
Experiment. 65					
C (control mice)	5	122.8	11.6	86.9[2]	5.2
D (immune mice)	5	70.0	43.1	48.7[2]	29.2

Statistical analysis of results by Student's t-test & Wilcoxon test.

1 $P < 0.001$ $P - 0.01$

2 $0.05 > P > 0.02$

cortisone treated donor mice, were transferred to four control mice, and four immune mice. The immune recipients (female CFLPb strain mice) received 500 eggs of A.tetraptera,39 days before the transfer and a further 500 eggs, 8 days later. Piperazine citrate was given 3 days prior to transfer. Control recipients were mice from the same litters, treated identically with the exception of the immunising infections. Both groups of recipients were killed 7 days after the transfer and the worm burdens were assessed.

In the second experiment, there were five mice to each group, the interval between the second immunizing infection and the transfer was increased to 40 days, the larvae were 8 days old on transfer and the antihelminthic was given a week later. Both immune and control mice were killed and the worm burdens were assessed as usual.

In Expt.64, immune mice lost 72.8% of the transferred larvae by day 7. In contrast control mice lost only 13.8%. The difference between the mean worm recoveries of these two groups was statistically significant ($P < 0.001$). A similar result was obtained in Expt.65, but the difference between the two groups was not as clear cut, although still significant ($P < 0.05$).

The transfer of 14 day 'damaged' worms to recipient mice.

Experiment 66 - 'Damaged' worms were obtained from five female mice, five weeks old at infection. These mice were killed 14 days after infection, when a large proportion of their worms were already passing down the colon. Control worms were obtained from four male mice similarly infected but also given hydrocortisone on days -2, +5, 7 and 9. The recipients were 7-8 week old female CFLPb strain mice. They were killed five days after transfer. The guts were frozen and examined

TABLE 69 The survival of 'normal' and 'damaged' worms after transplantation to recipient mice.
EXPT. 66

Group	No. mice	Worm recovery on day 5			
		Mean no. worm recovery.		Percentage worm recovery.	
		Mean	± s.d.	Mean	± s.d.
A (Normal worm)	6	36.5	2.7	88.7[1]	6.8
B (Damaged worm)	6	10.3	5.6	25.4[1]	14.0

Statistical analysis of results

1 $P > 0.001$

a week later.

The results clearly indicated that 'damaged' worms were less successful in establishing themselves in recipient mice as assessed by the number of worms remaining on day +5. There were at least two possible ways in which this result could have come about.

1). It could be that the 'damaged' worms were not capable of maintaining their position in the preferred region of the colon. It may have taken up to five days before most of the worms were lost.

2). Alternatively it may have been that the 'damaged' worms became established in the usual way, but the recipient mice responded to their presence and because the process had already begun in donor mice, recipient mice effected the expulsion earlier than with normal worms. In order to test these alternatives, the next experiment was carried out.

The effect of hydrocortisone on the survival of 'damaged' worms.

Experiment 67 - Normal worms were obtained from one female CFLPb mouse, five weeks old at infection, given 2,500 eggs, fourteen days before the transfer. This mouse received hydrocortisone on days-1, and +4. 'Damaged' worms were obtained from five female mice, treated identically with the exception of the hydrocortisone.

Fourteen female CFLPb mice, 7-8 wks old were divided into two groups. Four control recipients received 50 normal worms. The remaining ten mice were given 50 'damaged' worms and were then arranged into two groups of five, of which one group was injected with hydrocortisone on days 0, 2 and 4. All fourteen mice were killed on day 5.

The results (Table 70.) showed that the expulsion of

TABLE 70 The effect of hydrocortisone on the survival of "damaged" worms, transplanted to recipient mice.
EXPT. 67

Group	No. mice	Worms transferred		Worm recovery on day 5.			
				Mean worm recovery		Percentage worm recovery	
		Mean	± s.d.	Mean	± s.d.	Mean	± s.d.
A (normal worms)	4	49.8	0.5	42.8	4.6	86.0[1,2]	9.4
B (damaged worms.)	5	49.6	0.6	13.0	2.7	26.2[1]	5.4
C (damaged worms to hydrocortisone treated recipients.)	5	49.8	0.4	43.8	2.3	87.9[2]	4.1

Statistical analysis of results

1 $P < 0.001$
2 P = N.S.

'damaged' worms from the recipient mice was inhibited by hydrocortisone ($P < 0.001$). There was no difference between the survival of 'damaged' worms in hydrocortisone treated recipients and the survival of normal worms in untreated recipients.

The effect of irradiation on the survival of 'damaged' worms in recipient mice.

Experiment 68 - The normal worms used in this experiment were obtained from a single CFLPb female, 8 weeks old at infection, given 2,500 eggs of *A.tetraptera* and treated with hydrocortisone on days -4, 0, 4 and 7. These worms were 12-days old on transfer. 'Damaged' worms were obtained from seven female mice infected 2 days prior to the normal worm donors and with the exception of the hydrocortisone, treated identically. Recipient mice were fifteen female CFLPb mice, 6-wks old on the day of transfer. Six mice from this group were irradiated with 400 Rads two days before the transfer. Approximately 40 'damaged' worms were transferred to each of these six mice and also to five of the remaining 9 recipients. The last four recipients were given 50 normal worms. All the mice were killed on day 5 and were examined.

The transfer of 'damaged' worms was complicated by the fact that very few worms were obtained from the 7 donor mice. Clearly, a large proportion of the worms was lost earlier during the infection because the hydrocortisone treated mouse received the same dose of eggs and provided over 300 worms. Both the number of worms transferred and that recovered at autopsy were more variable than in the previous experiments and consequently the results must be evaluated with caution.

It appears fairly conclusive that 'damaged' worms did not survive well in normal recipients. The mean percentage recovery of 32.6 ± 20.4 was lower than in the other groups

TABLE 71 The effect of irradiation on the survival of 'damaged' worms transplanted to recipient mice.
EXPT. 68

Group	No. mice	Worm recovery on day 5.			
		Mean worm recovery.		Percentage worm recovery.	
		Mean	± s.d.	Mean	± s.d.
A (Normal worm)	4	31.3	8.5	64.1	16.8
B (Damaged worm)	5	11.6	7.4	32.6	20.4
C (Damaged worms to irradiated recipients.)	6	25.2	8.5	63.8	20.2

Statistical analysis of results by Student's 't' test and the Wilcoxon test.

Groups compared	
AXB	$0.05 > P > 0.02$
AXC	P= N.S.

($P < 0.05$). Two mice from the irradiated group lost a greater percentage of the worm burden but in the remaining four, irradiation affected the ability to reject 'damaged' worms. Surprisingly, the 12 day normal worms did not survive as well as normal worms from previous experiments. This could indicate that the younger worms (12 days old as against 14 days) at an earlier developmental stage were more susceptible to the varying conditions imposed by the transfer operation. In spite of this, two mice had 70.2% and 84% of the transferred normal worms on day 5 and this was as good a recovery as in either of the two previous experiments. It is more likely that the variable recoveries from this group reflected the overall variability which was a feature of all three groups in this experiment. It could be that some unidentified factor contributed to a general lower viability of the transferred larvae and hence the variable recoveries.

DISCUSSION.

When adult N.brasiliensis were transferred to non-immune rats, the worms survived for about a week after the transplantation, although the duration of their survival varied from experiment to experiment, expulsion commencing as early as on day five and at other times as late as on day ten (Ogilvie and Jones,1971). The transfer of mature worms to immune rats, however, resulted in an immediate response (Neilson,1969). Worm expulsion began within a day of the transfer operation and continued until all the worms were lost, this being achieved within a week of transfer.

In the previous chapter it was found that the survival of transferred mature A.tetraptera was not as successful in immune recipients as in recipients with no previous experience of infection. This conclusion, however, was not based

on unquestionable evidence, since it was difficult to interpret the somewhat variable results. When 8-9 day larvae were transferred to immune mice, however, the majority were lost within a week of infection. Thus whereas in control mice, approximately 86% of the transferred larvae were still present on day 7, the worm burden in immune mice declined to 27.4% and 48.7% in the two experiments (Expt.64 and 65). There can be little doubt, then that acquired immunity is effective against 9-10 day larvae and is capable of terminating such an infection. It would have been interesting to follow this elimination in detail, to determine the rate of rejection and the extent of the elimination process, compared to that in control mice.

In N.brasiliensis infected rats, immunity acts against the mature worms; in A.tetraptera infected mice immunity eliminates the larvae. In the latter case therefore 'normal' immunologically unaffected worms might have been expected to be found prior to the initiation of events leading to worm expulsion, during the first week of infection. If these worms were to have been transferred as control 'normal' worms and compared to 14 day 'damaged' worms, interpretation of results would have been complicated by a second variable, the difference in morphological development between 'normal' and 'damaged' worms. To overcome this problem, 'normal' worms were obtained from hydrocortisone treated donor mice and were the same age as 'damaged' worms.

When 'normal' and 'damaged' worms were transferred to recipient mice, not previously exposed to infection, the 'damaged' worms did not survive as successfully as the controls. Five days after infection, almost 75% of the 'damaged' worms were lost.

In order to distinguish between the two possible interpretations, the experiment was repeated and a third group was included, consisting of immunosuppressed recipients given 'damaged' worms. The result showed that 'damaged' worms could survive adequately in these mice. Irradiation of the recipient mice prior to the transfer of 'damaged'worms was equally effective.

It has been demonstrated, therefore, that the worms found 14 days after a primary infection, in spite of their inability to maintain their position in the preferred site in the intestine, can survive for at least an extra five days when transferred to immunosuppressed mice. These results show that the cause of the stunting and retarded development of 14-day larvae does not necessarily result in death before the fourth week of infection. Either the continuation of this same process, in recipient mice, is required to effect complete rejection, or alternatively subsequent events, dependant on the preliminary step are the direct cause of spontaneous self-cure. If the first explanation is valid, then it follow that the continuation of the process begun in donor mice, is stimulated and brought into maximum effect within five days of transfer. Unless 'damaged' worms are capable of initiating the process more effectively than the younger stages, or are more susceptible to its action, it seems unlikely that recipient mice, given only a fraction of the donor mouse worm burden should reject worms more successfully than mice infected by the eggs of the parasite, which usually take up to three weeks to terminate an infection.

If the second explanation is valid, the actual expulsion is achieved by events which follow the slower initial phase and complete spontaneous self-cure within a few days of the worms becoming available to their action. This would corres -

pond to the situation in N.brasiliensis where the first phase is achieved by protective antibodies and the second step may be dependant on the co-operation of activated lymphocytes. There are some similarities between the two situations, but it is still too early, to say with certainity whether the expulsion of the worms is brought about by a related mechanism in both of these host-parasite systems.

CHAPTER FIFTEEN

The effect of hydrocortisone, indomethacin and methotrexate on the survival of 7 day larvae in immune and uninfected recipient, CFLPb mice.

INTRODUCTION

While the major experiments involving the transfer system (Chapter thirteen and fourteen) were being carried out, there were several opportunities to try smaller experiments. The aim of this study was to test the effect of the three drugs in the worm transfer system, in the hope of obtaining evidence to support the hypothesis that the loss of 7 day transferred larvae was mediated by immunological phenomena.

RESULTS.

The effect of hydrocortisone on the survival of 7-day larvae in recipient mice.

Experiments 69 and 70 - In the first experiment 29-37 larvae were transferred to each of 12, four month old, female mice.Hydrocortisone acetate was given to six mice on days 0 (2.5 mg), 2 (1.88 mg), 4 (1.25 mg), 7(1.25 mg), 9 (1.25 mg), and 11 (1.25 mg). All the mice were killed on day thirteen.

In experiment 70, 25 larvae were transferred to each of 10 mice from the same group as the recipients in experiment 69. Hydrocortisone was given on days 0 (1.25 mg), 2 (2.5 mg), 4 (1.25 mg), 6 (1.25 mg), 8 (1.25 mg) and the mice were killed on day 21.

In both experiments hydrocortisone successfully delayed the onset of worm expulsion ($P < 0.001$/Expt.69/ and $P < 0.005$ /Expt.70/).

TABLE 72 The effect of hydrocortisone on the survival of 7-day larvae
EXPTS. 69 & 70 transplanted to recipient mice.

Group	No. mice	Day killed	Percentage worm recovery.	± s.d.
Experiment. 69				
A (control)	6	13	8.3[1]	9.2
B (hydrocortisone treated.)	6	13	50.3[1]	20.5
Experiment. 70				
C (control)	5	21	0	0
D (hydrocortisone treated.)	5	21	65.9	13.7

Statistical analysis of results by Student's t-test & Wilcoxon test.

1 $P < 0.001$ $P < 0.005$

TABLE 73 The effect of hydrocortisone on the survival of 7-day larvae transplanted to immune recipient mice.
EXPTS. 71 & 72

Group	No. mice	Day killed	Percentage worm recovery.	± s.d.
Experiment. 71				
A (control.)	5	10	5.3[1]	7.7
B (hydrocortisone treated.)	5	10	69.7[1]	35.7
Experiment. 72				
C (control.)	4	10	11.5[2]	7.1
D (hydrocortisone treated.)	5	10	65.4[2]	31.8

Statistical analysis of results by Student's t-test & Wilcoxon test.

1 $P < 0.01$
2 $P < 0.01$

The effect of hydrocortisone on the survival of 7-day larvae in immune recipient mice.

Experiments 71 and 72 - Recipient mice were immunized by three 21-day infections (500 eggs), the last of which was terminated with piperazine citrate. Three weeks after the last infection, approximately 100 larvae were transferred to each of the recipient mice. Five mice were then given hydrocortisone on days 0 (2.5 mg), 2 (1.25 mg), 4 (1.25 mg), 6 (1.25 mg), 8 (1.25 mg) and both groups were killed on day 10.

The plan for experiment 72 was similar. The same total dose of hydrocortisone was administered to the treated group, but the last dose was given a day earlier, on day 7. Both groups were killed on day 10.

Statistical analysis indicated that hydrocortisone was effective in delaying the progress of worm expulsion in both experiments (Table 73).

The effect of indomethacin on the survival of 7-day larvae in immune and in non-immune mice.

Experiments 73 and 74 - In experiment 73, 100 larvae were transferred to ten, 6-wk old, male mice. Five mice were given 0.1 mg of indomethacin in 0.2 ml of milk, every second day from day 1 to day 13. The mice were killed on day 14.

In experiment 74, 100 larvae were transferred to five immune and six control mice aged 4 months. Immunization was achieved by three 21 day infections, each of 500 eggs. The last infection was terminated with piperazine citrate and the larvae were transferred 4 days later.

Although in experiment 73, only three treated mice survived to day 14, there was no statistical difference, between the mean worm recoveries in control and experimental groups in either of these experiments.

TABLE 74 EXPTS. 73, 74, & 75. The effect of indomethacin and methotrexate on the survival of 7 day larvae transplanted to immune and control mice.

Group	No. mice	Day killed	Percentage worm recovery. Mean ± s.d.	
Experiment 73.				
A (Control mice)	5	14	11.4	17.2
B (Indomethacin treated.)	3	14	6.7	11.5
Experiment 74.				
C (Control immune mice.)	6	10	15.7	14.2
D (Immune mice treated with indomethacin)	5	10	10.6	8.4
Experiment 75.				
E (Control mice.)	5	14	18.3[1]	17.8
F (Methotrexate treated mice.)	3	14	43.6[1]	21.0

Statistical analysis of results.
Students t-test — all comparisons are non significant.
Wilcoxon test — 1. P = 0.05

The effect of sodium methotrexate on the survival of 7-day larvae in recipient mice.

Experiment 75 - One hundred larvae were transferred to each of 10 male mice. Five mice were injected with 0.1 ml of MTX (Dose as before, Chapter ten) and then with 0.05 ml on days 2,3 and 6. Two mice died before day 14 on which the remainder of the groups was killed. There was a borderline difference between the mean worm recoveries of the two groups on day 14 (P=0.05).

DISCUSSION.

In Chapter nine, it was shown that hydrocortisone was extremely effective in preventing the onset of primary self-cure. No attempt was made, however, to examine the effect of hydrocortisone in immune mice. In this chapter the action of hydrocortisone was further studied and its effect on the expulsion of transferred 7-day larvae from non-immune recipients, a week after transplantation and from immune recipients within the week, was investigated. It was found that hydrocortisone was equally effective in both immune and non-immune mice, delaying the onset of worm rejection beyond the day on which the animals were killed. MTX was only studied in one experiment in which two of the five treated animals died, but nevertheless there were more worms in the MTX injected mice than in controls. Indomethacin was not found to be effective in the two experiments. Indeed it seemed as though there were fewer worms in indomethacin treated mice than in the relevant controls.

These results must be considered with caution, since the mice used were not as carefully selected for uniformity as in the experiments in previous chapters. Hence there was an element of uncertainty here. It must be noted, however,

that in so far as hydrocortisone was effective in preventing worm loss during a primary infection, it was equally effective in preventing rejection of 7-day larvae from immune and non-immune mice. This finding must strengthen the hypothesis that transferred worms were rejected by an immunologically mediated self-cure response. In this context, MTX gave a valuable result, although the suppression of rejection was not entirely convincing. Whereas the antiinflammatory action of indomethacin did not inhibit worm rejection, hydrocortisone had a consistently powerful effect, indicating that the antiinflammatory component of the latter drug could only have been secondary to its more potent immunosuppressive effect.

CHAPTER SIXTEEN

The survival of Aspiculuris tetraptera in mice concurrently infected with Trichuris muris and Nematospiroides dubius.

INTRODUCTION

One of the original aims of this thesis was the investigation of possible antagonism between A.tetraptera and the other murine parasites. Antagonism, for instance, has been demonstrated between A.tetraptera and T.muris (Keeling,1961) and between A.tetraptera and S.obvelata (Stahl,1966 b). There are many ways in which antagonism is brought about (Listed below), but perhaps the most interesting mechanisms involve the host response. Where two parasitic species are related or have similar antigens, it is possible that cross-immunity may occur. H.diminuta, for example, will not grow well in rats previously infected by H.nana (Heyneman,1962). Another interesting mechanism, of excluding competing species is non-reciprocal cross-immunity, where the species initiating the host response persists, but others are prevented from becoming established (Schad,1966). Besides antagonism, enhancement of two species in a concurrent infection has also been recorded in the Literature. Thus Ewing and Todd (1961 a, 1961 b) found that unless Metostrongylus apri and M.pudendotectus were present together, few worms developed to adults, and it has been suggested that this type of synergism may result when one species depends for food on the pretreatment of the available food supply by the other species by passage through its gut, perhaps removing from it some materials, whilst enriching it with others (Holmes,1973). Although very little is known about the mode of interaction between different helminth species infecting the same host its consequences are

manifest in niche diversification, arising from a) competition leading either to dominance by one of the interacting species or to an opportunistic inferior competitor and b) interactive and selective segregation, exemplified by temporal separation, site segregation, resource segregation, or the host age-class parasitized (Ayala,1972; Holmes,1973). It has been suggested that there are at least five ways in which the parasites in concurrently infected animals can interact.

Enhancement.

1) Antigen competition
2) Other mechanisms (Holmes,1973)

Antagonism.

3) Inter-specific immune response Hunter et al,1967; Yoeli,1956; Cross,1934; Schad,1966.
4) Non-specific enhancement of innate immunity. Stahl,1966 b; Cox,1952.
5) Direct antagonism resulting from:
   1/ competition for nutrients (Holmes,1961)
   2/ mechanical interference and crowding (Holmes,1973)
   3/ secretion of substances by one species which are poisonous to the second (Keeling,1961).

It was not possible to carry out a detailed investigation into the existence of interactions involving A.tetraptera, because the biology of this parasite had been poorly documented in the past and the inconsistencies between the various authors and my own work precluded the arrangement of adequately controlled experiments. Four experiments were, however, completed, but, as in the previous chapter, they were not designed to entirely evaluate all the possibilities involved; rather these experiments were carried out as and when, time and materials were available.

RESULTS.

The effect of a Trichuris muris infection on a challenge with A. tetraptera.

Experiment 76 - Forty nine male CFLP mice were arranged into five groups, one of which had 9 mice and the remainder ten mice each. Groups A,C and E were given 500 eggs of T.muris on day 0. Groups B and C were then given 500 eggs of A.tetraptera on day four, and three groups (A,B,and C.) were killed on day 14. Groups D and E were given 500 eggs of A.tetraptera on day 14 and they were killed four weeks later.

The results showed that both the A.tetraptera and the T.muris infections in group C could be favourably compared to their respective control group. There was no reduction in the worm burden of the concurrently infected mice. Further more, when the T.muris infection was 14 days old and the mice were challenged with A.tetraptera, there was no detectable effect on the recovery of mature A.tetraptera four weeks after challenge.

Simultaneous infection with Aspiculuris tetraptera and Trichuris muris.

Experiment 77 - Twenty two female CFLPa mice were divided amongst two equal groups. One group received 1,000 eggs of T.muris on day -1 and then both groups were given 500 eggs of A.tetraptera twenty four hours later (day 0). The mice were killed four weeks after the challenge infection and the worm burdens were recorded. The degree of T.muris infection was estimated as nil, slight, moderate, heavy and very heavy. The results are shown in Table 76.

The difference between the mean numbers of A.tetraptera recovered from the two groups was found to have statistical

TABLE 75 The survival of the larvae of
EXPT. 76 A.tetraptera in mice infected with Trichuris muris.

Group	Number of mice	Duration of T.muris infection	Mean worm recovery			
			A.tetraptera		T.muris	
			Mean	± s.d.	Mean	± s.d.
A (T.muris control day 10)	10				409.5	196.8
B (A.tetraptera control day 10)	10		136.8	29.6		
C (Experimental day 10)	10	4 Days	132.2	43.5	397.3	190.4
D (A.tetraptera control day 28)	10		5.3	7.1		
E (Experimental day 28)	10	14 Days	1.7	1.1	0.4	1.3

TABLE 76 EXPT. 77 The survival of the larvae of A.tetraptera in mice infected with Trichuris muris

Group	No. of mice.	Mean worm recovery.	± s.d.
A (A.tetraptera controls day 28)	11	123.4[1]	34.5
B (Experimented day 28.)	11	75.2[1]	27.3

TABLE 77 EXPTS. 78a,b. The survival of the larvae of A.tetraptera after transplantation to recipient mice challenged on day 1 with 2,000 larvae of Nematospiroides dubius.

Group	No. of mice	Percentage worm recovery	± s.d.
Experiment.78a			
A (control.)	5	18.8[2]	22.1
B (N. dubius infected.)	6	27.5[2]	25.3
Experiment.78b			
C (control.)	5	17.3[3]	25.5
D (N.dubius infected.)	5	33.7[3]	27.6

Statistical analysis of results by Student's t-test and Wilcoxon test

1	$P < 0.01$	—
2	P = N.S	N.S.
3	P = N.S.	N.S

significance ($P < 0.01$). It was noted, however, that few worms had been rejected from either group, the mature worm burden being unusually high in both T.muris infected and in control mice. Moreover, the T.muris infection was not completely terminated in many of the mice, whereas normally there are few worms left by day 29. It would seem that the mice used in this experiment behaved atypically and the reason for this may be that CFLPa mice were bred in small numbers from commercially obtained CFLP mice. Nevertheless this does not invalidate the finding that when T.muris was allowed to develop for 2 to 3 weeks in large numbers it exerted an influence on concurrent A.tetraptera infections.

The effect of Nematospiroides dubius on the development of transferred 7-day A.tetraptera in recipient mice.

Experiment 78a and 78b - In the first of these two experiments, 100 7-day larvae of A.tetraptera, obtained in the usual way, were transferred to each of eleven female CFLPb strain recipients. On day 1, six of the mice were infected with 2,000 larvae of N.dubius, given in 0.25 ml of water. Control mice received 0.25 ml of water at the same time. Both groups were killed and autopsied on day 15. In the second experiment the infecting dose of N.dubius was increased to 5,000 larvae.

Although in both experiments the means were higher in the groups challenged with N.dubius, there was no statistically significant difference between the control and the experimental groups.

DISCUSSION.

The progress of experimental infections, compared to the relevant control groups, is normally assessed by one or more

of four criteria. The most important of these are the number and the size of worms recovered at autopsy. Of secondary importance, however, are the number of hosts infected and the fecundity of female worms. Where it is not possible to use all four of these criteria, an attempt should be made to evaluate which one is likely to be the most sensitive in detecting the effect of a given experimental variable.

An important prerequisite to the successful elucidation of interaction taking place between species in concurrently infected animals, is the understanding of the biology and the life-cycle of both helminths involved. Only with this knowledge is it possible to include appropriate control groups to distinguish between the influence of individual host variation, the effects of the host response and the contribution of the heterologous challenge to the progress of the original infection.

Keeling (1961) studied interactions between S.obvelata, A.tetraptera and T.muris in concurrently infected animals. The degree of antagonism was determined from the number of animals supporting an infection at autopsy; no mention was made of whether only mature worms were considered or whether stunted, and larval worms were included in the results. Keeling found that 35.5% of mice were infected with T.muris five weeks after infection and that this percentage could be increased by removing the pin-worm burden before infection with T.muris. It is surprising that so many mice were still infected at autopsy, since it has been shown that mice respond immunologically to T.muris and expel most of the worms during the third week of infection (Campbell,1963; Wakelin,1967). Although Keeling did not discuss the nature of the parasites recovered at autopsy or the significance of the presence of worms five weeks after

infection, in relation to the host response, it is possible that the strain of mice (Clarke) used, included immunologically non-reactive animals. Nevertheless Keeling claims to have shown that there is a strong interaction between T.muris, A.tetraptera and S.obvelata. There was a reduction in all three species in mice carrying mixed infections, the most successful species being the one which became established first.

If Keeling's criterion of antagonism had been applied in experiment 77, no interaction would have been demonstrated, since a 100% infection rate was achieved in both groups. The more sensitive method of comparing mean worm recoveries, however, gave a significant result. ($P < 0.01$). The importance of understanding the life cycle of both parasites before deciding when to autopsy groups was illustrated by experiment 76. The A.tetraptera challenge infection was given four days after T.muris and both experimental and control groups were autopsied on day 14. Since the day of autopsy came before the initiation of actual worm rejection, the influence of immunity, either specific for each parasite or non-specific would not have been expected to have played an important role here, unless the immune response was accelerated in mice infected with both species. Earlier expulsion could not be detected, there being no significant difference between the controls and the concurrently infected mice. Neither immunity, nor possible competition had an affect on the mean worm recovery.

Stahl (1966 b) examined the possibility of interactions between A.tetraptera, T.spiralis, S.obvelata and H.nana. Mice were autopsied twelve days after the A.tetraptera challenge and the results were thus not complicated by the individual variation in the degree of the host response. When mice

already infected with S.obvelata were given A.tetraptera eggs, the later infection did not become as successfully established as in controls. A twelve day period between a T.spiralis infection and the A.tetraptera challenge was equally effective in reducing the mean worm recovery of the latter. Less interaction was demonstrated when the challenge was given on day 4 or on day 20. Stahl explained this by considering the life cycle of T.spiralis:

> "In the 12 day interval experiment the period of time the mice were concurrently infected was from the twelfth day of the Trichinella infection to the twenty-fourth day. This is the time of maximum production of larvae by the adult Trichinella females, with a concomitant increase in intestinal mucosa penetration and blood migration by the larval trichinae. These larval activities, in addition to the longer adult Trichinella association with the mice, may have provided the necessary stimulation to account for the significantly increased resistance of the concurrently infected mice to Aspiculuris. When the Aspiculuris challenge was administered 20 days after the Trichinella infection, the pinworm burdens of the doubly infected mice returned to the control levels. The reason for this may have been the decrease in Trichinella activity in the host's intestinal mucosa, with a subsequent decrease in the inflammation and irritation of the gut wall".

There was no sign of antagonism between A.tetraptera and H. nana.

The second two experiments (78a and 78b) were carried out to determine whether the response to transferred 7-10 day larvae could be suppressed by a heterologous antigenic challenge, during the period of initial antigenic stimulation. Colwell and Wescott (1973) showed that a prior infection with N.dubius and challenge with N.brasiliensis 30 days later resulted in a delay of the expulsion of the latter infection. Egg production by N.brasiliensis continued well after the time that it ceased in controls.

The results obtained in experiments 78a and 78b showed no significant difference between the control and the concurrently infected groups.

A consideration, however, of the number of animals with > 20 worms at autopsy, suggested that there may have been some effect after all.

TABLE 78
Expt. 78 a and b.

A comparison of the no. of mice with more than 20 A.tetraptera in control and experimental mice.

	Control	N.dubius infected group.
Expt. 78 a -	1/5	3/6
Expt. 78 b -	1/5	3/5

It would be unwise to categorically state that enhancement was demonstrated, but the evidence nevertheless suggests that this may have been the case.

This aspect of interaction between species of parasites concurrently infecting a host has received little attention in the past, but it is conceivable that antigenic competition may be involved. When there is a long interval between the two infections, antigenic competition is not likely to be the explanation since antigenic competition is usually induced by antigens given either simultaneously or soon after one another (Taussig,1973). It is conceivable that in Expt.78, the administration of two infecting species within 24 hours of one another weakened the host response to one or both parasites, it being evident from the worm recoveries that the response was not entirely abolished.

Another concept may be proposed here to explain the prolonged N.brasiliensis infection in mice previously infected with N.dubius. The latter parasite is not expelled from mice in a primary infection and it is conceivable that the parasite in some way incapacitates the host response to

prevent the host from eliminating it. It may be that in this situation, the protection was also effective in preventing N.brasiliensis from being expelled, although the mechanism whereby this was achieved is unknown. Other mechanisms of enhancement were discussed in the introduction to this chapter.

The experiments carried out in this chapter did not greatly increase knowledge about the mechanisms involved in interactions between different species of parasites, concurrently infecting the host, but then this was not the main objective. As stated in the introduction, interference is real and it was successfully demonstrated in experiment 77. The main point is that interaction can depend on many factors and it can be influenced by the chronological order of infection, the period of time elapsing between infection and challenge, and the time of autopsy. Detection of interference, therefore, is dependant essentially on the inclusion of adequate control groups based on an understanding of all the other variables involved.

SECTION 4

Chapter seventeen

Final Discussion

As with any zoological project, an essential prerequisite to the successful completion of the work reported in this thesis, was the proper maintenance, the well-being and the health of the animals upon which the experiments were carried out. Not surprisingly, this aspect of the project was not problem-free, the acquisition of helminth-free stock constituting a major hindrance in this respect. A.tetraptera is a notoriously difficult parasite to eliminate from conventional mouse colonies, only S.P.F. mice being guaranteed free from infection. In spite of this, however, the first two dealers contacted, during the initial stages of the study, supplied animals infected with large numbers of worms. Carworth Europe Ltd., was the only organisation which successfully provided helminth-free mice, but even here, one particular order consisting of 160 mice, came with moderate infections. Protests from other customers about this point, however, resulted in a replacement order, the only setback being, in this instance, the delay and the necessity to prepare a new stock of infective material. One further frustration, when dealing with animal suppliers was the occasional inability of these organisations to meet the need of smaller buyers, because of contract commitments to much larger customers. On two particular occasions this caused delays which could not have been foreseen and were difficult to overcome.

Maintenance of infective material of the parasite also posed numerous problems. Although technically the preparation of eggs was an easy operation, to obtain a clean inoculum

required the separation of individual worms from gut contents, a process which could involve the autopsy of up to 40 TFI mice when large quantities of infective material were required. Hydrocortisone treated, heavily infected CFLP mice reduced the need to use so many donors, but the vigilance required in ensuring a constant source of injected, infected mice was not without problems itself. The situation was not aided by the fact that the eggs of A.tetraptera could not be kept at maximum viability for longer than a few weeks at a time, even at 4°C. The unpredictability of the infectivity in egg suspensions prepared in apparently identical ways was a constant source of anxiety, variation of 0-100% being just as common as a 50% infectivity. This aspect of egg preparation was never fully solved, although more reliable "takes" were achieved in the later experiments.

Although, as reported in Chapter three, helminth parasites were controlled in the animal room, the protozoan parasite Trichomonas muris could not be eradicated. This murine parasite is also a parasite of A.tetraptera and viable organisms can be recovered from the intestines of the worm (Theiler and Farber,1936). Whether it is actually pathogenic in A.tetraptera is uncertain, but it was suspected as the cause underlying some experimental infections which failed to establish. Dimethtridazole treatment of stock mice did not reduce the T.muris incidence. The observation was made, however, that in hydrocortisone treated mice, not only did A.tetraptera survive better than in controls, but T.muris also proliferated in numbers difficult to reconcile with any detrimental effect to the pinworm. Therefore, if it did affect the growth of A.tetraptera in normal mice, it must have done so by a process which was hydrocortisone sensitive, e.g. cross

immunity or other such effect.

It is clear by now that A.tetraptera is in many ways, an unusual parasite when compared to other model systems currently being investigated. Perhaps the first significant finding was the confirmation of Chan's (1955) work, in which it was suggested that the life cycle of A.tetraptera involved an emigration from a primary establishment site to a second preferred site in which the worms matured. Although it is reasonable to supposed that mature worms might find it difficult to survive in sections 5-7 in the colon, it is not altogether clear why the larvae do not enter the crypts in the anterior colon. Two possibilities can be proposed to answer this question. Firstly, it may be that the two phases of the parasite (i.e. 0-7 day larvae and 8 + day larvae) have different nutritional, respiratory or physiological requirements which can only be satisfied in the two preferred sites. If this is the true explanation then it follows that sections 5-7 of the colon must have some unique properties and it would be extremely interesting to carry out anatomical, histological and physiological studies, comparing this region to the remainder of the colon. From a parasitological point of view, the use of the transplant operation would facilitate the implantation of very young larvae to various regions of the alimentary canal, both anterior and posterior to the preferred site, in an effort to elucidate the nature and necessity of the requirement to enter the crypts is sections 5-7. Another useful tool here, may be the incorporation into such studies of infections in atypical hosts. For instance, it was shown (Chapter seven) that a similar preferred site was detected in rats and although an anterior emigration was attempted, it was unsuccessful presumably because the parasite could not maintain its position in the anterior colon. Indeed, trans-

ferred mature worms, though surviving for 2 days in the rat were lost in a manner entirely compatible with this hypothesis. Perhaps experiments in other strains of mice might also be informative.

The second possibility to explain the existence of two preferred sites, is the observation that a host response may occur in both regions, suggesting that the anterior emigration may be an escape mechanism to avoid a severe local response. The host response may cause the normal preferred site of the parasite to become unsuitable for further occupation, thus necessitating an emigration by the parasite (Crompton,1973). N.brasiliensis (Brambell,1965), T.colubriformis (Connan,1966) and N.dubius (Panter,1969), undergo such a change, revealing after the events of immunity a different distribution pattern in the host intestine, from that found in the non-immune host. It was found that both the crypt phase (Chapter eight) and the 7+day larvae (Chapter thirteen) stimulated and were subject to a host response in which they could be eliminated from the host.

The observation that larvae may be lost in immune mice and in atypical hosts at times different to that in a normal primary infection, gives rise to two possibilities; these being that either the normal response is accelerated under these circumstances or that different, though not unrelated, processes are responsible for the earlier loss of larvae. Jarrett et al, (1967) suggested that in the N.brasiliensis system, infection was followed immediately by Loss Phase 1 (LP1), in which a proportion of the larvae died before reaching the lungs. This was followed by a Plateau Phase (PP1) which lasted until day 11 when infection was all but terminated by an abrupt response, LP2, during which most of the remain-

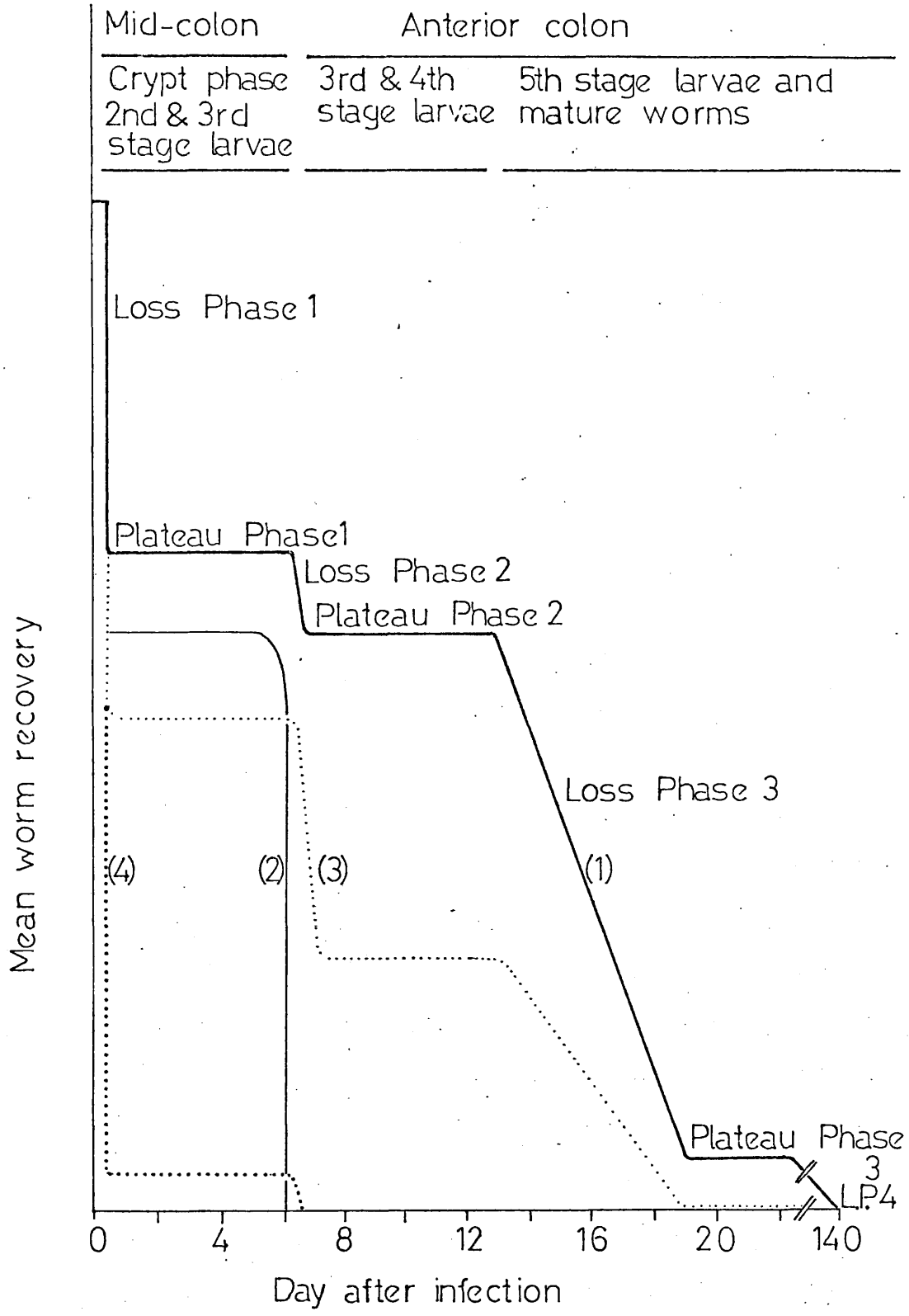


FIG. 64 A diagramatic representation of the survival of Atetraptera in young mice (1), immune mice (2), old mice (3), and abnormal hosts (4).

ing worms were eliminated. LP2 continued until only the threshold level was left, these worms surviving in a plateau phase, PP2, presumably until death from senility. This system, however, could be altered, as evident from immune rats in which LP1 was more severe, PP1 was shorter and LP2 was earlier and greatly accelerated reaching a lower threshold level.

In the A.tetraptera system LP1 was manifest by larvae which failed to hatch, hatched too late or failed to become established in the preferred site.Fig.64 shows a diagramatic representation of these events. PP1 corresponds to the crypt phase during which there is no change in numbers but it is followed by LP2 which is co-incident with the anterior emigration and is usually only marginal in young mice. PP2 lasts until day 12 when LP3 takes over (Comparable to the LP2 in the N.brasiliensis system), eliminating the bulk of the remaining worms by days 19-21. PP3 lasts for a long time gradually merging into LP4 which accounts for the last of the residual worms, dying from senility. As in the previous system, these events can be modified by various changes in the host. Thus although no experiments were carried out on age resistance, the work of Stahl (1962) and Mathies (1959) showed that in old mice proportionally more worms were lost either by LP1 or LP2, since age resistance was already seen in mice autopsied 12 days post infection. LP3 probably accounted for a larger proportion of the remaining worms since old mice autopsied after the third week harboured extremely small worm burdens.

It can be seen that LP1, the extent of which determines the level of the initial infection, is the overall result of many phenomena, some entirely host-independent,(e.g.ability to hatch, ability to interpret location in the gut etc.) others mainly host-dependent characteristics (e.g.contents of

the gut, availability of hatching stimulus, pH of gut contents etc.). Treatment of the host with immunosuppressants will only alter the last category and indeed it was found that hydrocortisone did not substantially decrease LP1. LP1 was, however, dramatically altered in atypical hosts where on day 1 only a fraction of the worm burden in control mice was recovered from these animals. In this situation, LP2 was responsible for the eventual worm loss. When A.sylvaticus were treated with hydrocortisone LP2 was entirely abolished and the worms survived until day 10, successfully completing the anterior emigration. LP2 was also abrogated in hydrocortisone treated CFLP mice as was LP3. Immune mice did not appear to be less susceptible than control mice and here LP1 was almost equivalent. As in the atypical host, however, LP2 was vitally important and accounted for the elimination of most of the worms by day 10.

In considering the overall situation in the A.tetraptera system, an important observation is the fact that three loss phases before day 24 cannot leave much hope for the survival of the parasite to patency and hence its ability to survive in nature appears to be unaccountable unless variation in host strain characteristics is a contributing factor. Clearly it is only the residual worm burden which can survive to produce eggs and frequently this is only a minor proportion of the original infection. In nature things appear to be similar. Thus it was found (Chapter two) that only 16-20% of mice harboured more than 10 mature worms (i.e. approx 6 female worms) and the reasons for this were discussed (Chapter two). The remainder of the population harboured smaller worm burdens, but this level was sufficient to ensure the survival of the parasite. Perhaps the few worms which became patent, survived long enough to ensure that enough eggs were returned to the

environment to similarly infect new hosts.

The hypothesis outlined above is complex, but it is thought that most of the observations can be fitted into such a general outline. If it is accepted that the function of a hypothesis is to explain as simply as possible all the known facts and then to promote further work by experiments testing the structure of the hypothesis, then the account above amply fulfills these criteria. Specific experiments are required to examine each LP, to distinguish between their separate characteristics and to identify the mechanism underlying each loss phase.

A.tetraptera proved a useful model in the study of sex resistance, a topic fully discussed in Chapter six. It was shown that in this relationship sex difference arose principally as a result of LP3, which was stronger in female mice. Orchidectomy altered the response in male mice, which then responded as well as females, suggesting that sex hormones were instrumental in bringing about the sex differences. Knowledge of the influence of sex hormones in immunity is still only fragmentary at the present time and it is perhaps worth suggesting that the A.tetraptera model may prove useful in increasing knowledge in this field.

Transplantation of larval and mature worms proved to be a highly successful procedure. With a little more experimentation it could become the basis of an entirely new approach to the assay of drugs for antihelminthic activity. At present pharmaceutical firms test their products by dosing large numbers of either naturally infected mice or reinfected mice. This has two basic setbacks. Firstly as shown in Chapter eight mice acquire resistance to A.tetraptera and this is reflected in the earlier expulsion of worms. Moreover worms which survive the immune response or develop in immune hosts may well be

different from primary infection worms (Ogilvie and Hockley, 1968; Ogilvie,1969; Jenkins and Phillipson,1970,1972a,1972b; Jenkins,1972), implying that the effect of the experimental drugs is not being tested on a uniform population of the parasite. Secondly the currently employed techniques require large numbers of mice to give meaningful results. The transfer system on the other hand, has been shown to establish uniform and consistant infections in all the mice tested. Thus, although a more laborious procedure, laparotomy would reduce the need for large numbers of experimental animals and would give precise information about even minimal effects.

The expulsion phase LP3 was studied in some detail, by the effect on it of various known immunosuppressive drugs and other agents. The effect of hydrocortisone was particularly interesting. Surprisingly ALS was found to be without effect and this experiment needs to be repeated. The use of specific antisera can give precise information on the development of immunity and is a very useful tool in this connection (Chapter ten). There are many more drugs which have specific activity and could usefully be tested in the A.tetraptera system e.g. some of the antiinflammatory agents and the antiamines.

The immunization of the host with various somatic antigens from specific parasite tissues has given important information in the past (Wakelin and Selby,1973). Unfortunately the various antigens are extremely difficult to prepare because of the need to dissect many hundreds of worms, but once accomplished microprecipitation techniques could be very useful here.

With the successful development of the transfer system it was hoped to initiate studies in which immune serum and lymphocytes could be transferred to recipient mice to determine the order and the importance of the various components of immune expulsion (Ogilvie and Jones,1971,1973; Selby and Wakelin,1973).

Unfortunately it was not found possible to commence such studies, principally because of the large number of animals required as donors (Currently a ratio of 2-1 or 4-1 donor to recipient is being employed in various laboratories) and also because of the need to use inbred mice. Nevertheless if such studies could be initiated they could prove extremely informative.

There are few nematode parasite model systems being studied at present in which the parasite does not at some stage undergo a phase of tissue penetration. Although A.tetraptera is one such parasite, it does undergo a period of very intimate contact with the host tissues, especially in the early infection when the larvae are located deep in the lumen of the crypts. It is at this stage that the antigenic stimulus is first recognised by the host defence system, since the initiation of the host response was evoked by larvae which were not allowed to develop longer than to day 6 (Chapter eight). The later stages, however, are also immunogenic, as demonstrated by the elimination of 7-day larvae transferred to recipient mice (Chapter thirteen). Even the mature worms, although unique in not being themselves affected by immunity, primed the host defense mechanisms sufficiently for these mice to be able to resist challenge infection with eggs. In summary, it seems that although all the stages stimulate immunity, only the 5-th stage and mature worms can resist actual expulsion. This type of stage specificity and stage specific privilege, has been reported in the past. Thus, Soulsby (1957) found that third stage larvae of Ascaris develop precipitates in immune serum but this did not apply to the second stage. Oesophagostomum radiatum only gave such a reaction after the third moult and Haemonchus contortus only after the fourth (Silverman and Patterson,1960). Other manifestations of immunity are also

stage specific. Schistosoma mansoni mature worms will survive for a long time in rhesus monkeys, but a state of concomitant immunity will ensure that all reinfecting larvae are killed (Smithers and Terry,1969).

Nematospiroides dubius will live for up to eight months in mice, but challenge infections are quickly terminated before the final ecdysis (Panter,1969). A.tetraptera, therefore, is not alone in showing this characteristic, but since the mechanisms underlying this type of privilege by certain stages in the life cycle of parasites are poorly understood, it is suggested here that A.tetraptera may present an opportunity to study the phenomenon in detail and so perhaps discover facts which may help to cure human parasitic diseases as well as those of domestic animals.

The A.tetraptera /mouse host-parasite relationship exhibited similarities to other models being studied. As in the murine parasite Trichuris muris and the rat parasite Nippostrongylus brasiliensis expulsion occurred at the end of the second week of infection during the third and was complete by day 21. Exposure to infection modified the response to further infection and the mechanism which caused LP3 was shown to affect worms (damage?) before the final rejection. The evidence for this and the possible explanations were discussed (Chapters 5,8,13,14). The technique used in this work, i.e.transfer of "damaged" worms, is thought to offer many possibilities for the elucidation of the mechanism of LP3.

In conclusion then it can be stated that the A.tetraptera model system is thought to be very useful for future work, not only in that unlike the human pinworm Enterobius vermiculuris, A.tetraptera is a pinworm which can be easily maintained and controlled, but also in that it is one example

of a gut-lumen dwelling parasite which may help to increase knowledge about the host response to nematode infection.

It is sincerely hoped that the laboratory and field observations made in this study, have shed some light on the nature of host-parasite relationship. The principles and the inferences which have been made here, may perhaps increase knowledge in this field and may draw, just a little bit closer, the ultimate goal of every parasitologist, the complete understanding of all aspects of parasitic diseases and the ability to control these. If the balance of this relationship could be altered in favour of the host, it would eliminate from this world, the source of much suffering, mortality and wasteful economic loss.

APPENDIX 1

Phosphate buffered saline (PBS)

PBS (pH 7.2 , 0.02M. PO_4 , 0.14M. NaCl) was prepared as follows :-

2.84 gms Na_2HPO_4 (anhydrous)
2.76 gms $NaH_2PO_4.H_2O$
8.50 gms NaCl
Deionized water to make 1 litre

Merthiolate was added in a final concentration of 1 : 10,000 to retard microbial growth.

Hanks's saline.

Hanks's saline was prepared from Hanks's stock solution granules (Oxoid).

1 litre of stock solution A contained :-

80.0 gms NaCl
4.0 gms KCl
1.0 gms $MgSO_4.7H_2O$
1.0 gms $MgCl_2.6H_2O$
1.4 gms $CaCl_2$
Phenol red

1 litre of stock solution B contained :-

1.52 gms $Na_2HPO_4.12H_2O$
0.6 gms KH_2PO_4
11.2 gms Dextrose

These solutions were stored at 4°C until required. Working Hanks's solution was prepared as follows :- 1 vol.sol.A : 1 vol.sol.B : 8 vols. deionized H_2O. This was autoclaved at 10 lbs/in^2 for 15 mins. $NaHCO_3$ was not included.

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The survival of *Trichuris muris* in wild populations of its natural hosts

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SUMMARY

The results of experimental infections of *Trichuris muris* in wild field mice (*Apodemus sylvaticus*) and laboratory-bred wild house mice (*Mus musculus*) showed that the parasite elicited an immune response similar to that previously described in strains of laboratory mice. Experiments in laboratory mice showed that the parasite was able to become sexually mature only when small single infections or repeated low-level infections were given. A survey of a population of 43 wild house mice naturally infected with *T. muris* showed that the pattern of small worm burdens in the majority of mice was consistent with a situation of repeated low-level infection, except in the case of six female mice which harboured larger mature worm burdens. It is suggested that in these mice pregnancy and/or lactation may have suppressed the immune response, allowing the accumulation of a worm burden in excess of the threshold for worm expulsion.

INTRODUCTION

An interesting and relatively unstudied aspect of helminth biology is the problem of survival in the wild of species which, when passaged in laboratory strains of their natural host species, provoke strong, protective immune responses. This problem is most acute in parasites such as *Nippostrongylus brasiliensis* and *Trichuris muris* which may, during a primary infection, elicit responses capable of bringing about the expulsion of the worms from the host's intestine. In *N. brasiliensis* adult worms developing from an initial infection are expelled after a short period of egg production (Mulligan, Urquhart, Jennings & Neilson, 1965; Ogilvie, 1965), but in *T. muris*, where the prepatent period is long, the worms are removed before maturation and egg-laying (Wakelin, 1967) and cannot, therefore, contribute to the survival of the species. In nature, however, *T. muris* continues to reproduce itself in populations of wild rodents and there must therefore be some means by which the apparent limitations imposed by the immune response are circumvented. Two such means can be proposed, namely that the host–parasite relationship between *T. muris* and wild rodents differs qualitatively from that which has been described in laboratory mice, and that protective immune responses do not occur to the same extent, or that the level and frequency of infection in the wild is such that protective responses are not elicited.

During the course of a study of nematodes of wild rodents the opportunity arose

of sampling a wild population of house mice (*Mus musculus*) in which *T. muris* was known to occur, and of trapping and infecting specimens of the long-tailed field mouse (*Apodemus sylvaticus*), a recorded host of *T. muris*, in which the parasite did not occur. In this way it was possible to add information to that available from infections of laboratory mice and laboratory-bred house mice in order to test the hypotheses outlined above.

MATERIAL AND METHODS

Laboratory mice

Male mice of the Carworth-Europe CFLP (specific pathogen free) strain were used and were infected when approximately 6 weeks of age unless stated otherwise. The mice were maintained on a sawdust litter and had access to pellet food and water *ad libitum*.

Field mice

The *Apodemus* were trapped live, using baited Longworth traps, in the grounds of Trent Park College, Middlesex. Traps were set in the evening and examined the following morning. Mice were removed to the laboratory, sexed, weighed and maintained as described above. To facilitate handling and infection the mice were lightly anaesthetized with ether. Only mature mice were used for the experimental infections and these were given piperazine citrate orally, at a dose rate of 500 mg/kg body weight, in order to remove existing burdens of the nematode *Syphacia stroma*. *Nematospiroides dubius*, *Corrigia vitta* and two species of cestodes were also present in individuals from the population, but *T. muris* was never recorded. The mice were infected 3 days after injection with piperazine citrate.

Laboratory-bred house mice

A number of male and female mice from a colony maintained in the Zoology Department of the University of Glasgow were kindly made available by Mr R. Stoddart. The colony was established from trapped wild mice and has been bred in the laboratory for more than 20 generations. The mice were lightly infected with *Aspiculuris tetraptera* and *Syphacia obvelata*, but no attempt was made to remove these before infection with *T. muris*. Anaesthesia with ether was used in order to facilitate handling.

House mice

These were caught in domestic premises using baited break-back and Longworth traps. The mice were sexed, weighed and the intestines removed and carefully examined for helminths under a binocular dissecting microscope. Infection with parasites other than *T. muris* was rare in the population; *Syphacia obvelata* and the strobilocercus larva of *Hydatigera taeniaeformis* were each recorded on one occasion.

Methods used in the maintenance of *T. muris* and for the infection and examination of mice have been described in a previous paper (Wakelin, 1967). Two cultures of the parasite were used in the infections, one recently isolated from naturally infected house mice, the other a long established laboratory strain, passaged in laboratory mice for over 20 years.

Table 1. *The development of* T. muris *in mice given single or repeated (trickle) infections of eggs*

Group	No. of eggs	Nos. of worms recovered: At 2 weeks Mean	At 2 weeks S.D.	At 6 weeks Mean	At 6 weeks S.D.	Analysis of worm recoveries at 6 weeks: No. of mice with worm burdens > 2-week mean for dose	No. of mice with adult worms and mean worm burden		No. of mice with larval worms and mean worm burden	
1	50 × 1	22·3	4·29	1·4	1·39	0/9	1/9	1·0	6/9	2·0
2	25 × 1	12·7	1·41	3·7	3·77	0/9	5/9	5·0	4/9	2·0
3	10 × 1	3·7	1·63	4·0	1·00	6/9	7/9	3·4	5/9	2·2
4	10 × 5	Not done		3·3	4·08	0/9	6/9	2·3	5/9	3·2
5	5 × 5	Not done		6·1	3·30	0/9	6/9	2·6	8/9	4·6
6	2 × 25	Not done		10·8	10·36	3/8	5/9	6·6	6/9	10·7
7	2 × 12	Not done		3·7	4·62	1/9	3/9	3·3	5/9	4·6

RESULTS

The establishment of single and repeated (trickle) infections in laboratory mice

It has been shown previously (Wakelin, 1973) that the worms which develop from a single low-level infection (< 15 eggs) are not removed from the host by a primary self-cure response, but persist and become sexually mature. When trickle infections of 10 or 5 eggs were given daily over a period of 2 weeks or so a primary response was elicited and the worms removed. A daily intake of 10 or even 5 embryonated eggs may be an unrealistic model of the natural situation and it was therefore decided to amplify the trickle infection experiment. Seven groups, each of 12 CFLP mice were infected with the laboratory culture of *T. muris* as follows:

Groups 1–3. A single infection of 50, 25 or 10 eggs.

Groups 4 and 5. Five infections of 10 or 5 eggs given at weekly intervals.

Groups 6 and 7. Daily infections with 2 eggs given for 25 or 12 days.

Thus three infection levels, namely 50, 25 and 10 eggs were tested, the first two levels being achieved both as single and as divided doses. Three mice from each of groups 1, 2 and 3 were killed 2 weeks after infection and all remaining mice were killed 4 weeks later. The results are given in Table 1.

The results confirm previous studies and show that a primary response was elicited when more than 10 eggs were given as a single infection. In groups 1–3 approximately 50% of the infection had become established by the second week, but in both groups 1 and 2 the mean worm recovery at 6 weeks after infection was considerably reduced. In group 1 the worms remaining after the immune response had taken place were small and stunted, the characteristic situation which occurs when self-cure is induced by much larger infections. The establishment and fate of worms from the trickle infection groups is less straightforward to interpret. Worms established from the first two infections in groups 4 and 5 and from the first 7 to 10 infections in groups 6 and 7 would have had sufficient time in which to become mature and thus, in the absence of an immune response, each mouse should have

harboured at 6 weeks a small number of adult worms (approximately equal to the 2-week mean of group 2 [25 eggs] in the cases of groups 4, 6 and 7, and of group 3 [10 eggs] in the case of group 5) and a larger number of immature worms, the total burden approximating to the 2-week mean of group 1 (50 eggs) in the cases of groups 4 and 5, and of group 2 (25 eggs) in the cases of groups 5 and 7. By this criterion none of the trickle regimes was successful in avoiding the stimulation of an immune response. The high mean burdens, both of adult and larval worms, in group 6 were the result of high counts from 3 mice, which contributed a total of 24 adults and 48 larval worms, and thus the mean values are not representative. This is borne out by the low values in group 7, although this group too contained two mice with relatively high burdens. The most successful regime in terms of adult and larval burdens established and of consistency of infection in the mice was that of group 4, namely five infections each of 5 eggs, although none of the burdens of individual mice was higher than the 2-week mean for group 2. It therefore seems reasonable to conclude that a threshold value for the stimulation of an immune response is reached even when small numbers of eggs are administered at intervals and that this threshold is reached when some 10 to 20 worms are established. However, there is some indication that the immune response stimulated by trickle infections is qualitatively different from that stimulated by single large infections, in that the first worms to become established from a trickle infection may mature even though worms established later are eliminated. For example in group 3, seven of the nine mice had mature worms, but only four of these had larval worms. The mean values for mature and larval worms are similar, although if there had been no response there should have been many more larvae. In group 4, six of the nine mice had mature worms, with a mean value indicating that almost all of the worms established by the first two infections had survived. In contrast, however, the mean number of larval worms was lower than the expected value. This differential survival may be dependent upon the frequency of infection and the total number of eggs given, as trickle infection regimes used previously have brought about a near-complete expulsion of the infection (Wakelin, 1973).

Comparison of the development of low- and medium-level infections in Apodemus sylvaticus *and CFLP laboratory mice*

Apodemus sylvaticus has been reported as a natural host of *T. muris* by several authors (Dujardin, 1845; Bernard, 1964; Wertheim, 1971). The population from which the experimental field mice were taken was adjudged to be *Trichuris*-free on the basis of examination of 26 animals. A total of 22 *Apodemus* and 19 CFLP mice were infected with eggs taken from the recently isolated culture of *T. muris* and two levels of infection were used: namely (*a*) 300 eggs, at which level a primary self-cure response is elicited in CFLP mice and (*b*) 5 eggs at which level no response is elicited. Non-pregnant female mice were used for the higher level infection and male for the lower; there is no sex difference in response to *T. muris* in laboratory mice. Five *Apodemus* and three CFLP mice infected with 300 eggs were killed 2 weeks after infection, in order to ascertain the size of the established worm

Table 2. *Comparison of the development of* T. muris *in* Apodemus sylvaticus *and CFLP laboratory mice given infections of* 5 *or* 300 *eggs*

Level of infection	Host	Number of worms recovered after infection: After 2 weeks Mean	After 2 weeks S.D.	After 5 weeks Mean	After 5 weeks S.D.	% adult worms at 5 weeks
5 eggs	CFLP	Not done		1·8	1·5	72
	Apodemus	Not done		1·9	1·2	74
300 eggs	CFLP	193·0	35·7	11·7	10·8	0
	Apodemus	167·4	87·4	3·7	5·6	0

population. The remaining mice from both infection levels were killed 3 weeks later. The results are shown in Table 2.

The infection of 300 eggs became established equally well in *Apodemus* and CFLP mice, but in both species the majority of worms were lost at some point after the second week. None of the surviving worms had reached sexual maturity or even the juvenile stage of development. In contrast, the majority of worms recovered from the *Apodemus* and CFLP mice infected with five eggs were sexually mature and eggs were present in faecal samples taken on day 35, the normal time of patency. Only one animal (an *Apodemus*) in the groups infected at this level was without worms. The behaviour of the two host species with respect to low- and medium-level infections of *T. muris* therefore seems to be comparable.

Development of Trichuris muris *in laboratory-bred wild house-mice*

Five male and 14 female mice were available for infection. All were given an infection of 400 eggs from the laboratory strain of *T. muris*. Four female mice were killed 14 days after infection, when the mean worm burden was 141·0; the remaining mice were killed 3 weeks later. None of the male mice had worms present and only one of the female mice was infected, although this mouse had a fully mature burden of worms. It appears, therefore, that an immune response to the parasite had developed after the second week of infection and had eliminated the worm population in the majority of the mice, a situation which is directly comparable to that in laboratory mice.

Survey of Trichuris muris *in a population of wild house mice*

T. muris has been recorded in populations of house mice on very few occasions (Fahmy, 1954; Roman, 1951). The discovery of a source of infected mice in domestic premises provided an opportunity to assess the incidence and intensity of infection under natural conditions in order to establish whether the distribution of the parasite would be as predicted from the results of the above experiments, namely:

(*a*) That mice would harbour small numbers of mature and/or larval worms, corresponding to a situation in which infection had been acquired by ingestion of a small total (subthreshold or threshold) number of eggs.

Table 3. *The occurrence of* T. muris *in a population of wild house mice*

		Distribution of infections						
		Uninfected	Infected < 20 worms			Infected > 20 worms		
	No. of mice examined	No. of mice	No. of mice	Mean no. worms	% adult worms	No. of mice	Mean no. worms	% adult worms
Mature ♂	8	1	7	8·6	43·3	0	—	—
Juvenile ♂	10	2	8	3·4	0·0	0	—	—
Mature ♀ (non-pregnant)	9	0	7	5·0	57·1	2	81·5	61·4
Mature ♀ (pregnant)	8	0	4	4·6	69·5	4	61·3	59·6
Juvenile ♀	8	2	6	1·9	0·0	0	—	—
Total mice	43	5	32	—	—	6	—	—

(*b*) That mice would harbour a few immature worms, corresponding to a situation in which sufficiently large numbers of eggs had been ingested to elicit a normal self-cure response and subsequent immunity.

The results of the survey are given in Table 3.

DISCUSSION

Experimental studies in laboratory mice have shown that *T. muris* is a strongly immunogenic parasite, capable of eliciting a self-cure response in its host (Wakelin, 1967). The response takes place during the second or third week of a primary infection (depending on the strain of mouse) and thus, as *T. muris* has a prepatent period of approximately 5 weeks, prevents the worm from reaching sexual maturity. Immunity once acquired is long-lasting and protects against subsequent infection for at least 3 months. In view of these facts the propagation of *T. muris* in wild populations of its host species becomes problematical. There are, however, a number of possible explanations for the survival of *T. muris*, which may be summarized as follows:

(*a*) Under natural conditions animals ingest relatively few embryonated eggs and do not acquire a worm population large enough to elicit an immune response.

(*b*) The laboratory situation is artificial and the response of wild hosts to wild strains of the parasite differs qualitatively from that of laboratory mice to laboratory-adapted strains of the parasite.

(*c*) Wild host species contain individual animals which are unresponsive to *T. muris* and in which the parasite can mature and reproduce.

The first experiment described in this paper demonstrates that there is indeed a threshold worm population size, below which a primary self-cure response does not occur, and it is suggested that this threshold lies between 10 and 20 worms. Worms established from a subthreshold infection mature and the infection becomes patent. Trickle infections, in which small numbers of eggs are given repeatedly, do not circumvent the development of a self-cure response, as is apparently the case in

Nippostrongylus brasiliensis (Jenkins & Phillipson, 1971), but elicit a response when the population threshold is reached. However, in this situation not all the worms are eliminated and a small number become sexually mature. This situation resembles that in *N. brasiliensis* when trickle infections of 50 larvae per day are given; a self-cure occurs, but some worms remain and resume egg production.

The development of experimental infections above and below the threshold level in wild *Apodemus sylvaticus* and laboratory-bred house mice seems to be directly comparable to the situation in laboratory mice. The infections in *Apodemus* were established using a culture of *T. muris* isolated from infected house-mice and passaged for only a short time in laboratory mice, so that there could be no question of using an atypical, laboratory-adapted strain of the parasite. The *Apodemus* responded uniformly to infection at both levels used; none of the seven mice infected with 300 eggs and killed after 5 weeks was found to be unresponsive, i.e. had allowed the worm population to persist and mature, as is the case with individual animals in certain strains of laboratory mice (Wakelin, 1970), although one unresponsive animal was revealed in the laboratory-bred house mice. The experiments suggest, therefore, that *T. muris* is unlikely to reach sexual maturity in wild populations unless infection rates are low or unless some other factor intervenes to suppress the animal's normal immune responses.

The results of the survey are striking, in that the majority (88%) of the wild house mice were infected with *T. muris* and that, in mature mice, the percentage of adult worms lay between 43 and 70%. Mature worms would not be expected in juvenile mice, less than 6 weeks of age, as it appears that mice cannot be infected before the age of 2 weeks (Wakelin, 1973). Most of the infected mice (84%) had burdens of less than 20 worms, a situation which, taken together with the proportion of mature worms, could be explained as a result of exposure to small numbers of embryonated eggs, leading to the acquisition of subthreshold infections, or as a result of exposure to somewhat larger numbers of eggs leading to the type of immune response that was demonstrated in experimental trickle infections. This view is supported by the fact that the juvenile mice had low worm burdens, as it is less likely that there would have been sufficient time for these mice to acquire burdens large enough to elicit a self-cure response and thus their burdens should reflect the level of infection to which the population was exposed. The existence of burdens greater than 20 worms in six mature female mice [non-pregnant 43 and 120; pregnant 29, 31, 54 and 131] could be consistent with this explanation if it is assumed that the females were either genetically unresponsive to the parasite or were physiologically unresponsive, as a result of pregnancy and lactation, and had therefore not limited the size of the population of parasites with which they were infected. The fact that only female mice were found to harbour large infections would suggest that physiological unresponsiveness was the more likely explanation. In wild mice adult females are normally pregnant and/or lactating [R. Stoddart, personal communication] and there is good evidence that these states suppress the immune responses of animals to helminth parasites (reviewed by Dineen & Kelly, 1972). In rats infected with *Nippostrongylus brasiliensis* lactation has the effect of delaying worm expulsion in both primary and challenge infections (Connan, 1970,

1972) and preliminary experiments in laboratory mice have shown that self-cure is suppressed when mice are infected with *T. muris* during lactation, although infections given during pregnancy appear to stimulate a normal immune response (G. R. Selby & D. Wakelin, unpublished). It is therefore obvious that a reproducing population of wild host animals may well contain a number of females carrying mature worm burdens adequate to provide sufficient infective eggs for the propagation of *T. muris*, and in this case the numbers of eggs produced by mature worms from mice exposed to subthreshold infections would be of minor importance. However, as the survey shows, not all mature females are heavily infected with the parasite and the eggs from other infected members of the population may well be the long-term means of survival of *T. muris*.

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