THE TRANSMISSION OF *SYPHACIA MURIS* (NEMATODA: OXYUROIDEA) IN THE LABORATORY RAT

by

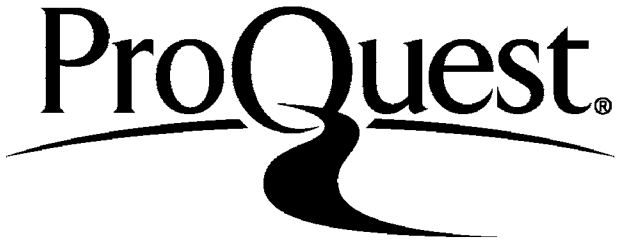
JOSEPH D'SILVA
M. Sc. (Dacca), M.S. (British Columbia)

Thesis submitted for the degree of
Doctor of Philosophy

at

Royal Holloway College
University of London
Egham Hill, Egham, Surrey.

November, 1981.



ABSTRACT

During its transmission, Syphacia muris Yamaguti 1935, (Nematoda: Oxyuroidea), undergoes 4 moults in the rat host to become an adult. Worms deposit up to 4000 eggs daily on the host's perianal region following a circadian rhythm. Most of the eggs are deposited in the day time, with a peak occurring around noon time. The rhythm is dependent upon the behaviour of the rat which is itself influenced by the lighting regime in the environment. Rats are nocturnal animals and normally feed and defaecate at night. Adult female worms of S. muris, therefore, release eggs during the day time when the rat is at rest so as to avoid the loss of eggs in the faeces at night.

A technique is described to collect eggs from the perianal region of the rat for experimental infections. Eggs collected from around the peak of egg-laying activity produce the most worms in isolator-raised rats. In single infections, the worm burden increases with the dose size of eggs administered. But worm size is reduced in larger infections. Fecundity remains unaltered. In multiple infections a severe expulsion phase occurs in male rats. Females bear smaller worm burdens.

An infection is transmitted to suckling rats about 12 days after birth so that litters already harbour worms on the day of weaning. The infection increases rapidly during the 32 days after weaning, thereafter it decreases and oscillates around a constant level. This oscillation may be attributed to periods of refraction and susceptibility to infection by the host. Infestation levels are remarkably similar in rats caged individually and in groups, indicating a degree of stability in the transmission strategy of Syphacia muris.

to my wife, Jane

ACKNOWLEDGEMENTS

It would have been impossible for me to begin and end this thesis had it not been for the support, encouragement and advice I received from Dr. John Lewis of Royal Holloway College. I acknowledge a deep debt to him here, fully aware that I shall never be able to repay it *in toto*. I thank him also for his permission to reproduce a photograph of the Lewis-Rentmore activity sampler shown on page 69a. Many thanks are due to Anne Lewis for her role during the course of this work.

Sue Haswell cared for the many rats I used throughout the three-year period. Rosemary Doran assisted me in the animal house and kindly translated some papers from French to English. Carol Spurdon from the Department of Botany coached me on the use of the transmission electron microscope. Morris Colthorpe and many others in the Department of Zoology at Royal Holloway College made my sojourn there a happy one for me and I shall forever remember this. I thank them all for the help they extended to me. I would also like to record a deep sense of appreciation to my Irish friends Noreen and Bob McNamara for the part they played in bringing this thesis to a conclusion.

Finally, work on this thesis has meant a great deal to my wife Jane and our families and I am happy to note that I have had their support while I was doing it.

The work was carried out during 1978 - 1980 on a Royal Holloway College Major Studentship.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The transmission of nematode parasites in vertebrate hosts includes several features. During transmission, a nematode proceeds through certain stages of development. Its entry into a host is determined in some cases by its behaviour and that of its host. The worm burden in the host is regulated by the age and sex of the host, the numbers of infective stages entering it, and immune responses as well as other factors. In spite of repeated infections, a remarkable stability may be produced whereby the worm burden within the host does not increase infinitely. References in support of these features are cited below. The present study investigates the transmission of the oxyuroid nematode Syphacia muris Yamaguti, 1935 from the laboratory rat in the context of the features mentioned.

(a) The development of a nematode proceeds from the egg through 4 larval stages to the adult form (Chitwood & Chitwood, 1974). Each larval stage is followed by a moulting of the cuticle and the growth of the worm. During growth, the worm increases in size and adds new structures to its body. The adult female then produces either larvae or eggs. These become infective when a suitable host is reached.

Among oxyuroid nematodes such as the human pinworm Enterobius vermicularis Linn, 1758, moulting occurs twice inside the egg (Chitwood, quoted by Otto, 1966). Two further moults occur in the caecum of man. The moulting stages for other oxyuroids is unknown. However, it is a general belief that at least one moult takes place while the larva is still within the egg shell (Otto, 1966).

(b) Many parasites possess adaptive features designed to facilitate transmission to their hosts. The circadian rhythm exhibited by several parasites is pertinent to this study.

It is known that a few parasites make their appearance during precise periods of the 24-hour day, and this is linked to the activity of their hosts. The human pinworm E. vermicularis migrates to the perianal area at night to deposit its eggs (MacArthur, 1930). The rat pinworm Syphacia muris oviposits on the perianal region during the day (Van der Gulden, 1967) as does the mouse pinworm Syphacia obvelata Rudolphi, 1802 (Lewis & Shava, 1977). All these worms deposit eggs during periods of their hosts' inactivity presumably to avoid being lost otherwise. Another mouse pinworm, Aspiculuris tetraptera Nitzsch, 1921 oviposits mainly at night so that it may be transmitted in the faeces which are deposited then. It, therefore, has a rhythm of oviposition at night (Phillipson, 1974) designed to allow eggs to be carried out in the faeces when mice are most active.

Many species of microfilariae exhibit circadian activity. Extensive work reviewed by Hawking (1967, 1975) has shown that Wuchereria bancrofti Cobbold, 1758, Brugia malayi Brugia, 1927, Dirofilaria corynodes Linstow, 1899 are transmitted by mosquitoes at night. To facilitate transmission, the parasites appear in the peripheral blood of the host so as to be picked up by the mosquito. Loa loa Guyot, 1778 on the other hand migrates to the peripheral blood system in the daytime because its vector Chrysops spp. is a daytime feeder.

Hawking (1967) showed that oxygen tension in the blood is linked to the migration of the filarial parasites. The lowered oxygen tension in man at night allowed for the migration of *W. bancrofti*, *B. malayi* and *Dirofilaria immitis* Leidy, 1856. An increase in oxygen tension during the day apparently causes *Loa loa* migration. Such evidence goes to suggest that worms are stimulated to begin a circadian rhythm by a factor in the host body.

(c) The age of a host influences the numbers of parasites that may become established. It is almost axiomatic that parasitic helminth numbers decrease with the increase of host age. This may be either due to innate resistance or acquired immunity on the part of the host.

Innate resistance has been demonstrated by Panter (1969) for *Syphacia obvelata* in mice, and for *Aspiculuris tetraptera* in the same rodent (Mathies, 1959a; Stahl, 1961 b) The situation in the case of *A. tetraptera* has been verified by Jacobson & Reed (1974). *Enterobius vermicularis* incidence occurs mostly among children (Cheng, 1973).

On the other hand, age resistance does not appear to operate in the establishment of *Oesophagostomum radiatum* Rudolphi, 1803, *Bunostomum phlebotomum* Railliet, 1900 (Mayhew, 1940). Neither is *Cooperia punctata* Linstow, 1907 in calves age dependent (Bailey, 1949). Sarles (1932) found *Trichostrongylus calcaratus* Ransom, 1911 to be equally infective in younger and older rabbits. Larsh & Hendricks (1949) found no difference in localization of *Trichinella spiralis* Owen, 1835 in young and adult mice. *Trichostrongylus colubriformis* Giles, 1892 in guinea pigs is not limited by age of host (Herlich, 1959). Boss & Olson

(1965) discovered that new born mice were more resistant to T. spiralis than 8 year old mice. Stoimenov (1976) stated that more Heterakis spp. survived in older hosts than in young. The incidence of Skrjabingylus nasicola Leuckert, 1842 in weasels increased with the age of the host in the wild (King, 1977). Taylor & Kilpatrick (1980) carried out experimental infections with Trichostrongylus vitrinus Looss, 1905 in lambs and found that older lambs harboured larger infections. But Dudzinski & Myktowycz (1963) noted no relation between age and level of infection in rabbits harbouring Trichostrongylus retortaeformis Zeder, 1900.

It has been suggested that innate age resistance has occurred in parasite-host systems as a result of the long association between the two organisms (Sandground, 1929).

(d) The transmission strategy of a parasite is to produce vast numbers of infective stages in order to ascertain that a few at least survive to become adults. Consequently, nematode parasites are prolific egg producers. The human pinworm E. vermicularis has the capacity to contain 4,600 to 16,000 eggs (Reardon, 1938). A female Ascaris lumbricoides Linnaeus, 1758 contains about 27 million eggs and deposits over 200,000 of them per day for several months (Brown & Belding, 1964). Necator americanus Stiles, 1902 deposits between 5,000 to 10,000 eggs daily (Cheng, 1973). The filarial nematode Wuchereria produces several million larvae during its lifetime while Ancylostoma duodenale Dubini, 1843 lays 25,000 to 30,000 eggs per day (Schmidt & Roberts, 1977). Although all the eggs or larvae produced by these parasites may not reach their respective hosts, the latter are, however, exposed daily to a large number of the infective stages.

The numbers of worms that become established may depend upon the size of dose of eggs or larvae that are administered. It has been demonstrated in many cases that worm burden varies inversely with the size of the infective dose that is administered, acting in a density-dependent manner. The work of Dorman (1928) on Heterakis papillosa Bloch, 1782 showed that a small dose of eggs allowed a higher worm establishment in chickens. Ackert, Graham, Nolf and Porter (1931) and Sadun (1949) found a lower dose of eggs of Ascaridia galli Freeborn, 1923 in chickens led to a higher proportion of adults becoming established. Fewer Nippostrongylus brasiliensis Travassos, 1914 adults were established from a higher dose than a lower dose of larvae administered to rats (Africa, 1931; Haley, 1958; Sey, 1969). Dobson (1965, 1974) demonstrated a similar dose size effect on the survival of Amplicaecum robertsi Sprent & Mines, 1960 in mice and Oesophagostomum columbianum Curtice, 1890 in sheep. Dose dependent worm establishment was also observed in the case of Ostertagia ostertagi Stiles, 1892 infections in calves (Michel, 1969).

A high dose of eggs affects the nematode in two other ways, namely by reducing the size and fecundity of worms establishing in the definitive host. Numerous examples of this can be cited from the literature. Africa (1931), Ogilvie & Hockley (1968) reported that size and fecundity of N. brasiliensis was reduced when the infective dose size was large in

comparison to a small one. Krupp (1961) found that high doses of A. caninum eggs yielded a low number of adult parasites. Dobson (1974) discovered a similar effect with doses of O. columbianum eggs. Reduction in size of worms and fecundity have been attributed to effects of crowding in large worm populations by Hill (1926), Sarles (1929), McCoy (1931), Chandler (1936), Michel (1963), Kassai & Aitken (1967). It is likely that competition for food and space are responsible (Read., 1951) as is the immune response.

(e) The immune response, depicted by the expulsion of worms from the intestine, may also be viewed as a measure to regulate the transmission of nematodes. Two expulsion phenomena are recognized in a major review by Wakelin (1978). In some nematode transmission cycles, worms are expelled spontaneously at the time of a primary infection. Spontaneous expulsion of worms has been noted in Trichinella spiralis (Larsh & Race, 1954), Trichostrongylus colubriformis (Herlich, Douvres & Isenstein 1956), Nippostrongylus brasiliensis (Haley, 1962), Trichuris muris (Wakelin, 1967), Aspiculuris tetraptera (Behnke, 1975), Nematospiroides (=Heligmosomoides polygyrus) dubius (Cypess et al., 1977) Strongyloides ratti (Moqbel & Denham, 1977).

A second type of expulsion occurs when a secondary infection is superimposed on a primary worm burden. This commonly-occurring phenomenon is observed in the laboratory and in the wild. Stoll (1929) termed it 'self-cure' when he observed the abrupt elimination of Haemonchus contortus in sheep previously exposed to an infection. The expulsion of

Ostertagia ostertagi (Michel, 1963; 1969) in calves is also a self-cure phenomenon.

Worm expulsion operates as a negative feedback, restraining the worm burden from increasing to levels lethal to the host. But this is not deleterious to the transmission of the worm. On the contrary, by limiting its burden, the parasite avoids causing death to its host and ensures its own survival.

(f) It is reasonable to suggest that if all the infective stages reaching a host were to become established, these would lead to an exponential growth in the worm burden similar to the way an animal population would increase. However, as mentioned earlier, parasite numbers are regulated by the age of the host, the dose size of the infective stage, immune responses and other factors, such as competition. These factors produce a stabilising effect on the worm burden in the individual host so that after an initial rise in numbers of worms, there exists a remarkable state of equilibrium. Both Crofton (1971) and Anderson (1976) arrived at this conclusion using mathematical models. In the presence of regulating factors, the stabilised state is reflected by a plateau in the growth of worm burden (Anderson, 1976). When oscillations occur in worm burden, they do so around a mean level. Regulatory mechanisms are cited as being responsible for the stability in many nematode populations found in mammalian hosts in the

wild (Grundmann, Warnock and Wassom, 1976).
In general, the persistence of many parasites in their hosts in spite of repeated infections, suggests that regulation of their numbers is a normal feature.

The present work on the transmission of S. muris in the laboratory rat was undertaken for several reasons. Although S. muris has been reported universally both in the wild and in the laboratory situation, little work has been done on its transmission. Yamaguti (1935) first described S. muris from the 'large intestine' of Rattus norvegicus var albus as Enterobius muris (Oxyuridae). He later (1941) emended it and transferred it to the genus Syphacia. Since then it has been reported in breeding colonies in the United States by Prince (1950), Hussey (1957), Stahl (1961 a), Blair and Thompson (1969); in the United Kingdom by Owen (1972), Sparrow (1975), Taffs (1975); in France by Roman (1969); Austria (Muller, 1976); Germany (Strasser & Tiefenbach, 1977); Japan (Tanaka, Ohshima & Fujinami, 1974). Perhaps there are no animal houses in the world where S. muris does not occur.
Although S. muris is not known to be pathogenic, its presence in animal breeding houses is a nuisance to breeders (pers. comm.) because worm-free animals cannot be supplied to customers for research.
Efforts to eliminate and curb S. muris infections with the use of chemotherapeutic drugs has met with little success. For example, Blair and Thompson (1969) used pyrvinium pamoate against the worm but reported that reinfections appeared 3 weeks after treatment. Strasser and Tiefenbach (1977) treated S. muris infected rat colonies with fenbendazole for 5 months

before clearing the infection. However, the infection reappeared within 7 months. Although Thompson et al. (1962) reported that after piperazine treatment they were able to clear S. muris infection, and Van den Bossche (1972) stated that worms were expelled 3 days after treatment with mebendazole, the efficacy of these drugs is doubtful. D'Silva (unpublished data) autopsied apparently worm-free rats after treatment with piperazine and mebendazole, only to find ovigerous females in the host caecum. Outbreaks of S. muris infections are known to occur (Strasser & Tiefenbach 1977) and these are especially a threat to animals being raised in sterile conditions (Owen & Turton, 1979).

In spite of the easy availablilty of S. muris in the laboratory and considering the problems posed by it in animal houses, very little is known about the rat pinworm. Prince (1950) published a study on the life cycle of Syphacia obvelata in rats but these specimens were later reidentified as S. muris in 1951 (see Hussey, 1957). The latter author established distinctive morphological differences between S. muris which occurred in the rat, and S. obvelata which was found in the mouse caecum. A scanning microscope description of S. muris from a rat host in Spain was published by Tenora et al. (1978). An electron microscope study in Japan was also reported briefly by Wakai, Kikuchi and Hayashi (1975) but no micrographs were published.

The 8-day life cycle was reported by Stahl (1961a, 1963). He showed that gravid S. muris from the rat caecum migrated down to the colon to deposit eggs on the peri-anal region of the host. When these eggs were picked up by the rat, apparently in the act of grooming, and were ingested, the eggs became infective immediately. The male disappeared within 72 hours of development. A study on experimental infection of S. muris in mice was carried out by Fukui, Adachi & Hayashi (1973); unfortunately no translation of the paper was available.

Singhvi & Johnson (1976) and Ashour (1980) found wild rats to be infected with S. muris in India and Egypt respectively. The intensity of infection increased with age (Singhvi & Johnson, 1977; Ashour op. cit.). Roman (1969), Roman & Kientruong (1973) found that the course of S. muris infection in laboratory rats was sporadic. There were periods when the hosts displayed a high infection followed by refraction. But these periods exhibited no sustained patterns.

The thesis of Van der Gulden (1966) was a significant contribution to the study of S. muris; Van der Gulden demonstrated that S. muris deposited large numbers of eggs on the perianal region of the host. The deposition followed a circadian rhythm, with most of the eggs being laid during the day time. Egg deposition was stimulated by lower temperature and higher oxidation : reduction potential in the perianal area. The results of his work were published in two papers in 1967, 1976.

The paucity of knowledge regarding the transmission of S. muris led to the present investigation. It was expected that S. muris would proceed through 4 larval stages of development and these could be studied with the aid of the electron microscope. The diurnal rhythm of egg deposition might be linked to the activity of the host. The establishment of S. muris might be regulated by density-dependent factors. The age and sex of the host was likely to influence the worm burden and whether or not continuous autoinfection might lead to an exponential increase in worm burden required further study.

-ooOoo-

CHAPTER II

MATERIALS AND METHODS

The present work required the use of worm-free rats, and the handling and collection of Syphacia muris eggs. Both these presented problems initially.

(1) General Husbandry of Rat Hosts:

Worm free rats were required throughout the course of this work for experimental purposes. The Sprague-Dawley rats in the animal houses were, however, generally infected with Syphacia muris. This was demonstrated by applying a 1" x 1" piece of Sellotape to the perianal region of the host, and pressing it thereon 5 times. Examination of the Sellotape under a dissecting or compound microscope revealed the presence of S.muris eggs. Therefore, attempts were made to make the rats worm-free by using two anthelmintics, namely, piperazine citrate (Citrazine (J.M. Loveridge Ltd.)) and mebendazole ('Equiverm' (Crown Chemical Co., Ltd.)). Piperazine citrate was given in the water at the rate of 100 mg/kg body weight to freshly weaned, 3 week old litter pups for 3 days. Thereafter the animals were sampled by Sellotape for the presence of eggs on the perianal region. The absence of eggs implied no worms were present. However, as a precautionary measure, several rats which did not have eggs on the perianal region at the end of the treatment were autopsied and the caeca examined. Autopsies revealed that compared to infection in untreated animals, the worm burden in treated animals became reduced sharply. Worms in treated animals were, therefore, never completely eliminated and rats were never worm-free.

A .003% solution of mebendazole mixed in 1% Tween 80 was also administered to 3 week old infected rats in the drinking water. The treatment was given over a 13-day period before worm elimination could be observed, both by the perianal area sampling, and by host autopsy.

In sharp contrast to piperazine citrate, mebendazole allowed a better elimination of worms. Only one or two female worms were found in treated animals. Therefore, .003% mebendazole was used in the initial period of this study to make rats worm-free. In all cases, only 3 week old, freshly-weaned rats were dosed. These rats were used to study (i) the life cycle of S. muris and (ii) its pattern of egg deposition.

The use of mebendazole on the other hand had two drawbacks. After treatment of the hosts, its residual effect on the re-infection experiments were unknown. Mebendazole is known to remain in the host body several hours after treatment (Van den Bossche, personal communication.). The second drawback was the long length of time the treatment took in order to eliminate worms in infected animals. By the time the treatment was terminated, the animals had grown in age, thus making it impractical to use them for age-related studies. To overcome these problems, it was decided that isolator-raised, germ-free rats should be used.

Two canopy isolators (Vickers Medical, Vickers Ltd., Basingstoke) attached to a fan blower were used to house and raise the germ-free rats. The method for maintaining these is described in some detail below. Each canopy (Fig. 1) was made of polyvinyl chloride film and measured 2' x 2' x 5'. The canopy was mounted on a foam sheet laid on a wooden base. Air from the fan blower entered the canopy through an inlet filter attached on one end of the broader side. The core of the filter was made of stainless steel mesh and was cylindrical. The ends of the cylinder were snugly fitted to PVC bases. A PVC pipe protruded from the base at the top. It was bent at a right angle and fitted into an inlet sleeve in the canopy.

Fig. 1. Isolator unit used to raise worm-free Wistar rats.

FIG 1

The stainless steel mesh was wrapped 3 times with glass wool, and fastened to the PVC bases at each end by several turns of a pressure sensitive Scotch tape (3M). The glass wool was covered by a layer of ordinary surgical gauze. A loose plastic jacket covered the entire cylinder and was secured to the PVC bases by tape and jubilee clips to make it air tight. Air entered the jacket from the fan blower via a plastic reinforced hose.

The air outlet filter was fitted on the same side of the canopy as the inlet filter. Its metal core was wrapped once right around the fibre glass wool, and surgical gauze. There was no plastic jacket cover. Air was let out through a right angle PVC pipe fitted on to the top of the filter. Air filters were removed every two months and the glass wool and the gauze replaced.

Two heavy duty gloves were mounted on the same side of the canopy as the filters. The gloves were used to manipulate animals, cages, food, water bottles, saw dust, filter papers which were placed inside the isolators. Gloves had to be occassionally replaced due to wear and tear.

There was a port-hole on the canopy side facing the gloves. The port hole was 9" deep and 12" in diameter. It was kept covered on the inside and outside by snugly fitting plastic covers. The cover inside was held in place by a rubber band; on the outside by several layers of pressure sensitive Scotch tape. Animals and materials entering and leaving the isolator were held in the port hole briefly before the covers were removed.

Materials that were to be introduced into the isolators were always autoclaved. These included cages with lids, water bottles and tops, rolls of paper towel, 2 litre

flasks of water. All such materials were double wrapped in autoclavable polyurethane paper bags which were fastened with autoclavable masking tapes. These were autoclaved under steam at 15 lbs per square inch pressure for 20 minutes. Irradiated rat food pellets were double wrapped and supplied by Dixon and Sons, Ware, Herts. Since the vitamins in irradiated diets were destroyed, vitamin K ('Konakion' Roche Products Ltd.) was supplied from ampoules. Autoclaved materials were introduced into the canopy through the port hole. The interior of the canopy was thoroughly sprayed with a solution of freshly prepared 2% peracetic acid in distilled water mixed with a few drops of Teepol. The solution is a disinfectant and is a bactericide and is used routinely in isolator units (Medical Research Council Laboratories, Carlshalton). After spraying, the port hole was sealed inside and outside, and the canopy allowed to inflate. The interior was left to dry overnight. Any remaining water was mopped up with paper towelling. The room in which the isolator was held was first cleaned with Teepol.

Two pairs of breeding and pathogen-free 4 star Wistar rats were originally purchased from the Medical Research Centre, Carlshalton and introduced into two isolators. The animals were placed on dust-free saw dust in plastic cages. They were maintained on irradiated Diet 86 rat pellets. The drinking water was provided with vitamin K at 1 ml/litre of water. Paper towelling was put into the cages for nesting material. Cages were cleaned every week and food and water were replenished as required.

At any one time, one breeding male and four breeding females were kept in each isolator. The male was held in a 17.5" x 11" x 8" plastic cage (North Kent Plastic Cages Ltd., Dartford, Kent), and the females

each in 15" x 10" x 7" cages. Male and female were allowed to remain together in a cage for 8 days for mating and then separated. A litter was produced approximately 26 days later. Litters were weaned on the 21st day after birth and removed from the isolator unless needed for age-related studies. Each breeding female was allowed to produce at least 5 litters before being replaced by fresh breeding females. At the end of the five reproductions, litter sizes were reduced from an average of 8 pups to 5. Litters were removed from the isolator in polyurethane bags which had been autoclaved and put into the isolator. The bags were briefly put in the port hole, and the cover removed to isolate them from the canopy.

Such litters were put into thoroughly washed and heat dried plastic false bottom cages measuring 15" x 10" x 7" on saw dust. The cages were fitted on the top with a perspex cover to prevent aerial contamination of the animals with S. muris eggs. On one half of the perspex top, 3 mm. diameter air vents were bored in 4 rows 1 inch apart for ventilation. It could be argued that such vents could allow S. muris eggs to reach the rats placed within the cage. However, autopsy of rats maintained in this way for several days showed no infections to occur. Experience therefore, showed that such filter top cages could be used for housing rats without fear of infection by chance from the air.

After litters were put into perspex top cages, they were fed on ordinary Diet 86 rat pellets contained in a food hopper beneath the top. Tap water was provided via a plastic tubing introduced through a 5 mm. diameter vent on the perspex top from a master water bottle. Here it

could be argued that both the ordinary diet and the tap water could have been a source of aerial infection for the pathogen free litters. However, after maintaining rats in this manner for several days, autopsy revealed no S. muris in the caecum. Moreover, it was necessary to feed ordinary food and water to the gnotobiotic animals in order to allow the growth of microfauna in the caecum. Initial investigation revealed that S. muris larvae would not establish in litters freshly removed from the isolators. Litters needed to be fed on an ordinary diet and water at least two days before experimental infection with S. muris eggs could be carried out.

(2) The Collection of Eggs of S. muris:

Eggs for experimental infections were obtained from a strain of S. muris which was maintained in Sprague Dawley rats in a separate animal house from that which housed the isolators. Normally a piece of cellophane tape is applied to the perianal area of the host and is pressed several times for eggs to adhere to the adhesive surface, (Chan, 1952). The cellophane is mounted on a glass slide and examined under a microscope. Eggs can then be identified and/or counted. This method was used to collect S. muris eggs to note patency of an infection and to count the number of eggs in certain experiments. The method is not good however, if eggs are to be used for re-infection purposes. For, eggs adhere to the sticky surface of the tape and cannot be dislodged for further use. To overcome this problem, Stahl (1961) followed Chan's (1952) technique for the collection of S. obvelata eggs by incubating eggs in 0.85% saline from S. muris which were in the migratory phase in the colon and he found that these were good for infection studies.

Stahl's technique was tested on 3 occasions. Infected rats were autopsied and gravid females removed from the colon. Worms were placed in a petri dish of 0.85% saline and the eggs teased out with a fine needle. The eggs were incubated for 4,5,6 hours at 37°C and thereafter fed to worm-free rats. Autopsy revealed that no worms became established.

Stahl's technique suffered from one other defect: an infected rat needed to be autopsied each time eggs were needed for incubation and experimental work. Stahl's technique was therefore abandoned, and other methods were tried. Emphasis was placed on collecting eggs from the perianal area of the host.

(i) Cellophane smear technique (Chan, 1952):

Strips of cellophane tape were cut and floated on water with the adhesive surface on top. Contact with the water caused the tape to curl up and the adhesive layer to come free of the cellophane. The cellophane was then applied to the perianal area and examined under the microscope. Eggs could be seen to adhere to the tape but the number of eggs was always insufficient for carrying out experimental infections.

(ii) Egg albumin smear:

A glass slide was smeared with egg albumin and applied to the perianal area of infected rats. Eggs were not, however, picked up by this method.

(iii) White adhesive labels:

The labels were moistened and applied to the host's perianal region but, again, results were not satisfactory. Additionally, the white background of the paper made it difficult to observe any eggs.

(iv) Rice smear technique:

In this method a smear was made onto a glass slide using glutinous, boiled rice (Tolly Boy and Co.). The slide which was pressed against the perianal area of infected rats at least 3 times, was examined under a stereodissecting microscope and was found to have sufficient number of eggs for experimental use. The method is herewith described in some detail below because of its suitability.

Rice grains were washed and boiled until the coleoptile areas were soft. This was easily detected when a longitudinal furrow appeared along the grain. The softness can also be ascertained by pressing a few grains between thumb and forefinger. The rice should have a soft consistency and should be drained of the water. Freshly drained rice is not only warm but contains moisture which does not allow eggs to adhere to the surface of the slide when making a smear. The rice was therefore cooled overnight and a thin smear was made on the glass slide with the thumb. Before becoming dry, the smear was pressed onto the host's perianal area and the eggs recovered in this way. Although the quantity of eggs obtained was not as much as that gathered by an adhesive Sellotape, the number was nevertheless sufficient for experimental handling. Up to 2000 eggs may be recovered in addition to entire gravid female worms about to deposit eggs.

The eggs were removed from the slides by immersion in a petri dish of warm saline. Petri dishes containing a 0.65% $NaCl$ solution were warmed on a hot plate maintained at 31°C, which was observed to be the rectal temperature of the rat hosts using a Grant thermistor model S. Warming the saline prior to collecting eggs allowed any air bubbles in it to be removed. The presence of air bubbles

in the saline had one disadvantage: when bubbles floated to the top, eggs were carried away and could not be collected from the water surface. Warmth also allowed the glutinous rice to become brittle and dislodged from the slide surface. This facilitated teasing away eggs for counting and for collecting in a syringe via a blunted hypodermic needle.

Throughout this work, eggs were collected by the rice smear technique. Slides were left immersed in 0.65% saline solution in petri dishes maintained at 31°C. The petri dish was placed under a Zeiss stereomicroscope IVB and eggs were teased from the rice smear with a fine needle mounted at the end of a glass rod. Known quantities of eggs were picked up through a 50 mm. number 19 G x 2 (Gillette) hypodermic needle attached to a 1 ml. syringe (Rocket). The syringe contained warm tap water so as to further dilute the salt solution in which eggs were picked up.

Prior to infection rats were gently held in the neck region between thumb and forefinger. Each rat was cupped in the palm of the hand with the tail secure between the little and fourth finger. The hypodermic needle was introduced into the oesophagus and the contents of the syringe plunged in and washed down with one ml. of tap water.

(3) Collection of S. muris worms:

Worms were removed from experimentally infected rat hosts by a modified Baermann technique. A strainer was made by fastening a piece of nylon gauze to a perspex cylinder with araldite. The mesh size was 444 per square inch. The perspex cylinder was 2.5 inches in diameter and one inch deep. The strainer was placed in a 4 inch wide funnel to the stem of which was attached a 4 inch long

rubber tubing hose. The hose was clamped with a screw clip and the funnel filled with a 0.65% saline solution until it reached the level of the strainer.

The stem of the funnel was lowered into a bath of water, maintained at 37°C, and the funnel held in place by a ring clamp attached to a clamp stand. The size of the bath, 1' x 2' x 1' was large enough to accommodate 4 funnels on clamps.

The intestine, caecum, or colon of infected rats was removed after autopsy, slit open and placed in the strainer. Worms followed the warm gradient in the saline solution and were collected in the clamped hose. Worms were allowed to collect for 30 minutes in this manner.

By unclamping the screw clip around the hose, worms were allowed to drain into 3" x 1" glass vials. After worms had settled to the bottom of the vials, excess saline solution was decanted with a pasteur pipette. This had the advantage of removing any stomach contents and debris which had passed through the strainer, and which would have made sorting more difficult. Some worms were immediately fixed in a warm 4% formalin solution and stored for counting later. Other fresh worms were fixed for electron microscopy.

-ooOoo-

CHAPTER III

The Growth and Life Cycle of S. muris

The growth of nematodes proceeds in stages. It follows the pattern:

Embryo $\longrightarrow$ 1st larval stage $\xrightarrow{\text{moult}_1}$ $L_2 \xrightarrow{M_2} L_3 \xrightarrow{M_3} L_4 \xrightarrow{M_4}$ adult.

At each moult, the cuticle is shed and is replaced by a new one. Growth may occur at each moult, and may be measured by observing changes in the length of the nematode. From this data, growth curves can be constructed to reveal whether growth is continuous, or is interrupted by a period of lethargus (Rogers & Sommerville, 1960). During lethargus, a nematode stops growing shortly before, during, and just after moulting (Lee & Atkinson, 1976) and approximates a hypothetical growth curve.

The growth and life-cycle of S. muris was first described by Stahl (1961a, 1963). The developing embryo hatches out of the egg inside the rat intestine. Larvae become established in the caecum and develop to egg producing females in 8 days. Male worms leave the host on the 4th day after hatching. In order to ascertain the developing stages in the life cycle of S. muris in the rat and to further extend Stahl's (op. cit.) studies, an investigation was carried out on the egg, larval stages and adults using light and electron microscopy. Particular emphasis was paid to moulting since this aspect had not hitherto been studied in S. muris.

MATERIALS AND METHODS

Eggs which were collected by the Sellotape method from the perianal region of previously infected hosts were examined microscopically and measured with an oculometer.

Three week old rats were removed from the isolator and infected with 100 eggs by oral intubation. Rats were sacrificed at intervals of 18, 20, 24, 40, 48, 60, 66, 72, 96, 120, 144, 168, 192 hours post infection. The caecum was removed and slit open in a warm 0.65% saline solution. Worms were collected by placing the caecum in the Baermann apparatus. From day 5 onwards, the perianal area of the rat hosts was examined before autopsy by Sellotape for the likely presence of eggs being deposited by migrating females.

Worms and Sellotape were at first examined and counted under a Zeiss Stereomicroscope IVb. Using a Leitz orthoplan microscope, a second examination was carried out in which worms were drawn and measured. Measurements were taken from drawings by placing a scale on the paper from a stage micrometer, also drawn under the same magnification as the worms. A cartographer's wheel was used to measure worms against this scale, so that a growth curve was constructed for each day of the life cycle.

For scanning electron microscopy, eggs from the perianal region, and worms collected at 24 hour intervals from the caecum, were washed several times in .65% saline. These were fixed for 2 hours in 5% glutaraldehyde made up in sodium cacodylate buffer maintained at pH 7.2. Specimens were post-fixed in osmium tetroxide for 1 hour, and washed several times in the cacodylate buffer. Eggs and worms were dehydrated in graded series of ethyl alcohol. Specimens were critically point dried, coated with gold and scanned under the Cambridge Stereoscan S4-10.

For transmission electron microscopy, eggs and worms collected at regular intervals of growth were fixed, post-fixed and dehydrated as above. Eggs fixed in this way did not embed in the resin media. Therefore, whole female worms containing eggs, and in a state to deposit them, were removed from the colon of rats for fixation and sectioning.

Specimens were processed through a graded series of ethanol-Spurr's mixture before being placed in 100% Spurr's resin (Reynolds, 1963). They were cured overnight at 65°C under 15 lbs/sq. in. in vacuum.

Sections were cut using glass knives on a LKB Ultramicrotome III. Silver-grey sections were picked up on formvar coated copper grids of 200 mesh size, and stained in uranyl acetate followed by lead citrate. Sections were viewed under a Leitz 95 Transmission electron microscope at 60 kV.

RESULTS

The development of Syphacia muris is described by a series of photomicrographs and illustrations.

Eggs: The eggs (Fig. 2) measured 78 µm x 30 µm and were ellipsoid in shape, the anterior end being slightly more obtuse than the posterior. The anterior end bore an operculum through which the larva escaped during hatching. One face of the egg was flattened and the opposing side was curved. The surface contained minute pits (Fig. 3) which were observable under the scanning electron microscope. These pits were more densely situated on the flattened surface towards the operculum.

Encased within the transparent shell was a larva, its head lying close to the operculum. This was the first stage larva. It had a fully formed oesophagus, with characteristically shaped oxyuroid oesophageal bulb. No intestine was visible, although the intestinal area was marked by granular material which appeared to be in constant motion. The larval tail tapered to a point posteriorly in the egg shell. As revealed by transmission electron microscopy, the larva was surrounded by a loose thin cuticle within the egg (Fig. 4). The cuticle was shed as the larva escaped from the egg (Fig. 5). This was the first moult to occur and it produced the second larval stage.

18, 20, 24 hour larvae: The worms recovered from infected rats at these periods revealed that the second stage larvae underwent a moult to give rise to the third larval stage. The moulting cuticle covered the entire worm (Fig. 6) which showed a depression in the body wall about 0.10mm. from the head in the males and 0.13 mm. in the females. Superficially, the cuticle was wrinkled and divided into broad, transverse annuli (Fig. 7). Between the annuli, there were deep furrows. The moulting cuticle stretched over the mouth opening and appeared transparent.

Fig. 2. The ellipsoid-shape eggs of S. muris.

FIG 2

The moulting cuticle as observed under the transmission electron microscope possessed 3 layers: outer cortical, median, and inner basal (Fig. 8). The cortical layer measured 0.14 um. and was itself composed of 3 layers: an outermost electron dense layer, a transparent mid layer and a thin inner electron luscent layer. The cuticular median layer, which was the broadest of the 3 layers, varied in thickness according to the height of the ridges and furrows. Most of the space in the ridges was filled up by the median layer. Only a thin median layer was found under the furrows. The median layer was flocculent and granular in nature, and did not stain with osmium. The basal layer consisted of a single band of fibrous material about 0.7 µm. thick. The cuticular layers rested on a basal lamella. Moulting occurred when a new cuticle developed from beneath the basal lamella. As seen under the light microscope, the old **cuticle** broke into two halves around the depression which was observed in the body wall of the worm. The larva first threw off the anterior part of the cuticle before escaping from the remaining half.

Some basic adult features could be recognised in the head region of the emerged third stage larva. These included 4 corners where papillae would appear eventually (Fig. 9). The triangular mouth opening was small, and led to a buccal capsule which housed 3 teeth. Three leaf-like structures arose from within the buccal capsule in some specimens (Fig. 10). In other specimens these structures were not visible. Instead, only a hollow space was observed where the buccal capsule arose. From this it may be surmised that the leaf-like structures arose from within the buccal capsule and were attached to the oesophagus. The appearance of the structures indicated they were pushed up by a movement of the oesophagus and that the larva was capable of feeding at this stage.

The oesophagus possessed an oesophageal bulb which led to an intestine containing granular material. Males could be differentiated from females by the short, sharp tail as seen under a light microscope. In some specimens primordial genitalia were observed and a depression occurred in the body wall where the gubernaculum and spicules would develop.

40-48 hours: Some significant changes occurred in 'male' and 'female' larvae as the 3rd larval stage underwent a moult to produce the 4th stage. The mouth parts, the intestine, and the genitalia became more prominent. Males could be easily distinguished from females by the shorter length. The moulting cuticle, enveloping the entire larva, was taut around the mouth (Fig. 11). Otherwise, it was loose throughout the length of the worm (Fig. 12). The cuticle was wrinkled and annulated as before.

The ultrastructure of the moulting cuticle showed a cortical, median and basal layer. While there was a slight increase in the thickness of the cortex compared to the earlier moulting cuticle, the median layer increased considerably in thickness (Fig. 13), mainly due to an increase in the amount of flocculent material. Two bands of thin fibre occupied the basal layer. (In contrast, the new cuticle of the 4th larval stage developed 3 bands of fibres in the basal layer - see Fig. 14). The shedding of the old cuticle was observed by electron microscopy. It took place when the cuticle broke open around the mouth opening (Fig. 15). The larval head was then pushed through this opening and the old cuticle was cast off (Fig. 16). The head which emerged was knob-like. The triangular rim around the mouth opening was thick (Fig. 17) and the three buccal teeth were more conspicuous. Two fleshy bosses surrounded the rim of the mouth from opposite sides.

The bosses were separated from each other by deep spaces which led to the buccal capsule but disappeared in later stages of growth. The intestine was composed anteriorly of a trough of cells which became aggregated in the posterior region. An excretory pore appeared below the oesophageal bulb.

66-72 hours: The 4th moult occurred at this time. The moulting cuticle, which was observable by transmission electron microscopy, was composed of the 3 basic layers: cortical, median and basal (Fig. 18). The cortex was limited to an outer thin electron luscent and an inner electron dense layer. It was thrown into folds and valleys due to variations in the amount of granular, flocculent material in the median layer. The median layer occupied more space than in previous specimens. The basal layer consisted of 3 bands of fibres (Fig. 18). The old cuticle was never observed to be shed in specimens examined at 66 and 72 hours of development. However, a new cuticle growing beneath the basal layer was evident from electron micrographs (Fig. 18), and it was likely that the old cuticle was resorbed. The new cuticle had a cortical, median and basal layer. The median layer was thicker due to an increase in granular material. The basal layer had 3 bands of fibres. In males, the outermost band separated in places, crossed the median layer to come and lie against the cortical layer (Fig. 19).

The adult worm which emerged showed 3 well-formed lips and teeth (Fig. 20). The lips occupied the deep spaces seen in earlier development. Two papillae were positioned lateral to the dorsal lip, and two below the ventro-lateral lips. Amphids appeared laterally. In both male and females, two spongy areas appeared in the head region, one being situated above the dorsal lip, the other across from it from the ventro-lateral lips. The

spongy areas (Fig. 21) were reported by Tenora et al. (1978) for S. muris and by Ogden (1971) for S. citteli. The function of these is unknown. These structures were prominent and did not develop further in later stages. The appearance of the spongy structures in males just before they copulated may suggest a sensory chemotactic function to aid copulation. Alternatively, because the intestine at this stage was completely bifurcated, thus allowing more space for food contents, the spongy areas could be absorptive surfaces for nutrient uptake.

At 66 hours, cephalic alae appeared. Namelons were seen on the males (Fig. 22). In females a dark plug occluded the vaginal pore (Fig.23). In addition, the reproductive organs were completely formed. The testes, vas deferens, spicule, gubernaculum were laid down. Ovaries, oviducts, and uteri became differentiated.

96 hours: One male was recovered from 3 autopsied rats. The females grew longer in size (Fig. 24). Several gland cells appeared around the oesophageal bulb. The nerve ring was clearly visible whilst the excretory pore and excretory bladder also became more obvious. The oviduct was composed of long rows of elliptical cells. Eggs were seen for the first time in the anteriormost part of the uterus which lay close to the vulva.

120 hours: Both uteri were completely filled with eggs. One uterus reached the junction of the oesophageal bulb and intestine. Eggs contained morula states of the embryo. Rats sampled for the presence of eggs on the perianal region showed gravid females had not begun oviposition.

144 hours: The plug on the vaginal pore disappeared (Fig. 25). The excretory bladder contained an irregular-shaped small body, the nature of which could not be determined. Worms were narrower in shape being stretched antero-posteriorly.

Fig. 3. Pits seen on the egg surface of S. muris.
x 45,000

Fig. 4. Section through the egg of S. muris.
Note larva covered by thin cuticle (arrows)
x 2310

FIG 3

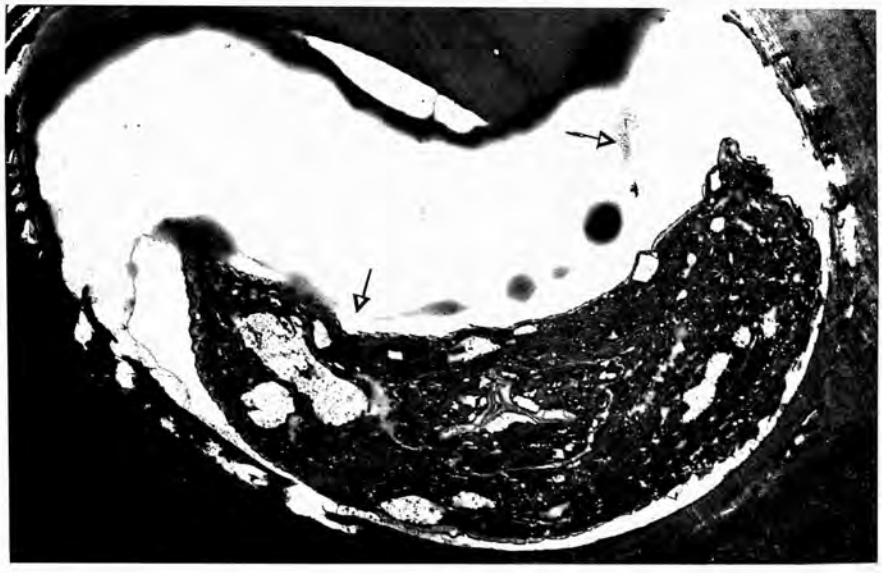


FIG 4

Fig. 5. Egg with exsheathed cuticle (star) of larva. x 4,500

Fig. 6. Second stage (L_2) female larva with exsheathed cuticle. Note depression (arrow) on body wall where cuticle will rupture. (10 x 40)

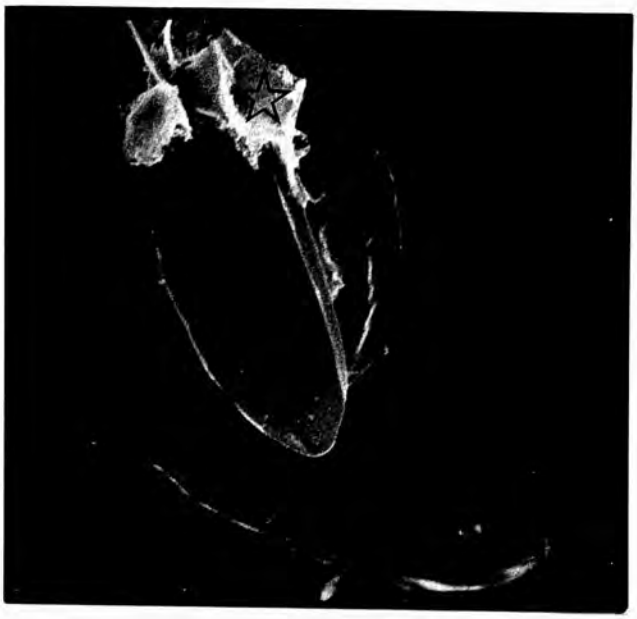


FIG 5

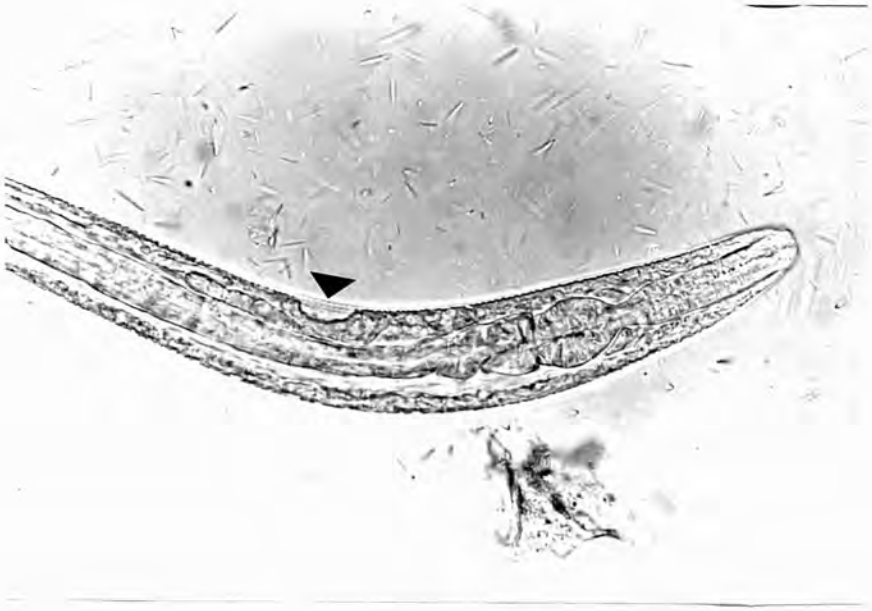


FIG 6

Fig. 7. Second stage larva showing surface cuticle with wrinkled annuli and furrows.
x 22,500

Fig. 8. T.S. Cuticle of second stage larva.
Note ridges and furrows. The cortical layer is thin (arrow).
M - median layer
B - basal
BL - basal lamella.
×4000

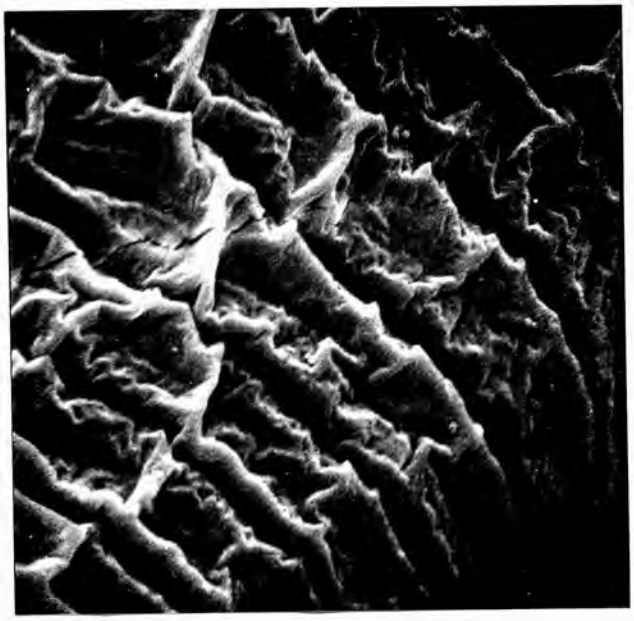


FIG 7

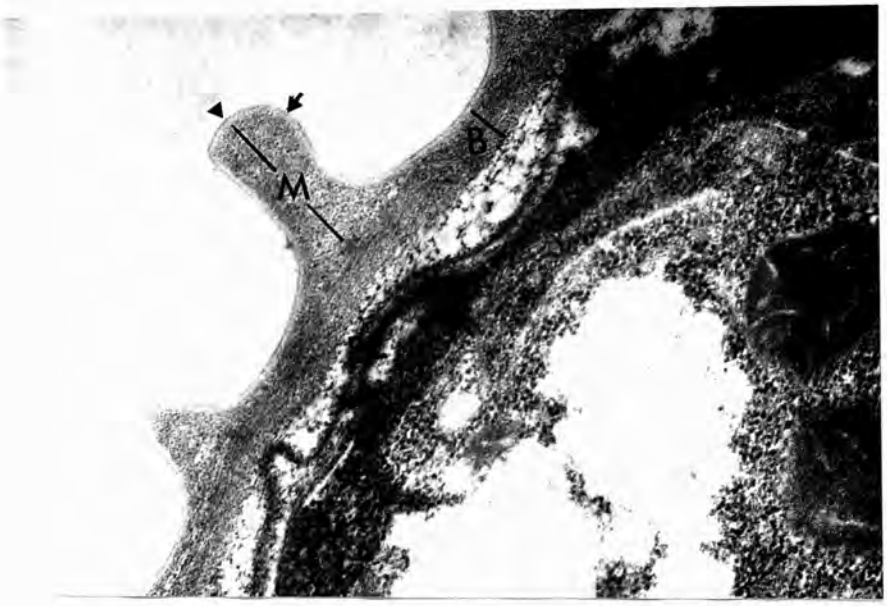


FIG 8

Fig. 9. *En face* view of L_2 larva.
Thin cuticle envelopes the mouth and rest of larva and is almost invisible. Three teeth are visible through the mouth opening.
x 32,000
■ - site where papillae appear in later stages.

Fig. 10. *En face* view of L_3 larva showing foliate structures arising from the mouth opening.
x 32,000.

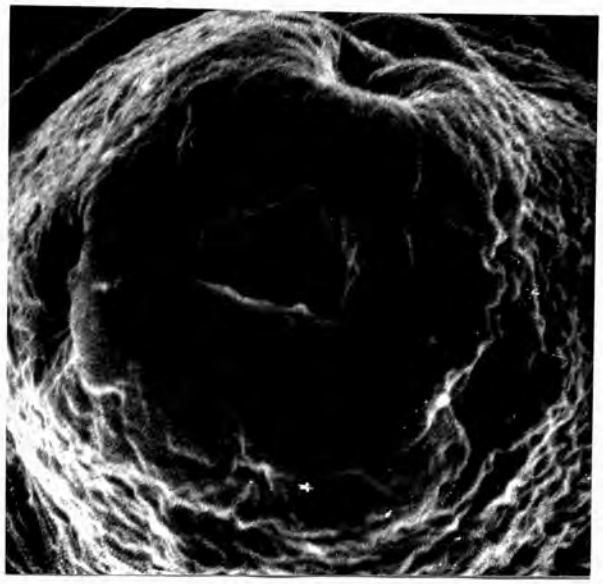


FIG 9

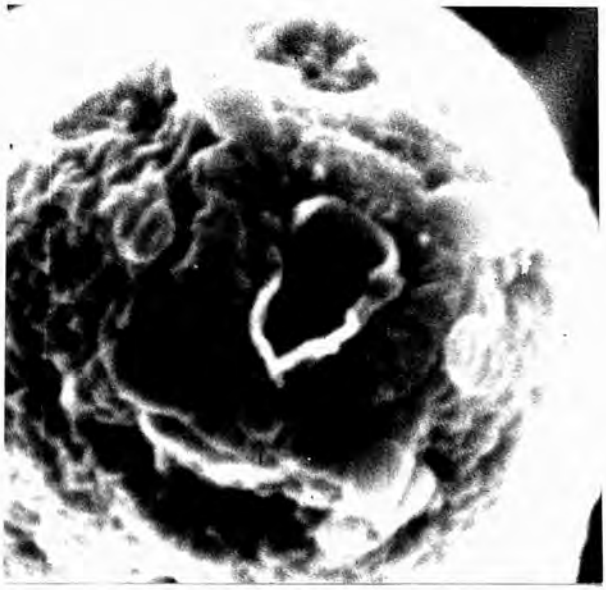


FIG 10

Fig. 11. *En face* view of third stage (40 hour) larva - note cuticle stretching over mouth opening and head.
x 20,000

Fig. 12. Surface cuticle of L_3 at the time of moulting. Annuli are less wrinkled than before moulting.
x 15,600.

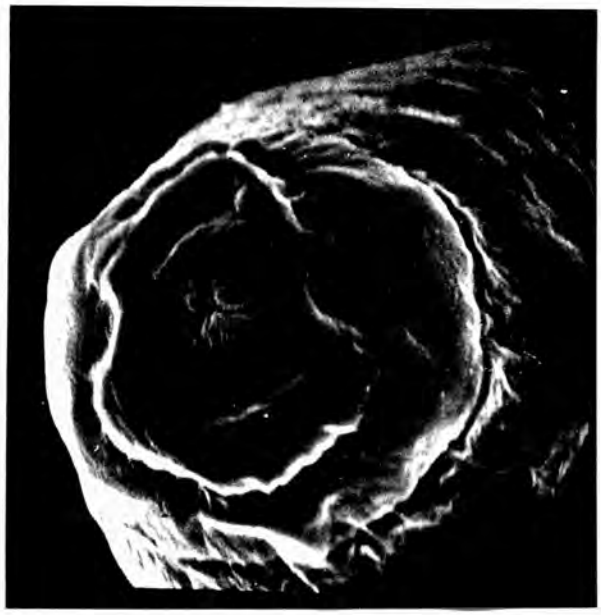


FIG 11

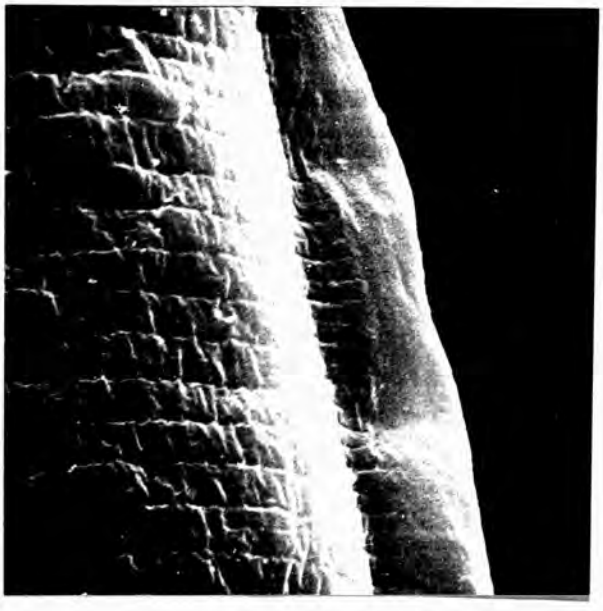


FIG 12

Fig. 13. T.S. Cuticle of moulting L_3 larva. The old cuticle shows 2 bands of fibres in basal layer. New cuticle (N.C.) arises below basal layer.
x 4,800

Fig. 14. Fourth stage (40 hour) larva. The band of fibres in basal layer have increased to three.
x 9.000.

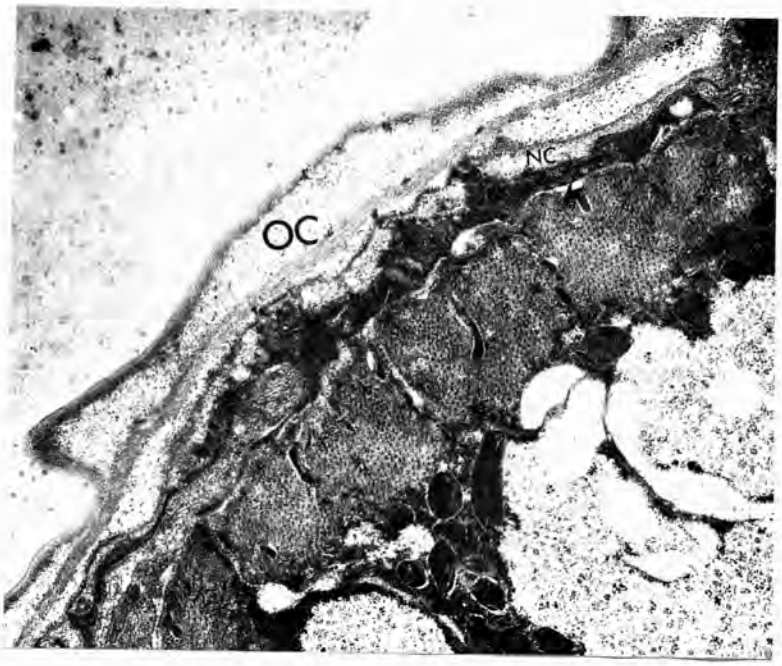


FIG 13

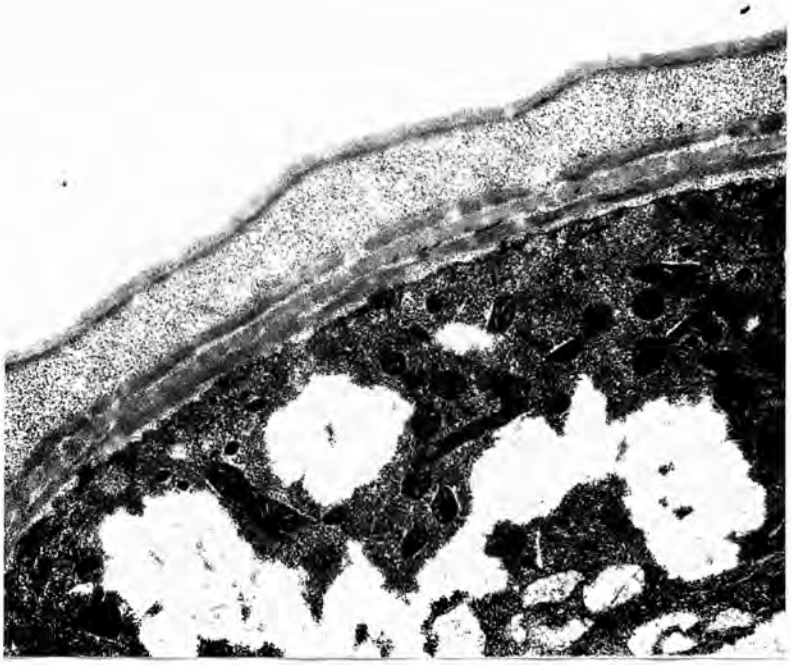


FIG 14

Fig. 15. *En face* view of L_3 larva.
Note head pushing through shedding cuticle (S.C.).
x 22,500

Fig. 16. Cast off cuticle of 'male' L_3 larva.
(10 x 40)

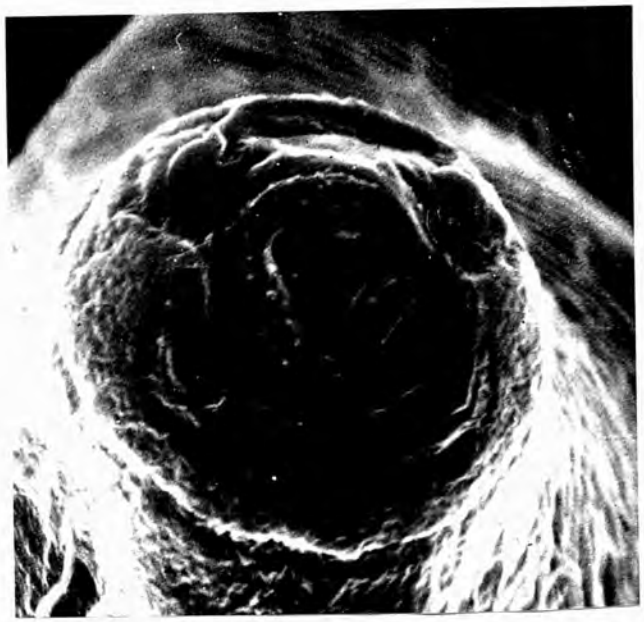


FIG 15

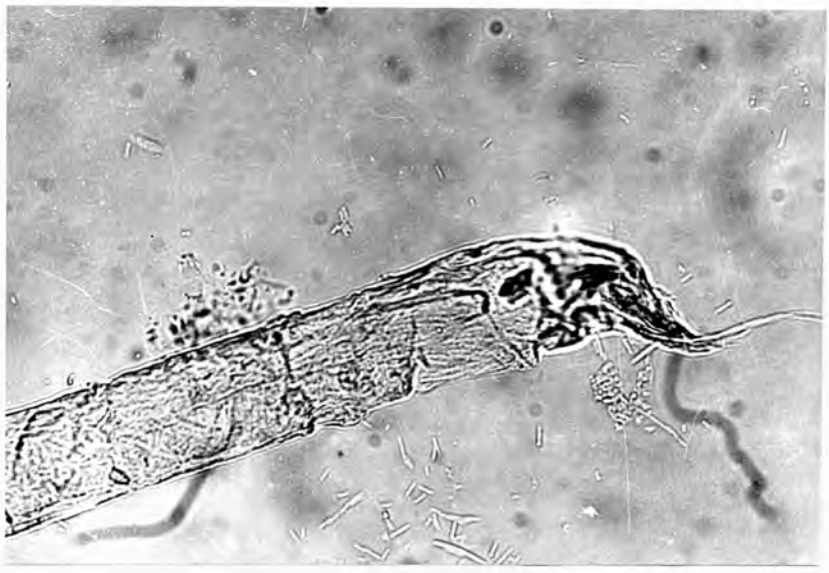


FIG 16

Fig. 17. En face view of moulted L_4 larva showing 2 fleshy bosses (B), cavernous space,(*) mouth opening and 3 teeth.
x 40,000.

Fig. 18. T.S. Fourth stage (72 hour) male larva. Note old cuticle (O.C.) lying on top of new cuticle (N.C.). F - three fibrous bands in basal layer of old cuticle.
x 4,000

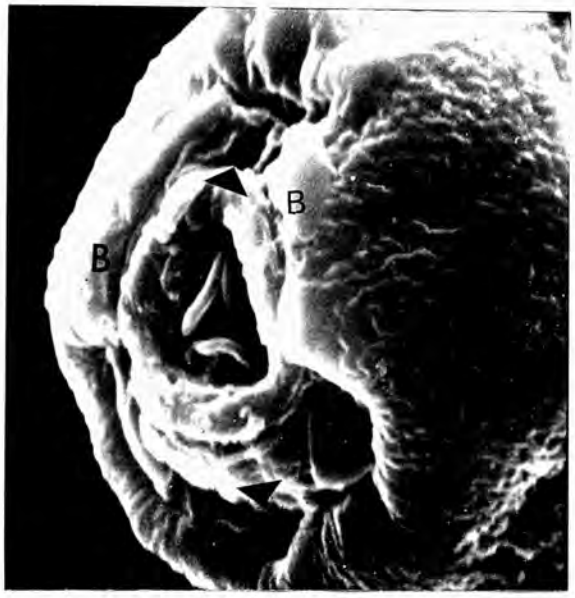


FIG 17

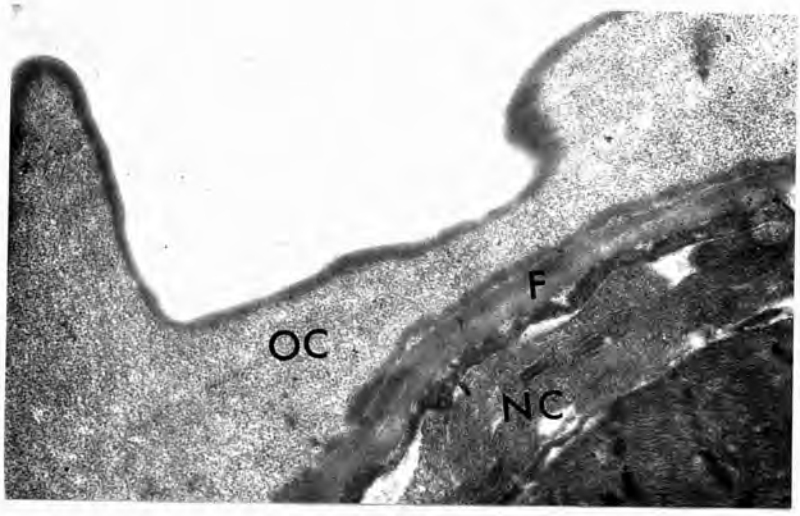


FIG 18

Fig. 19. T.S.Cuticle of moulted adult.
Note the band of fibre (arrows) crossing median layer to lie underneath the cortex (C).
M - median layer
B - basal
x 11,660

Fig. 20. En face view of moulted adult female larva.
Note 2 lateral spongy areas (star).
x 45,000.

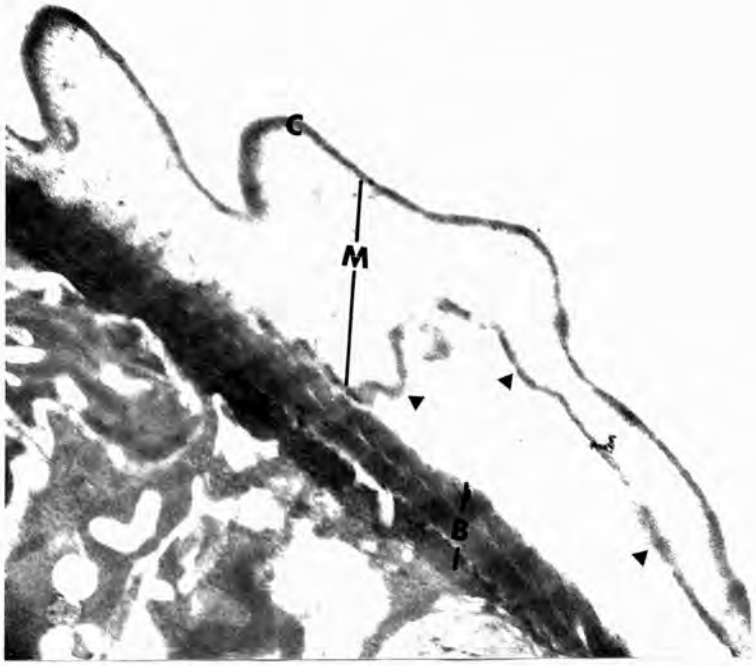


FIG 19

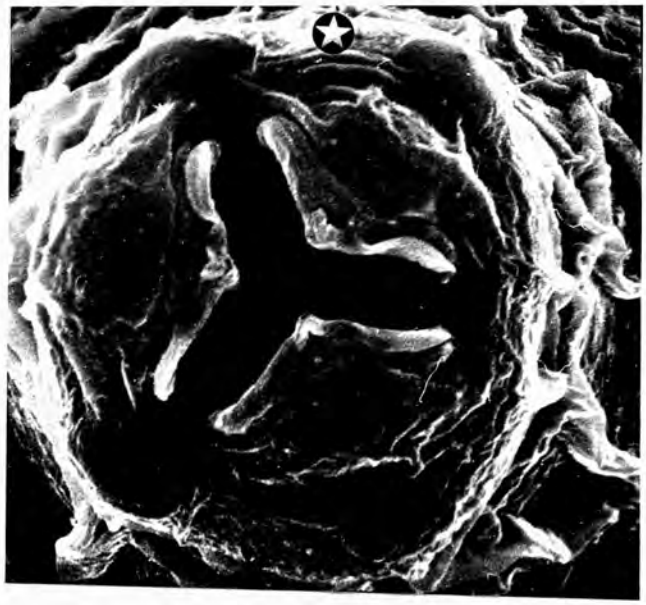


FIG 20

Fig. 21. Spongy area on the head of 72 hour male.
x 23,000

Fig. 22. Adult male showing mamelons (arrows) and cephalic alae (C.A.).
x 320.

FIG 21

FIG 22

Fig. 23. Adult female showing plug over vaginal pore.
x 9,000

Fig. 24. Adult female showing excretory pore (e.p.), excretory bladder (e.b.) and plug (arrow) over vaginal pore.
x 160

FIG 23

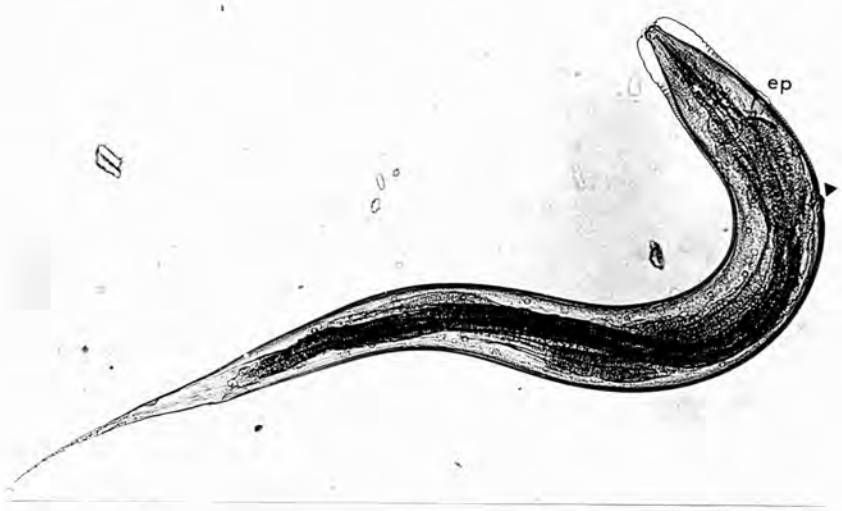


FIG 24

Fig. 25. Adult female showing absence of vaginal plug.
x 9,000

Fig. 26. Surface cuticle of ovigerous female - note cuticle has stretched to accommodate eggs in uterus.
Small papillae, seen as white spots, cover surface.
x 9,000.

FIG 25

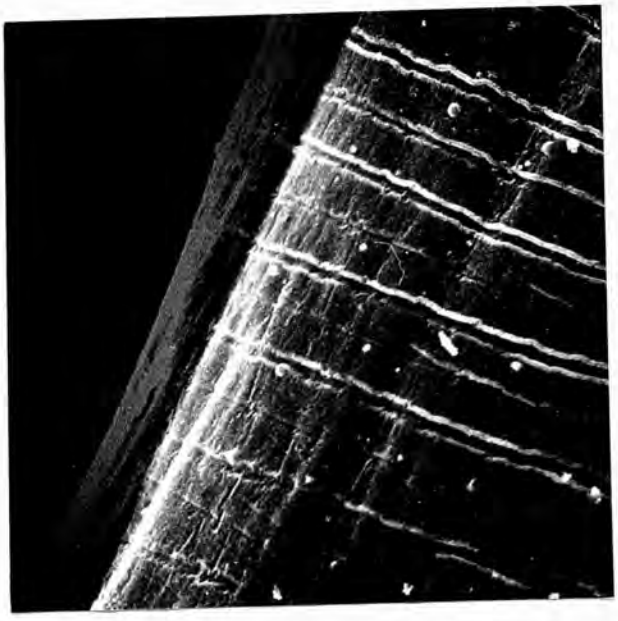


FIG 26

The annuli on the cuticle were stretched and each annulus now bore a narrow depression which surrounded it. Deep ridges appeared between the annuli (Fig. 26). Small papillae dotted the cuticular surface and were observed by scanning electron microscopy.

168 hours: Eggs appeared on the perianal region of the host for the first time, indicating egg deposition by worms. Oviposition occurred immediately after gravid females had reached the perianal region. Eggs were released through the vaginal pore in rapid succession. The spent female shrank in size, caused by contraction of the longitudinal muscles. The cuticular median layer remained unchanged in thickness. The numbers of eggs recovered from gravid females which had been removed from the colon ranged between 421 and 542 in 50 worms. The mean number of eggs per female was 440. Most of the eggs contained fully developed larvae while others showed morula and gastrula stages of the embryo. At 192 hours no worms were recovered from the caeca of autopsied rats, thus showing that the life cycle of S. muris was completed in 7 days.

The number and size of the worms that were recovered daily after the hosts were initially infected with 100 eggs is given in Table I. The mean number of female worms found in 24 hour old infections per host was 12. Female worm recovery on the following days varied only slightly from this number. The lowest recovery occurred in 144 hours when the mean female burden was 9.6 worms per host. Otherwise, the number of worms did not decline until after 168 hours when no more females were found. Fewer male worms were recovered compared to females, the ratio of females to males (F:M) ranging between 2.26 and 42.4. The highest F:M occurred when most of the males had left

TABLE I. The number and size of worms recovered up to 192 hours post-infection (Dose = 100 eggs; No. of rats used per day = 3).

PERIOD POST-INFECTION (HRS)	MEAN NUMBER OF WORMS RECOVERED			MEAN LENGTH OF WORMS (mm.)	
	Female	Male	FMR		
24	12	5.3	2.26	0.44	not measured
48	14.6	2.3	6.34	0.73	.50
72	13.3	3.3	4.03	1.21	.35
96	14.00	0.33	42.42	2.05	not measured
120	13	1.00	13.00	2.50	1.30
144	9.6	0.33	2.90	2.84	not measured
168	12	0.33	36.36	3.44	1.30
192	0	0	0		

the rat in 96 hours and at the end of their life cycle. In contrast to female worms, the number of males declined in infections older than 24 hours. After 24, 48 and 72 hours, the mean number of male worms per host was 5.3, 2.3 and 3.3 respectively. In 96 hour infections, only one male worm was recovered from the 3 autopsied rats, thus indicating most of the males were lost 72 hours after the life cycle began. No males were found in 192 hour infections.

The size of the 24-hour female was 0.44 mm. At the end of its life-cycle in 168 hours, the average size of a female worm was 3.44 mm. Its growth (Fig. 27) shown by the daily increase in length, was similar to that reported by Stahl (1963). Growth was continuous and smooth and was not interrupted between periods of moulting. Twenty-four hour old male worms were not measured, being lost during transfer to a slide. The 48-hour male at 0.59 mm. length was slightly smaller than the female, suggesting growth rate was similar for both male and female, worms up to that time. The largest male was 1.30 mm. long but only half the size of the fully grown, egg-depositing female.

DISCUSSION

The foregoing study reports several features in the development of S. muris for the first time. These include moulting, changes in the cuticle, the appearance of several structures at varying developmental times, and the numbers of worms established at 24-hour intervals from an initial dose of 100 eggs.

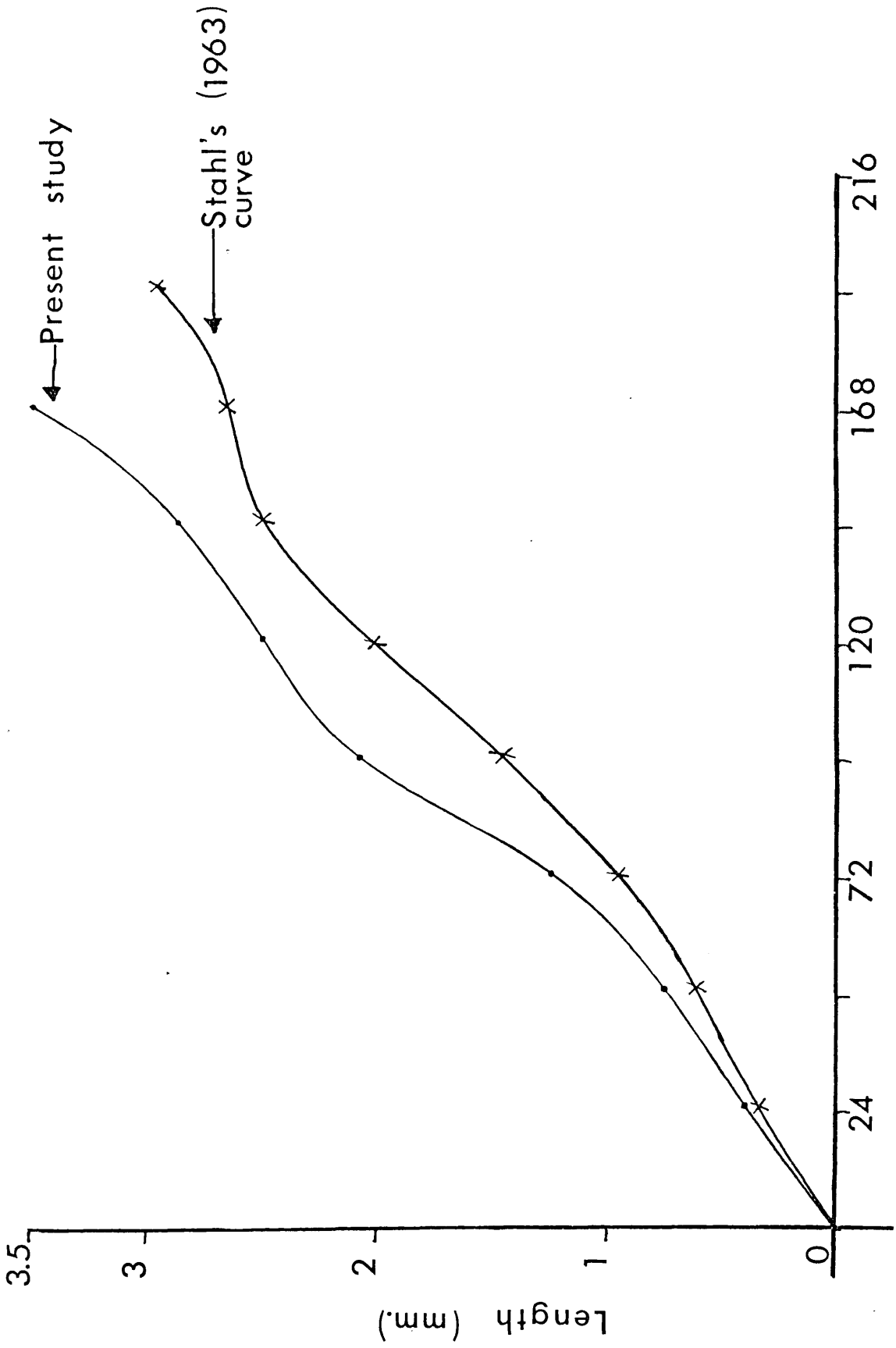


Fig. 27. THE GROWTH CURVE FOR S. MURIS

Syphacia muris undergoes 4 moults during the completion of its transmission cycle. In this respect it is no different from other nematodes. However, dissiliarities occur in the way moulting takes place. The first moult occurs within the egg. Chitwood (vide Otto, 1966) on the other hand found that two moults took place within the egg of the oxyuroid worm Enterobius vermicularis. The rest of the moults in S. muris occur in the caecum of the host at approximately 20. 40. 72 hours post-infection. The shedding cuticle which Stahl (1963) observed in 24-hour old worms was no doubt the second moult. While the 20 and 40-hour moults can be observed under the light microscope, the 72-hour moult is not so obvious. Electron micrographs, however, indicate that a new cuticle grows under the old, thus suggesting that the old cuticle is perhaps never shed and is in fact resorbed by the worm. Resorption of the cuticle has been cited in Trichinella spiralis (Lee, 1966), Ascaris lumbricoides (Thust, 1966), and Haemonchus contortus retains its cuticle after the second moult (Cheng, 1973). Cuticle resorption is, however, rare in animal parasitic nematodes (Bird, 1971).

Changes take place in the cuticle of the moulting Syphacia muris. The cortical layer grows only slightly in thickness. The median layer, however, grows considerably. The basal layer adds two bands of fibres to its initial one band as the worm completes its third moult. In males, the outermost band of fibres crosses the median layer to lie beneath the cortex perhaps to give support to the body of the worm, similar to the case where striations found in the median layer of Syphacia obvelata are believed to give support to it (Dick & Wright, 1973). The surface of the cuticle of S. muris is annulated and wrinkled in pre-adult stages. In gravid females, the wrinkles disappear and annuli become stretched. This situation no doubt aids the accommodation of the uteri which gradually become filled with eggs.

The mouth undergoes remarkable changes during the development of the worm. A mouth opening and teeth are present at birth. Tri-radiate lips, 3 teeth, 4 papillae and amphids become prominent by 72 hours of development. Two spongy areas appear near the head region laterally. These may function as chemotactic or absorptive organs.

Most of the male worms disappear after 72 hours in contrast to 96 hours as previously reported by Stahl (1963). Females begin developing eggs after 96 hours. The rest of the life cycle of the female is taken up by the development of the embryos within these eggs.

The present study suggests that S. muris in this laboratory has a 7-day life cycle. Organs such as the cephalic alae, the vaginal plug, reproductive organs, appear one day earlier in S. muris under study compared to that of Stahl (1963) and this might be due to differences in the strain of the worm or of the host.

The growth curve compares favourably with that drawn by Stahl (1963). The curve shows a continuous growth for S. muris and does not follow the 4 steps of the hypothetical curve for nematode growth suggested by Rogers (1962) and Lee & Atkinson (1976). Unlike the hypothetical curve which assumes interruptions in growth to occur between moults, the curve for S. muris is smooth at 20, 40 and 72 hours. But an accelerated growth occurs between 72 and 96 hours, and 144-168 hours during the course of maturation.

The numbers of S. muris established from an initial dose of 100 eggs over a period of 168 hours (Table I) suggests that establishment is never 100%. Between 9.6 and 14.6 female worms become established out of 100 eggs. But this number remains more or less constant from the day worms first become established to the time the life cycle is completed. Most males are, however, lost after

72 hours of development. Stahl (1963) noted that males were lost in 96 hours post-infection, having inseminated the females. While the loss of male worms at the end of either 72 or 96 hours can thus be explained, it is not known why a large number of males and females fail to establish in the host. It is quite possible that the loss of worms may be due to a number of reasons, namely, larvae may not all hatch because these are not fully embryonated or they may be mechanically displaced from the caecum in the faeces or it is probable eggs may be removed from the alimentary canal. It is, however, certain that S. muris lays many more eggs than is necessary in order to ensure a small percentage of establishment of worms. The large numbers of eggs found in each gravid female suggests only a few worms are necessary to lay sufficient numbers of eggs on the perianal region for transmission to continue.

CHAPTER IV

PERIODICITY OF EGG DEPOSITION

It was shown in the last chapter that each gravid female Syphacia muris contained 425 - 542 eggs all of which were deposited on the perianal region of the host by worms that had migrated there on completion of the life cycle in the day time. Van der Gulden (1966) showed that in excess of 1,800 eggs (155-3,955 range) were deposited per rat host over a 24-hour period. The eggs were laid on a rhythmic pattern, most of the deposition taking place the day time with a peak occurring around noon. When the lighting regime in the animal house was reversed so as to turn day into night, S. muris followed a reversed pattern of egg deposition and laid eggs when the host was facing the lit up situation.

No confirmation of Van der Gulden's (op. cit.) results exist in the literature. However, Lewis & Shava (1977) reported that a diurnal pattern of egg deposition was followed by S. obvelata in mice. Most of the eggs were laid in the day time with a peak of deposition taking place at noon. Like S. muris, it continued to lay eggs in the lit-up period when lighting conditions were reversed. In contrast, Enterobius vermicularis laid its eggs at night (MacArthur, 1930) as did Aspiculuris tetraptera (Phillipson, 1974).

Previous reviews by Hawking (1967; 1975) showed that the circadian rhythm which is followed by some microfilariae, was linked to the hosts' period of activity or inactivity. Wuchereria bancrofti, Brugia malayi and Dirofilaria immitis appeared in the peripheral blood of their hosts at night when hosts were at rest. However, it was at this time that mosquito vectors were active and feeding. Hence the transfer of microfilariae was facilitated by the nocturnal feeding behaviour of the vector. In contrast, Loa loa microfilariae

appeared in the host blood in the day time simply because its insect vector Chrysops spp. fed actively in the day time when it was thus able to be transmitted to a fresh host. The appearances of the worms, during the night or during the day, in the blood was, therefore, an adaptation to facilitate transmission. The cue for migration of W. bancrofti, B. malayi, D. immitis came from a lowered oxygen tension in the blood at night while an increase during the day time induced Loa loa migration (Hawking, 1967; 1975). In the case of pinworms migrations, Hawking suggested lowered rectal temperature may be responsible.

From these considerations, it occurred that S. muris egg deposition may be linked to the host's activity (Lewis & D'Silva, 1980). Rats were nocturnal animals, and fed and defaecated mostly at night (Richter, 1922; Barnett, 1975; Morimoto & Yamamura, 1979). Therefore, it was likely that S. muris found it most convenient to deposit eggs in the day time when its hosts were least active so as to avoid them being passed out in the host faeces at night. The observations by Van der Gulden (1967) and Lewis & Shava (1977) that egg deposition occurred in the lighted period when the day-night situation was reversed may possibly be explained by the altered feeding pattern of the hosts. As soon as the day-night situation was reversed, the rodent hosts switched feeding habits so that feeding still occurred when the animal was in darkness.

In order to pursue this line of reasoning, first Van der Gulden's (1967) experiments were repeated. Next, host activity and rectal temperature were recorded under normal daylight and night conditions to ascertain whether these were related to S. muris egg deposition rhythm.

Thereafter, host feeding activity was forcibly altered in infected rats to note if this affected the pattern of egg deposition.

MATERIALS AND METHODS

1. Recording S. muris egg deposition in rats:

Two experiments were carried out initially to verify the circadian rhythm established by Van der Gulden (1967) for S. muris in rats.

(a) Thirteen male and 13 female Sprague-Dawley rats, weaned on the 21st day after birth, were sampled with Sellotape and found to be infected. The infection was cleared by dosing all the animals with .003% mebendazole in 1% Tween 80 for one week. After allowing 3 days for the residual effects of the anthelmintic to subside, each rat was dosed with 100 eggs from S. muris and caged individually. The infection was allowed to build up for 24 days. All rats were maintained on a 6 a.m. to 6 p.m. lighting regime and allowed to feed ad libitum on Diet 86 rat pellets.

The perianal region was sampled using a 1" x 1" Sellotape every 2 hours for 24 hours. Each piece of Sellotape was pressed on the perianal region 5 times to remove the eggs deposited thereon. The Sellotape was mounted on a 3" x 1" glass slide which had been etched with grids 1 mm. x 1 mm. in size to facilitate counting the eggs. Sampling was carried out during the night under bare minimum illumination in the animal house.

A 40-watt anglepoise lamp was shaded off and kept at a distance from the rats while they were being sampled. Eggs on the Sellotape and slide were counted under a Watson stereomicroscope.

(b) This experiment was designed to record the egg deposition of S. muris when the light was reversed in the animal house. Six male and seven female rats which had been cleared of an infection immediately after weaning by the method above were each dosed with 100 S. muris eggs. The rats were individually caged and allowed to feed ad lib. The lighting regime was changed to 6 p.m. - 6 a.m. in the animal house and the rats allowed to remain under these conditions for 24 days. The perianal region was sampled on the 24th day every two hours for 24 hours using Sellotape.

2. Recording host activity:

The activity of hosts was recorded by using an activity sampler (Fig. 28) designed by Lewis & Rentmore (1968, 1979). The sampler consisted of a wire cage suspended by a light steel frame on top of a free-running roll of PVC sheet and a 9-channel activity recorder. Eight of the channels were attached to the sides of the finely balanced cage. The 9th channel led to a food hopper. All the channels were fitted with touch sensitive probes. Animal movement within the cage triggered the probes which scored marks on a recorder paper according to the channels that were touched. Food was provided in a hopper, and water out of a bottle attached to the top of the cage. The probe in the food hopper recorded feeding activity when the animal consumed any food. The recorder could be left on to run for continuous periods of up to 72 hours.

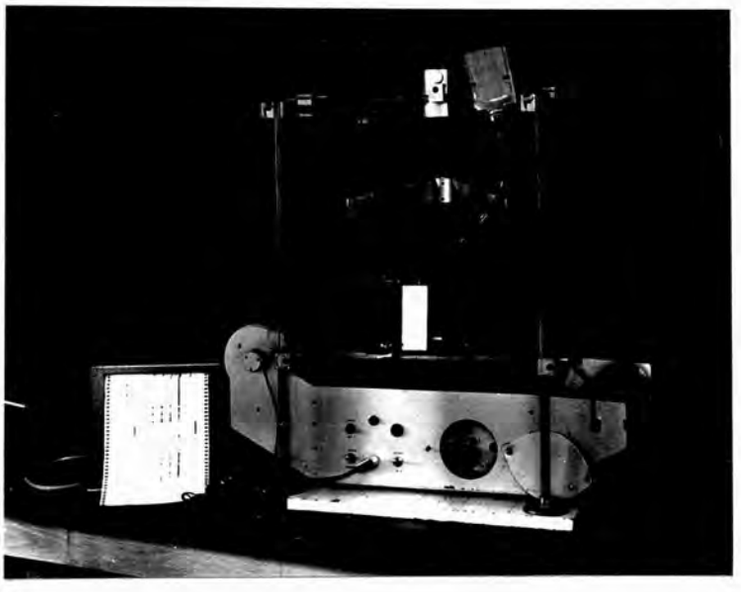


Fig. 28. The Lewis-Rentmore Activity Sampler.

faecal pellets dropped through the cage floor to the PVC sheet below. The PVC was attached to a steel shaft at one end, and a revolving aluminium drum at the other. The sheet was then wound from the shaft to the drum automatically on a pre-determined time scale which was set by a timer switch attached to the base of the sampler. The PVC roll was returned to the shaft by throwing a reverse switch. In the experiments below, the timer switch was set so as to allow the PVC sheet to roll every two hours. Thus a faecal deposit was collected every two hours.

Three male and 3 female Sprague-Dawley rats, each 3 weeks old, were used to record host activity. A rat was placed each time in the activity cage in the evening and provided with food and water. It was allowed to acclimatize itself until morning when the recording channels were switched on. The rat was left in the cage for another 24 hours before it was removed. The lighting regime was maintained on a 6 a.m. to 6 p.m. light/dark cycle.

By applying the reverse switch at the end of the experiment the PVC roll was returned to the shaft. Each automatic roll back constituted a faecal deposit for 2 hours, starting with the last deposit first, the penultimate second, and so on. The feeding and movement activity of the rat was simultaneously obtained from the scores marked on the 9 channels of the recorder paper.

3. Egg deposition of S. muris and host/feeding activity:

The following experiments were an attempt to correlate the egg deposition pattern of S. muris with the feeding activity of the host.

(a) Six male and 6 female Sprague-Dawley rats weaned on Day 21 after birth were cleared of an infection as described above. Rats were each dosed with 100 eggs, and the infection allowed to build up for 16 days. Rats were fed ad lib and maintained on a 6 a.m. - 6 p.m. light/dark cycle. Animals were sampled for eggs deposited on the perianal region every two hours for 24 hours so as to ascertain if the normal circadian rhythm of deposition was present. At the same time, rectal temperature of each animal was recorded using a Grant thermistor Model S. Every effort was made to minimise the effect of handling on the body temperature. Rats were sampled as quickly as possible. During night sampling, they were kept at a distance from a shaded 40-watt table lamp.

(b) The same rats were fed and watered from 8:30 a.m. to 5 p.m. for 10 days. The lighting regime was maintained from 5 a.m. to 5 p.m. Each day, for 10 days, the amount of food, the volume of water and the weight of the rats was recorded to make certain rats were feeding and in good health.

(c) Six male and 8 female Wistar rats were removed from the isolator on Day 21 after birth. Each animal was housed individually, and given food and water between 8 a.m. and 8 p.m. only when lights were left on in the animal house. One week later, each animal was infected with 100 S. muris eggs. The patency of the infection was checked 7 days later by smearing of the perianal region of each animal with Sellotape. The infection was allowed to build up for a further 3 week period after which the rats were sampled for eggs deposited on the perianal region every two hours over a 24 hour period.

(d) Approximately 1000 eggs of S. muris were collected by the rice smear technique, washed thoroughly in water and swallowed by the author on 3 separate occasions in order to observe whether or not man was susceptible to infection with S. muris and if so to determine whether the timing of egg deposition would be influenced by man's feeding and resting habits. On the 7th day post-infection, the perianal area was sampled with Sellotape attached to a glass slide. The slide was examined under a stereomicroscope.

RESULTS

1. Egg deposition in rats:

(a) Of the 26 rats infected, 1 male and 1 female did not appear to harbour any worms at the end of 24 days of infection as no S. muris eggs were recovered from the perianal region during the 24-hour sampling period. The numbers of eggs deposited per day ranged from 75 to 6643 in the 12 male rats and 2 to 1844 in 12 females (Table II). The total number of eggs deposited in the male rats amounted to 19,970, making an average oviposition of 1,536.15 eggs per rat per day. In female rats, 6723 eggs were deposited in all and the mean per rat was 560.25. Thus, significantly (Student's t-test, $p = .05$) more eggs were collected from male than from female rats.

Egg deposition occurred primarily in the day time (Fig. 29 (a), Table II). It began to rise at 0600 hours in both male and female rats. Of the total number of 26,693 eggs deposited in male and female rats, 23.53% were laid at 0800 hours. But oviposition peaked at 12 noon when 31.73% of the total eggs were laid. Thereafter, egg production declined.

TABLE II

The number of eggs deposited by S. muris in male and female rats subjected to a 6 a.m. - 6 p.m. lighting period.

TIME (HOURS)	MALES	FEMALES	TOTAL	%
0200	75	2	77	.288
0400	46	10	56	.209
0600	408	700	1108	4.150
0800	5318	964	6282	23.534
1000	1931	1488	3419	12.806
1200	6643	1844	8487	31.794
1400	1298	509	1797	7.918
1600	583	381	964	3.611
1800	647	128	775	2.903
2000	1869	252	2121	7.945
2200	612	159	770	2.884
2400	550	287	837	3.135
Grand Total	19970	6723	26693	

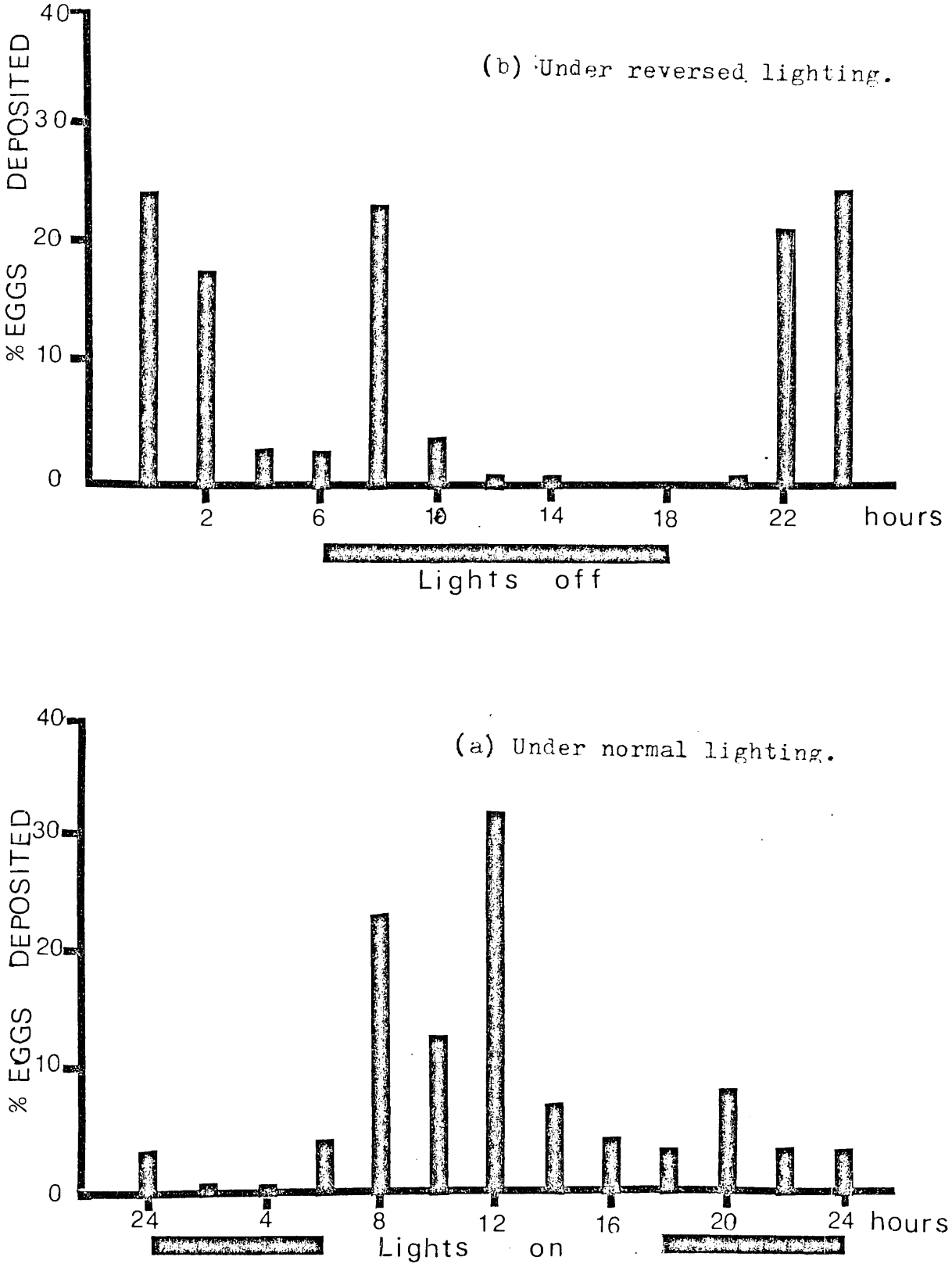


Fig. 29. The pattern of oviposition by Syphacia muris worms when the hosts are subjected to (a) normal, and, (b) reversed lighting in the animal house.

However, a small peak appeared at 2000 hours. From then on fewer eggs were recovered from the perianal region until the lights in the animal house were turned on at 6 a.m. These results confirm the existence of a diurnal rhythm of egg deposition in S. mansoni which was first reported by Van der Gulden (1967) and form the basis of comparing the results of experiments described below. The numbers of eggs collected in the present sample are, however, small compared to that shown by Van der Gulden (1967). It is very likely this was due to the insufficient period of time which was allowed for the infection to build up. The worm-free rats in this study were allowed to build up an infection for 24 days whereas Van der Gulden sampled naturally infected rats.

(b) The eggs deposited on rats subjected to a reversal of the lighting period is presented in Fig. 22 (b), Table III. As expected, with reference to Van der Gulden's (1967) results, most of the egg deposition occurred between 1800 and 0600 hours when rats were facing a lit up environment in the animal house. Egg deposition rose to 24.87% of the total number laid at 2400 hours. Little or no egg deposition occurred during the dark period which began at 0600 hours, although there was a peak at 0700 hours when 23.56% eggs were laid. Between 0800 hours and 2000 hours, egg deposition was minimal. Thus reversing the lighting period had the effect of reversing the circadian rhythm which was demonstrated on a 6 a.m. to 6 p.m. light/dark cycle in Experiment 1 (a) above. As before, more eggs were recovered from male than female rats.

TABLE III

The number of eggs deposited by S. muris when male and female rats are subjected to a reversed lighting regime between 6 p.m. and 6 a.m.

TIME (HOURS)	MALES	FEMALES	TOTAL	%
0200	1943	858	2801	17.97
0400	318	132	450	2.88
0600	408	41	449	2.88
0800	2622	1050	3672	23.56
1000	464	87	571	3.66
1200	119	25	144	.92
1400	80	10	90	.57
1600	12	3	15	.09
1800	24	0	24	.15
2000	76	4	80	.51
2200	2940	470	3410	21.88
2400	3869	8	3877	24.87
Grand Total	12895	2688	15583	99.94

2. Host feeding activity and egg deposition of S. muris:

The feeding activity and time of defaecation of 3 male and 3 female rats are indicated in Table IV, along with the normal egg deposition rhythm of 6 male and 6 female rats from Experiment 3(a). It is quite evident that most of the feeding activity occured in the dark period after 1900 hours, although it began to an extent at 1600 hours in the male rats. After 0600 hours, feeding activity was reduced in male and females. Rats began to defecaete at 1600 hours and continued to do so mostly at night. When considered along with the egg deposition pattern which began at 0800 hours and subsided at 1600 hours after peaking at noon, it will be seen that feeding and defaecation are synchronised to egg deposition behaviour.

3. It has been shown in Table IV that 6 male and 6 female rats used in Experiment 3(a) demonstrated that S. muris followed a normal circadian rhythm of egg deposition. When these rats were forced to feed between 5:30 a.m. to 5:00 p.m. for 10 days, the pattern of egg deposition changed.

(a) The circadian rhythm of egg deposition by S. muris in 6 male and 6 female rats is shown in Fig. 30 (a). As already shown in Table IV, egg deposition began at 0800 hours, peaked at noon and declined thereafter. Little or no deposition occurred at night. Rectal temperatures recorded over a 24-hour period ((Fig. 30 (b)) showed oscillation between 30°C and 34°C. There was no apparent diurnal rhythm in the temperature. Females showed a lowered rectal temperature compared to males.

TABLE IV

The percentage of Syphacia muris eggs deposited every two hours in male and female rats exhibiting a normal feeding and defaecation period. (+ denotes faeces present).

Time of day (Hours)	% eggs deposited/rat		% feeding activity /rat/hour		Timing of defaecation
	Male	Female	Male	Female	
0200	0	0	13.2	21.6	+
0400	0	0	8.4	2.1	+
0600	0	0.1	0	4.6	+
0800	9.8	21.6	1.3	0	-
1000	12.9	26.0	2.4	1.8	-
1200(noon)	47.0	28.1	0	0	-
1400	27.0	9.8	0.2	0	-
1600	2.7	11.0	14.0	0	+
1800	0.6	3.4	9.4	15.6	+
2000	0	0	11.8	13.2	+
2200	0	0	13.1	18.2	-
2400 (midnight)	0	0	4.2	5.2	-

Fig. 30. (a) The pattern of egg deposition by Syphacia muris worms when rats are subjected to normal lighting and feeding conditions daily.

(b) The rectal temperature of female and male rats over a 24-hour period.

(c) The pattern of egg deposition by S. muris worms in rats subjected to a restricted feeding period between 8:30 a.m. and 5:00 p.m. for 10 days.

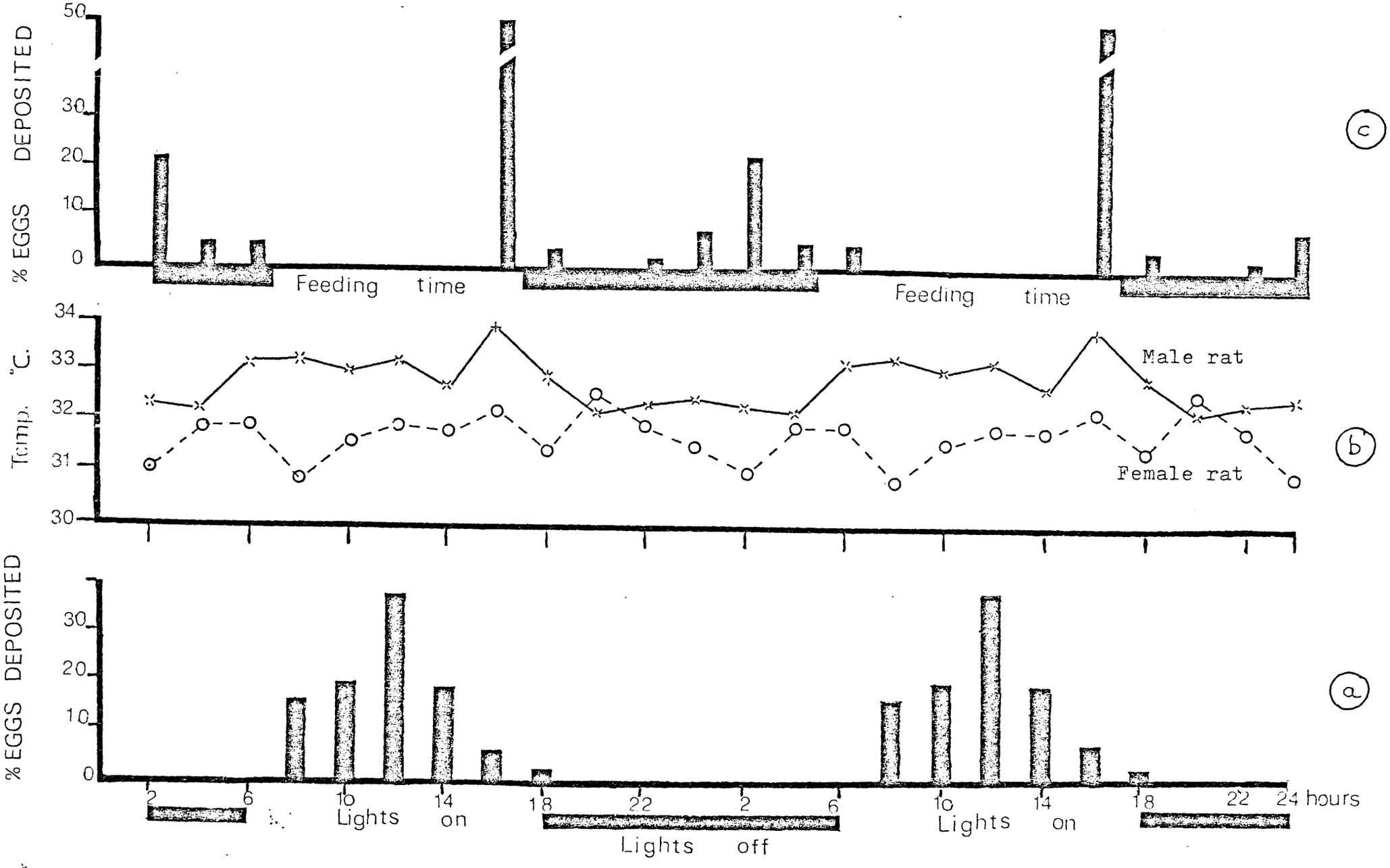

% EGGS DEPOSITED
50
30
20
10
0
Feeding time
Feeding time
c
Temp. °C.
34
33
32
31
30
Male rat
Female rat
b
% EGGS DEPOSITED
30
20
10
0
2
6
10
14
18
22
2
6
10
14
18
22
24 hours
Lights on
Lights off
Lights on
a

Fig. 30.

(b) The same animals when forced to feed abnormally between 8:30 a.m. and 5:00 p.m. for 10 days showed that the pattern of egg deposition had changed. Egg deposition peaked towards the end of the day-time feeding period at 1600 hours. However, most of the eggs were laid at night ((Fig. 30 (c)) when rats were not feeding. In contrast, little egg deposition occurred during feeding time. Furthermore, by forcing rats to feed between 8:30 and 5:00 p.m. the numbers of eggs laid by S. muris was reduced considerably (Table V).

(c) When 6 male and 8 female Wistar rats were maintained for a longer period of time on a day time feeding regime, that is between 8 a.m. and 5 p.m. for 21 days, the results of egg deposition were quite different to that reported under 3(b) above. Only 3 rats produced significant numbers of eggs over a 24-hour sampling period. Six rats did not have any eggs on the perianal region while the remaining 5 indicated there were few eggs. These results can only be taken to mean that S. muris did not establish itself in most of the rats, perhaps due to inimical conditions within the host gut which was brought on by an abnormal feeding regime. The egg deposition recorded for 8 rats, however, revealed that it occurred mainly at night between 5 p.m. and 8 a.m. when the rats were not feeding (Table VI). Although some deposition occurred between 8 a.m. and 5 p.m., this was not as much as that which occurred during the night. Peak deposition occurred at 0600 hours, two hours before rats resumed feeding.

TABLE V

The number of eggs deposited every 2 hours in male and female rats subjected to an abnormal feeding period for 10 days.

TIME (HOURS)	MALES	FEMALES	TOTAL	%
0200	1505	781	2286	23.21
0400	474	27	501	5.08
0600	501	0	501	5.08
0800	31	0	31	.31
1000	3	0	3	.03
1200	2	0	2	.02
1400	0	0	0	0
1600	3121	1900	5021	50.99
1800	257	195	452	4.59
2000	42	9	51	.51
2200	0	211	211	2.14
2400	485	303	788	8.00
Grand Total	6421	3426	9847	99.96

TABLE VI

The number of eggs deposited every two hours in male and female rats subjected to an abnormal feeding period for 21 days.

TIME (HOURS)	MALES	FEMALES	TOTAL	%
0200	91	1005	1096	10.92
0400	703	700	1403	13.99
0600	1543	992	2535	25.26
0800	797	272	1069	10.65
1000	1373	286	1659	16.53
1200	705	0	705	7.02
1400	191	0	191	1.90
1600	0	0	0	0
1800	26	0	26	.26
2000	318	513	822	8.19
2200	219	0	219	2.18
2400	311	0	311	3.10

(d) In self-infection studies, S. muris appeared on the perianal region after 7 p.m. It was accompanied by itching of the perineum similar to that experienced by the author during infections with Enterobius vermicularis as a child. The infections became patent on each occassion on the 7th day post infection, and disappeared after two days. Thereafter no further infections were detected.

DISCUSSION

There is evidence from the foregoing study that the circadian rhythm of deposition by S. muris as first reported by Van der Gulden (1967) is linked to the activity of the host. The worm migrates to deposit eggs on the perianal region during the day time when its host is at rest. It avoids egg deposition at night in all liklehood to prevent eggs from being passed out in the faeces. That the rat host is feeding actively at night and also defaecating is shown by the results of the host activity experiment. It may be observed that both the worm and its host undergo two separate circadian rhythms, one of depositing eggs, the other of feeding and defaecating. The circadian rhythm of the worm is synchronized with that of the host and operates on a pattern designed to avoid the loss of eggs.

Evidence is presented for the feeding and defaecating activity of the rat at night and this is supported by the studies of Richter (1922, 1927); Teitelbaum & Campbell (1958); Bare (1959); who showed that the feeding habit of the rat is confined to the dark period of the 24-hour day.

Margules et al. (1972) reported that laboratory rats consume 80% of their food at night. But originally it was Richter (op. cit.) who showed that laboratory rats followed a rhythm of active and non-active periods. Activity, such as feeding, defaecation and running was limited to the night while resting behaviour was confined to the day time.

When the feeding habit of the rat is experimentally restricted to the day time, the deposition of eggs by S. muris occurs mainly at night. This indicates egg deposition is linked to the feeding habits of the host. It is possible that S. muris females receive a signal to migrate from the digestive processes that appear with the onset of host feeding. It is likely that during feeding activity muscle contraction and flow of digestive juices (Richter, 1927) stimulates the female S. muris to leave the host. Further indication of the link between feeding and defaecating and resting behaviour of the host to S. muris migration is seen from the poor adaptation in the host which the worm experiences when the rat is put on a restricted feeding pattern. The results show that a restricted feeding pattern lowers the numbers of eggs deposited at night and this may be the result of fewer females migrating to the perianal area. Fewer females may become established in the host due to the host being subjected to a restricted feeding pattern. Indeed, data indicates that fewer rats harbour an infection when rats are kept on a limited feeding schedule for a prolonged period. Loss of worms could occur in the faeces during the night and day because rats defaecate at both times when put on a restricted feeding schedule. The worms, unless adapted to the

feeding regime of the host, would have passed out in the faeces in the day time. They may also have been removed at night because of the nocturnal defaecating behaviour of the rats. For, although the feeding behaviour may have been restricted to the day time, rats also actively defaecate at night.

The final indication that egg laying behaviour is interlinked comes from the ability of S. muris to adjust to the periods of activity and rest in man. In self-infections carried out by the author, S. muris always appeared on the perianal region towards the evening which was the normal time of rest. The itching behaviour felt on the perineum being similar to that felt when infected with the human pinworm shows that S. muris and S. vermicularis are more alike in their transmission strategies than has been realised.

Although Hawking (1975) suggested that S. muris migration could have been stimulated by a lowering of the rectal temperature of the host, this does not appear to be likely. As shown in this study, rectal temperature oscillates throughout a 24-hour period and does not show a day/night periodic rhythm, perhaps due to handling during the sampling period.

It is concluded that the circadian rhythm of egg deposition followed by S. muris is linked with the host's feeding and defaecation activities and favours its transmission. By laying eggs during the day S. muris avoids being lost in the faeces at night. This allows eggs a period of incubation during the day time after which it is ready to be swallowed during grooming. In its strategy for transmission, S. muris is no different from those other worms which exhibit circadian rhythms and which depend upon host behaviour to continue the process of infection.

CHAPTER V

WORM ESTABLISHMENT

In view of the large numbers of eggs deposited by Syphacia muris on its host's perianal region and the certainty of the contact between these eggs and the host, many eggs may be presumed to be ingested by the rat every day. Van der Gulden (1966) estimated that 90% of the eggs deposited around the perianal region were swallowed at night, most certainly in the act of grooming. Experiments were therefore, desirable to ascertain whether or not the numbers of S. muris that became established in the host were related to the number of eggs ingested, the time of the day and age of the host. Also the number of eggs ingested might influence the size and fecundity of worms subsequently established.

It is a generally held belief that density dependence factors influence the establishment of the numbers of worms in a host system. Dorman (1928) found that as the size of the infective dose of Heterakis papillosa was increased, the numbers of worms that became established in chickens decreased. The work of Ackert et al. (1931) with Ascaridia lineata in chickens produced similar results as did that of Winfield (1935) with Heterakis spumosa in rats. Nippostrongylus brasiliensis establishment in rats also decreases with increasing larval dose (Haley & Parker, 1961). Recently Michel (1969) showed that as the Ostertagia ostertagi infection dose was increased in calves, so did the numbers of worms recovered post infection decrease. Both Ackert et al. (1931) and Winfield (1935) reported respectively that with increasing dose size of larvae administered, the lengths and fecundity of A. lineata and H. spumosa decreased. Michel (1969) made comparisons of the egg output by Ostertagia ostertagi in calves receiving a

light and heavy infection and found that egg production decreased in hosts that were given a heavy infective dose. The work of Michel, Lancaster & Hong (1977) showed that the length of O. ostertagi was inversely related to the initial worm burden. D'Silva & Lewis (1979) and Lewis & D'Silva (1980) indicated that the time of the day eggs were laid by S. muris and subsequently ingested by the rat host would affect worm establishment in the host.

On the effect of host age on worm establishment, it is generally regarded that infection decreases with increasing age. Notably, Enterobius vermicularis infection is found in children (Cheng, 1973). Aspiculuris tetraptera recovery from mice (Mathies,1959a) and Syphacia obvelata from the same host (Panter, 1969) decline with increasing host age. However, data from Blair & Thompson (1969), Singhvi & Johnson (1977), Ashour (1980) indicate that S. muris numbers increase with host age.

Against this background of information experiments were carried out with S. muris to determine its establishment in the rat host.

EXPERIMENTAL DESIGN & MATERIALS AND METHODS

The two doses of eggs used in this study were 100 and 1000, the eggs being collected at 10 a.m. and 2 p.m. The rats dosed with the eggs were divided into 3 age groups: 3-, 7- and 10- weeks old. Each age group of rats was given a dose of eggs at the time a collection was made, that is, at 10 a.m. and 2 p.m. The 10 a.m. dose was also administered to rats maintained on a dark/light cycle during the day. This was designed to simulate night time conditions and to test the effect of darkness on the establishment of worms. The over-all experimental pattern was as follows:

Size of Dose (Eggs)	Time of egg collection (Hours)	Time of infection (Hours)	Age of Rats (Weeks)
100/1000	10:00	10:00	3
			7
			10
	14:00	14:00	3
			7
			10
	10:00	Darkness	3
			7
			10

Eggs for infection were collected on rice covered glass slides from the perianal region of rats previously infected and maintained on a 6 a.m. - 6 p.m. light/dark cycle. The slides were immersed in 0.65% saline in petri dishes and kept warm on a hot plate at 31°C until needed. Eggs were individually counted under a stereo dissecting microscope and the requisite amount was picked up in a syringe with a blunted 25 gm. hypodermic needle. The

syringe was filled with tap water to dilute the saline in it to prevent rats from voiding the solution. Eggs were then delivered to the rat by oral intubation. No more than a millilitre of liquid was used to suspend the delivered eggs. Immediately after delivery, an additional millilitre of water was delivered to the rat oesophagus to ensure all eggs in the syringe were washed down. The syringe and hypodermic needle were also washed immediately afterwards and the washings examined under a microscope to ascertain that all the eggs had been delivered.

The rats were divided into 3 age groups of 3, 7 and 10 weeks. The rationale behind this was to determine worm establishment in pre-pubertal, pubertal and post-pubertal rats. Breeding experiments had earlier shown that rats in this laboratory were capable of reproducing litters at 7 weeks after birth. Five male and 5 female rats were used for each experimental infection, all of the 10 rats coming from the same litter. This was done in order to minimise genetic differences between host. Whenever a litter did not bear such numbers of animals of the two sexes, rats were added from a second litter so as to make up the difference. Rats were killed 72 hours after infection in order to ensure the collection of male worms which leave the host after that time. Rats were killed with carbon monoxide gas or chloroform vapour. When these were not available, rats were killed by a sharp blow to the head. Immediately after autopsy, worms were gathered from the caecum by the Baermann technique as previously described in Chapter II. Worms were fixed in warm 4% formalin and stored in vials for later counting.

In order to determine the effect of egg density on the length of worms, 3 week old rats were dosed with 100, 500 and 1000 eggs at 10 a.m. Three female rats were used in each experimental dose. Rats were sacrificed 72 hours post infection. Worms were collected by the Baermann technique, fixed and drawn with the aid of a draw tube fitted to a Leitz microscope.
Drawings were made on graph paper. A scale was drawn from a stage micrometer under the same magnification as the worms. A cartographer's wheel was used to measure the worms against this scale.

To determine the effect of size of dose of infection on the fecundity of S. suris, each group of 3 three-week old rats was dosed with 100, 500 and 1000 eggs collected from the perianal region at 10 a.m. Rats were killed and autopsied 7 days later. Worms were removed by the Baermann technique and fixed in 4% formalin. The gravid females were dissected under a stero dissecting microscope and the eggs from the uterus removed with a fine needle for counting.

-ooOoo-

RESULTS

The numbers of Syphacia muris that became established from a dose of 100 or 1000 eggs administered in the morning, afternoon and 'darkness' in 3 differing age groups of rats are given in Table VII (a)(b), Figs. 31, 32. The number of male and female worms that became established from 100 eggs was lower than that from 1000 eggs. In terms of actual numbers, there was a 10 fold increase in the number of worms recovered from rats receiving 1000 eggs. The mean number of worms established, for example, from 100 eggs fed at 10 a.m. to 3 week old male rats was 10.40 (8.60 female worms + 1.80 males), compared with a mean of 142.8 (84.44 females + 58.4 males) in rats dosed with 1000 eggs. There was, however, little difference in the percentage of worms that became established from the two doses. For example, in 10 week old males dosed at 10 a.m., it was evident that 33.8% worms were established from 100 eggs compared with 35.12% from a 1000 egg dose. There also appears to be no significant difference in worm establishment (mean number of worms and % 'take') if eggs were collected from rats and administered to them at 2 p.m. The mean worm count from 100 eggs fed at 2 p.m. to 3 week old rats was 12.20 whereas from a 1000 egg dose it was 157.3. However, only 6 rats harboured an infection from 100 eggs whereas all 30 rats dosed with 1000 eggs were infected.

Significantly (Student's t-test; $P = .05$) more worms became established in more hosts if eggs were collected and administered at 10 a.m. rather than at 2 p.m. The worms recovered from 100 eggs fed at 10 a.m. were 10.4, 16.4 and 33.8 in 3-, 7- and 10-week old male rats respectively and all the hosts were infected. In contrast, the 2 p.m. infection of 100 eggs resulted in mean numbers of 12.20, 0.40 and 1.4 worms becoming established in the 3 age

TABLE VII (a)

The establishment (mean worm numbers) of adult worms of Cyphacia muris in 3 age groups of rats, administered with 100 eggs in the morning, afternoon and darkness.

Size of Infective Dose (eggs)	Time of Egg Collection; Infection	Worm Sex	Mean Number of Worms® Recovered from				Age of Rats (Weeks)
			5 Males	F M R	5 Female rats	F M R	
100	10 a.m.	Female	8.60 ± 3.51	4.77	8.20 ± 3.70	6.83	3
		Male	1.80 ± .84		1.20 ± 1.30		
		Female	13.80 ± 3.70	5.30	12.80 ± 2.28	5.81	7
		Male	2.60 ± 1.52		2.20 ± 1.92		
		Female	23.60 ± 9.02	2.31	27.40 ± 3.21	2.70	10
		Male	10.20 ± 3.1		10.00 ± 1.58		
	2 p.m.	Female	7.60 ± 7.83	1.65	1.00 ± 2.24		3
		Male	4.60 ± 4.39		0		
		Female	0.40 ± .89		0		7
		Male	0		0		

® ± standard deviation

Contd. over/.

Table VII(a) cont'd.

Size of Infective Dose (Eggs)	Time of Egg Collection; Infection	Worm Sex	Mean Number of Worms⊗ Recovered from				Age of Rats (Weeks)
			5 Males	F M R	5 Female rats	F M R	
100	2.p.m.	Female	0.80 ± 1.10	1.3	.80 ± 1.79		10
		Male	0.60 ± .55		0		
	Darkness	Female	6.40 ± 2.06	2.83	6.00 ± 2.25	3.85	3
		Male	2.40 ± 1.14		1.60 ± .89		
		Female	10.60 ± 3.05	2.52	14.80 ± 8.32	3.70	7
		Male	4.20 ± 1.64		4.00 ± 2.24		
		Female	16.20 ± 5.35	2.61	18.00 ± 9.22	3.10	10
		Male	6.20 ± 3.11		5.80 ± 1.48		

⊗ ± standard deviation

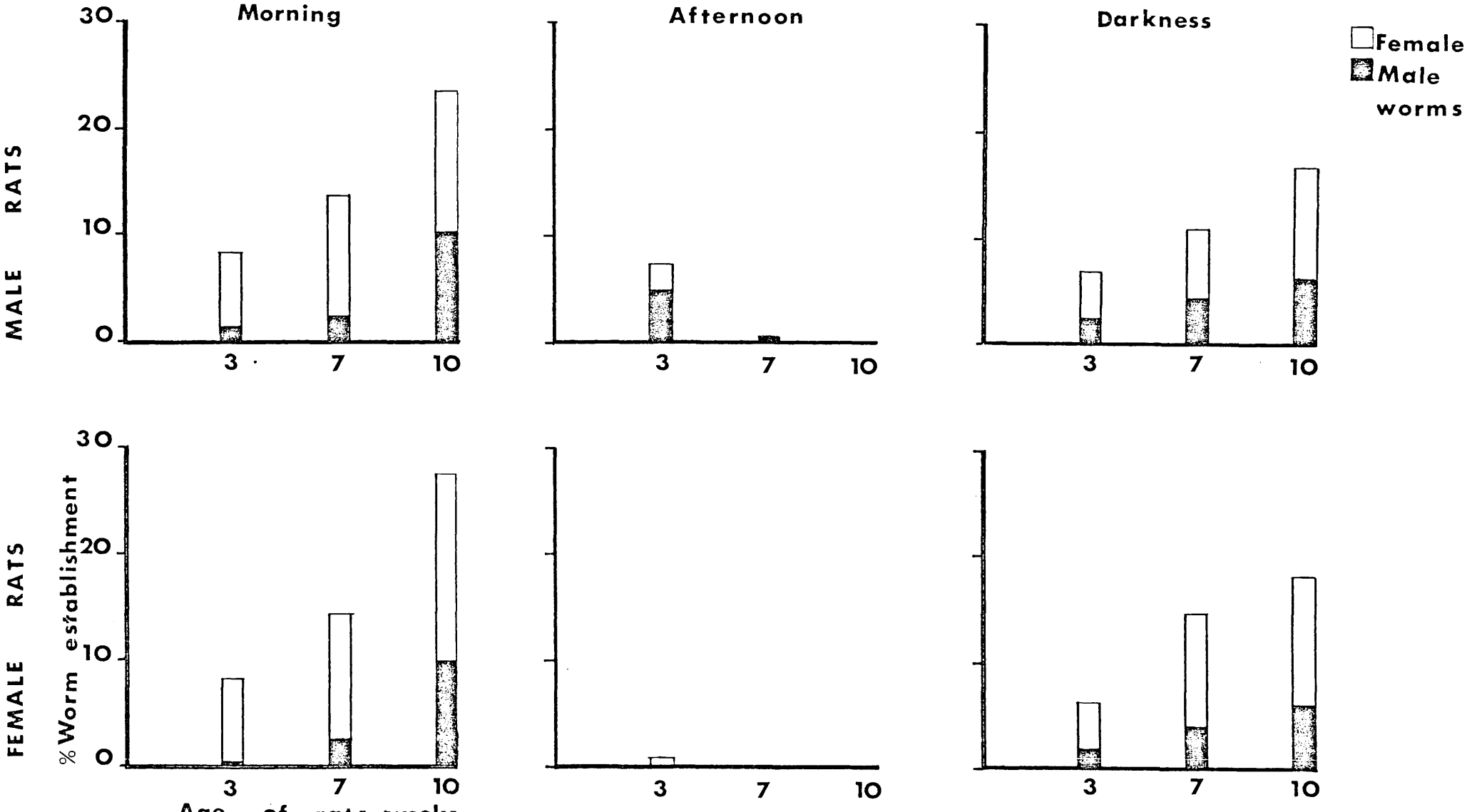


Fig. 31. The establishment (% take) of adult worms of Syphacia muris in 3 age groups of rats, each administered with 100 eggs in the morning, afternoon and in darkness.

TABLE VII(b)

The establishment of (Mean worm numbers) of adult worms of Syphacia muris in 3 age groups of rats, administered with 1000 eggs in the morning, afternoon and darkness.

Size of Dose (Eggs)	Time of Egg Collection; Infection	Sex of Worms Recovered	Mean Number of Worms⊗ Recovered from				Age of Rats (Weeks)
			5 Males	F M R	5 Female rats	F M R	
1000	10.a.m.	Female	84.40 ± 50.84	1.44	102.8 ± 62.58	1.70	3
		Male	58.40 ± 62.52		59.20 ± 66.19		
		Female	192.0 ± 47.83	1.78	177.6 ± 93.25	2.06	7
		Male	107.6 ± 36.46		86.20 ± 60.06		
		Female	262.60 ± 66.55	2.99	190.6 ± 59.24	3.17	10
		Male	88.60 ± 76.03		60.00 ± 30.23		
	2 p.m.	Female	134.6 ± 32.93	5.96	99.0 ± 39.60	2.91	3
		Male	22.6 ± 8.62		33.8 ± 16.33		
		Female	106.2 ± 62.54	1.49	63.20 ± 27.12	2.92	7
		Male	71.60 ± 41.91		33.60 ± 8.17		

⊗ ± Standard deviation

Contd. over/....

Table VII(b) contd.

Size of Dose (Eggs)	Time of Egg Collection; Infection	Sex of Worms Recovered	Mean Number of Worms⊗ Recovered from				Age of Rats (Weeks)
			5 Males	F M R	5 Female rats	F M R	
1000	2 p.m.	Female	124.8 ± 54.78	2.06	71.60 ± 6.27	1.98	10
		Male	62.20 ± 43.26		37.0 ± 4.80		
	Darkness	Female	62.40 ± 37.23	1.16	63.8 ± 34.11	1.41	3
		Male	53.2 ± 34.23		45.20 ± 21.99		
		Female	132.8 ± 43.38	1.67	94.4 ± 44.94	1.77	7
		Male	79.2 ± 32.07		51.60 ± 52.19		
		Female	266.6 ± 102.31	2.07	175.20 ± 130.69	2.54	10
		Male	96.8 ± 49.29		68.8 ± 27.76		

⊗ standard deviation

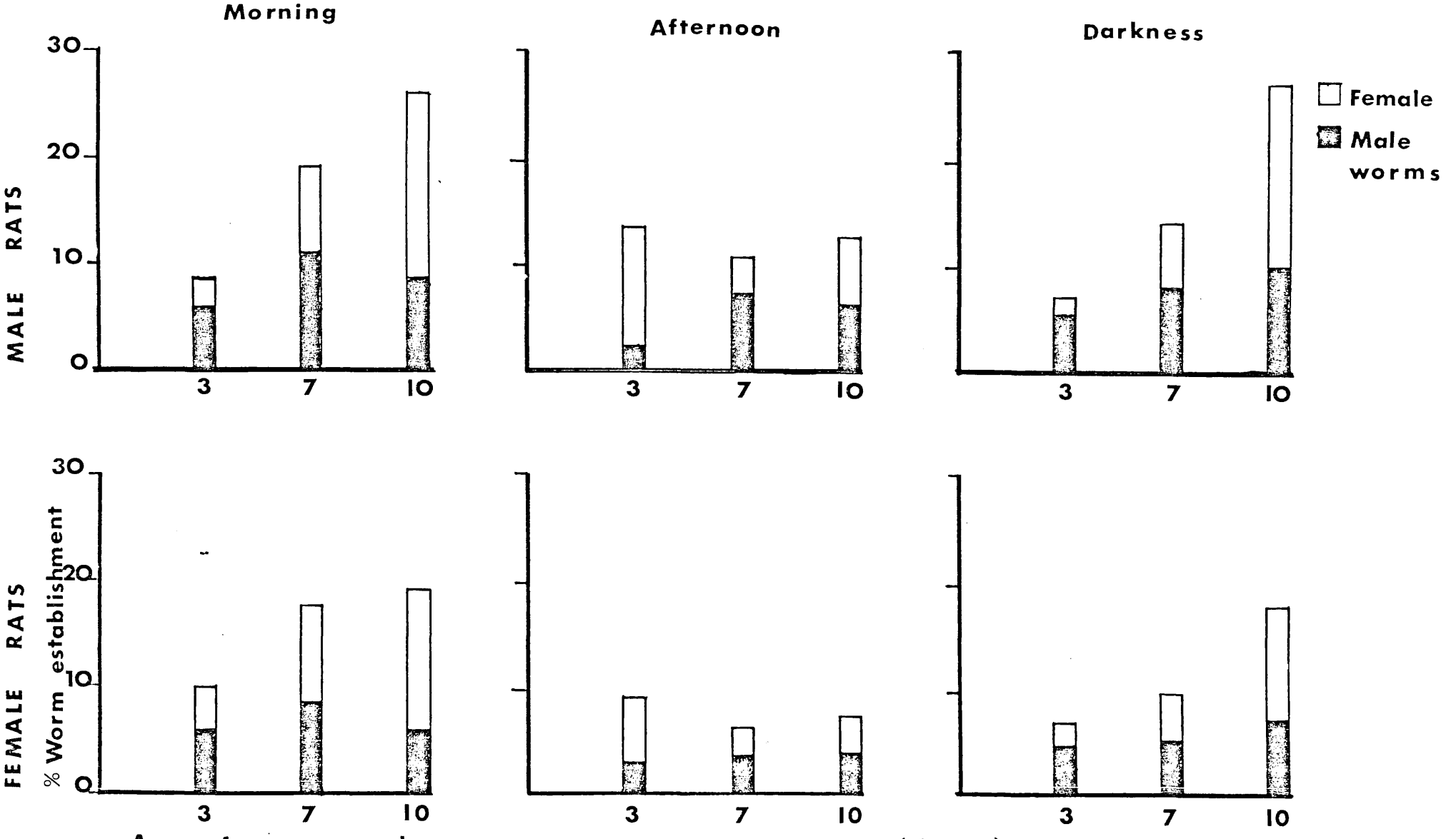


Fig. 32. The establishment (% take) of adult worms of Syphacia muris in 3 age groups of rats, each administered with 1000 eggs in the morning, afternoon and in darkness.

groups of male rats respectively. In female hosts, the figures were similar for the two time periods under consideration; fewer worms were established and fewer rats became infected from a 2 p.m. inoculation. If 1000 eggs were fed at 2 p.m., the recovery of worms was lower than if it was given at 10 a.m. The range of worms developed from the 10 a.m. dose in male rats was 142.8 to 251.2 and a range of 162.0 - 263.8 in females. Comparable numbers for the 2 p.m. infection were 157.2 - 196.0 in male rats and 96.8 - 132.8 in females.

Worm establishment appears unaffected whether eggs from the morning collection are administered in daylight or darkness in all 3 age groups of rats. The 100 eggs fed in daylight show an establishment of 10.40, 16.4 and 33.8 worms in 3, 7 and 10 week old male rats respectively. When fed to rats in the dark, the establishment is 9.8, 14.8 and 22.4 in the 3 respective age groups. From 1000 eggs given in daylight, 142.8, 296 and 351.2 worms were found in 3, 7 and 10 week males respectively. The establishment in rats in the dark was 115.6, 212.0 and 363.4. In female rats, similar patterns of establishment occurred. Therefore, the numbers of worms that are found to establish in the hosts does not appear to be influenced by the time when eggs are ingested so much as by the period these are deposited on the perianal region.

Except in two instances, the worms established appeared to be correlated with the age of the host. Whether rats were infected at 10 a.m., or in darkness, either with 100 eggs or 1000 eggs, more worms were found in older rather than in young animals. In 3, 7 and 10 week old male rats given 100 eggs at 10 a.m., the mean numbers of worms found were 10.4, 16.4 and 33.8 respectively compared with mean numbers of 9.4, 15.37 and 37.4 in female rats. The

increase was also evident in rats infected with 1000 eggs. Hence, it is suggested that for the ages considered, resistence factors do not operate to limit T. muris establishment in the rat. Only when rats were infected at 2 p.m. did worm establishment imply decrease with host age. But even this conclusion is doubtful because only a few of the total number of rats used in the 2 p.m. experiment showed a patent infection. Only 5 of 15 male rats, and 2 of 15 females were infected. Three of the males were 3 weeks old and had a mean of 12.6 female worms in them. One male was 7 weeks old and the other 10; each harboured 2 female worms only. The two female rats were 3 and 10 weeks old. Whilst only 5 female worms were recovered from the former, 4 female worms were found in the latter age group.

The size of worms recovered from an infective dose of 100 eggs ranged between 1.00 - 1.39 mm. (females) and 0.80 - 1.09 mm. (males) in 72 hours. The worms from a dose of 500 eggs varied between 0.60 - 1.49 mm (Table VIII). Most of the female worms from this group fell into size range of females from the 100 egg dose, but a few measured below 1.00 mm. With the exception of 5 males from the 500 dose falling in the 1.10 - 1.39 mm. class size, all the others ranged between 0.60 to 1.09mm. The range covered by the 6 males recovered from 100 eggs was 0.80 - 1.00 mm. In contrast, the males from 1000 eggs measured between 0.40 and 1.29 mm., with most of them below 0.70 mm. in length. It may be observed, however, that the number of worms recovered from infection doses of 100, 500 and 1000 eggs increased with size of infective dose.

TABLE VIII

The size and number of worms of *Syphacia muris* established after 72 hours in rats administered with 100, 500 and 1000 eggs.

Worm Length (mm.)	Females	Males	Females	Males	Females	Males
1.40-1.49			3	0		
1.30-1.39	3		6	2		
1.20-1.29	13		32	0	2	4
1.10-1.19	15		33	3	6	1
1.00-1.09	4	1	37	36	2	1
0.90-0.99		3	9	37	7	1
0.80-0.89		2	15	23	14	10
0.70-0.79			4	3	9	27
0.60-0.69				1	12	5
0.50-0.59					11	12
0.40-0.49					1	4
No. of worms measured	35	6	139	104	71	76
No. worms Recovered	35	6	182	123	401	139
% worm recovery	11.6	2	12.13	8.6	13.36	6.63
Initial Dose per rat (eggs)	100		500		1000	

The egg-bearing capacity of a female S. muris worm was not correlated with the size of an infective dose as shown by Table IX and the number of eggs to be found per gravid female varied. The mean number of eggs collected was 408.93 (350 - 489 range), 426.25 (332-487), and 417.6 (319-514) from the females that became established from 100 , 500 and 1000 eggs respectively. The worms bearing more than 350 eggs appeared unusually stout under the microscope.

In 168 hour old infections, the number of male and female worms declined as the size of the infective dose was increased (Table IX). The mean percentage of worms that became established from 100, 500 and 1000 eggs were 13.76 11.8 and 9.47 respectively. On the other hand, the mean length of worms from all 3 infective doses remained similar, indicating that smaller sized worms recovered from 1000 eggs in 72 hour infections grew in 168 hours.

TABLE IX. The number of eggs contained in the uterus of 168 hour old worms of S. muris that became established from 3 different infective doses.

Size of infective dose (Eggs)	Number of eggs collected		Mean Number of Worms established (%)	
	Range	Mean ± standard deviat.	Females	Males
100	350-489	408.93 ± 53.65	13.7	3.8
500	332-487	426.25 ± 54.47	11.8	1.62
1000	319-514	417.6 ± 52.52	9.47	1.41

DISCUSSION

In view of the large numbers of *Syphacia muris* eggs that are ingested by the rat host daily, a lower number of worms was expected to be established from a larger dose of eggs as opposed to a smaller dose. The experiments carried out suggest that this does not happen in 72 hour old infections when male worms have not yet left the host and can be considered together with females. Hence, more worms are established from a larger dose. Such a result is unexpected to occur because density-dependent factors normally limit parasite burden in a host. The work of Dorman (1928) on *Heterakis papillosa* and that of Ackert et al.(1931) Sadun, (1949) on *Ascaridia galli* in chickens demonstrated that a higher proportion of worms became established from a lower dose of eggs. A similar conclusion was reached by Africa (1931); Haley (1958), Sey (1969) experimenting with *Nippostrongylus brasiliensis* in rats. Dobson (1965, 1974) reported a similar dose size effect on the survival of *Amplicaecum robertsi* in sheep as did Michel (1969) with *Ostertagia ostertagi* in calves. However, references in the literature also show that worm burden may increase proportionate to the size of the infective dose, although such cases may not be frequently observed. Chan & Kopilof (1958) infected two strains of mice with *Syphacia obvelata* eggs and found that one strain harboured 32.2% worms from a dose of 100 eggs; the same strain showed 40.7% worms establishment from 200 eggs. The second strain of mice was infected with 4.4 and 9.5% worms from the two respective egg doses quoted. Tongson and McCraw (1963) discovered that in 2-4-8 week old chickens dosed with 500, 2000 and 5000 *Ascaridia galli* eggs, there was a gradual increment in the percentage of second stage larvae in the hosts. According to Michel (1969), a larger dose of *O. ostertagi* larvae resulted in

a greater establishment of worms in calves, and worm burden declined only after repeated doses of larvae were administered. In the case of S. muris, it may be speculated that the dose of 1000 eggs was not large enough to produce the kind of result expected, that is, a reduction in the number of worms did not occur from a large dose of eggs as compared to a small dose. Normally the rat host is exposed to as many as 6000 eggs daily (Chapter IV) and about 90% of the eggs are ingested by it (Van der Gulden, 1966). When this is considered, the dose size of 1000 eggs used in the present study is indeed small. However, other regulating mechanisms operate in order to restrict the numbers of S. muris that occupy the host caecum. The time related experiments establish that more eggs are infective if these are laid around the peak of egg deposition which occurs around noon when hosts are kept on a 6 a.m. to 6 p.m. light/dark environment. The eggs deposited after peak hour deposition yield few worms in the host. These results were earlier reported by D'Silva & Lewis (1979), Lewis & D'Silva (1980). The specific period of high infectivity may be cited as a transmission strategy on the part of the worm. By restricting more of its viable eggs to be laid midway in its circadian rhythm of egg deposition, the worm ensures that a certain period of incubation takes place on the host perianal region before they are ingested by the host. Therefore, a restricted period of more viable egg deposition is linked to host activity. From the data presented on the activity of rats, it is clear that feeding and defaecation of the hosts begins at 18:00 hours. This is also the period when intense grooming activity of the rat begins (personal observations), and because S. muris eggs are ingested by the host during grooming, a maximum number of viable eggs are available and ensured at this time.

The differing infectivity with respect to time shown in the case of S. muris has not been recorded before in helminth literature. However, Garnham & Powers (1974) noted a similar occurrance in the formation of oocysts by Plasmodium cynomolgi Mayer gametocytes. These gametocytes follow a circadian rhythm by appearing in the blood of monkey (the host) at night when they are normally picked up by the mosquito vector. If the gametocytes are fed to mosquitoes at mid-night, the result is the formation of more oocysts than if experimentally infected during mid-day. Perhaps the case of S. muris eggs and P. cynomolgi gametocytes are alike.

The age of the rats used in this study does not appear to have a limiting influence on S. muris burden in the caecum and the number of worms increase with host age. This is in contrast to the studies carried out with 3 other pinworms. Panter (1969) working with S. obvelata in mice, and Mathies (1959a) with Aspiculuris tetraptera in the same host, found that the worm burden decreased with increasing host age. The human pinworm Enterobius vermicularis is only found in children (Cheng, 1973). The laboratory rats which Blair & Thompson (1969) used, however, show that S. muris burden increased with host age. In the wild, adult rats harboured more S. muris worms than sub-adults (Singhvi & Johnson, 1977) in India. Ashour (1980) working on wild rats in Egypt found the same age-dependent phenomenon with S. muris. Therefore, the present studies reflect a true situation. The lack of age resistance may be indicative of a long association between S. muris and its host during which barriers to establishment have been overcome. The present data also shows that male rats harbour a larger worm burden than females and this too has been recorded by Singhvi & Johnson (1977) in the wild.

Although the density of the infective dose does not regulate the worm burden in 72 hour infections, it is apparent that worms recovered from 1000 eggs are reduced in size at this time. In 168 hour old infections, however, worm numbers decline with increasing dose size; but worm length remains the same irrespective of dose size. This suggests that a density dependent phenomenon operates to inhibit length in 72 hour old infections. It is possible that the presence of male worms at this time reduced the amount of space available in the caecum and thereby contributed to the decrease in length, similar to the concept of the crowding effect advocated by Read, (1951). But following 72 hours of infection, the loss of male worms (Chapter II) combined with growth of the caecum allows the remaining worms to increase growth. The resumption of growth after a period of inhibition has been shown to occur in Ostertagia ostertagi (Michel, 1963). The decline in worm numbers from a larger infective dose at the end of 168 hours, though not significant, suggests another density-dependent phenomenon and a trend that worms are lost in the course of infection. Larger infective doses of S. muris could possibly establish this further. It is likely that larger doses could provide sufficient antigenic stimulus in the host and thereby elicit an immune response to lower worm burdens.

A density-dependent phenomenon --- the reduction in the fecundity of worms with increasing worm burden --- has not been demonstrated in the case of S. muris, although the worm does show that its egg-bearing capacity may vary. Variation in fecundity is normally associated with the density of a population or the immune response (Anderson, 1976). Reduced fecundity due the 'crowding effect' has been observed in Ancyclostoma caninum by Hill (1926),

Krupp (1961), in Haemonchus contortus by Whitlock (1966). However, no correlation was observed between worm burden and fecundity in O. ostertagi (Michel, 1969), and Oesophagostomum columbianum (Dobson, 1974). In the case of S. muris since no reduction in fecundity was observed when rats were given a dose of 1000 eggs, it is possible that other unknown factors, such as poor worm development, inherent genetic causes, are likely to be responsible for varying egg burden in individual worms.

-ooOoo-

CHAPTER VI

WORM EXPULSION

In the course of routine sampling for eggs deposited on the perianal region of infected rats, by using the Sellotape or rice smear technique, it was occassionally observed that in addition to normal egg laying females, other stages of S. muris also left the host, generally en masse. These worms were larval and/or mature males and females, and appeared on the anal region clumped together. From these observations, it became apparent that an expulsion of worms occurred in the host and this might be similar to that seen in some other nematode life histories.

Some worm expulsion models include Nippostrongylus brasiliensis from the rat (Haley, 1962; Jarrett et al. 1968); Trichinella spiralis from the rat (Larsh, 1963); Trichuris muris from mice (Wakelin, 1967); Nematospiroides dubius from mice (Cypess & Van Zandt, 1973); Aspiculuris tetraptera from mice (Behnke, 1975); Strongyloides ratti from mice (Dawkins et al. 1980). When these models are examined, it appears that an expulsion can occur at the end of a primary infection and also after a secondary infection although the time period of expulsion after a secondary infection is generally shorter than that following a primary one. Three examples are cited, namely, Nippostrongylus brasiliensis is expelled within 8 - 11 days after a primary infection; but within 5 - 10 days after a secondary. Similarly, T. spiralis is lost between 9 and 12 days of the first infection and 6 and 10 days after the second (Larsh, 1963). Behnke (1975) observed the loss of A. tetraptera to occur 17 days after the primary infection, and in 12 days following a secondary dose.

It now remained to be determined whether S. muris was expelled after a primary or secondary infection. For a working hypothesis, it was assumed that expulsion of S. muris would occur due to the existence of a prior infestation since expulsions had been observed in rats which had always harboured infections. The following experiments were therefore conducted to determine the nature of S. muris expulsion in the rat host.

Experimental Design

In experiments designed to elicit an immune response, such as worm expulsion, host animals are first given a primary infective dose of eggs or larvae and this is followed by a challenge infection. In between the two infective doses, self-infection is prevented among the experimental animals by placing them in individual cages or on wire mesh floors. For example, Jarrett et al. (1968) placed rats infected with N. brasiliensis in single cages on mesh floors to prevent self-infection. There are 3 criteria used to determine that expulsion has occurred and any one of these may be employed. First, infected hosts may be autopsied to determine if a decrease in worm load has occurred, as for example in the case of mice infected with N. dubius (Cypess & Van Zandt, 1973). Or, expulsion may be followed by reduced egg deposition on the part of the worm. Sey (1969) observed that the expulsion phase in rats infected with N. brasiliensis was accompanied by a reduction in the number of eggs found in the faeces. Thirdly, the number of larvae appearing in the stool of infected hosts may also be used to judge expulsion has taken place. Dawkins et al. (1980) observed that S. ratti larval excretion ceased at the end of 10 days after mice were initially infected.

In the case of S. muris, 1000 eggs were administered on the 21st day after birth, to each of 5 male and 8 female Wistar rats belonging to one litter and 3 males and 3 females to another. No other eggs were administered to the 3 male and 3 female rats and these acted as controls. During the course of the experiment, all rats were put on saw-dust and individually caged. The saw dust was changed daily. The rats were allowed to feed ad libitum and maintained on a 6 a.m. to 6 p.m. day/light cycle. The rats were not prevented from autoinfecting themselves because a previous attempt to prevent autoinfection by placing funnel-shaped cardboard collars around the neck of the rat was detrimental to the health of the animals. Although these funnel-shaped collars prevented the rat from licking its anal region, the animal in an attempt to dislodge the collar, was placed under considerable stress. In view of the long period of time in which the infection experiments would be conducted, it was decided not to use a collar to prevent rats from re-infecting themselves.

On the 7th and 14th day post-infection, the 5 male and 8 female experimental rats were each challenged with a dose of 1000 eggs. All the rats, including the control group, were sampled for the presence of eggs and worms on the perianal region by using Sellotape from the 5th day onwards after the initial infective dose was administered. Daily sampling was carried out every 2 hours from 8 a.m. to 6 p.m. The sampling was terminated on Day 28 post infection when animals became ill due to continuous Sellotape application on the perianal area.

The numbers of eggs and worms on the Sellotape were counted. Worms were placed in 3 groups: non-gravid and gravid females, and those not in an egg-laying stage.

* "Male and female rats, including tests and controls, were sacrificed after the experiments were terminated. The caeca were removed and worms collected by the Baermann technique for counting.

The gravid females included spent females. When no spent females were found, all the eggs on the Sellotape were counted to arrive at an estimate of the females laying them. Egg counts in intact females had previously shown that there was an average of 440 eggs present in each female. The number of eggs found on the Sellotape was divided by this figure to estimate the number of females laying them. *

RESULTS

The worms recovered as a result of a single infection and two challenge infections is given in Table X for male rats and in Table XI for female rats. For convenience, all the worms found at the end of each 7 day period are totalled and presented graphically in Fig. 33.

No eggs were recovered before the 7th day post-infection, but eggs were found on the Day 7 p.i. indicating that the infection had become patent. The number of eggs were few in both male and female rats, only 3 gravid females being recovered from the 3 male control rats and 10 from the five used as tests (Table X). Between the 7th and 14th day post-infection, 34 gravid and 23 non-gravid females were found from the control males. Since these animals were not experimentally infected again after the initial infective dose was given, it may be safely assumed that these worms were produced from eggs that were swallowed by the hosts during auto-infection from Day 7 onwards. Auto-infection also produced 88 gravid and 36 non-gravid females found at the end of 21 days of infection. The 36 non-gravid females were recovered on 2 days of the week only. From then on, non-gravid worms did not appear on the perianal region and the number of gravid females declined to 24 on Day 28 post infection.

TABLE X

The numbers of Syphacia muris worms recovered from MALE rats after primary and challenge infections with 1CCC eggs (* days challenged).

Days Post-Infection	Primary Infection				Challenge Infection			
	Gravid Females	Total	Non-Gravid Females	Total	Gravid Females	Total	Non-Gravid Females	Total
1 - 6								
7	3	3			10	10		
8					2			
9					2			
10					5			
11	3		3		1			
12								
13			9					
14	31	34	11	23	*60	70		
15	13		10		31		12	
16	17		26		102		271	
17	14				31		74	
18	23				53		501	
19	7				7			
20	4				230			
21	10	88		36	*12	526	101	959
22	5				36		5	
23	4				185		5	
24	3				50			
25	2						202	
26	4				7			
27	0				19		5	
28	6	24			16	313		217
29	3				5		4	
30	0				29			

TABLE XI

The numbers of S. muris worms recovered from FEMALE rats after primary and challenge infections with 1000 eggs (*days challenged).

Days Post Infection	Primary Infection				Challenge Infection			
	Gravid Females	Total	Non-Gravid Females	Total	Gravid Females	Total	Non-Gravid Females	Total
1 - 6								
7	4	4			24	24		
8	9				8			
9	3				4		3	
10					1		57	
11			60					
12								
13			1	61	1			
14	10	22			*56	70		60
15	4				68			
16	2		2		54			
17	3				19			
18	3				2			
19	1							
20	4				6			
21	9	26		2	*18	167		
22	0				27			
23	3				21			
24	4				13			
25	4				3		1	
26	5				9		10	
27	2				1			
28	4	22			5	79		11
29	1				7		9	
30	0				6			

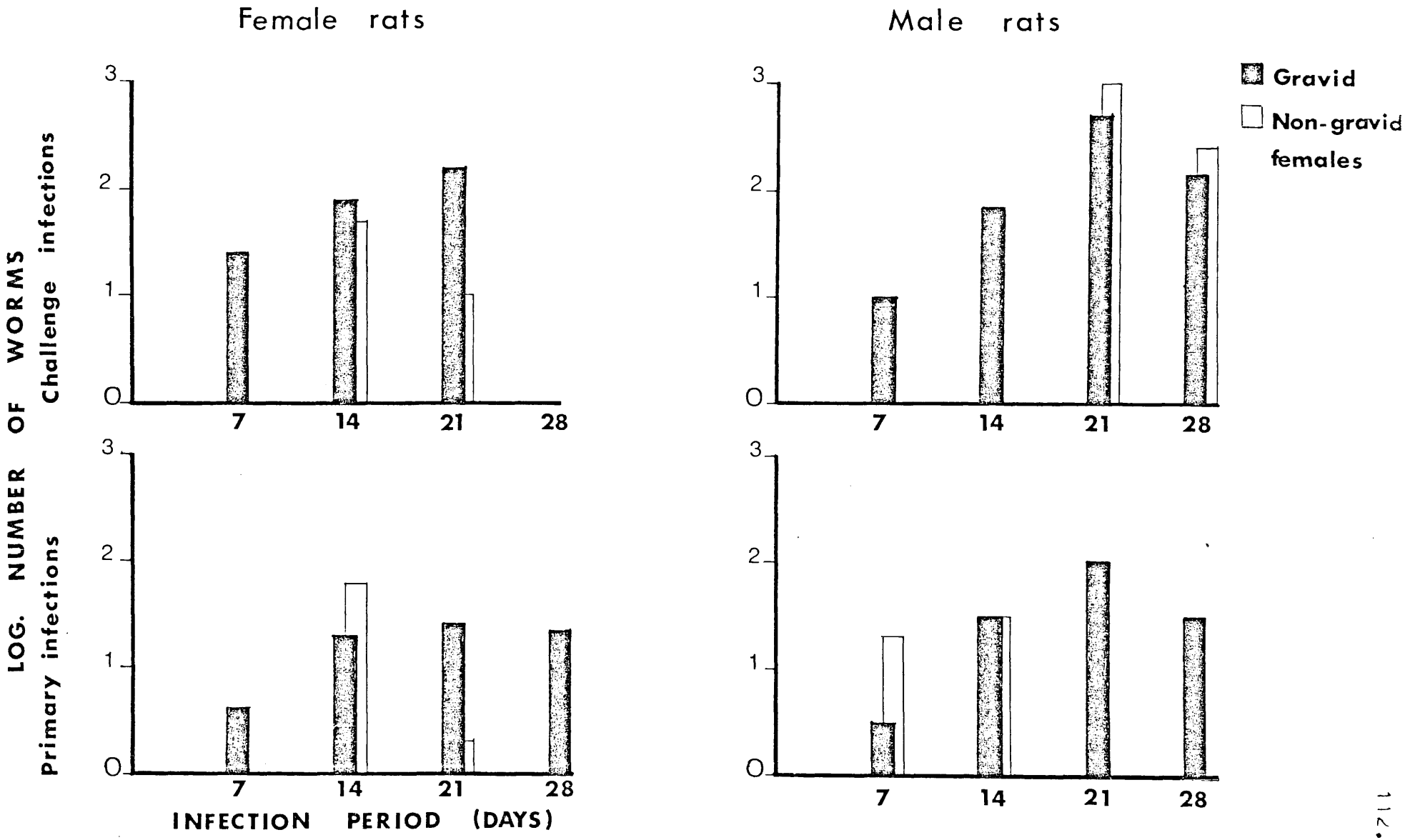


Fig. 33. WORM EXPULSION OF S. MURIS

In the 5 male rats given challenge doses of S. muris eggs, the course of infection was similar to that in control animals (Table X) in that after Day 14 post-infection the worm recovery peaked on Day 21 and declined on Day 28 (Fig. 33). Seventy gravid worms were recovered, making a mean of 14 worm recoveries per rat host. It is evident that the first challenge infection was not expelled as was expected. However, following a second challenge, large numbers of worms were expelled daily. By Day 21 of the infection, 526 gravid and 959 non-gravid females had been expelled. Most of these worms were discharged in large masses. The loss was often accompanied by slurry.

After Day 21, gravid and non-gravid females were produced daily in varying numbers. Nevertheless, worms expulsion occurred en masse frequently in all infected rats. By day 28, the numbers declined to 313 gravid and 217 non-gravid worms.

In the female rats, the infection followed a parallel course (Table XI, Fig. 33) to that observed in males, except that significantly fewer worms were recovered. In the 3 control rats, only 4 gravid females appeared at the end of the first infection period on Day 7. Gravid worms were found on 3 other days subsequently, bringing the total to 22 worms on Day 14 post-infection. At the same time, 61 non-gravid worms were recovered; thereafter only 2 non-gravid worms were found. Gravid females passed out daily after Day 14 and amounted to 26 at the end of the 3rd week of infection. The total number of worms remained surprisingly at 22 by Day 28, all of which were gravid. But a severe expulsion was not observed at any time.

A total of 24 gravid worms were found from the 8 female rats used as experimental hosts at the end of the first infection period on Day 7. The challenge infection produced a sum of 70 gravid and 60 non-gravid worms by

*

The mean recovery from dissected rats was:-

	WORMS			WORMS	
	♀ ♀	♂ ♂		♀ ♀	♂ ♂
Male rats (Challenge	261.6 (157-349 range)	92.6 (34-91 range)	Male rats (Control)	404.0 (393-415 range)	108 (88-123 range)
Female rats (Challenge	203.50 (159-252 range)	53.82 (20-91 range)	Female rats (Control)	237.03 (154-266 range)	47.30 (32-61 range)

Day 14. Of these 56 were produced on one day alone. An expulsion of worms would appear to occur after the second challenge dose. Sixty-eight and 54 worms were found on the two days following the challenge. This contributed to the total of 167 worms released on Day 21. However, it must be remembered these were shed by a total of 8 female rats. In contrast to the heavy expulsion of non-gravid females in male rats, no such losses were observed in the female hosts. Worm release remained high for two days after Day 21 but gradually declined. On Day 28, only 79 gravid worms were found. At this time, 11 non-gravid worms were also recovered. *

DISCUSSION

When the results of the control and experimental groups of the infected animals are compared, it is clear that S. muris expulsion occurs in the rat host. The expulsion is characterised by an en masse exodus of worms from the male rats. Both larval and adult (male and female) worms are lost. The loss cannot be predicted as to time of occurrance. It does not occur after the first challenge dose has been administered. In the above experiment, expulsion occurred only after a second challenge had been given to the hosts. In contrast, it will be seen that expulsion did not take place in male rats which were not given a challenge infection but were nevertheless allowed to become autoinfected, even though these had acquired a secondary and tertiary infections. It is suggested that rat hosts must acquire certain optimal number of worms before expulsion can be elicited. It is not too difficult to envisage such a situation because under normal circumstances rat hosts are exposed to and ingest many more S. muris eggs than were used in the experiments above.

Syphacia muris expulsion does not occur at the end of a primary infection as is the case with N. brasiliensis (Haley & Parker, 1962; Jarrett et al., 1968); T. spiralis (Larsh, 1963); A. tetraptera (Behnke, 1975). Nor can it be said that expulsion occurs after a second challenge infection has been given which occurs in the case of N. brasiliensis, T. spiralis and A. tetraptera. Rather it may be speculated that expulsion of the nature reported here may occur only after a certain number of worms become established in the host caecum. In nature this occurs as a result of continuous exposure to eggs deposited on the perianal region. This may point to a situation where expulsion may occur in S. muris due to a 'crowding effect' . Such a view may be more likely given the short (8 day) life cycle of S. muris. There is a rapid turnover of parasite burden and excess worms in the caecum are simply expelled as a result of overcrowding. An immune reaction could also elicit a response leading to expulsion, but this was not investigated in the present study. However, if an immune reaction did exist, a large number of S. muris would probably be required to elicit such a response considering that it was only after a tertiary dose of 1000 eggs that expulsion occurred in the experiments described here.

The lack of severe expulsion reaction in the female rats may be due to the lower number of S. muris that occupy the caecum (see Chapter V). It was also shown that fewer eggs were deposited on the female perianal region than on the male (Chapter IV). The smaller worm burden in female rats may be due to the smaller size of its caecum in comparison with that of the male (personal observations). In addition the female perianal region is smaller in surface area compared to that of the male. Consequently, fewer eggs can

be accommodated on its perianal surface. Therefore, severe expulsion of S. muris may not occur because of the limitation of space in the caecum and perianal area of the female host.

It is of interest to note that male rats harbour more S. muris than females and that severe expulsion occurs in the male only. Male hosts, in general, are more susceptible to nematode infections than female hosts (Solomon, 1969). The expulsion of S. muris from males, on the other hand, is perhaps more significant with respect to the pinworm's epidemiology. It is known that male rodents forage over wider distances than females, the latter being more confined to the burrows on account of litter-bearing activities. Hence an expulsion of S. muris from the male rat would allow the dissemination of the parasite in space. Lewis (1968) established a case where Apodemus sylvaticus males are disseminators of N. dubius in the wild. The female rat, on the other hand, may not be totally relied upon to transmit S. muris over distances because the female rat is more likely to pass on the S. muris infection directly to her litters.

The expulsion may also be regarded as a means of regulating the worm burden of S. muris in its host. When a comparison is made of the higher number of worms expelled in the experimental group of rats with that in controls, the figure becomes significant. In the absence of density-dependent factors operating to limit the size or fecundity of S. muris, perhaps expulsion is the only way worm burden is regulated in the rat host.

-ooOoo-

CHAPTER VII

THE COURSE OF INFECTION

In a study of the transmission of Syphacia muris, it is desirable to know the onset of an infection and its course thereafter. It may be assumed that litter pups first pick up an infection while they are still suckling so as to harbour the parasite once they are weaned. After weaning, the infection may be influenced by auto-infection and the behaviour of the host. Due to daily auto-infection, the worm burden in a host may be cumulative in time, particularly as large numbers of eggs are deposited on the perianal region. If according to Van der Gulden (1966), 90% of these eggs are ingested, continued exposure might increase the worm burden in a host. However, it is unlikely that accumulation of worms can continue indefinitely. Regulatory mechanisms, such as the host immune response, are likely to operate to ensure that the host is not killed and that S. muris survives. Roman (1969) and Roman & Kientruong (1973) recorded S. muris egg deposition on the perianal region of rats over a long period of time. The authors found that the infection was sporadic and that rats displayed varying periods of infection and resistance, indicating that some form of regulation occurred.

Host behaviour may influence infection because it is generally regarded that S. muris transmission occurs by contact (Stahl, 1961, 1963; Van der Gulden, 1966; Roman, 1969; Roman & Kientruong, 1973). Eggs deposited on the perianal region are ingested while the rat is grooming. In a typical animal house, rats are caged singly, or sometimes in groups.

Both these situations may affect the progress of infection. In individually caged rats, contact will be limited to the perianal region of one host. In rats held in groups, contact will occur, in addition, between individuals due to rodent grooming behaviour. Consequently, infection as defined by the worm burden in a host, may be expected to be higher in a group of rats than in singly caged animals, as there will be the chance to contact-infect from more than one host. Experiments were carried out, therefore, to determine the onset of an infection in litter pups and its progress thereafter.

MATERIALS AND METHODS

Experiment 1:

To determine the onset of the first infection in suckling rats, the perianal region of litters was sampled for *S. muris* eggs on the days following their birth. The time eggs were first ingested could then be determined by considering that an infection became patent on the 7th day post-infection.

Nine 7-week old Wistar female rats in the isolator were allowed to become impregnated by association with males for 5 days, since females ovulate once in 5 days of an oestrus cycle. Impregnated rats were removed to individual cages and placed on saw-dust and water and allowed to feed *ad libitum*. All the rats were infected with 100 *S. muris* eggs two days later. After 7 days the perianal region of each rat was sampled with Sello-tape to ensure patency had occurred. Thereafter, rats were allowed to build up the worm burden in the caecum by auto-infection. During the 21-day gestation period, rats were provided with sheets of paper towelling for

nesting material. After litters were born, a record was maintained of the number of pups born per rat. Each pup belonging to a litter was then sampled by applying Sellotape on the perianal region once daily from day 7 after birth. Pups were returned to the litter after sampling which was terminated on the day pups were weaned, usually day 21 post birth.

Experiment 2:

Nine female rats were allowed to become impregnated as before while remaining within the isolator. After removal to outside cages, they were infected with 100 S. muris eggs. The infection from these eggs was allowed to build up during the period of gestation by autoinfection. When litters were born, a number of pups were removed from each litter at two-day intervals from the 7th day after birth and autopsied by cervical dislocation. The alimentary canal was removed and slit open in 0.75% saline in a petri dish. The contents were teased into the saline and examined under a dissecting microscope for larvae at 10 x 10 magnification.

Experiment 3:

The course of infection in rats caged singly and in groups was determined by the following:
(i) Thirty male and 30 female rats freshly weaned and 21 days old were removed from the isolator and caged individually in 15" x 10" x 7" plastic cages. Each rat was infected with 100 S. muris eggs two days later and allowed to build up an infection through auto-infection. In addition, all the animals were infected with 100 eggs at 7 day intervals. Rats were maintained on saw dust and allowed to feed ad libitum. The bedding material was cleaned every week. The lighting regime

in the animal house was maintained on a 6 a.m. - 6 p.m. light/dark cycle. After 8, 16, 32, 48, 64, and 80 days, 5 male rats and 5 females were autopsied after being killed by carbon monoxide gas fuming. The caecum was removed and worms collected by the Baermann technique. Worms were fixed in warm 4% formalin and counted under a Zeiss stereoscan microscope.

(ii) Thirty male and 30 female weanlings were removed from the isolator, infected with an initial dose of 100 S. muris eggs and caged individually. The infection was allowed to build up through auto-ingestion of eggs. Animals were maintained as above. At intervals of 8, 16, 32, 48, 64 and 80 days, 5 male and 5 female rats were killed and autopsied to determine the worm burden in the caecum. Worms were collected by the Baermann technique, fixed in 4% warm formalin and counted under a steroscan dissecting microscope.

(iii) Thirty suckling female rats, each 21 days old, were removed from the isolator and infected with 100 S. muris eggs. The rats were divided into 6 groups of 5 each. A group of rats was put into a 17.5" x 11" x 8" plastic cage and the infection was allowed to build up. Rats were placed on saw dust which was changed weekly. They were fed ad libitum and kept on a 6 a.m. - 6 p.m. light-dark regime. A group of rats was removed at intervals of 8, 16, 32, 48, 64, and 80 days and autopsied for the collection of worms from the caecum by the Baermann technique. Worms were fixed in 4% warm formalin and counted under a dissecting microscope.

RESULTS

Experiment 1;

The sizes of the litters born to each of the 9 impregnated rats and the number of eggs found in each litter after their birth is presented in Table XII. It is apparent that eggs first began to appear on the perianal region of a litter 10 days after birth. Eggs were first observed in litter sizes of 8, 10 and 12 on Day 10 after birth, with eggs being recovered daily thereafter. The first eggs in a litter size of 13 pups appeared on Day 11 while that in a size of 6 was seen on Day 13. The 11 pup litter first showed an infection on Day 17. Three other litter sizes, viz., 4,4, and 7 showed patency after Day 19.

There was a great variability in the number of eggs that were deposited in each litter. The total number of eggs found at the time of weaning ranged between 3 and 1,311 (Table XII). The 3 eggs were deposited in a litter bearing 4 rats and 1,311 in a litter size of 11. A litter size of 4 also produced 150 eggs and that of 8 rats 59 eggs. On the other hand, 1,242 eggs were found on 6 pups in a litter and 250 on 7 pups in another. Thus the numbers of eggs deposited could not be correlated with the size of the litter. Neither could the mean number of eggs collected per sample be related to the size of the litter. However, the total number of eggs found each day on all litters showed an increasing trend as the time of weaning approached (Table XII). On Day 21 when litters were weaned, there was a large increase in the number of eggs.

TABLE XII

The number of eggs laid by each litter of rats after birth.
(-) denotes days on which litters were not sampled.
(w) denotes days litters were weaned.

LITTER SIZE

Days Post Birth	4	4	6	7	8	10	11	12	13	Total Eggs Deposition	$\bar{x}$
10					5	6		2		13	4.33
11					1	12		1	12	36	9
12			0		-	-		7	8	15	5
13			1		1	12		6	1	21	4.2
14			0		0	9	-	-	0	9	2.25
15			1		7	21		9	1	39	7.80
16			2		0	8		29	21	60	12
17			0		1	2	3	111	-	117	23.4
18			-		1	4	6	21	27	59	11.80
19		1	22		2	5	-	6	23	59	9.83
20		1	548		41	50	80	4	5	729	104.14
21	0		660	9	0	w	35	5	277	994	142.0
22	150	1	w	160	w	w	1187	w	220	1718	245.60
Total	150	3	1242	250	59	129	1311	201	595		
No. Days Sampled	2	3	9	2	11	11	5	11	11		
Mean No. Eggs/ Sample	75	1	138	125	5.36	12.9	262.2	28.27	54.9		

Not all the rats belonging to a litter were infected on the day a litter was first found to be infected (Table XIII).

TABLE XIII

The days after birth when litters were first found to bear eggs.

Litter Size	4	4	6	7	8	10	11	12	13
Days Post-Birth Eggs Found	10	21	13	21	10	10	17	10	11
No. Rats Infected	1	1	1	2	4	5	2	2	6

In a litter size of 4, the first infection was detected in one rat on Day 21 after birth. But 6 out of 13 rats in a litter were infected on Day 11. Although all the litter pups did not become infected at the same time, by Day 21 all of them harboured an infection.

Experiment 2:

To confirm the results of Experiment 1, litter pups were autopsied several days after birth. The immature worms recovered are given in Table XIV.

The first larvae were detected in the caecum as late as Day 12 after birth in some pups. In others, these were found on Day 14 and 16. The immature female worms from the 12th and 14th day of collection measured between 0.41 - 0.47 mm.

TABLE XIV

The number of larvae of *Syphacia muris* recovered from suckling rats prior to weaning.

Larvae Recovered									
Male		0		1			1 3	0 2	2 5
Female		2		4			0 6	2 4	6 15
Number of pups Infected	0 0	0 0 1	0 0 0	0 0 2	0 0 0	0 0 2	0 0 1 2	0 0 1 2	0 0 3 4
Age at Autopsy (Days)	8 10	8 10 12	8 10 12	8 10 12	8 10 12	10 14 16	8 10 12 14	10 12 14 16	10 12 14 16
Number of pups Autopsied	3 2	3 3 2	3 3 3	3 3 3	3 3 4	3 3 4	3 3 2 3	3 3 3 3	3 3 3 4
Litter Size	5	7	9	9	10	10	11	12	13

A depression appeared on the body wall of both the male and female worms recovered at this time. Both these characters indicated that these immature worms were 24 hours old. The worms collected on Day 16 showed a mixed infection of 24, 48 and 72 hour olds. The 48-hour worms could be distinguished by the absence of a depression on the body wall and by their longer lengths. The 72 hour worms had developed alae. Mamelons were found on the males while females bore dark plugs on the vaginal pore.

It is readily observable that the number of worms recovered were few in number. Each pup harboured for the most part two female worms and rarely three. The male worms were fewer in number. The number of worms found did not depend upon the size of the litter. Nor did the size of the litter influence the time when worms first appeared in the caecum. But there was a trend for worms to increase in number and to be found in more pups as the time of weaning approached. This was apparent from the numbers of worms found on Day 16 after birth.

The interesting feature was that worms were detected in caeca which contained bedding material in the ingesta. Normally caeca contained curdled milk and no worms were found in these. Several conclusions can be drawn from these observations. First, worms could not be detected under the microscope for the very reason that they were masked by the milk and escaped detection. This was unlikely because curdled milk was diluted with water and examined in small quantities under a 10 x 10 magnification. It is more likely that worms could not survive in the caecum due to a property in the milk which might be anthelmintic. Secondly, worms were detected

in ingesta containing bedding material because this provided a growth medium. Thirdly, it is tempting to postulate that pups swallowed eggs contained in the bedding material, although it is more likely that pups swallowed eggs from the mother whilst suckling for milk or licking as part of the weanling behaviour. Litter pups were found to be in searching and licking situations on the body of a mother until the time of weaning.

On account of the 7 day life cycle of Syphacia muris, at the end of which the female worm oviposits on the perianal region of the host, the appearance of eggs in litter pups may be used to determine the establishment of the first infection in unweaned litters. On such a basis, litter pups in Experiment 1 first became infected as early as Day 3 after birth. However, in Experiment 2 it was shown that the first 24-hour worms were not detected in the caecum until Day 12 or thereafter post-birth. Neither were any ovigerous females recovered from the caecum at this time. Since detection of worms by autopsy is a better method to determine the first infection, eggs found on the rats in Experiment 1 could not have come from worms residing in the litter pups. These eggs must have therefore originated in the mother and appeared on the body of pups due to contact with the mother. The body surface of the pups is naked for sometime after birth and the eggs of S. muris have an adhesive property.

Experiment 3:

All worms collected in the three experiments were divided into males and females. No distinction was made between immature and adult worms for two reasons. Firstly,

some of the female worms which appeared to be immature proved to be ovigerous upon dissection. As the dissection of worms suspected to be ovigerous would be tedious, all immature worms were counted together with mature worms as one. Secondly, the immature male could not be separated from the adult on the basis of morphological characters. The adult male possesses fully developed mamelons and is longer in length than immature males. However, it was difficult to determine whether or not mamelons were fully developed since immature males also posses these mamelons. Male and female worms were however, easily separable on the basis of the sharp, short tail end present in the male.

(i) In animals which were dosed with 100 eggs each week and caged individually, the worm recovery was as shown in Table XV. At the end of the first infection period on Day 8, an average of 17.8 female worms were present per male rat, the range being 10 - 24. In female rats, the average was 9.60 female worms, and the range 7 - 13. All the worms were adults and had reached the egg-releasing stage. No immature worms were present. The worm burden rose rapidly by Day 32 to 631.8 (481 - 1278 range) in male rats and to 345.4 (185 - 501 range) in females. But on Day 48 the infection declined to 543.00 (37 - 2000) in male rats and to 163.2 (89 - 207 range) in female rats. A second decline occurred on Day 64 in both male and female rats. But on Day 80 the worm burden peaked sharply. The wide range was characteristic of all infections and showed that individual rats could harbour light as well as heavy infections. Where the infection was light as in the case of the male rat bearing 37 female worms on the 48th day of an infection, worms were visibly ovigerous. In heavier infections, only a small proportion of the worms were egg-bearing, the majority appearing to be immature.

Although the combined worm burden was cumulative as the infection grew older, even in old infections several individual rats harboured light infections.

The male worm burden was always less than the female (Table XV). The female to male ratio (FMR) reached proportions between 1.8 and 13.7. The FMR range of 2.00 - 4.5 was the most frequently observed. The FMR fluctuated from one infection period to the next. In general, the male worm burden increased or decreased as in the females. In a few older infections, males were completely absent.

The relative rate of infection may be expressed simply by

$$r = \frac{X_t}{X_{t-1}} \quad ,$$

where, X_t is the number of worms in time t,
and, X_{t-1} is the number of worms in time $t-1$.
The relative rate of infection, determined by the increase or decrease in female worms from one infection period to the subsequent showed a constancy after an initial period. In male rats, the pattern appeared as follows: On day 16, there was a 10-fold increase in female worm numbers over the last infection period observed on day 8. The increase on day 32 was 4 times that on day 16. However, the infection decreased once on day 48 and thrice on day 64. When it peaked on day 80, the increase was 4.5 fold (Fig. 34 a). In female rats, with the exception of the 10-fold increase on day 80, the rate and pattern of infection was similar to that in males (Fig. 34b).

Female rats harboured fewer worms than males, but there was no significant difference between the mean worm burdens in the two sexes of hosts ($P = .05$; t-test). In general, female rats tended to harbour fewer immature stages than adult worms, perhaps due to the smaller size of the caecum.

TABLE XV

The number of worms recovered from individually-caged rats subjected to a weekly dose of 100 *S. muris* eggs.

WORM RECOVERY

MALE RATS:

Days Post-Infection	Worm Sex	1	2	3	4	5	Mean	Standard deviation	FMR
8	Female	23	15	17	10	24	17.8	5.80	2.69
	Male	0	3	18	12	0	6.6	8.04	
16	Female	391	5	56	75	284	162.2	166.41	9.77
	Male	38	0	3	4	37	16.6	19.11	
32	Female	481	284	1278	536	580	631.8	378.53	2.17
	Male	160	72	753	256	211	290.4	267.48	
48	Female	2000	434	37	167	77	543.0	829.05	2.02
	Male	1044	239	5	55	0	268.6	444.23	
64	Female	74	158	53	241	413	187.8	146.24	4.38
	Male	7	7	0	95	105	42.8	52.41	
80	Female	359	311	57	2000	1346	814.6	825.03	1.80
	Male	183	76	18	1322	663	452.4	548.52	

TABLE XV contd: The number of worms recovered from individually-caged rats subjected to a weekly dose of 100 S. muris eggs.

WORM RECOVERY

FEMALE RATS:

Days Post Infection	Worm Sex	1	2	3	4	5	Mean	S.D.	F.M.R.
8	Female	7	11	9	8	13	9.6	2.40	9.60
	Male	0	0	0	0	5	1.00	2.23	
16	Female	191	50	47	112	148	109.6	62.4	13.70
	Male	23	1	0	3	13	8.0	9.84	
32	Female	185	488	348	205	501	345.4	149.9	2.69
	Male	26	159	92	101	263	128.2	88.90	
48	Female	195	127	207	89	198	163.2	52.33	2.01
	Male	66	33	90	19	63	54.2	28.22	
64	Female	47	7	436	468	9	193.4	236.87	4.51
	Male	0	0	82	128	4	42.8	59.08	
80	Female	2000	1031	861	320	843	1011.0	613.74	3.06
	Male	650	335	422	8	231	329.9	236.83	

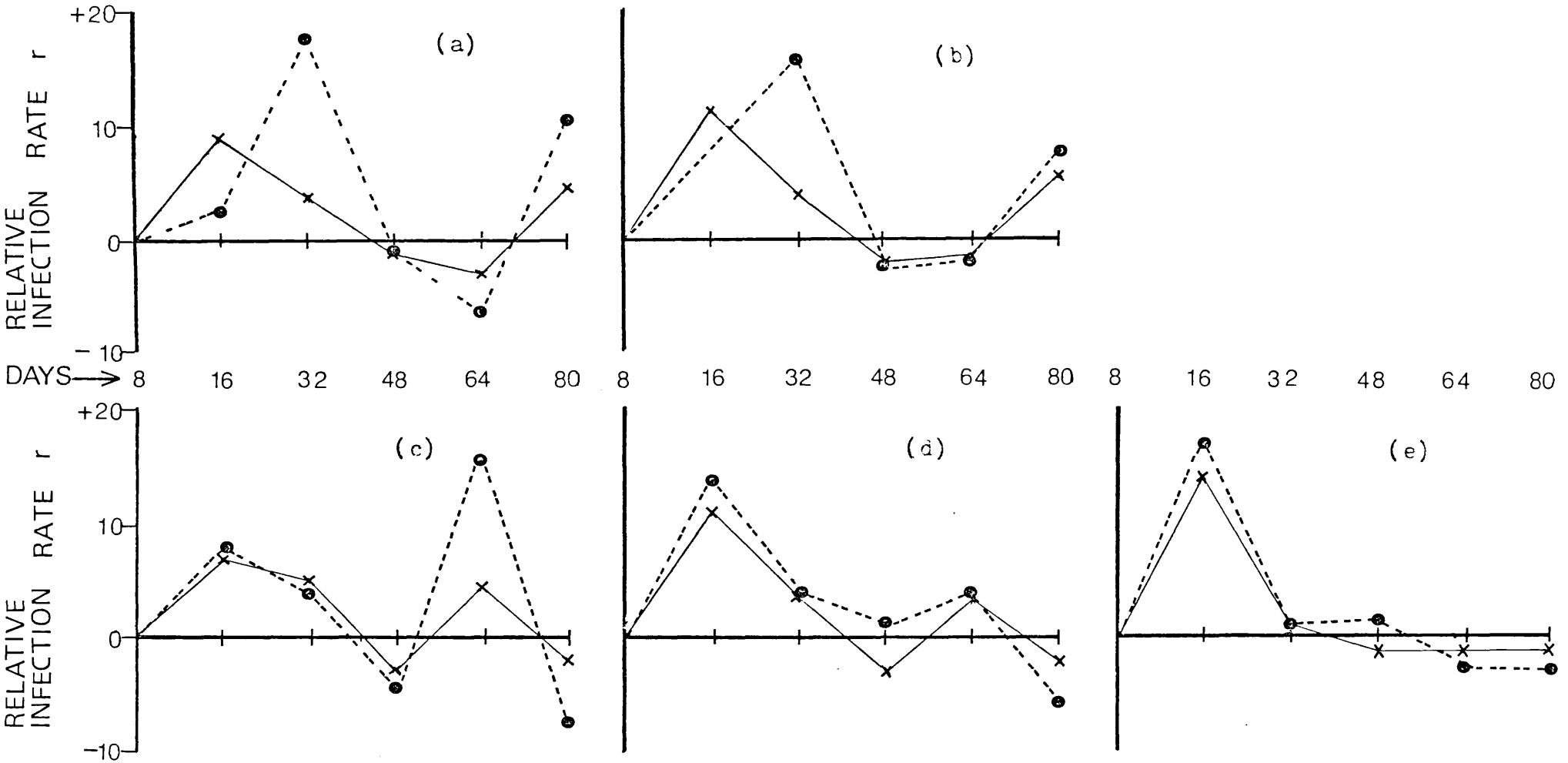


Fig. 34. The infection rate of Syphacia muris females (×——×) and males (o——o) in rats subjected to varying experimental conditions, viz:-
(a) Male rats infected weekly with 100 eggs, caged singly;
(b) Female rats infected weekly with 100 eggs, caged singly;
(c) Male rats infected once with 100 eggs, caged singly;
(d) Female rats infected once with 100 eggs, caged singly;
(e) Female rats infected once with 100 eggs, caged together.

(ii) In animals which were allowed to build up a worm burden through auto-infection after they were dosed with 100 eggs initially, while being caged individually, the infection rate was in general similar to that described above (Table XVI). After the first week of infection, rats harboured a mixed infection of immature and adult worms. Individual rats showed a light or heavy infection. In heavy infections, the immature worms constituted the majority of S. muris burden. In light infections, most of the female worms were ovigerous. Even in older infections, a few individual rats harboured a light infection. However, in contrast to experiment (i) above, the range of worm numbers was narrower, perhaps reflecting the condition in the wild.

Worm burden rose sharply in the first 32 days of the infection. (Fig. 34 (c)). It declined to a mean of 158.8 worms in the male hosts and 195.7 worms in the females on Day 48 (Fig. 34 (d)). However, unlike results in (i) it did not decline on Day 64. Instead, the infection dropped slightly on Day 80 when the average female worm burden was 243.0 and 213.0 in male and female rats respectively.

As before, the number of female worms was greater than that of male worms with the FMR ranging between 2.7 and 11.50 with the more frequent ratios being between 2.7 and 4.4 (Table XVI). In female hosts, the FMR was between 1.6 and 11.2. The FMR in female rats varied greatly and in this respect was no different from that shown in the female rats of experiment (i). Male rats again tended to harbour more worms than females, although there was no significant difference between the worm burdens in the two sexes.

TABLE XVI

The number of worms recovered from individually-caged rats subjected to an initial dose of 100 S. muris eggs.

WORM RECOVERY

MALE RATS:

Days Post Infection	Worm Sex	1	2	3	4	5	Mean	S.D.	F.M.R.
8	Female Male	23 0	15 3	17 18	0 12	24 0	17.8 6.6	5.80 8.04	2.7
16	Female Male	232 56	153 19	75 9	69 13	99 33	125.6 26.0	68.08 19.07	4.8
32	Female Male	432 79	462 67	332 50	321 71	281 32	365.6 59.8	77.4 18.80	6.1
48	Female Male	154 15	135 10	144 9	202 18	159 17	158.8 13.8	25.85 4.08	11.50
64	Female Male	715 152	960 325	303 94	617 276	915 216	702.0 212.6	263.87 92.74	3.3
80	Female Male	35 6	247 47	311 53	537 166	85 3	243.0 55.0	199.56 66.13	4.4

TABLE XVI Contd: The number of worms recovered from individually-caged rats subjected to an initial dose of 100 S. muris eggs.

WORM RECOVERY

FEMALE RATS:

Days Post Infection	Worm Sex	1	2	3	4	5	Mean	S.D.	F.M.R.
8	Female Male	7 0	11 0	9 0	8 0	13 5	9.60 1.0	2.4 2.23	9.60
16	Female Male	29 6	119 16	51 4	49 10	390 15	127.6 11.4	150.57 4.09	11.19
32	Female Male	265 54	256 43	108 13	291 38	392 66	262.4 42.8	101.85 19.8	6.13
48	Female Male	351 93	235 20	256 15	219 26	302 79	195.7 46.6	85.96 36.5	4.19
64	Female Male	160 26	845 254	75 7	1447 645	332 119	345.66 210.2	437.14 261.96	1.64
80	Female Male	236 85	412 71	144 11	190 5	83 24	213.0 39.2	124.81 36.41	5.43

TABLE XVII

The number of worms recovered from individual female rats initially infected with 100 S. muris eggs and caged in groups.

Days Post Infection	Worm Sex							Mean	S.D.	F.M.R.
8	Female	11	6	7	16	13	9	10.33	± 3.7	7.76
	Male	2	0	3	0	1	2	1.33	± 1.21	
16	Female	153	244	151	60	165	95	144.66	± 63.28	6.42
	Male	24	59	37	5	3	7	22.5	± 22.23	
32	Female	297	105	454	68	172	303	233.16	± 145.02	7.21
	Male	33	14	61	8	35	43	32.33	± 19.34	
48	Female	167	286	337	125	168	201	214.0	± 80.90	3.38
	Male	78	73	84	71	68	75	63.16	± 29.04	
64	Female	57	171	388	92	300	255	210.0	± 126.17	7.59
	Male	2	13	16	1	44	90	27.66	± 34.27	
80	Female	33	645	104	191	55	43	178.5	± 235.84	20.21
	Male	1	0	31	12	5	4	8.83	± 11.65	

The infection rate shows that in male rats the increase on Day 16 in female worms was 7.5 times the number on Day 8 (Fig. 35(c)). This was followed by a 3 fold increase on Day 32. A decrease first occured on Day 48. But the female worms rose 4 times on Day 64 compared to that in Day 48. It declined on Day 80 to three times the figure on Day 64. In female hosts, the female worms on Day 16 rose by 14 times the amount on Day 8. Two-fold increases were seen on Day 32 and 64. The decrease on Day 48 and Day 80 was of the order of 1.5 (Fig. 35 (d)).

(iii) The female rats caged together, after initially being dosed with 100 eggs, produced worm burdens which were allowed to build up through auto-ingestion (Table XVII). Overall there was little variation from the data shown for experiments (i) and (ii) above. The female worms which were recovered at the end of the first week of infection were found to be adult, with a mean number of female worms = 10.33. When this number increased 14 fold to 144.66 (60-244 range), the infection consisted of adult and immature worms, with the latter forming the greater majority. The next increase on Day 32 was smaller in comparison and was only 1.5 times that on Day 16. The female worm burden began to decline from Day 48 until the end of the experiment on Day 80. The order of the decline was gradual and the magnitude was small, being limited to one fold in each infection period compared to the last period (Fig. 35 e). More females harboured light infections as the infection grew older. On Day 64, three of 6 rats carried a light infection ranging between 57 and 171 worms. In contrast, the remaining three carried infections ranging between 255 and 388. On Day 80, four of the 6 rats carried infections ranging from 33 to 104 worms. The remaining two rats harboured 191 and 645 worms respectively.

The male worm burden was lower but followed the pattern of increases and decreases shown by the female worms. The FMR was between 3.4 to 20.2 and reflected (Table XVII) the wide range seen in the FMR of the female rats in experiments (i) and (ii). In 64 and 80 day infections, the male worm burden was considerably reduced.

DISCUSSION

The transmission of Syphacia muris begins in litter pups as late as Day 12 after birth. No infection occurs prior to this date presumably because the small size of the caecum and the anthelmintic effect of the milk diet would prevent the establishment of adult worms which can reach a size of 3.5 mm. when fully ovigerous. It is also likely that an environment other than a milk diet is required for the first worms to become established. The presence of bedding material in the ingesta of the litter pups along with milk strongly suggests that this provides the necessary medium for the growth of the first worms.

The discovery of immature worms in the caecum from Day 12 onwards indicates that rats already harbour an infection at the time of weaning on or around Day 21 post birth. The numbers of these worms are few, considering that the pups are exposed to a large number of eggs originating from the infected mother from which many more worms might have become established. But it is these few worms which provide the eggs for the next generation of worms after weaning.

The pattern of infection in weaned rats is that of a rapid growth in worm burden for the next 32 days. This pattern, observed in Experiment 3 (i), (ii) and (iii), suggests that it is a characteristic feature of

S. muris infections. The infection reaches a degree of constancy after the 32 days and is thereafter characterised by short fluctuations in the worm burden. From these results it is clear that while there is no barrier for the growth of worms in the caecum for 32 days following weaning, there is a regulation thereafter. The rapid growth is most certainly due to the number of worms entering the host being in excess of those leaving. It is facilitated perhaps by the absence of any anti-worm responses on the part of the host at this time. It may be that insufficient numbers of worms are present in the host to stimulate a regulatory mechanism such as an immune response. This would imply that a 'threshold' level of worms is required in the host before anti-worm responses can be elicited. Dineen (1963), working with Haemonchus contortus infections in sheep, first proposed that a 'threshold' level of worms was required before a host could be stimulated to bring on the immune response. Subsequently it was shown by Jarrett et al.(1963), Jarrett (1971) that sufficient numbers of Nippostrongylus brasiliensis were required in the rat host before an expulsion phase could set in. This was confirmed by Jenkins & Phillipson (1970) who carried out repeated infections of rat with small doses of N. brasiliensis. Michel's (1963) experiments with Ostertagia ostertagi in cattle showed similar results, though he differs with Dineen's view (op. cit.) and suggests it is in fact the rate at which the infection occurs which determines the onset of an immune response. But Ikeme (1971) reported that in chickens infected with Ascaridia galli a threshold level of worm burden was required to stimulate the expulsion phase. It is, therefore, more than likely that a threshold level of S. muris in the rat caecum is reached in 32 days after weaning for on Day 48 the infection declines. This decline is quite severe (Table XVII).

The level of constancy reached by the infection after 32 days has been ascribed to short decreases and increases in the worm burden. Constancy would in itself imply that the rate at which worms became established depended upon the numbers that entered and left the host. To account for decreases, the numbers leaving must be in excess of those entering. Adult males leave the host 3 days after becoming established in the caecum; gravid females in 7 days at the end of their respective life cycles. While these may contribute to the decrease in the worm burden, they may not be expected to contribute to that extent whereby the numbers leaving the host are lower than those entering. The decrease must be of a higher magnitude. In the male rats this is achieved by a severe expulsion of worms. In the female rats where an expulsion phenomenon has not been observed, it is suggested that fewer worms inhabit the caecum and this is the basis of a regulatory mechanism. But it is doubtful that this is the only mechanism and that other mechanisms which have not been observed are also responsible for decreases of S. muris burdens in the female rat. To account for the increases in worm burden following a decrease, the numbers of worms entering the host must be in excess of those leaving the host. Considering that the rat is exposed to large numbers of eggs from the perianal region, an increase may not be difficult to achieve. However, for such an increase to occur, it must also mean that a partial relapse takes place in the immune response of the rat host to allow worm burdens to build up again. The increase is caused relatively by the large numbers of immature worms that begin to occupy the caecum 32 days after the initial infection and it has been shown that in heavier infections the majority of the worms are immature.

It cannot be concluded at present that worm loss occurs because of a crowding effect or to an immune response alone. A combination of both factors may be responsible. In Chapter (Ch. VI) it was shown that a crowding effect may influence the numbers of worms residing in the caecum. This could be true for worms in the female rats where worms are fewer in comparison to those in males. On the other hand, it may be argued that an immune response operates to limit the number of larvae that may hatch from ingested eggs in female hosts. In male rats, although a crowding effect could be responsible for worm loss, it is likely that an immune response is the more plausible factor. While worms are lost suddenly in male rats, older infections in some individual male and female rats are characterised by light worm burdens, thereby pointing to periods of immunity. The caecum of rats in old infections is neither limited for space nor nutrients and, therefore, a crowding effect could not account for the paucity of worms there. Moreover, Panter (1969) showed that S. obvelata in mice are controlled by host age. Jacobson & Reed (1974) found that athymic (nude) mice were heavily infected with S. obvelata in contrast to thymic mice. There is thus a possibility that humoral or cell-mediated immune responses operate in rats against S. muris. The sporadic periods of infectivity that Roman (1969) and Roman & Kientruong (1973) observed may be explained by the light and heavy worm burdens which are found in older infections.

Whatever the regulatory mechanism may be, it produces a constancy in the S. muris infection after 32 days in weaned rats. The level of constancy reached after an initial period of rapid increase is a feature which appears common to helminth infections (Anderson, 1976). Ostertagia ostertagi infection in calves reaches a plateau following an initial increase (Michel, 1963) as does Nippostrongylus brasiliensis (Brambell, 1965;

Jarrett et al. 1968; Alphey, 1970) in rats and Ascaridia galli in chickens (Ikeme, 1971). The studies by Michel (1969), Jenkins and Philippson (1970) and Ikeme (1971) are particularly significant to the present investigation because hosts were infected repeatedly and paralleled the autoinfection that occurred in rats from S. muris. The worms are not expelled entirely from the host caecum after experiencing the immune state and it is believed that a quantity of worms remaining in the host may become adapted to the immune state (Jenkins & Phillipson, 1970). In fact, the immune state may be suppressed by a parasite (Keller et al. 1971).

In general, the male worm burden follows a similar course of infection to the female worms. A rapid increase is followed by decreases and increases, pointing to a similarity in the maintainance of the infections. The same features that regulate the female worm burdens probably operate on male worms. The male worms never reach proportions in excess of the female, showing that a few males are sufficient to inseminate a large number of females. The FMR observed (1.2 - 12.0 and above) is consistent with data reported in Chapter V and with that obtained from wild rats (Singhvi & Johnson, 1977; Ashour, 1980). One explanation for the high FMR might be that males leave the host earlier than females, the life cycle of the male being 3 days and that of the female, 7. Jenkins & Phillipson (1970) indicated that the FMR in Nippostrongylus brasiliensis was biased in favour of males when females were lost more rapidly. The opposite may be the case for Syphacia muris, thus contributing to a high FMR.

The surprising result that transmission of S. muris is similar in rats allowed to be caged individually compared to grouped rats may be interpreted in two ways. The first is that worm burdens in singly caged animals compare favourably with those in grouped animals because only self-infection occurs. In other words, rats grouped together do not pick up an infection from fellow rats, even though this might be expected of grooming behaviour. There is no experimental evidence at hand to prove this. Therefore, it is more likely a second explanation is more reasonable, namely S. muris builds up an infection and maintains it at a suitable level in the interests of its own survival. This suitable level is an inherent feature of S. muris.

-ooOoo-

CHAPTER VIII

CONCLUSIONS

During transmission, Syphacia muris goes through four moults before reaching the adult stage. The use of the electron microscope was required to detect two moults while two others were observed under the light microscope. After the final moult which occurs in the host about 66 hours after hatching, the remaining four days of the life cycle of the female S. muris is occupied by growth and maturation of the eggs in the uterus.

The present study shows that in rats 3 to 10 weeks old, the number of Syphacia muris that become established increases with host age. This is supported by studies carried out on S. muris infections in the wild by Singhvi & Johnson (1977) and Ashour (1980) who showed that older rats harboured more Syphacia muris worms than young rats. The lack of age resistance to S. muris in rats is at variance with that shown by many nematodes in their hosts, e.g., Panter (1969) showed that mice developed a striking age resistance to a related species, Syphacia obvelata, although no other specific resistance or immune responses were detected. Jacobson & Reed (1974) on the other hand, showed that athymic (nude) mice were more heavily infected with S. obvelata than thymic mice and thus linked humoral and/or cell-mediated immune responses to the resistance demonstrated by athymic mice. In view of these conflicting reports, more work on the immune response in mice to S. obvelata is needed.

However, the absence of host age resistance has been observed in other nematodes and hence, the lack of age resistance to S. muris is not an isolated case. Sandground (1929) suggested that age resistance may not

CHAPTER VIII

CONCLUSIONS

During transmission, Syphacia muris goes through four moults before reaching the adult stage. The use of the electron microscope was required to detect two moults while two others were observed under the light microscope. After the final moult which occurs in the host about 66 hours after hatching, the remaining four days of the life cycle of the female S. muris is occupied by growth and maturation of the eggs in the uterus.

The present study shows that in rats 3 to 10 weeks old, the number of Syphacia muris that become established increases with host age. This is supported by studies carried out on S. muris infections in the wild by Singhvi & Johnson (1977) and Ashour (1980) who showed that older rats harboured more Syphacia muris worms than young rats. The lack of age resistance to S. muris in rats is at variance with that shown by many nematodes in their hosts, e.g., Panter (1969) showed that mice developed a striking age resistance to a related species, Syphacia obvelata, although no other specific resistance or immune responses were detected. Jacobson & Reed (1974) on the other hand, showed that athymic (nude) mice were more heavily infected with S. obvelata than thymic mice and thus linked humoral and/or cell-mediated immune responses to the resistance demonstrated by athymic mice. In view of these conflicting reports, more work on the immune response in mice to S. obvelata is needed.

However, the absence of host age resistance has been observed in other nematodes and hence, the lack of age resistance to S. muris is not an isolated case. Sandground (1929) suggested that age resistance may not

occur in host-parasite systems that have evolved over a long period of time. Wakelin (1978), perhaps following Dineen (1963), suggested that such an evolution may be the result of reduced immunogenicity. While no attempt has been made to understand the lack of host age resistance to S. muris in the present study, it is suggested that due to the short life span of S. muris at the end of which female worms leave the host, the rat does not mount any age resistance. Moreover, no pathogenic effects were found in infected rats, indicating that S. muris has little reason to be dislodged due to host age.

Although age resistance does not limit the worm burden of Syphacia muris in the rat, other forms of resistance are evident. The female rat harbours fewer worms than males. This may be an adaptation to further transmission in the wild via litter rats and is a feature which has remained in the laboratory rat. In the wild, male rats cover greater distances than females during foraging activity (Wood, 1971; Barnett, 1976; Nass, 1977). The female is also confined to its nesting area due to its litter-bearing activities. Therefore, it is easier for S. muris to be transmitted longer distances by the male rat. The severe expulsion of worms which occurs in the male rat no doubt enhances this form of transmission, and since the eggs of S. muris remain viable for several weeks (Van der Gulden, 1966), it is a good strategy for transmission. But it is not known why male rats are capable of harbouring more S. muris than females. The review by Solomon (1969) suggests that hormonal factors may be involved. Mathies (1959 a, b) found male mice harboured twice as many Aspiculuris tetraptera than females and attributed this to the level of gonadal hormones with the onset of the

oestrus cycle in the female. **Stahl (1969b)** agreed with Mathies (*op. cit.*) that female sex hormones reduced worm burdens. Behnke (1975) suggested that sex hormones caused an immunodepression in male mice infected with *A. tetraptera*, thereby allowing more *A. tetraptera* to become established. Dobson (1961) originally stated that oestrogens stimulated the immune response and lowered *Nematospiroides dubius* burdens in female mice. Reappraisal of his data established that testosterone in males may suppress reactions to *N. dubius* (Dobson & Owen, 1978). According to Solomon (1969), testosterone can influence the infection in favour of male hamsters dosed with *Nippostrongylus brasiliensis* larvae. Thus evidence seems to favour the suppression of the immune response in male hosts and allow for a higher build up of parasites in them. Such a suppression of the immune system by testosterone in the rat may allow the accumulation of *S. muris*.

The rhythmic egg deposition of *S. muris* during the day time is clearly an adaptation designed to take advantage of the host's behavioural activity and to further transmission. Instead of depositing its eggs throughout the night and thereby endangering their loss in the faeces, *S. muris* lays its eggs during the day when the host defaecates little and is least active. By laying most of its viable eggs at a particular time, that is at noon, it not only allows a period of incubation for the larva in the egg, but also maximises the chances of infecting the rat when the latter begins grooming activity once dusk sets in. By this means *S. muris* is assured of being ingested, since about 90% of the eggs are picked up by the host at night (Van der Gulden, 1966). It is also significant in that the circadian egg deposition of *S. muris* is linked with the rhythmic maternal behaviour

of the rat. The lactating female spends the greatest amount of time with its litter midway through the day, (Grota & Ader, 1969; Ader & Grota, 1970). This suggests that peak egg deposition and pronounced viability at noon time is synchronized with maternal behaviour in order to further transmission in litter rats. But egg deposition in the day time and the period of incubation required has meant that adaptations have had to be made to avoid the loss of eggs from the perianal area. To overcome this, S. muris eggs have an adhesive property which makes them attach to the skin firmly.

In contrast to the day-time deposition rhythm of Syphacia muris, the trichostrongyle Hematospiroides dubius which is found in mice, lays most of its eggs between 19 and 24 hours (Lewis & Shava, 1977). Another pinworm in mice, Aspiculuris tetraptera, lays most of its eggs towards dawn (Phillipson, 1974) when most of the faeces are deposited. Both these worms are no doubt adapted to transmitting their eggs in the faeces of their respective hosts, and perhaps depend upon coprophagy for re-infection to take place. Syphacia cutamii Tiner, 1948 and S. peromysci Harkema, 1936 are not known to have a circadian rhythm of oviposition. Eggs are instead passed out in the faeces with transmission occurring by coprophagy and host contact (Parry, 1968). It will be interesting to observe whether Syphacia citelli Tiner & Rausch, 1950 worms which are found in the caecum of ground squirrels undergo a rhythmic migration in order to deposit eggs. Since the worms do not rupture to discharge eggs when exposed to air (unlike S. muris and S. obvelata), eggs mature outside the host and are likely to be transmitted when squirrels eat whole worms (Noble, 1965). Such a

mechanism would imply that S. citelli leaves the host in the faeces.

The fact that egg deposition is linked to the feeding regime of the host raises interesting problems. The feeding habit of the rat, like many other physiological functions, is a circadian activity (Sulzman et al., 1977). Many of these functions are endogenous (De Coursey, 1960) but they are likely to be influenced by the feeding habits of the experimental animal. But the mechanism by which feeding time is linked to the circadian rhythm is unknown. However, circadian pacemakers (linked with neurotransmitters) are known to reside in the hypothalamus (Stephen & Zucker, 1972) and these may be affected by the feeding habit of the animal; it is also possible that a food component may affect the pacemakers according to Stephen & Zucker, (1972). On the other hand, the nature of the circadian rhythm in S. muris is unknown. It is possible that the rhythm is exogenous and depends upon an external stimulus to become operative because the experiments on rats forced to feed during the day time showed that the normal day time rhythm of egg deposition was shifted to the night time. Moreover, in self-infection studies S. muris worms deposited eggs at night, presumably as a result of receiving a stimulus from the host's resting period. Again, Van der Sulden (1966) observed that only egg-depositing females followed a circadian rhythm whereas non-laying females appeared on the perianal region during the night and day. This was also seen in the present investigation (personal observations) so that the rhythm is not inherently present in S. muris but develops in the females when it is egg-depositing time. Further research is required to determine whether or not female S. muris develop a sensory mechanism capable of receiving a stimulus to migrate only when the female is close to the end of its life cycle. By sampling rats entrained to continuous

periods of light or darkness, the nature of the S. muris egg deposition rhythm may be determined further.

Syphacia muris is a prolific egg producer, but in comparison with most nematodes, female S. muris lay a smaller number of eggs, which is perhaps suited to the short life cycle. For example, Enterobius vermicularis has a 2 - 4 week life cycle, although it may be longer (Reardon, 1938) and lays 11,000 eggs per day. Egg deposition in Ascaris lumbricoides begins 2 months after a host is infected and can continue for 12 - 18 months, with as many as 200,000 eggs being laid daily (Brown & Belding, 1964). Ancyclostoma duodenale matures in 5 - 6 weeks time and deposits 25,000 to 30,000 eggs daily (Schmidt & Roberts, 1977). On the other hand, female worms of the mouse pinworm, Syphacia obvelata, which has a 10 day life cycle, produces about 250 eggs per female (Shava - pers. comm.). The rat pinworm has little need to produce any more eggs than necessary. By releasing most of its eggs at a particular time of the day and providing these adhere firmly to the perianal region, S. muris is assured that most of its eggs will be transmitted to its host by contact. But in spite of these safety measures, fewer than 30% of the worms become established in the host. It may be presumed that many eggs are lost after ingestion, although the number that survive is sufficient to maintain the population at a level which is not detrimental either to the host or to the parasite.

Density-dependent factors affect the number of worms that survive. From a large dose of eggs, few worms are produced. But the crowding effect does not limit the fecundity of S. muris, although worm size is reduced in 72-hour large infections. The similar worm lengths in 7-day

old infections resulting either from a small or large infective dose of eggs suggests that normal growth is resumed following the crowding effect. Retarded growth has been observed in Ostertagia ostertagi (Michel, 1969) and at least 30 other species of nematodes according to Michel (1974). The time at which eggs are laid appears to have a greater effect on the number of worms that will develop rather than the number of eggs that are ingested, e.g. more worms of S. muris develop from eggs laid around noon time.

The expulsion phenomenon is no doubt the manifestation of an immune response. Its role in the transmission of S. muris through the male rat has already been cited. The severe expulsion in the male rat and expulsion in both male and female rats which occurs only after a tertiary dose of 1000 eggs is administered implies that the immune response is dose-dependent. According to Dineen (1963), a small or moderate infection would evoke no immune response in a host and would be subliminally tolerated. But a minimum threshold level of antigenic information would be necessary to stimulate the response. If the antigenic information was large enough, the immune response would operate to reduce the worm burden to a point tolerable to the host. Donald et al., (1964), and Dineen et al. (1965 a), produced considerable data in support of this hypothesis by working with Nematodirus spathiger and Haemonchus contortus in sheep. It has also been reported that a certain amount of antigenic stimulus is required for expulsion to occur in rats infected with Nippostrongylus brasiliensis (Jarrett et al., 1968; Jenkins & Phillipson, 1970. Wakelin (1973) found that a sufficient number of Trichuris muris was required to stimulate its expulsion in mice. But Wakelin & Lloyd (1973) found that Trichinella spiralis expulsion could occur with as little as 10 worms or with 45 worms. Borgsteede and Hendriks (1979) found that calves would expel Cooperia oncophora only at high

levels of infection, while at low levels there was hardly any observable reaction. The expulsion of Trichostrongylus colubriformis was dependent upon a threshold level of antigenic stimulation (Chiejina & Sewell, 1974). Thus most of the evidence from the literature tends to favour the requirement of a sufficient antigenic stimulus to elicit an immune response, such as the expulsion of worms. In the case of S. muris the expulsion phase may also depend upon a sufficient number of worms being stimulated. But it may also be argued that worms were not expelled prior to a third infection dose due to immunological incompetence of the young rats used in the experiments. For example, lambs less than six months old do not acquire immunity against H. contortus (Urquhart et al., 1966) and rats against N. brasiliensis (Jarrett, 1971). Nematospiroides dubius fails to establish in young mice perhaps due to lack of functional immunological competence (Cypess et al., 1973).

The immune response to S. muris needs further investigation to determine whether it is a cell or antibody-mediated response. Both factors have been cited in nematode literature as being responsible for the expulsion phenomenon. Love (1975) clearly stated that both anti-bodies and lymphocytes were necessary for the expulsion of N. brasiliensis in mice. Wakelin (1975) linked genetic factors in mice influencing the expulsion of Trichuris muris but he also considered that anti-body mediated and lymphoid cell-mediated components were necessary. Dawkins et al., (1980) suggested that Strongyloides ratti expulsion was T-cell dependent. The immunity to T. muris was thymus dependent according to Wakelin & Selby (1974). Earlier, Wakelin (1970) had found that by suppressing the immune system of mice after administering them with cortisone acetate, the infection level of T. muris could be raised. By using/

corticosteroid drugs to suppress the immune response in the host, several investigators have indirectly linked the immune response to cell-mediated factors. Ogilvie (1965) studied the fate of N. brasiliensis in cortisone-administered rats and recovered higher worm burdens from them than from non-treated controls. Mathies (1962) found a lowering of resistance in mice infected with A. tetraptera due to cortisone. Behnke (1975) suppressed immunity to A. tetraptera by injecting mice with hydrocortisone. While Olson & Schillier (1975) used corticosteroids to suppress expulsion of S. ratti in rats. Moqbel & Denham (1978) achieved the same effect by injecting hosts with betamethasone. Michel & Sinclair (1969) found cortisone depressed the immune response in calves and allowed C. ostertagi burdens to increase. Thus, in view of the large evidence available for expulsion occuring in other nematode-host systems due to suppression of the immune response, a cell-mediated factor may operate in rats. But antibodies may also be involved; as S. muris is found in the mucous lining of the caecal wall and does not pass through a tissue phase, the components necessary for expulsion may be found in the mucus. Dobson (1966) demonstrated that antibodies were present in intestinal mucus of sheep infected with Oesophagostomum columbianum.

The course of infection followed by the three groups of rats under differing experimental conditions showed remarkable similarity. After increasing for the first 32 days of the infection period, worm burdens fluctuated around an equilibrium level. Since the conditions of the experiments closely approached those in laboratories in animal houses, the results are a reflection of the normal course of events. The accumulation of worms in the 32 days indicated lack of

resistance on the part of the host. At this stage either the host was immunologically incompetent, as has been cited in the case of H. contortus infections in lambs (Urquhart et al., 1966) and N. dubius in mice (Cypess et al., 1973), or not enough worms of S. muris were present to elicit an immune response of the type suggested by Dineen (1963).

The small decreases and increases in the rat of infection which characterise the steady state after 32 days may be explained by two references available in the literature. According to the 'threshold level' of antigenicity described by Dineen (1963), sufficient antigenic information must have been present in the host 32 days after the infection was initiated in order to stimulate an immune response and thereby lower the worm burden to a tolerable level. The lowered worm burden was, however, insufficient to maintain the antigenic stimulus and, therefore, the burden was allowed to rise to a level which again elicited a host reaction. But Michel (1963, 1969) argued that the course of infection of Ostertagia ostertagi in calves was maintained by a turnover of worms. A number of worms of O. ostertagi was lost during the course of infection and this depended upon the number present in the host, with no immune responses being involved in the regulation of worms. There is no other reference available in the literature to support Michel's hypothesis. In the case of S. muris regulation in the rat, a steady state is probably maintained by an immune response and a fast turnover of worms because it is apparent that a large number of worms does elicit a host resistance response, and daily autoinfection coupled with the short (3-day) life-cycle provides the fast turnover that is necessary.

The stability depicted by S. muris in its course of infection has been apparent in other nematode transmission cycles. But only recently have serious attempts been made to analyse the factors involved in maintaining this stability. Crofton (1971) used a mathematical model to study host-parasite relationships, and he suggested that population densities of parasites fluctuated around equilbrium levels. Essentially the equilibrium was achieved by the parasites regulating their own populations through such mechanisms as the host immune response. Bradley (1972, 1974), maintained that density-dependent factors, such as competition, the immune response, regulated parasite populations and produced stability in the system. Grundmann et al. (1976) decided on the basis of extensive data that was collected from the wild that no less than eleven factors contributed to the stability of parasite populations. Anderson (1976), basing his studies on mathematical models, concluded that parasite populations reached equilibrium levels as a result of four rate parameters: (a) birth, (b) emigration, (c) immigration, (d) death. In order to reach equilibrium levels, anyone of these rate parameters was needed to be regulated by density-dependent factors. In the absence of density-dependent constraints regulating a rate, for example, birth, the population could proceed unchecked and rise exponentially. In nature, however, density-dependent factors do operate and control populations. For example, egg output per worm in heavy infections of O. ostertagi in calves is depressed in comparison with light infections (Michel, 1967). The immune response of the rat is likely to be one of the density-dependent factors that regulate S. muris population and thereby contribute to its stability. The role played by other density-dependent factors, such as overcrowding, competition, need to be determined.

To conclude, Syphacia muris and the rat host present us with a model for further study of host-parasite relationships. In particular, it needs to be determined whether or not the circadian rhythm of egg deposition by migrating females is exogenous or endogenous in nature, and if it is linked to other host activities besides feeding and defaecation. Work is required on the population dynamics of S. muris in terms of the four rate parameters: birth, immigration, emigration and death, and the relevance of density-dependent processes. Some problems associated with Syphacia muris - infected rats have perhaps deterred workers in the past from using them for extensive studies. These problems have been overcome during the course of the present work. By using sterile isolators, worm-free rats have been raised in the laboratory. Rats have been maintained in ventilated, perspex-top cages outside the isolators for relatively long periods of time without becoming infected from the air. A method has been described for the collection of S. muris eggs from the perianal region. These measures and the short, direct life cycle of S. muris worms, their easy availability in the laboratory makes the rat pinworm ideal for the study of infection processes. The application of these results to an understanding of the nature of the pinworm infection in man should prove invaluable since there is little knowledge available about Enterobius vermicularis which is found in children universally and which cannot be cultured in laboratory hosts.

-ooOoo-

REFERENCES

ACKERT, J.E., Graham, G.L., Nolf, L.O., and Porter, D.A. (1931): Quantitative studies on the administration of variable numbers of nematode eggs (Ascaridia lineata) to chickens.

Trans. Amer. micr. Soc., 508: 206-214.

ADER, R. and Grota, L.J. (1970): Rhythmicity in the maternal behaviour of Rattus norvegicus.

Anim. Behav., 18:144-150.

AFRICA, C.M. (1931): Studies on the host relations of Nippostrongylus muris with special reference to age resistance and acquired immunity.

J. Parasit.: 18:1-13.

ALPHEY, T.J.W. (1970): Studies on the distribution and site location of Nippostrongylus brasiliensis within the small intestine of laboratory rats.

Parasitology: 61: 449-460.

ANDERSON, R.M. (1976): Dynamic aspects of parasite population ecology. In Ecological Aspects of Parasitology, C.R. Kennedy, Editor. Pp. 431-462. North Holland Publishing Co., Amsterdam.

ASHOUR, A. (1980): Ultrastructural and other studies on intestinal nematodes of small mammals from Egypt. Ph.D. Thesis, Ain Shams University, Cairo.

BAILEY, W.S. (1949): Studies on calves experimentally infected with Cooperia punctata (v. Linstow, 1907) Ransom, 1907.

Amer. J. vet. Res., 10: 119-129.

BARNETT, S.A. (1976): The Rat: A Study in Behaviour University of Chicago Press.

BEHNKE, J.M. (1975): Immune expulsion of the nematode Aspicularis tetraptera from mice given primary and challenge infections.

Int. J. Parasit.: 5: 511-515.

BIRD, A.F. (1971): The Structure of Nematodes. Academic Press, New York.

BLAIR, L.S. and Thompson, P.E. (1969): Effects of pyrvinium pamoate in the ration or drinking water of rats against the pinworm Syphacia muris.
Lab. Anim. Care 19:639-643.

BORGSTEEDE, F.H.M., and Hendricks, J. (1979): Experimental infections with Cooperia oncophora (Railliet, 1918) in calves. Results of single infections with two graded dose levels of larva.
Parasitology 78:331-342.

BOSS, G.K. and Olson, L.J. (1965): Trichinella spiralis in newborn mice: Course of infection and effect on resistance to challenge.
J. Parasit., 51: 640-644.

BRADLEY, D.J. (1972): Regulation of parasite populations. A general theory of the epidemiology and control of parasitic infections.
Trans. R. Soc. trop. med. Hyg., 66: 697-708.

BRADLEY, D.J. (1974): Stability in host-parasite systems. In Ecological Stability, M.B. Usher and M.H. Williams, Editors., Chapman and Hall, London.

BRAMBELL, M.R. (1965): The distribution of a primary infestation of Nippostrongylus brasiliensis in the small intestine of laboratory rats.
Parasitology 55: 313-324.

BROWN, H.W. and Belding, D.L. (1964): Basic Clinical Parsitology, Appleton-Century-Crofts, New York.

CHAN, K.F. (1952): Life cycle studies of the nematode Syphacia obvelata.
Amer. J. Hyg., 56: 22-30.

CHAN, K.F. and Kopilof, S. (1958): Susceptibility of two strains of white mice to Syphacia obvelata infections.

J. Parasit., 44: 451-452.

CHANDLER, A.S. (1936): Studies on the nature of immunity to intestinal helminths. III. Renewal of growth and egg production in Nippostrongylus after transfer from immune to non-immune rats.

Amer. J. Hyg., 23: 46- 52.

CHENG, T. (1973): Textbook of General Parasitology. Academic Press, New York.

CHIEJINA, S.N. and Sewell, M.M.H. (1974): Experimental infection with Trichostrongylus colubriformis (Giles, 1892) Looss, 1905 in lambs: worm burden, growth rate and host resistance resulting from prolonged escalating infections.

Parasitology 69: 301-314.

CHITWOOD, B.G. and Chitwood, M.B. (1974): Introduction to Nematodology, University Park Press, Baltimore.

CROFTON, H.D. (1971): A model of host-parasite relationships. Parasitology 63: 343-364.

CYPESS, R.H. and Van Zandt, P. (1973): Time studies on the immune expulsion of Nematospiroides dubius in the mouse.

J. Parasit., 59: 579-580.

CYPESS, R.H., Lucia, H.L., Zidian, J.L. and Rivera-Ortiz, C.I. (1977): Heligmosomoides polygyrus: temporal, sptatial and morphological population characteristics in LAF1/J mice.

Expl Parasit. 42: 34-43.

DAWKINS, H.J.S., Grove, D.I., Dunsmore, J.D., Mitchell, G.F. (1980): Strongyloides ratti: susceptibility to infection and resistance to reinfection in inbred strains of mice as assessed by excretion of larvae.

Int. J. Parasit., 10; 125-129.

DE COURSEY, P.J. (1960): Day light sensitivity rhythm in a rodent.

Science 131: 33-35.

DICK, T.A., and Wright, K.A. (1973): Ultrastructure of the cuticle of the nematode Syphacia obvelata (Rudolphi, 1802). I. The body cuticle of larvae, males, and females, and observations on its development.

Can. J. Zool., 51: 187-196.

DINEEN, J.K. (1963):Immunological aspects of parasitism.

Nature, London, 197:471-472.

DINEEN, J.K., Donald, A.D., Wagland, B.M., and Offner, J. (1965): The dynamics of the host-parasite relationship. III. The response of sheep to primary infection with Haemonchus contortus.

Parasitology 55: 515-525.

DOBSON, C. (1961): Certain aspects of the host-parasite relationship of Nematospiroides dubius (Baylis). I. Resistance of male and female mice to experimental infections.

Parasitology 51: 173-179.

DOBSON, C. (1965): The relationships between worm population density, host survival and the growth of the the 3rd stage larva of Amplicaecum robertsi Sprent & Mines, 1960 in the mouse.

Parasitology 55: 183-193.

DOBSON, C. (1966): The demonstration of antibodies in the intestinal mucus of sheep infected with Oesophagostomum columbianum by means of the percutaneous anaphylaxis.

Austln. Jnl Biol. Sci., 19:339-340.

DOBSON, C. (1974): Studies on the immunity of sheep to Oesophagostomum columbianum: effects of different and successive doses of larvae on worm burdens, worm growth and fecundity.

Parasitology 68:313-322.

DOBSON, C. and Owen, M.E. (1978): Effect of host sex on passive immunity in mice infected with Nematospiroides dubius.

Int. J. Parasit., 8: 359-364.

DONALD, A.D., Dineen, J.K., Turner, J.H., and Wagland, M. (1964): The dynamics of the host-parasite relationship.I. Nematodirus spathiger infection in sheep.

Parasitology 54: 527-544.

DORMAN, H.P. (1928): Studies on the life-cycle of Heterakis papillosa (Bloch).

Trans. Am. Micrs. Soc., 47: 379-413.

D'SILVA, J. and Lewis, J.W. (1979): The transmission of the rat pinworm, Syphacia muris.

Parasitology 79: xxi.

DUDZINSKI, M.L. and Myktowycz, R. (1963): Relationship between sex and age of rabbits, Oryctolagus cuniculus (L.,) and infection with nematodes Trichostrongylus retortaeformis and Graphidium strigosum.

J. Parasitol., 49: 55-59.

FUKUI, M., Adachi, J., and Akaike, I. (1973): Studies on experimental infection of rat pinworm, Syphacia muris, to mouse for research. Part 2.
Jap. J. Parasit., 22'(1, Suppl) 7.

GARNHAM, P.C.C., and Powers, K. (1974): Periodicity of infectivity of plasmodial gametocytes: the "Hawking phenomenon."
Int. J. Parasit., 4: 103-106.

GORDON, H. McL. (1948): The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep.
Aust. Vet. J., 24: 17-45.

GROTA, L.J. and Ader, R. (1969): Continuous recording of maternal behaviour in Rattus norvegicus.
Anim. Behav. 17: 722-729.

GRUNDMANN, A.W., Warnock, R.G., and Wassom, D.L. (1976): Some mechanisms of natural regulation of parasitic helminth populations.
Amer. Midl. Naturalist 95: 347-360.

HALEY, A.J. (1958): Host specificity of the rat nematode, Nippostrongylus brasiliensis.
Amer. J. Hyg., 67: 331-349.

HALEY, A.J. (1962): Biology of the rat nematode Nippostrongylus brasiliensis (Travassos, 1914). II. Preparasitic stages and development in the laboratory rat.
J. Parasit., 48: 13-23.

HALEY, A.J., Parker, J.C. (1961): Effect of population density on adult worm survival in primary Nippostrongylus brasiliensis infections in the rat.
Proc. Helm. Soc., Wash., 28: 176-180.

HAWKING, F. (1967): The 24-hour periodicity of microfilariae: biological mechanisms responsible for its production and control.

Proc. Roy. Soc., B 169: 59-76.

HAWKING, F. (1977): Circadian and other rhythms of parasites. In Advances in Parasitology, Vol. 13, Ben Dawes, Editor, pp. 123-182. Academic Press, New York.

HERLICH, H. (1958): Further observations on the experimental host-parasite relations of the guinea-pig and the ruminant parasite Trichostrongylus colubriformis.

J. Parasit., 44: 602.

HERLICH, H., Douvres, F.W., and Isenstein, R.S. (1956): Experimental infections of guinea-pig with Trichostrongylus colubriformis, a parasite of ruminants.

Proc. Helm. Soc., Wash., 23: 104-105.

HILL, R.P. (1926): The estimation of the number of hookworms harboured by the use of the dilution egg count method.

Amer. J. Hyg., 6:19-41.

HUSSEY, K.L. (1957): Syphacia muris vs. S. obvelata in laboratory rats and mice.

J. Parasit., 43: 555-559.

IKEME, M.M. (1971): Effects of different levels of nutrition and continuing dosing of poultry with Ascaridia galli eggs on the development of parasite populations.

Parasitology 63: 233-250.

JACOBSON, R.H. and Reed, N.D. (1974): The thymus dependency of resistance to pinworm infection in mice.

J. Parsit., 60: 976-979.

JARRETT, E.E.E. (1971): Diminished immunological responsiveness to helminth parasites. The effect of repeated reinfection of rats from an early age with Nippostrongylus brasiliensis.
Clin. expl Immun., 8: 141-150.

JARRETT, E.E.E., Jarrett, W.H., and Urquhart, G.M.(1968): Quantitative studies on the kinetics of establishment and expulsion of intestinal nematodes in susceptible and immune hosts.
Parasitology 58: 625-639.

JENKINS, D.C. and Phillipson, R.F. (1970): The kinetics of repeated low-level infections of Nippostrongylus brasiliensis in the rat.
Parasitology 62: 457-465.

KASSAI, T., and Aitken, I.D. (1967): Induction of immunological tolerance in rats to Nippostrongylus brasiliensis infection.
Parasitology 57: 403-.

KELLER, R., Ogilvie, B.M. and Simpson, E. (1971): Tumour growth in nematode-infected animals.
Lancet 1 (7701): 678-680.

KING, C. M. (1977): The effects of the nematode parasite Skrjabingylus nasicola on British weasels (Mustela nivalis).
J. Zool., Lond. 182: 225-249.

KRUPP, J.M. (1961): Effects of crowding and of superinfection on habitat selection and egg production in Ancylostoma caninum.
J. Parasit., 47: 957-961.

LARSH, J.E., Jr. (1963): Experimental trichiniasis. In Advances in Parasitology, Vol. I., Ben Dawes, Editor. pp. 213-286. Academic Press, New York.

LARSH, J.E., and Hendricks, J.R. (1949): The probable explanation for the difference in the localization of adult Trichinella spiralis in young and old mice.

J. Parasit., 35: 101-106.

LARSH, J.E., Jr., and Race, A.G. (1954): A histopathological study of the anterior small intestine of immunized mice infected with Trichinella spiralis.

J. Inf. Dis., 94: 262-272.

LEE, D.L. (1966): The structure and composition of the helminth cuticle. In Advances in Parasitology, Vol. 4, Ben Dawes, Editor. pp. 187-254. Academic Press. New York.

LEE, D.L. and Atkinson, H.J. (1976): Physiology of Nematodes, Macmillan Press Ltd., London.

LEWIS, J.W. (1968): Studies on the helminth parasites of the Long tailed field mouse, Apodemus sylvaticus sylvaticus from Wales.

J. Zool., Lond., 154: 287-312.

LEWIS, J.W. and Rentmore, G. (1968): A method for collecting faecal samples from small mammals over a continuous period.

J. Zool., Lond., 156: 537-540.

LEWIS, J.W. and Shava, F.H.M. (1977): Rhythmic egg deposition by intestinal nematodes of laboratory mice.

Parasitology 75: iv.

LEWIS, J.W. and Rentmore, G. (1979): A method for recording general and feeding activity of small mammals.

J. Zool., Lond., 189: 553-557.

LEWIS, J.W. and D'Silva, J. (1980): Rhythmic egg deposition by the oxyurid nematode Syphacia muris in the rat.

J. Zool., Lond., 191: 429-433.

LOVE, R.J. (1975): Nippostrongylus brasiliensis infections in mice: the immunological basis of worm expulsion.

Parasitology 70: 11-18.

MACARTHUR, W.P. (1930): Threadworms and pruritis ani.

Jl. R. Army vet. Cps., 55: 214-216.

MCCOY, O.R. (1931): The egg production of two physiological strains of the dog hookworm Ancylostoma caninum.

Am. J. Hyg., 14: 194-202.

MARGULES, D.L., Lewis, M.J., Dragovich, J.A., and Margules, A.S. (1972): Hypothalamic norepinephrine: circadian rhythms and the control of feeding behaviour.

Science, 178: 640-643.

MATHIES, A.W. (1959a): Certain aspects of the host-parasite relationship of Aspicularis tetraptera, a mouse pinworm. I. Host specificity and age resistance.

Expl Parasit., 8: 31-38.

MATHIES, A.W. (1959b): Certain aspects of the host-parasite relationship of Aspicularis tetraptera, a mouse pinworm. II. Sex resistance.

Expl Parasit., 8: 39-45.

MATHIES, A.W., Jr., (1962): Certain aspects of the host-parasite relationship of Aspicularis tetraptera, a mouse pinworm. III. Effect of cortisone.

J. Parasit., 48: 244-248.

MAYHEW, R. (1940): Immunity to hookworm and nodular worm infections in calves. Immunity to the stomach worm.

J. Parasit., 26: 345-347

MICHEL, J.F. (1963): The phenomenon of host resistance and the course of infection of Ostertagia ostertagi in calves.

Parasitology 53: 63- 84.

MICHEL, J.F. (1969): Some observations on the worm burdens of calves infected daily with Ostertagia ostertagi.

Parasitology 59: 575-595.

MICHEL, J.F. (1974): Arrested development of nematodes and some related phenomena.
In Advances in Parasitology, Vol. 11. Ben Dawes, Editor. pp. 279-366. Academic Press, New York.

MICHEL, J.F., and Sinclair, I.J. (1969): The effect of cortisone on the worm burdens of calves infected daily with Ostertagia ostertagi.

Parasitology: 59: 691-708.

MICHEL, J.F., Lancaster, M.B., and Hong, C. (1978): The length of Ostertagia ostertagi in populations of uniform age.

Intl. Jnl Parasit., 8: 437-441.

MORIMOTO, Y., and Yamamura, Y. (1979): Regulation of circadian adrenocortical periodicities and of eating-fasting cycles in rats under various lighting conditions. In Biological Rhythms and their Control Mechanisms. Masami Suda, Osamu Hayaishi, Hachiro Nakagawa (Editors). pp. 177-255. The Nato Foundation, Elsevier/North Holland Biomedical Press.

MOQBEL, R., and Denham, D.A. (1977): Strongyloides ratti: 1. Parasitological observations on primary and secondary infections in the small intestines of rats.

J. Helminth., 51: 301-308.

MOQBEL, R., and Denham, D.A. (1978): Strongyloides ratti: The effect of betamethasone on the course of infection in rats.

Parasitology 76: 289-298.

MULLER, H. (1976): Zur Parasitenfauna der Labortiere.2. Die Parasitenfauna der weissen Ratte (Rattus norvegicus var albinus). Thesis abstract.

Wiener Tierarzthiche Monatschrift. 63:31.

NASS, R.D. (1977): Movements and home ranges of Polynesian rats in Hawaiian sugarcane

Pacific Science: 31: 135-142.

NOBLE, G.A. (1965): Ground squirrel pinworm infection.

J. Parasit., 51: 267.

OGDEN, C.G. (1971): Observations in the systematics of nematodes belonging to the genus Syphacia Seurat, 1916.

Bull. Brit. Mus. (Natl. Hist.) 20:253-280.

OGILVIE, B.M. (1965): Use of cortisone derivatives to inhibit resistance to Nippostrongylus brasiliensis and to study the fate of parasites in resistant hosts.

Parasitology 55: 723-730.

OGILVIE, B.M., and Hockley, D.J. (1968): Effects of immunity on Nippostrongylus brasiliensis adult worms: reversible and irreversible changes in infectivity, reproduction and morphology.

J. Parasit. 54: 1073-1084.

OTTO, G.F. (1966): Development of parasitic stages of nematodes. In Biology of Parasites. E.J.L. Soulsby, Editor. pp. 85-99. Academic Press, New York.

OWEN, D. (1972): Common parasites of laboratory rodents and lagomorphs. Laboratory Animals Centre Handbook.1. Her Majesty's Stationery Office, London.

OWEN, D., and Turton, J.A. (1979): Eradication of the pinworm Syphacia obvelata from an animal unit by anthelmintic therapy.

Lab. Anim.. 13:115-118.

PANTER, H.C. (1969): Studies on host-parasite relationships: Syphacia obvelata in the mouse.

J. Parasit., 55: 74-78.

PARRY, J.E. (1968): Transmission studies of nematodes with direct life histories in selected Utah mammals.

Diss. Abstr. 29(4): 1532-1533.

PHILLIPSON, R.F. (1970): Experiments on the reproduction of Nippostrongylus brasiliensis in the rat.

Parasitology 61: 317-322.

PHILLIPSON, R.F. (1974): Intermittent egg release by Aspicularis tetraptera in mice.

Parasitology 69: 207-213.

PRINCE, M.J.R. (1950): Studies on the life cycle of Syphacia obvelata, a common nematode parasite of rats.

Science 111: 66-67.

READ, C.P. (1951): The 'crowding effect' in tapeworm infections.

J. Parasit., 37: 174-178.

REARDON, L. (1938): Studies on oxyuriasis. XVI. The number of eggs produced by the pinworm, Enterobius vermicularis and its bearing on infection.

Publ. Health Reprint. 53: 978-984.

REYNOLDS, E.S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy.

J. Cell Biol., 17: 208-212.

RICHTER, C.P. (1922): A behaviouristic study of the activity of the rat.

Comp. Psychol. Monogr. 1: 1-55.

RICHTER, C.P. (1927): Animal behaviour and internal drives.

Q. Rev. Biol. 2: 307-343.

ROGERS, W.P. (1962): The Nature of Parasitism: The relationship of some metazoan parasites to their hosts. Academic Press. New York.

ROGERS, W.P. and Sommerville, R.I. (1969): Chemical aspects of growth and development. In Chemical Zoology. Vol III. M. Florkin and B.T. Scheer, Editors. pp. 465-499. Academic Press, New York.

ROMAN, E. (1969): Modalites d'infestation et resistance au parasitisme dans l'oxyurose due rat a Syphacia muris.

Bull. Ass. Dept. Microbiol., Nancy, No. 114.

ROMAN, E. and Kientruong, T. (1973): Comportement du rat a l'egard des infestations par l'oxyuride Syphacia muris aux differents ages de la vie.

Bull. de la Soc. Pathologie Exotique 66: 178-183.

SADUN, E.H. (1949) The effect of single infections of variable size on the resistance of chickens to the nematode, Ascaridia galli.

Am. J. Hyg., 49: 117-126.

SANDGROUND, J.H. (1929): A consideration of the relation of host specificity of helminths and other metazoan parasites to the phenomenon of age resistance and acquired immunity.

Parasitology 21: 227-55

SARLES, M. (1932): Development of an acquired resistance by repeated infection with Trichostrongylus calcaratus.

J. Parasit., 19:61-83

SCHMIDT, G.D., and Roberts, L.S. (1977): Foundations of Parasitology, The C.V. Mosby Co., Saint Louis.

SEY, O. (1969): The influence of size and duration of infection on changes in the sex ratio of Nippostrongylus brasiliensis populations in rats.
Parasit. Hung., 2: 45-54.

SINGHVI, A., and Johnson, S. (1976): The systematics, distribution, population dynamics and seasonal variation of the helminth parasites of the common house rat, Rattus rattus.
Zeitsch Angew Zool., 63: 469-496.

SINGHVI, A., and Johnson, S. (1977): The female to male ratio (FMR) in dominant nematode population in the house rat, Rattus rattus.
J. Parasit., 63: 858-860.

SOLOMON, G.B. (1969): Host hormones and parasitic infections.
Int. Rev. Trop. Med., 3: 101-158.

SPARROW, S. (1976): The microbiological and parasitological status of laboratory animals from accredited breeders in the U.K.
Lab. Animals 10: 365-373.

STAHL, W. (1961a): Syphacia muris, the rat pinworm.
Science 133: 576-577.

STAHL, W. (1961b): Influence of age and sex on the susceptibility of albino mice to infection with Aspicularis tetraptera.
J. Parasit., 47: 939-941.

STAHL, W. (1963): Studies on the life cycle of Syphacia muris.
Keio Jnl Medicine: 12: 55-60.

STEPHEN, F.K., and Zucker, I. (1972): Circadian rhythms in drinking behaviour and locomotor activity of rats and eliminated by hypothalamic lesions.

Proc. Natl. Acad. Sci., 69: 1583-1586.

STEWART, D.F. (1950): Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infestation. II. The antibody response to infestation with H. contortus.

Aust. J. agric. Res., 1: 301-321.

STOIMENOV, K. (1976): The survival and pathogenecity of Heterakis galinnae related to infection intensity and to the age of chicks.

Vet.-Medit. Nauki., 13: 48-54
(Helm. Abstr. (1977), Vol. 46, Abstr. No. 2976.)

STOLL, N.R. (1929): Studies with the strongyloid nematode Haemonchus contortus.I. Acquired resistance of hosts under natural conditions out of doors.

Amer. J. Hyg., 10: 384-418.

STRASSER, H., and Tiefenbach, B. (1977): Treatment of a Syphacia muris infection with fenbendazole in a rat breeding colony.

Deut. Tierarztliche Wochenschrift 84: 479-480.

SULZMAN, F.M., and Fuller, C.A. (1977): Feeding time synchronizes primate circadian rhythms.

Physiol. Behav., 18: 775-779.

TAFFS, L.F. (1976): Pinworm infections in laboratory rodents: a review.

Lab. Anim., 10; 1-13.

TANAKA, H., Ohshima, S., and Fujinami, F. (1974): A survey of parasites in commercially available small laboratory animals.

Exptl Anim., 23: 15-30.

TAYLOR, S.M., and Kilpatrick, D. (1980): Trichostrongylus vitrinus: The influence of age of sheep and population size on the intestinal distribution.
J. Helm., 54: 1-6.

TEITELBAUM, P. and Campbell, B.A. (1958): Ingestion patterns in hyperphagic and normal rats.
J. comp. physiol. Psychol., 51: 135-141.

TENORA, F., Barus, V., Wiger, R., and Mas-Coma, S. (1978): Scanning electron microscopic study of Syphacia muris and Syphacia arvicolae (Nematoda: Oxyuridae).
Acta Universita Agric., 26: 195-207.

TETLEY, J.H. (1949): Rhythms in nematode parasitism of sheep.
Bull. 96. Dept. Scient. and Indust. Res., New Zealand. 214 pp.

THOMPSON, P.E., Worley, D.E., and Meisenhelder, J.E. (1962): Anthelmintic studies on pyrvinium pamoate (Povan) and other drugs in rodents, dogs, and monkeys.
Am. J. Trop. Med. Hyg., 11: 89-95.

THUST, R. (1966): Electronenmikroskopische Untersuchungen uber den Bau des larvalen Integumentes und zur Hautungsmorphologie von Ascaris lumbricoides.
Zool. Anz., 188:411-417.

TONGSON, M.S., and McCraw, B.M. (1967): Experimental ascaridiosis: influence of chicken age and infected egg dose on structure of Ascaridia galli populations.
Expl Parasit., 160-172.

URQUHART, G.M., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.M., and Mulligan, W. (1966): Immunity to Haemonchus contortus infection: relationship between age and successful vaccination with irradiate larvae.
Am. J. vet. Res., 27: 1645-1648.

VAN DER GULDEN, W.J.I. (1966): The rat pinworm Syphacia muris (Yamaguti, 1935).
Published Doctoral Thesis, University of Utrecht.

VAN DER GULDEN, W.J.I. (1967): Diurnal rhythm in egg production by Syphacia muris.
Expl Parasit., 21: 344-347.

VAN DER GULDEN, W.J.I. (1976): Syphacia muris: water permeability of eggs and its effect on hatching.
Expl Parasit., 39: 40-44.

VAN DEN BOSSCHE, J.H. (1972): Biochemical effects of antihelmintic drug Mebendazole.
In Comparative Biochemistry of Parasites, Van Den Bossche, Editor. pp. 139-157.
Academic Press, New York.

WAKAI, R., Kikuchi, S., and Hayashi, S. (1975): Scanning electron microscopic observations on Syphacia obvelata and S. muris.
Jap. Jnl. Parasit., 24 (1, Suppl) 11.

WAKELIN, D. (1967): Acquired immunity to Trichuris muris in the albino laboratory mouse.
Parasitology 57: 515-524.

WAKELIN, D. (1970): Studies on the immunity of albino mice to Trichuris muris. Suppression of immunity by cortisone acetate.
Parasitology 60: 229-237.

WAKELIN, D. (1973): The stimulation of immunity to Trichuris muris in mice exposed to low-level infections.
Parasitology 66: 181-189.

WAKELIN, D. (1975): Immune expulsion of Trichuris muris from mice during a primary infection: analysis of the components involved.
Parasitology 70: 397-405.

WAKELIN, D. (1978): Immunity to intestinal parasites.
Nature, London 273: 617-620.

WAKELIN, D., and Selby, G.R. (1974): Thymus dependency of the immune response of mice to a primary infection with the nematode Trichuris muris.
Int. J. Parasit., 4: 657-661.

WAKELIN, D., and Lloyd, M. (1976): Immunity to primary and challenge infections of Trichinella spiralis in mice: a re-examination of conventional parameters.
Parasitology 72: 173-182.

WHITLOCK, J.H. (1966): The environmental biology of a nematode. In Biology of Parasites, E.J.L. Soulsby, Editor. pp. 185-197. Academic Press, New York.

WOOD, D.H. (1971): The ecology of Rattus fuscips and Melomys cervinipes (Rodentia: Muridae) in a South-east Queensland rain forest.
Aust. J. Zool., 19: 371-392.

YAMAGUTI, S. (1935): Studies on helminth fauna, Japan. Part 13.
Jap. J. Zool. 6: 433-457.

YAMAGUTI, S. (1941): Studies on helminth fauna of Japan. Part 35. Mammalian nematodes.
Jap. J. Zool. 9: 397-408.

The Transmission of the Rat Pinworm, *Syphacia muris*. By J. D'Silva and J. W. Lewis (*Department of Zoology, Royal Holloway College, University of London, Englefield Green, Surrey*)

Adult females of the pinworm *Syphacia muris* (Nematoda: Oxyuridae) migrate from the caeca of infected rats to deposit their eggs on to the host's perianal region primarily at noon (van der Gulden (1967). *Experimental Parasitology* **21**, 344–7). The noon-time periodicity of egg deposition is also shown by the mouse pinworm *S. obvelata* (Lewis & Shava (1977). *Parasitology* **75**, iv) whereas the human pinworm *Enterobius vermicularis* lays its eggs at night (MacArthur (1930). *Journal of the Royal Army Medical Corps*, **55**, 214–16).

The present study confirms the existence of a diurnal rhythm in egg production by *Syphacia muris*. By monitoring the feeding activity of Sprague-Dawley laboratory rats using an activity/feeding cage linked with an automatic faecal sampler (Lewis & Rentmore (1968). *Journal of Zoology, London* **156**, 537–40), the peak of daily egg production by *S. muris* occurs around mid day, when the rat shows minimum activity but following an active feeding period.

It is likely that the transmission of *Syphacia muris* in the rat is dependent upon the timing of daily egg deposition as (i) the degree of hatching of eggs produced around mid-day is significantly greater than those deposited by female worms at any other time of day and (ii) the majority of males in worm populations develop from those eggs deposited around the host's perianal region between 12 and 15 h. In addition the timing of the peak of egg production by female worms is influenced by changes in the feeding behaviour of the rat, and this was demonstrated by reversing the feeding and daylight conditions of the host over 24 h periods.

Reprinted from *J. Zool., Lond.* (1980) **191**, 429–433

Rhythmic egg deposition by the oxyurid nematode *Syphacia muris* in the rat

Introduction

Adult male and female worms of *Syphacia muris* (Yamaguti, 1935), commonly known as pinworms (Nematoda: Oxyuroidea), inhabit the caecum of most laboratory-bred rat colonies (Stahl, 1961; Taffs, 1976). Gravid female worms migrate from the caecum of the rat to the anus and deposit their embryonated eggs on the host's perianal skin. The rat becomes infected by ingesting eggs directly from the perianal region primarily whilst

grooming itself. The life cycle from embryonated egg to egg-producing females in the caecum of the rat is completed within eight days (Prince, 1950; Stahl, 1961, 1963).

van der Gulden (1967) showed that gravid females of *Syphacia muris* follow a diurnal rhythm when laying their eggs on the perianal skin of the rat, most of the eggs being deposited during the daytime with a peak occurring around noon. Lewis & Shava (1977) observed a similar rhythmic pattern of egg deposition in female *S. obvelata* (Rudolphi, 1802) in the laboratory mouse. In contrast the migration by females of the human pinworm *Enterobius vermicularis* (L., 1758) follows a rhythm but egg laying takes place at night (MacArthur, 1930). Because man is active during the day and rodents are nocturnal animals (Richter, 1922, 1927; Barnett, 1976) it seems reasonable to suggest that the migration of female pinworms along the gastro-intestinal tract may be linked with the host's activity or inactivity, eggs being laid when the mammalian host is least active. By using the method described by Lewis & Rentmore (1979) for recording the general and feeding activity of small mammals, the present paper therefore considers the role of feeding activity in the rat in the rhythmic deposition of eggs by female *Syphacia muris*.

Materials and methods

Six male and six female 3-week-old Sprague-Dawley laboratory rats, which had been experimentally infected with *Syphacia muris* were sampled for eggs deposited by female worms on the perianal skin using sellotape. Sampling was carried out throughout a 24-hour period, each sample representing the total number of eggs released per rat/2 hours. The rats were allowed to feed *ad lib* and the timing of defaecation and feeding activity (expressed as a percentage of the overall activity of the rat/hour) was recorded over a continuous 24 hour period using a faecal sampler and activity cage previously described by Lewis & Rentmore (1968, 1979).

Nine male and 9 female 3-week old rats infected with *Syphacea muris* were also conditioned for 10 days to feed during the daytime and to abstain at night. The above egg sampling and host recording procedures were repeated with the view to investigate the effect of a reversal in the feeding behaviour of the rat on the timing of egg deposition by *Syphacia muris*.

Furthermore, in order to determine whether the timing of daily egg deposition might influence the hatching success and subsequent development and maturation of *Syphacia muris* in the rat, samples of 150–715 eggs were collected at 1000, 1200, 1400 and 1600 hr from the perianal region of rats exhibiting a normal feeding and defaecation pattern; these eggs were fed to groups of three male and three female 3-week-old rats. The latter were autopsied 72 hours after initial infection and the caecum examined for numbers of adult worms established.

Results

The egg laying pattern of female *Syphacia muris* in normally fed male and female rats is shown in Table I. Eggs primarily appear on the perianal region between 0800 and 1800 hr, which is also the period when both male and female rats show minimum feeding activity and no defaecation. The peak of egg deposition occurs at noon time when no feeding takes place. On the other hand few or no eggs are laid by *Syphacia muris* when the rat is actively feeding and defaecating in the evening and early morning between 1800 and 0600 hr (Table I). If however rats are conditioned to feed during the daytime from 0800 to 1600 hr instead of from 1800 to 0600 hr, the pattern of egg laying in *Syphacia muris* also changes (Table II). The peak of egg deposition, following an active feeding period by the rat, shifts from noon to 1600 hr with a second peak re-appearing later at 0200 in male and from 0200 to 0400 hr in female rats.

The present work also shows that the timing of the peak of egg deposition is likely to influence the transmission of *Syphacia muris* in the rat as eggs deposited by female worms at 1200 hr in normally fed male rats and at 1400 hr in female rats appear to be more infective than egg samples taken at either 1000 or 1600 hr (Table III). Infectivity here is expressed as the percentage of adult worms establishing themselves in the caecum of the rat following ingestion of known doses of eggs from the perianal region.

TABLE I

The percentage of ***Syphacia muris*** *eggs deposited every two hours in male and female rats exhibiting a normal feeding and defaecation period (+ denotes faeces present)*

Time of day (hr)	% eggs deposited/rat/hour		% feeding activity rat/hour		Timing of defaecation
	Male	Female	Male	Female	
0200	0	0	13·2	21·6	+
0400	0	0	8·4	2·1	+
0600	0	0·1	0	4·6	+
0800	9·8	21·6	1·3	0	−
1000	12·9	26·0	2·4	1·8	−
1200 (noon)	47·0	28·1	0	0	−
1400	27·0	9·8	0·9	0	−
1600	2·7	11·0	14·0	0	+
1800	0·6	3·4	9·4	15·6	+
2000	0	0	11·8	13·2	+
2200	0	0	13·1	12·9	−
2400 (midnight)	0	0	4·8	5·2	−

TABLE II

The percentage of ***Syphacia muris*** *eggs deposited every two hours in male and female rats subjected to an abnormal feeding period (+ denotes the period of feeding activity)*

Time (hours)	% eggs deposited/rat/hr		Feeding period
	Male	Female	
0200	14·3	24·1	−
0400	0·5	21·6	−
0600	0	5·8	−
0800	1·0	0·4	+
1000	0	0	+
1200 (noon)	0	0	+
1400	0	0	+
1600	66·1	37·1	+
1800	4·5	3·0	−
2000	0·5	0·4	−
2200	3·5	0·1	−
2400 (midnight)	9·6	7·3	−

TABLE III

The relationship between worm establishment and timing of egg deposition by ***Syphacia muris*** *in female and male rats*

Timing of egg deposition (hours)	% worms established in caecum of rats	
	Male	Female
1000	26·0	7·1
1200	57·9	23·8
1400	13·3	47·9
1600	0	0

Discussion

Rats are nocturnal animals and their feeding pattern is circadian (Richter, 1922, 1927). Margules *et al.* (1972) stated that when laboratory rats are maintained on a 24 hour light/dark cycle, approximately 80% of their total food intake occurs in the dark. In the present study laboratory-bred Sprague-Dawley rats infected with *Syphacia muris*, also show peaks of feeding activity primarily between 1800 and 0200 hr, with the feeding stimulus likely to be the result of rhythmic muscle contraction in the gastro-intestinal tract (Richter, 1927).

Although few or no eggs of *Syphacia muris* are present on the perianal region of rats during the period of darkness, it is possible that gastro-intestinal muscle contraction, followed by feeding activity and flow of digestive juices, may stimulate mature females of *Syphacia muris* to commence migration from the rat's caecum during this nocturnal period. By the time the rat is about to enter a resting period around 0800 hr, female worms are ready to deposit their eggs around the perianal skin reaching a peak at noon in both male and female hosts (Table I). Eggs are therefore present on the host from 0800 to 1800 hr at a time when little feeding activity nor defaecation takes place. This could be advantageous to the pinworm because in the absence of faecal production there is less likelihood of eggs being lost or dislodged from the host's perianal region. Furthermore the prolonged inactive period during the daytime allows sufficient time for the eggs to incubate and become infective before the rat's nocturnal activities (including defaecation, feeding and grooming) begin at 1800 hr. Transmission of *Syphacia muris* in the rat is therefore likely to take place at night when infective eggs are ingested during the process of grooming. Not all the eggs produced during the day are equally infective to the rat since eggs deposited at 1200 and 1400 hr in male and female rats respectively were more infective than those deposited by female worms earlier or later in the day (Table III).

The present work has also shown that a change in the timing of egg deposition by female *Syphacia muris* can take place by reversing the feeding pattern of male and female infected rats (Table II). A small peak of egg deposition occurs at 0200 hr in male and from 0200 to 0400 hr in female rats, which corresponds to the time of *muris* egg output in rats exhibiting a normal feeding pattern. This suggests that female worms are able to adjust their intestinal migration with subsequent rhythmic deposition of eggs to a change in the host's feeding activity to ensure successful transmission. Although maximum egg deposition in the present experiments occurs at 1600 hr, following

an active feeding period, it is possible that had the rats been acclimatized to a reversed feeding regime for longer than 10 days the optimum peak in egg deposition might have shifted to 0200 or 0400 hrs. This would of course correspond to the pinworm's noon-time rhythm in normally fed rats (Table I). Further studies are now in progress to determine whether or not the peak in egg deposition at 1600 hr remains or disappears in rats acclimatized for at least a month to a reversal in their feeding pattern.

REFERENCES

Barnett, S. A. (1976). *The rat: a study in behaviour*. Chicago: University of Chicago Press.

Lewis, J. W. & Rentmore, G. (1968). A method for collecting faecal samples from small mammals over a continuous period. *J. Zool., Lond.* **156:** 537–540.

Lewis, J. W. & Rentmore, G. (1979). A method for recording general and feeding activity of small mammals. *J. Zool., Lond.* **189:** 553–557.

Lewis, J. W. & Shava, F. H. M. (1977). Rhythmic egg deposition by intestinal nematodes of laboratory mice. *Parasitology* **75:** iv.

MacArthur, W. P. (1930). Threadworms and pruritis ani. *Jl R. Army vet. Cps* **55:** 214–216.

Margules, D. L., Lewis, M. J., Dragovich, J. A. & Margules, A. S. (1972). Hypothalamic norepinephrine: circadian rhythms and the control of feeding behaviour. *Science, Wash.* **178:** 640–643.

Prince, M. J. R. (1950). Studies on the life cycle of *Syphacia obvelata*, a common nematode parasite of rats. *Science, N.Y.* **111:** 66–67.

Richter, C. P. (1922). A behaviouristic study of the activity of the rat. *Comp. Psychol. Monogr.* **1:** 1–55.

Richter, C. P. (1927). Animal behaviour and internal drives. *Q. Rev. Biol.* **2:** 307–343.

Rudolphi, C. A. (1802). Beobachtungen über die Eingeweidewürmer. *Weigmanns Archiv. Zool.* **2, SS:** 1–67.

Stahl, W. B. (1961). *Syphacia muris*, the rat pinworm. *Science, N.Y.* **133:** 576–577.

Stahl, W. B. (1963). Studies on the life cycle of *Syphacia muris*, the rat pinworm. *Keio J. Med.* **12:** 55–60.

Taffs, L. F. (1976). Pinworm infections in laboratory rodents: a review. *Lab. Anim.* **10:** 1–13.

van der Gulden, W. J. I. (1967). Diurnal rhythm in egg production by *Syphacia muris*. *Expl Parasit.* **21:** 344–347.

Yamaguti, S. (1935). Studies on the helminth fauna of Japan. Part 13. Mammalian nematodes. *Jap. J. Zool.* **6:** 433–457.

J. W. LEWIS AND J. D'SILVA, Department of Zoology, Royal Holloway College, University of London, Englefield Green, Surrey.

Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset

Regulation of Intestinal Nematode Populations in Laboratory Rodents. By J. W. Lewis and J. D'Silva (*Department of Zoology, Royal Holloway College, University of London, Egham, Surrey*)

The present study considers whether density-dependent processes are responsible for regulating the populations of an oxyuroid nematode, *Syphacia muris* in laboratory rats and a trichostrongyle nematode *Nematospiroides dubius* in laboratory and wild mice.

Mature female worms of *Syphacia muris* migrate from the rat caecum during daylight hours to deposit eggs on the host's perianal region on day 8 post-infection (D'Silva & Lewis (1979), *Parasitology* **79**, xxi). Following a short embryonation period these eggs are directly infective to susceptible hosts and when groups of male and female Wistar rats are experimentally infected with individual doses of 100, 500 and 1000 eggs, more worms survive in male than in female hosts. From days 2 to 8 there is overall a decline in the number of worms establishing themselves in the caecum. This loss of infection is also dose-dependent, with less worms surviving in rats given larger initial inocula. With the exception of days 2 and 3 post-infection, there is little variation in worm size and egg production in primary infections of *S. muris* and on day 8 the majority of worms are expelled from the caecum. When rats are challenged with known doses of eggs on days 8 and 16, *en masse* and intermittent worm expulsion occurs in male and female hosts respectively.

In ASH/CS1 laboratory mice infected with *Nematospiroides dubius*, on the contrary, worms are not expelled in primary infections; neither is there any clear indication, at least up to day 100 post-infection, of the existence of density dependency in the survival of worm populations. However, as the size of the initial larval dose increases, adult worms do migrate to a more posterior position in the upper small intestine from day 40 onwards, accompanied by some reduction in worm size and egg production. Following challenge with large larval doses of *N. dubius*, worm establishment in the small intestine of laboratory mice is reduced. On the other hand the generation of an immune response in wild mouse populations is likely to be dependent upon the density of free-living infective larval stages of *N. dubius*, the survival of which is in turn influenced by density-independent processes. In *Syphacia muris*, where free-living larval stages are absent, the population is regulated by some density-dependent constraints primarily operating on developing worms in the caecum of the rat.

RHYTHMIC EGG RELEASE BY INTESTINAL NEMATODES IN RODENTS

J. W. LEWIS & J. D'SILVA, DEPARTMENT OF ZOOLOGY,
ROYAL HOLLOWAY COLLEGE, UNIVERSITY OF LONDON, EGHAM, SURREY, U.K.

The present study confirms that mature females of the rat pinworm Syphacia muris (1) and the mouse pinworm S.obvelata migrate from the caeca of infected hosts during daylight hours to deposit eggs on the perianal skin. By recording the activity of Sprague-Dawley laboratory rats infected with S.muris and MFI laboratory mice with S.obvelata (2), it was shown that the peak of daily egg release occurs around noon when the rodents are least active, following an active feeding period overnight. In addition a change in the timing of the peak of egg release also takes place by reversing the host's feeding pattern and daylight conditions (3).

The success of Syphacia eggs hatching in the caecum is also dependent upon the time when the eggs are deposited and ingested by the host. In S.muris, eggs deposited around noontime are more viable than at any other time of day. As rodents feed and defaecate mostly at night, it is likely that the infective eggs are ingested in daylight hours primarily during the act of grooming to avoid being passed out in the faeces.

Unlike Syphacia, eggs of the trichostrongyle nematode Nematospiroides dubius from MFI mice do pass out in the faeces with a peak occurring at 19.00 - 24.00 hours. This enables the eggs to leave the host at its most active feeding and defaecating period and to allow further development of eggs into free-living infective larval stages in the soil or vegetation. Therefore changes in the timing of the peak of egg release by female pinworm (oxyuroid) and trichostrongyle nematodes is linked with differences in the transmission of infective stages to the definitive rodent host.

REFERENCES: (1) VAN DER GULDEN, W.J.I. (1967) Expl. Parasit. 21, 344.

(2) LEWIS, J.W. & RENTMORE, G. (1979) J.Zool.Lond. 189, 153.

(3) LEWIS, J.W. & D'SILVA, J. (1980) J.Zool.Lond. 190, 3.

Abstract of the 3rd Multicolloquim of Parasitologists
Cambridge, 1980