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STUDIES ON THE HOST-PARASITE RELATIONSHIP
OF STRONGYLOIDES RATTI IN RATS

A thesis submitted for the degree of Doctor of Philosophy
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by

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I dedicate this thesis
to my mother,
as a token of my
loving devotion

ABSTRACT

Studies were conducted on the host-parasite relationship
in Strongyloides ratti infections in rats. During initial infec-
tion, rats acquired a strong immunity against this parasite and
caused a drop in larval production and the expulsion of adult
worms on day 25 (PI. Infected rats were strongly resistant to
reinfection and this immune response lasted for a period of
three months, independent of the residual worm population. The
immune response during initial infection caused a reduction in
the size of adult worms after day 18 PI. Adult worms, which
occupied the anterior half of the small intestine in the first 18
days, shifted to the posterior half before they were expelled.
The immune response affected the adult worm in the intestine
during a primary infection, the migratory larvae in the lung during
a secondary infection and the skin stage following multiple infec-
tions. In heavily infected rats, larvae disseminated and were
detected in the brain tissue.

A full infection with S. ratti induced complete resistance to
reinfection, but adult worms were a major source of protective
antigens, while migratory larvae did not stimulate an immune res-
ponse against reinfection. "Normal" worms obtained on days 8 - 16
PI were able to continue their larval production when transferred
to new, clean, rats; "damaged" worms obtained on days 18 - 25 PI
were unable to do so. Worm damage during initial infection was
irreversible. Short non-fertile worms found in rats early after
challenge were able to resume their egg production and survive in
new hosts. The possibility of adaptation was discussed.

22 day-old worms were examined under the electron microscope
and were compared with 10 day-old worms. Damaged worms were smaller
in size and showed vacuolation and tissue disorganisation,
particularly the gut cells. Peculiar whorls were observed inside
the gut lumen of damaged worms, and were suggested to be immune
complexes. Precipitates were persistently observed surrounding the
mouths of damaged worms. It was proposed that worm damage is insti-
gated by immune complexes plugging the mouth and causing a state of

starvation through inhibition of food intake.

Worm expulsion was demonstrated to be an immunological event. Antibody mediated worm damage, in <u>S. ratti</u>, as an essential step in worm expulsion, was argued. Immune (MLNC) ^{mesenteric lymph node cells} alone, given on days 6 or 12 PI, were capable of inducing worm expulsion. Serum alone repeatedly induced auto-infection. A variety of factors were suggested to explain the action of trans- ferred antibodies. Co-operation between immune MLNC and serum in inducing worm expulsion was only evident when MLNC were given on day 12 PI.

The tissue eosinophils increased in the mucosa of infected intestines immediately before worm expulsion, during initial and challenge infections.

The percentage of degranulating mesenteric mast cells increased during the period of expulsion, and the introduction of histamine into the gut of infected rats caused a significant acceleration of worm damage and expulsion. Aspirin, a potent inhibitor of prostaglandins, delayed the action of expulsion for 5 days suggesting a possible role of prostaglandins in this pro- cess.

Corticosteroid treatment caused the long persistance of <u>S. ratti</u> worms in the rats and indicated that worm damage was due to an immune response, and not to the worm's senescence. Levels of tissue eosinophils and degranulated mesenteric mast cells were also suppressed. No auto-infection was demonstrable when cortico- steroid treatment commenced with the infection, but it was evident when the treatment started immediately prior to worm expulsion. Corticosteroids suppressed all manifestations of acquired immunity to reinfection. An attempt was made to render rats unresponsive to <u>S. ratti</u> infection by treating with corticosteroids at strategic periods during an initial infection.

ACKNOWLEDGEMENTS

My family, particularly my mother, whose loving encouragement during a period of severe difficulty, has been a constant source of inspiration to me.

To my wife Ann for her love, understanding and unfailing support, I wish to express my heartfelt thanks.

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CONTENTS

CHAPTER 1

Introduction

Strongyloidiasis is a generalised term referring to
infections by different species of Strongyloides which parasitize
the intestinal mucosa of vertebrate hosts, including man.
Infections with these soil-transmitted nematodes are cosmopolitan
in distribution. A few species, especially those which infect
sheep and swine, have veterinary importance as they produce loss
of weight, diarrhoea and enteritis. About 3% of the world popula-
tion is infected with Strongyloides stercoralis. This infection,
which is frequently innocuous, is usually acquired by people, such
as miners and farmers, whose professions involve contact with soil.

Interest in human strongyloidiasis has increased recently
following numerous reports that a mild infection with this parasite
can turn into a serious, and often fatal, infection under certain
conditions. It has been found that patients asymptomatically har-
bouring this parasite, who are treated with corticosteroids or
X-ray therapy, as immunosuppressive agents, developed a sudden and
overwhelming infection. In many cases dissemination of larvae
occurs and they invade many internal organs causing severe damage
and often death (Rogers and Nelson, 1966; Cruz et al., 1966; Willis
and Nwokolo, 1966; Civantos and Robinson, 1969; Yim et al., 1970;
Fagundes et al., 1971; Bitoun et al., 1972; Cookson et al., 1972;
Neefe et al., 1973; Purtilo et al., 1974).

As experimental studies on S. stercoralis were not possible ,
the present research work adopted the rodent species Strongyloides
ratti as an alternative to investigate the following points:

1. Aspects of acquired resistance which develop in rats following
    initial, second and multiple infections with S. ratti.
2. The immunogenic stage(s) of infection.
3. Effects of the immune response on adult S. ratti.
4. Mechanisms involved in the immune expulsion of adult S. ratti
    from the rats.

5. Alterations in the equilibrium between host and parasite following immunosuppression by corticosteroids.

Taxonomical Review

The members of the suborder Rhabdiata of the order Rhabdiasidea (Yamaguti, 1961) are of particular interest since they manifest a gradual evolutionary trend from free-living organisms to facultative parasites. The main genera of this suborder are placed in two major families: Rhabdiasidae and Strongyloididae. Worms belonging to these two families form a link between free-living and parasitic forms by manifesting the phenomenon of alternations of generations. The free-living adult generation consists of male and female worms, while only parthenogenetic females are found in the parasitic generation.

Family Rhabdiasidae

Nematodes belonging to this family, some of which are considered below, are parasites of reptiles, amphibians and mammals, but they also exhibit free-living activities. Rhabditis strongyloides live in soil, organic matter or water, but their larval forms are also found on the hairs of the perianal region of dogs where they cause itching pustules (Chandler and Read, 1961). Rhabditis pellio was recovered from the human vagina and urine (Chandler and Read, 1961). Another species Diploscapter coronata was found in the aspirated stomach contents of patients suffering from a total lack of gastric hydrochloric acid (Chandler, 1938a), and it is possible that this worm is only able to parasitize such patients.

Family Strongyloididae

This family was established by Chitwood and McIntosh (1934)

with only two genera, Strongyloides and Parastrongyloides, as
representatives. Chitwood and Chitwood (1950) defined the
essential diagnostic criteria for this family, the summary of
which is:
"Parasitic generation parthenogentic or dioecious, filariform,
stoma cup-shaped or greatly reduced. Females with 2 divergent
uteri, ovary reflexed, vulva near middle of body. Live in
gastro-intestinal tract of vertebrates".

Species of Strongyloides

Strongyloides spp. were discovered in animals early in the
second half of the 19th century, in both sheep and swine. The dif-
ferentiation of species presented a formidable task since morpho-
logically similar worms were reported from a wide range of
vertebrate hosts (Grassi, 1878; Looss, 1911; Bayliss, 1923).
S. stercoralis-like worms were recovered from frogs (Alfieri, 1908)
and this led to a temporary belief that the human infection was a
zoonosis, with frogs acting as reservoir hosts. Attempts to classify
the different species of Strongyloides were made, utilizing various
systems and criteria (Chandler, 1925; Sandground, 1925; Henry, 1931;
Noto-Soediro, 1933; Desportes, 1945; Basir, 1950). Little (1966a,
b) used the following criteria for the specific identification of
the various species of Strongyloides:

(1) The shape of the stoma in the parasitic females viewed en face;
(2) The shape of the ovary in the parasitic female;
(3) The developmental stage, or stages, passed in host's faeces.

The first of these criteria was found to be the most consistent
and accurate.

Over 40 species of Strongyloides are known to exist, and table
(1) lists some of the better known species found in vertebrates.

Table 1 :   A list of some of the parasitic species

of the genus _Strongyloides_

PARASITE	HOST
S. _stercoralis_ (Bavay, 1876) Stiles and Hassall, 1903	Man
S. _fülleborni_ Von Linstow, 1905	Old-world primates, not infrequently, man
S. _papillosus_ (Wedle, 1856) Ransom , 1911	Sheep and rabbits
S. _cebus_ Darling, 1911	Rhesus monkeys
S. _ransomi_ Schwarz and Alicata, 1930	Swine
S. _mycpotami_ Artigas and Pacheco, 1937	Nutria
S. _westeri_ Ihle, 1917	Horses
S. _agouti_ Griffith, 1940	Agouti and guinea-pigs
S. _ratti_ Sandground, 1925	Rats
S. _venezuelansis_ Brumpt, 1934	Rats
S. _lutrae_ Little, 1966	Otters
S. _dasypodis_ Little, 1966	Armadillos
S. _ardea_ Little, 1966	Herons
S. _serpentis_ Little, 1966	Snakes
S. _procynosis_ Little, 1966	Raccoons
S. _physali_ Little, 1966	Toads
S. _elephantis_ Greve, 1969	Elephants
S. _avium_ Cram, 1929	Fowls and Turkeys

Strongyloides stercoralis:

This species was discovered by Normand, a French Army
surgeon, in 1876, from the stools of French soldiers who died
from diarrhoea in Cochin-China. At autopsy, minute adult worms
were found attached to the mucosa of the ileum, and these were
morphologically different from those found in the faeces. The
worm of the faeces was named Anguillula stercoralis and the
intestinal worm A. intestinalis by Bavay in 1876. Further studies
by Grassi (1879), Perroncito (1880) and Leukart (1882) revealed
that these two worms were separate stages of the life cycle of one
species. The name Strongyloides stercoralis (Bavay, 1876) Stiles
and Hassall, 1903 was later adopted. Initial studies on the mode
of infection with this parasite were carried out by Zinn (1899),
Askanazy (1900), Van Durme (1902) and Ransom (1907). Looss (1905)
concluded that the migration of the infective stage of this species
was similar to that of Ancylostoma duodenale. Fülleborn (1914),
working on experimental S. stercoralis infections in dogs, contri-
buted greatly to our present knowledge of the route of infection
and the invasion of the skin by infective filariform larvae. He
also proved that their migration, within the host en route to the
intestine, was obligatory. These findings were confirmed by
Sandground (1926, 1928). Further elucidation of the life cycle in
experimental strongyloidiasis was presented by Faust (1933) who
described the successive developmental stages of the parasitic phase.
The filariform infective larvae penetrated the skin and migrated,
via the blood, the lungs where they developed into postfilariform
larvae; which migrated through the trachea and oesophagus to the
small intestine of the host and were then called preadolescent
females. Adult females were found buried in the mucosa and these
produced embryonated eggs which usually hatched into rhabditiform
larvae in the lumen of the gut, from where they were carried out
with the faeces.

The parasitic male enigma:

Several authors have recognized that parasitic adult females of *Strongyloides* spp. were parthenogenetic (Rovelli, 1888; Brumpt, 1911; Ransom, 1911; Chitwood and Graham, 1940). However, parasitic males have been found in man and experimentally infected animals (Kreis, 1932; Sotolongo Guerra *et al.*, 1971; Sotolongo Guerra, 1972). Unlike female worms, parasitic males were considered to be temporary residents which were expelled following fertilization of the females (Faust, 1933). On cytological evidence, Sandground (1926) claimed that the worms were hermaphroditic. Graham (1935, 1936, 1938a, 1938b) established infections with *S. ratti* from a single larva which indicated that parasitic adult females did not require conventional fertilization by "parasitic" males to produce new progeny. No reduction division was noticed by Chang and Graham (1957) during oogenesis in the free living female of *S. papillosus*, and they proposed that this species was pseudogamous. In pseudogamy the sperm does not penetrate the egg but rather activates it to divide. Bolla and Roberts (1968) investigated gametogenesis in the free living form and the chromosomal complement in both forms of *S. ratti*. They refuted Sandground's claim that the worms were hermaphroditic and instead confirmed the phenomenon of pseudogamy in the free living forms and parthenogenesis in the parasitic females. However, they did not offer any explanation for the occasional finding of parasitic males in human strongyloidiasis.

Direct and indirect modes of development:

Most *Strongyloides* spp. follow one of two methods of development outside their hosts, namely direct (homogonic) or indirect (heterogonic) development (Figure 1). This phenomenon suggests that this group of nematodes has only recently evolved to be parasites of vertebrates.

Attempts were made to explain the mechanisms which control these two modes of development. Internal and external environmental conditions, age of the worms and separate biological strains were

Figure 1 : Heterogamy and homogamy in <u>Strongyloides</u> sp.,

modified from Sandground, 1926

L3 ─────────────→ Parasitic adults (usually
↑   parthenogenetic females) ←─┐
│   │
L2   │   │
↑   ↓   │
Rhabditiform L1 larvae ─┐   eggs   │
↑   │   │
│   │   ↓   │
eggs   │   │
↑   └→ Rhabditiform L1 larvae
Free living bisexual ←──   │
adults   ↓   │
                          L2 ──→Infective
                              filariform L3
                              larvae

Indirect (heterogonic)   Direct (homogonic)

Development   Development
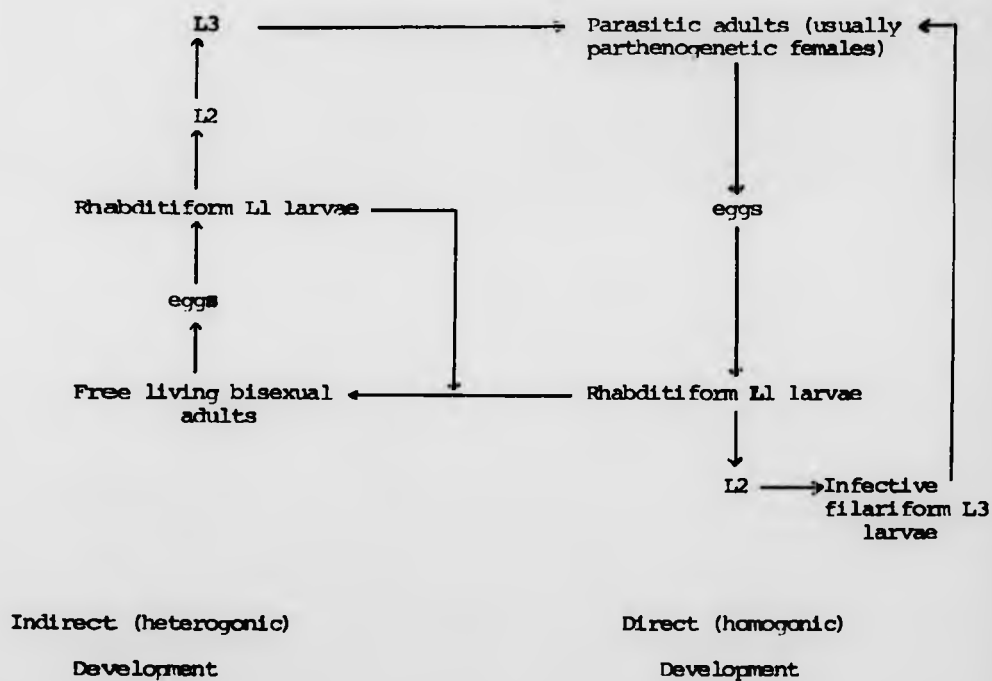
suggested (Leichtenstern, 1899; Darling, 1911a, b; Sandground, 1926). Beach (1935, 1936) stressed that favourable nutritional conditions outside the host induced the rhabditiform larvae to develop into free-living, heterogonic, dioecious adult worms; while unfavourable surroundings induced the direct homogonic path.

Graham (1935, 1936, 1938a, b, 1939a, b) selected pure homogonic and heterogonic lines by breeding from single filariform larvae, but certain homogonic lines produced heterogony and vice versa (Graham , 1940), which indicated that this phenomenon was not, totally, genetically controlled.

As yet, the exact factors, which determine whether or not a strain of Strongyloides spp. develop homogonically or heterogonically, are not fully elucidated. However, it seems that a combination of genetic and environmental factors are responsible for the switch over from one mode of development to another (Smyth, 1962).

Auto-infection in Strongyloidiasis

Most species of Strongyloides possess the rare ability to multiply within the host. Under certain circumstances the rhabditiform larvae moult twice in the lumen of the host, become infective larvae and penetrate the host. This can occur in two ways (Faust and de Groat, 1940):

1. Internal auto-infection: Infective larvae invade the intestinal mucosa and travel, via the portal circulation, to the lungs, migrate to the gut and develop into adult females.
2. External auto-infection: Some rhabditiform larvae moult to become infective larvae near the perianal region and these invade the epithelial lining of the anal sphincter and/or the skin of the perineum, repeating the same migratory cycle as above.

This kind of development might explain how S. stercoralis survives in humans for up to 36 years (Brown and Perna, 1958). It is suggested that auto-infection occurs in people with a depressed cell-mediated immune response (Purtilo et al., 1974). This aspect and the

clinical and pathological picture of human strongyloidiasis will
be further discussed in Chapter 8 .

Human strongyloidiasis as a zoonosis:

Natural infections with Strongyloides spp. occur in dogs
(Brumpt, 1922; Chandler, 1925) and although the human species
S. stercoralis was successfully established in dogs (Tashiro, 1912;
Chira, 1918; Sandground, 1928; Faust, 1933), there is insufficient
evidence for the existence of a zoonosis between dog and man.
However, human infections with the simian species, S. fülleborni
occur in Africa (Pampiglione and Ricciardi, 1971a, 1972a), and experi-
mental infections with this species were successfully established
in a human volunteer (Pampiglione and Ricciardi, 1971b, 1972a, b).

A few communities on the Fly River in New Guinea harbour an
unidentified species of Strongyloides for which no animal host has
been found. It has been suggested that this species is probably
S. fülleborni, which is interesting since there is no simian fauna
on the Island (Knight, 1976, personal communication).

Strongyloides ratti Sandground, 1925:

Grassi and Segre (1887) found a distinct species of Strongyloides
in wild mice, Mus sylvatris and M. decumanus. Hall (1916) included
the strain which he recovered from rats with S. papillosus of sheep
and rabbits, while Sandground (1925) proposed that this was a new
species and named it S. ratti.

The adult females of S. ratti (Plate 1) range between 1.85 -
3.03 mm (average: 2.35 mm). The cuticle has fine transverse striation.
The buccal cavity is shallow and surrounded by six minute papillae.
The oesophagus is 22-33% of the total body length. The anal orifice
is situated 32 - 50 μ from the posterior end of the body, and the
vulva 1.27 - 1.73 mm from the anterior end of the body. The ovaries
are directly recurrent, bending close to the oesophagus and the
posterior end of the intestine. As many as 11 eggs are present in

15

Plate 1 : Adult female of S. ratti (x 80)

Plate 2 : Egg of S. ratti, phase contrast (x 160)

Plate 3 : Rhabditiform, first stage (L1) larva (x 250)

Plate 4 : Filariform, infective, third stage (L3) larva (x 200)

Plate 5 : Trifurcated tail of third stage larva (x 250)

Plate 1 : Adult female of S. ratti   (x 80)

Plate 2 : Egg of S. ratti, phase
contrast (x 160)

Plate 3 : Rhabditiform, first stage
(Ll) larva (x 250)

Plate 4 : Filariform, infective, third
stage (L3) larva (x 200)

Plate 5 : Trifurcated tail of
third stage larva
(x 250)

Plate 1 : Adult female of S. ratti (x 80)

Plate 2 : Egg of S. ratti, phase contrast (x 160)

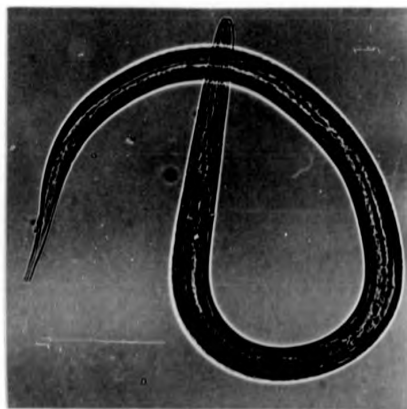Plate 3 : Rhabditiform, first stage (L1) larva (x 250)

Plate 4 : Filariform, infective, third stage (L3) larva (x 200)

Plate 5 : Trifurcated tail of third stage larva (x 250)

both uteri. The tail is finely tapered. The eggs (Plate 2)
(measuring 47-52 x 28-31 μ) are usually embryonated when leaving
the female, and are normally laid in a strand deposited deep in
the mucosa, where they hatch. First stage (Ll) rhabditiform
larvae (Plate 3) average 420 μ in length with a maximum width of
17-21 μ. They are similar to first stage larvae of hookworms
except for their short buccal cavity. The third stage (L3)
infective filariform larvae (Plate 4) are extremely active. They
average 650 μ in length and show two distinctive, equal, parts of
the gut; the oesophagus and the intestine. The tip of the tail
is characteristically trifurcated (Plate 5).

Life cycle of S. ratti in the rat (Figure 2):

Infective larvae enter the body through the hair follicles.
Invasion of the cutaneous tissue is rapid and larvae reach the
venules and lymphatics within 3 hours, and large numbers of larvae
are found in the blood 10 hours after exposure. The majority
(Spindler, 1958) arrive in the lungs about 16 hours post-infection
(PI) and grow into post-filariform larvae, which migrate to the
intestine about 23 hours PI (Abadie, 1963; Wertheim and Lengy, 1965).
No moulting occurs during this phase except in rare cases where
larvae are trapped in the lungs (Wertheim and Lengy, 1965). Upon
reaching the intestine, 34 hours PI, larvae burrow into the mucosa
and moult twice at 47 and 74 hours into adult females. Female worms
reach their full size 120 hours PI (Wertheim and Lengy, 1965) and
egg formation commences at 81 hours. Eggs and larvae can be
detected in the intestinal wall and faeces 85-92 hours PI.

Sheldon (1937c) compared subcutaneous, oral, intramuscular,
intracolonic, intraperitoneal, intragastric and intravenous routes
of S. ratti infection. The oral, where larvae were allowed to pene-
trate the pregastric mucosa, and the subcutaneous routes gave higher
yields of adult worms.

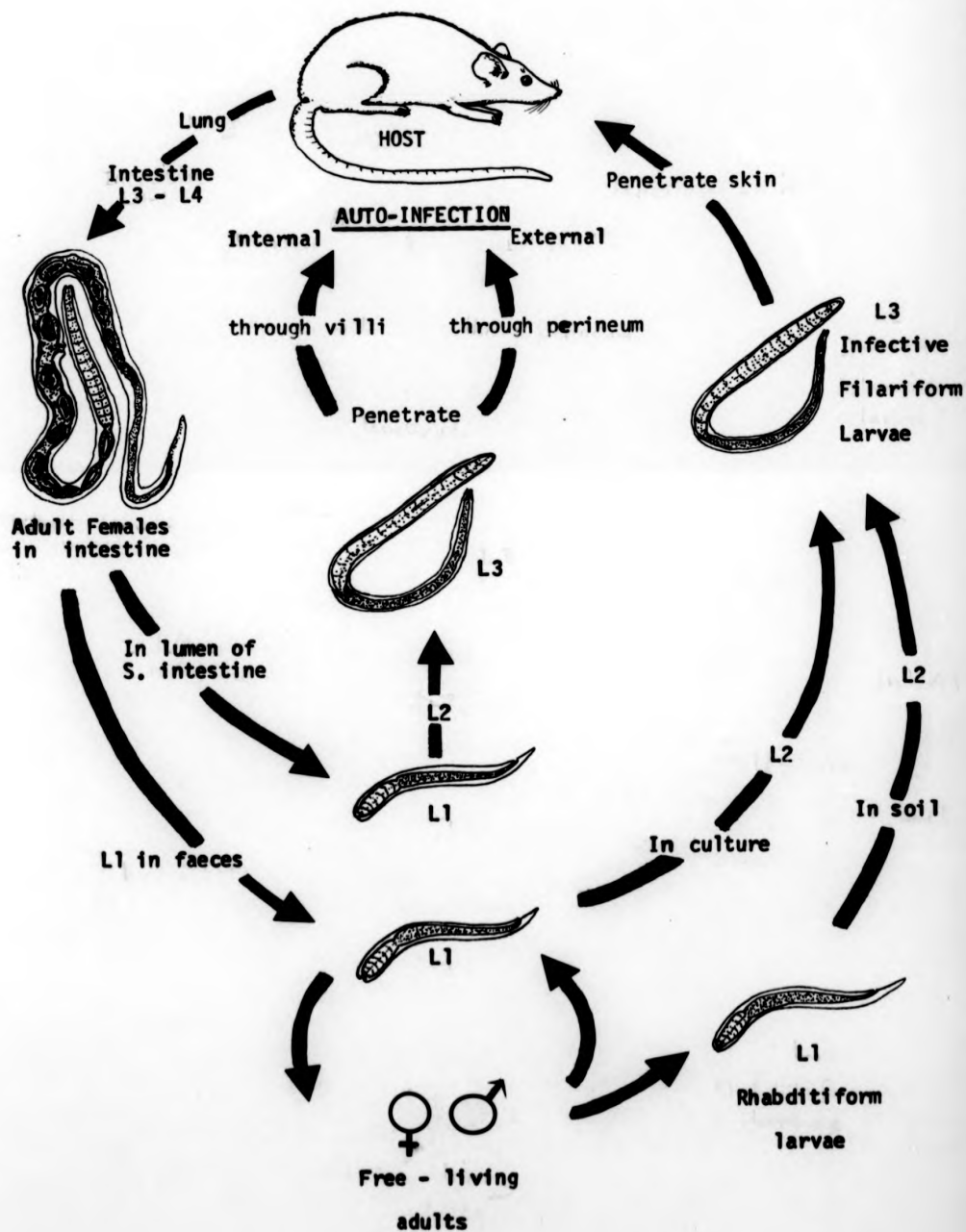Figure 2. Life cycle of <u>Strongyloides ratti</u> Sandground, 1925

S. ratti and S. venezuelensis:

Concurrent infections with these two nematodes occur in wild rats (Little, 1961; Wertheim and Lengy, 1964; Araujo, 1967), but they have different distributions along the small intestine (Araujo, 1967). Adult S. ratti mainly occupied the anterior 20 cm of the small intestine but stretched in their distribution as far as 60 cm, distally. They were found buried in the crypts of Lieberkühn where they usually deposited their eggs. S. venezuelensis, on the other hand, occupied the anterior 15 cm of the small intestine, with the majority in the proximal 5 cm. The females of this species were found near the surface of the villi where they laid their eggs. S. ratti reached the intestine about 20 hours before S. venezuelensis (Wertheim, 1970a, b). Wertheim (1970b) concluded that each species, when occurring concurrently, behaved as if present alone and demonstrated its own characteristic distribution in the intestine.

Host specificity of S. ratti

S. ratti was found in 45-70% of sewer rats (Sandground, 1925; Tanabe and Takaishi, 1936; Nashimura, 1943). Amano et al. (1958) and Abe et al. (1960) showed that Rattus norvegicus had a higher prevalence rate of S. ratti than R. rattus.

Attempts to infect mice with S. ratti have yielded a variable degree of success. Brackett and Bliznick (1949) passaged S. ratti through mice 19 times without increasing its infectivity to this host. It appears that different strains of mice react differently to such infections. CF1 mice were more susceptible to S. ratti (Goldgraber and Lewert, 1965) than other strains (Sandground, 1925; Sheldon, 1937d; Brackett and Bliznick, 1949).

There are no reports of human infections with this parasite, but there is some evidence that repeated exposure to its infective larvae produces an immediate type hypersensitivity reaction at the site of exposure.

Host sex differences in S. ratti infections:

This aspect of the host – parasite relationship in this system is still controversial. Roman (1951) noticed a slightly higher incidence of S. ratti in female R. norvegicus, Apodemus sylvaticus and Clethrionomys glareolus but concluded that the sex of the host had no effect on the recovery rate. Schacher and Chee-Hock (1960) and Wertheim and Lengy (1964) obtained equal distribution of S. ratti in male and female hosts. Abe et al. (1960) and Wertheim (1963) reported that male rats had a higher rate of infection than females. Katz (1961, 1962, 1963, 1967) investigated this problem and the effects of sex hormones on S. ratti infections. He concluded that female rats had a greater worm burden during the first three weeks of infection, while male rats showed a higher burden at the end of 4 weeks, but no definite role of sex hormones was demonstrated.

This introduction has reviewed various features of the different species of Strongyloides generally and S. ratti particularly. Other more specific aspects of the host – parasite relationship, in this parasite and other related nematodes, are reviewed in their relevant chapters.

CHAPTER 2

General Materials and Methods

The Parasite:

The original culture of <u>S. ratti</u> was obtained from the Wellcome Laboratories, Beckenham, Kent. This strain did not develop free-living adult forms in culture.

Laboratory Hosts:

Random bred, female albino Wistar rats (Tuck & CO.), at 100 - 150 gms (8 weeks old) were used to maintain the parasite, and for most of the experimental work. An inbred stock of the same strain was used for experiments involving passive and adoptive transfer of immunity.

Maintenance of the Parasite:

10 - 12 gms of freshly passed infected faecal pellets were collected once a week by giving a gentle squeeze to the perianal area which stimulated the passing of faeces. Fresh pellets were also collected from the cages. The faecal pellets were weighed and washed 3 - 4 times with distilled water. The faeces were left in a beaker with a few drops of distilled water for 30 - 60 minutes and were thoroughly mixed.

Test-tube/filter paper method for culturing infected faeces:

This method was introduced originally by Harada and Mori (1951) for the diagnosis of hookworms. It was improved by Sasa

Plate 6 : The test tube/filter paper (Harada-Mori) culturing
method.

Plate 6 : The test tube/filter paper (Harada-Mori) culturing method.

Plate 6 : The test tube/filter paper (Harada-Mori) culturing method.

et al. (1957) and was recommended by the W.H.O. expert committee
for the diagnosis of soil transmitted helminthiasis (W.H.O., 1963).
18 cm x 18 mm test tubes were placed in special racks and 7 ml
of distilled water added. 0.5 gm of the moistened faeces was
smeared onto 15 cm x 15 mm filter paper strips using a wooden
tongue depressor (Lewis). The smeared strip was inserted into
the test-tube leaving 7 cm clear at one end with the clean end
touching the bottom of the distilled water in the tube (Plate 6).
The tubes were sealed with aluminium foil and kept in an incubator
at 28°C for 3 - 4 days. During this period the first stage
rhabditiform larvae moulted twice and migrated towards the water
in the bottom of the tube. The filter papers were removed and
the infective larvae collected and concentrated by centrifugation
at 1000 rpm for 5 minutes. The larval suspension was stirred with
a teflon-coated magnet on a magnetic stirrer (Voss). Aliquots were
taken with a Pasteur pipette into a McMaster type counting chamber
(Figure 3). If the larval suspension was too concentrated,
dilutions of 1:10 or 1:20 were made. A total of 5 sequential
counts of live larvae were made in the chamber under a dissecting
microscope at x 20 magnification and the number of larvae calculated
from the mean of the 5 counts and the volume of the suspension.

Methods of infection:

A. Subcutaneous inoculation: Each infection was of 1000 larvae
per rat, in 0.2 ml of distilled water, injected subcutaneously in
the inguinal area.

B. Percutaneous infections: The rats were anaesthetized with a
mixture of air and Penthrane (Abbott Laboratories). The fur was
clipped from the abdomen, or the back, and the animals were placed
on a flat surface. The skin was moistened with distilled water
and a stainless steel ring (1.5 cm in diameter and 1 cm in depth)
placed in position. 1000 infective larvae in 0.5 ml of water
were pipetted into each ring (Plate 7). Infective larvae invaded
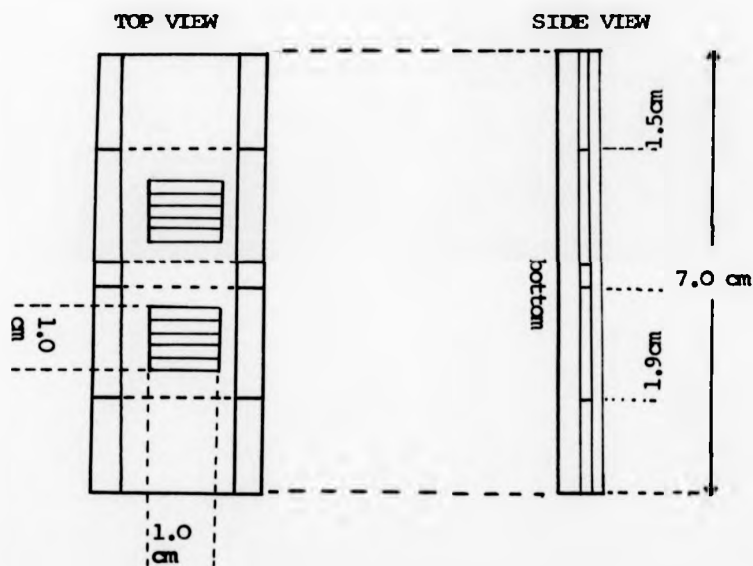the skin within 2 - 3 minutes, but the rings were left for 10

Figure 3 :   McMaster's type counting chamber

minutes. Larvae were rarely recovered from the distilled water inside the rings following this period of exposure. This method was introduced by Smithers and Terry (1965) for exposing rats and other rodents to cercariae of <u>Schistosoma mansoni</u>.

Recovery of adult worms:

The rats were killed by injecting Nembutal (Abbott Laboratories) intraperitoneally. The small intestine was removed, immediately, and slit using a blunt-nosed pair of scissors. The intestine was shaken in a jar of water at room temperature to remove the digesta. This resulted in a loss of 0.5% of the adult worms, but the absence of ingesta made subsequent examinations much easier and more accurate. The intestine was transferred to a jar containing 25 - 30 ml of 0.85% saline and incubated at $37^{\circ}C$ for 2 - 3 hours. Subsequently, the intestine was removed from the jar to a ruled petri dish and the adult worms counted under a dissecting microscope (x 15). The lining of the gut was scraped with the edge of a clean microscope slide, the mucosa squashed between two glass plates and the number of undetached worms counted. 2 - 3% of the worms did not detach.

A modified Baermann funnel was used for the mass collection of adult worms. The intestine was slit, washed and placed on a 50 mesh sieve (pore size 300 microns, Endecotts Test Sieves Ltd.) in a funnel filled with saline and left for 3 hours at $37^{\circ}C$. The worms migrated through the sieve to the bottom of the funnel. They were collected and washed twice with fresh saline.

Measuring of worms:

Adult worms were fixed in 10% formol saline and kept in Bijoux bottles. A sample of 30 - 80 worms was pipetted onto a slide and covered with a coverslip. The fresh preparation was examined immediately under x 300 magnification on a Nikon microscope with a built-in Camera Lucida. This magnification was ideal for drawing the lengths of worms on white paper. The overall lengths of drawn

Plate 7 : Percutaneous infection with S. ratti

Plate 8 : Preparation of mesentery for mast cell staining

Plate 7 : Percutaneous infection with S. ratti

Plate 8 : Preparation of mesentery for mast cell staining

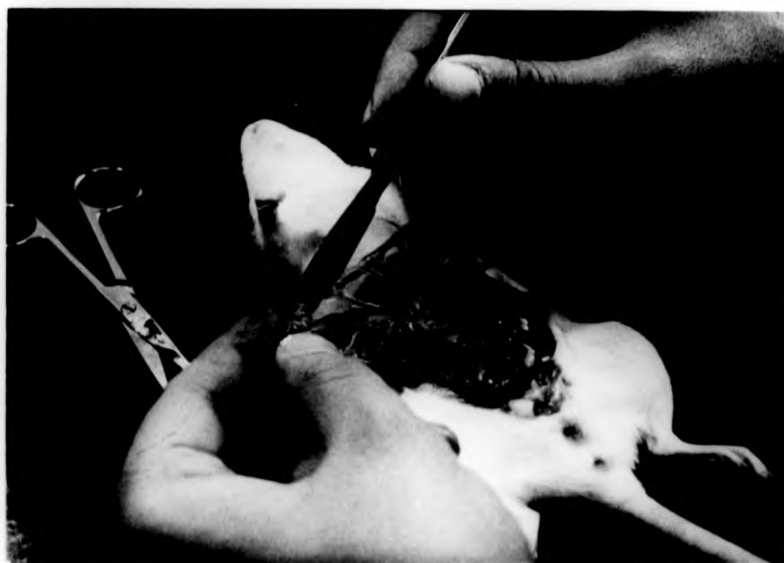Plate 7 : Percutaneous infection with S. ratti

Plate 8 : Preparation of mesentery for mast cell staining

worms were determined with a roller-head map measurer and later
converted into millimeters.

Histology:

Relevant tissues were collected and fixed immediately in
fixatives depending on the type of staining required. The most
frequently used fixative was 10% formol-saline. Bouin's and
Carnoy's solutions were used to fix tissues prior to staining with
specific dyes.

For the fixation of intestinal tissue, the small intestine was
removed and 1 cm segments, each inclusive of a Peyer's patch, were
taken. Each segment was slit and placed between two small sheets
of glass which were kept together with a rubber band, to avoid
curling of the tissue when placed in the fixative. The segments
were sectioned longitudinally, passing through the middle of the
Peyer's patches. The tissues were dehydrated, cleared and embedded
in paraffin wax in a Histokinette (British American Optical Co.).
The blocked tissues were sectioned at 5 μm on a rotary microtome
(Jung AG Co., Heidelberg) and stained in Haematoxylin and Eosin
(H. & E.), which was used routinely with most of the tissues.
Carbol Chromotrope (Lendrum, 1944) was used for staining tissue
eosinophils. Sections were stained with Astra-blue (Raymond Lamb)
adjusted to pH 0.25 with concentrated HCl (Bloom and Kelly, 1960)
and counter stained with Safranin O for intestinal mast cells
(Enerback, 1966). Pericapillary mesenteric mast cells were stained
for 1 min. in 0.25% acidic Toluidine blue (Smith and Atkinson, 1956)
after fixing the stretched mesentries on a slide (Plate 8) with
10% formol saline for 5 - 10 mins.

Methyl green - pyronin Y was used to stain Peyer's patches
in the intestine.

Statistical analysis of data:

The calculation of the mean and standard error was performed

on an Olivetti Programma 101. The results of the majority of experiments are expressed as mean (standard error). Student's 't' test was used to assess the significance of some of the results. Significantly different results had to show a probability P value of less than 0.10.

Other specific materials and methods, used during the course of this study, are presented separately in their relevant chapters.

CHAPTER 3

The parasitological response of rats to primary
and challenge infections with Strongyloides ratti

A worm population is expelled much earlier in rodents which
have been given a primary nematode infection and are then challenged with
more larvae. This has been shown to occur in Nippostrongylus
brasiliensis in rats (Ogilvie, 1969), Trichinella spiralis infec-
tions in mice, rats and guinea-pigs (McCoy, 1931, 1940; Roth, 1939;
Culberston, 1942 ; Larsh et al., 1952; Zaiman et al., 1953; Denham,
1968), Trichuris muris infection in mice (Wakelin, 1967) and
Trichostrongylus colubriformis in guinea-pigs (Herlich et al.,
1956; Herlich, 1963; Rothwell et al., 1973).

The immune response in different hosts, against parasitic
nematodes, causes a variety of changes,in addition to the early loss
of worms (Ogilvie and Jones, 1973). In some filarial infections,
the adult worms show a decline in the number of microfilariae pro-
duced in immune hosts (Bertram, 1966; Denham et al., 1972). Micro-
filariae of Litomosoides carinii remain arrested in the pleural
cavity of immune cotton rats and are almost unable to enter the
blood circulation (Bagai and Subrahmanyam, 1970). Adult worms are
also morphologically affected by the immune response of the host,
and show a delay in their migration, moulting and growth and this
produces irrevocably stunted worms (Soulsby, 1962; Bertram, 1966;
Sinclair, 1970; Jarrett et al., 1971; Denham et al., 1972; Ogilvie
and Jones, 1973).

Early work by Sheldon (1937a, b) on experimental S. ratti
infections revealed that rats became resistant to reinfection fol-
lowing an initial infection. The development of this resistance in
a primary infection caused a sharp drop in the larval output 19 days
post-infection and infection was terminated after day 26 (Sheldon,
1937a, c) although a few larvae were still produced. Sheldon (1937a)
conferred a marked degree of protection against S. ratti by serial

injections of heat-killed infective larvae, but Griffin (1972) was unable to repeat this result. This sensitization was specific as no resistance against S. ratti was stimulated by heat-killed infective larvae of S. stercoralis (Sheldon, 1939). Thomas (1970) showed that different strains of rats developed similar marked resistance to S. ratti following initial infection and that low-level "trickle" infections caused a delay in the expulsion of the worms for up to 8-10 weeks.

The movement of worms in the gut during the course of infection has been investigated in different rodent nematode systems, e.g. N. brasiliensis and T. spiralis (Brambell, 1965; Larsh et al., 1952). There is no published information on such movement in S. ratti, but the majority of adults occupy the anterior 15-20 cm of the small intestine and, in decreasing numbers, the remaining 45-60 cm, during the first few days after their arrival in the intestine (Abadie, 1963; Wertheim, 1970).

In this chapter an investigation is made of the effects of the rat's response on initial and subsequent infections with S. ratti.

Experiment 3.1:

This experiment was designed to study the development of resistance to primary and secondary infections. Fifty five female Wistar rats were separated into two groups: Group A, 30 rats, were infected subcutaneously with 1000 ($\pm$ 73) L3 each on day 0. Group B, 25 rats, were left uninfected.

Preliminary cultures were made from a random sample of five rats from Group A on days 6, 10, 18, 20 and 26 PI, to monitor larval output during the course of primary infection. On day 30 PI, both groups were treated with thiabendazole (TBZ) at 100 mg/kg for two consecutive days. TBZ is highly effective against human and experimental strongyloidiasis (Franz, 1963; Jorge and Barbosa, 1964; Chaia and Cunha, 1966; Bezjack, 1969). On day 32 PI five rats from group A were autopsied to ascertain the efficacy of TBZ. No adult worms were recovered from any of these rats. On day 34 PI, the remaining rats of groups A and B were challenged with 1000 ($\pm$ 51)

infective larvae of S. ratti per rat. Faecal cultures from both
groups were made every two days, commencing on day 6 post-challenge.
5 rats from each group were autopsied on days 10, 14, 18, 21 and
26 after challenge to count the number of adult worms. The results
are presented in Tables 2 and 3, and Figures 4 and 5.

During initial infection, larval output sharply increased
from 3415 to 7770 L3/gm of faeces between days 6 and 8 PI. There
was then a plateau for approximately 8 days followed by a sharp
drop in the number of larvae/gm of faeces reaching to 108 L3/gm on
day 26 PI. Following a challenge infection, larval output did not
exceed 3 L3/gm of faeces.

The highest number of adult worms was recovered on day 14 PI
and decreased from then on until it reached a mean of 32 worms per
rat on day 26 PI. The number of adult worms recovered following a
challenge infection was low with a maximum mean of 20 worms on day
10 PI. The worms were rapidly expelled around day 10 post-challenge
and an average number of approximately 2.7 worms was maintained
between days 18-26 post-challenge.

The worms recovered from the challenged group on day 10 post-
challenge were 54.7% shorter than those recovered from the challenge
control on the same day (Figure 6). Following a challenge infection,
female worms were unable to produce the expected number of eggs.
Adult worms recovered from the challenge control group on day 26 PI,
were shorter and less active than those recovered at an earlier
stage of the same infection.

Experiment 3.2:

This experiment was a repeat of experiment 3.1, with special
attention being paid to the changes in the size of worms during
primary and secondary infections, and the timing of the occurrence
of these changes. Seventy seven female Wistar rats were divided into
two major groups, A and B:

The 31 rats of group A were infected with 1000 ($\pm$ 88) larvae
each on day 0, while group B was left uninfected. On day 28 PI,
both groups were treated with 2 x 100 mg/kg of TBZ. Three rats from

Table 2, Exp. 3.1:

Mean number of larvae produced during initial and challenge infections with S. ratti

Day post-challenge	Mean (SE) number of larvae/gm of faeces	
	Challenge infection	Initial infection
	Group A	Group B
6	3	3415 ($\pm$ 138)
8	2.3	7770 ($\pm$ 481)
10	1.83	5733 ($\pm$ 129)
12	1	3909 ($\pm$ 215)
14	1.5	2866 ($\pm$ 129)
16	0.6	2619 ($\pm$ 225)
18	0.5	1211 ($\pm$ 71.3)
20	1.7	299 ($\pm$ 23)
22	0.3	533 ($\pm$ 30.8)
24	0.5	202 ($\pm$ 23.8)
26	0.5	108 ($\pm$ 9.8)

Table 3, Exp. 3.1:

Mean number of adult worms recovered during initial and challenge infections with S. ratti

Days post-challenge	Challenge infection		Initial infection	
	Number of rats	Mean (SE) number of worms	Number of rats	Mean (SE) number of worms
10	5	20 ($\pm$ 6.4)	5	452 ($\pm$ 24)
14	5	4.4 ($\pm$ 2)	5	614 ($\pm$ 45)
18	5	2.6 ($\pm$ 1)	5	217 ($\pm$ 36)
21	5	2.8 ($\pm$ 1.3)	5	102 ($\pm$ 10.5)
26	5	2.8 ($\pm$ 1.1)	5	32 ($\pm$ 16.5)

Table 3, Exp. 3.1:

Mean number of adult worms recovered during initial and challenge infections with _S. ratti_

Days post-challenge	Challenge infection		Initial infection	
	Number of rats	Mean (SE) number of worms	Number of rats	Mean (SE) number of worms
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21	5	2.8 ($\pm$ 1.3)	5	102 ($\pm$ 10.5)
26	5	2.8 ($\pm$ 1.1)	5	32 ($\pm$ 16.5)

Figure 4, Exp. 3.1:

Larval production during the course of initial and challenge
infection with S. ratti.
●———● Group A, initially infected and challenged
○———○ Group B, challenge control.
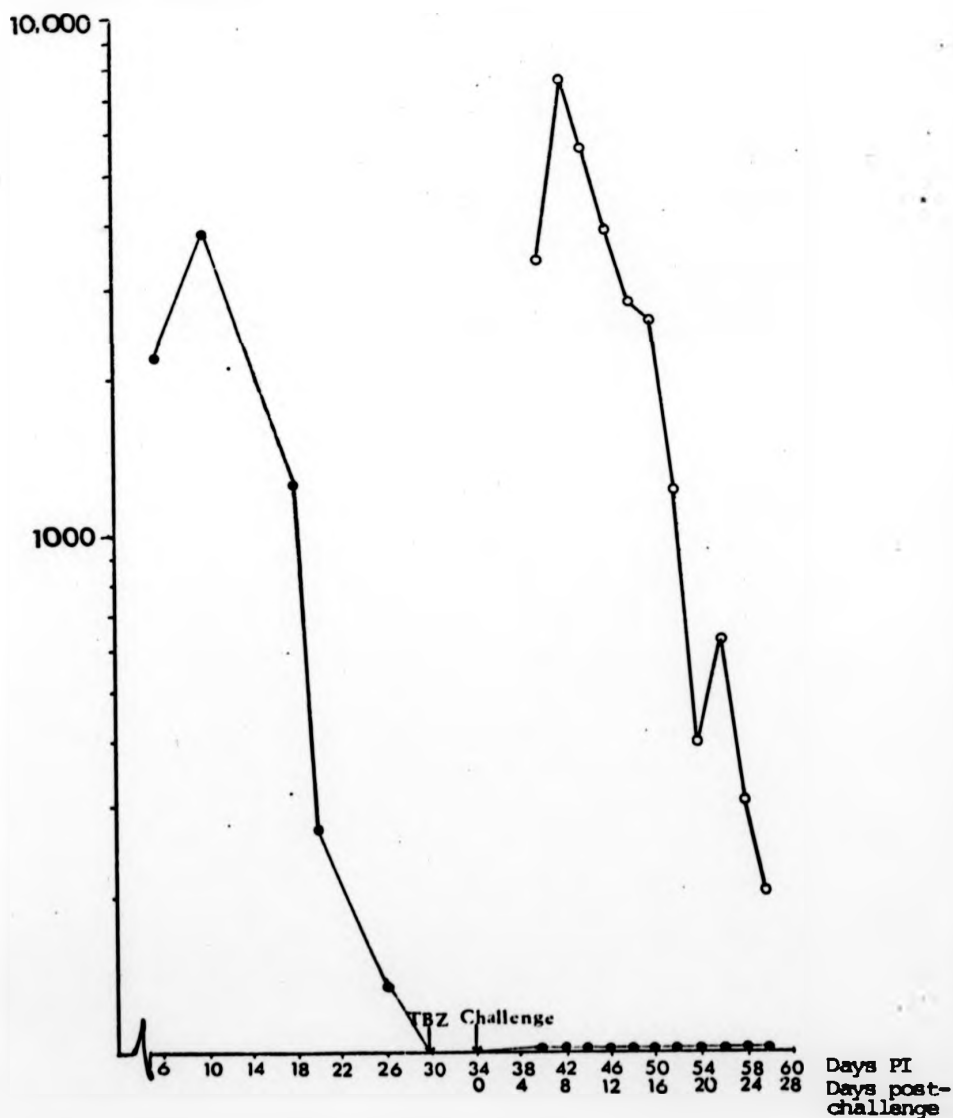
Larvae/gm of faeces

Figur e 5, Exp. 3.1:

   Mean number of adult worms recovered from two groups of rats.
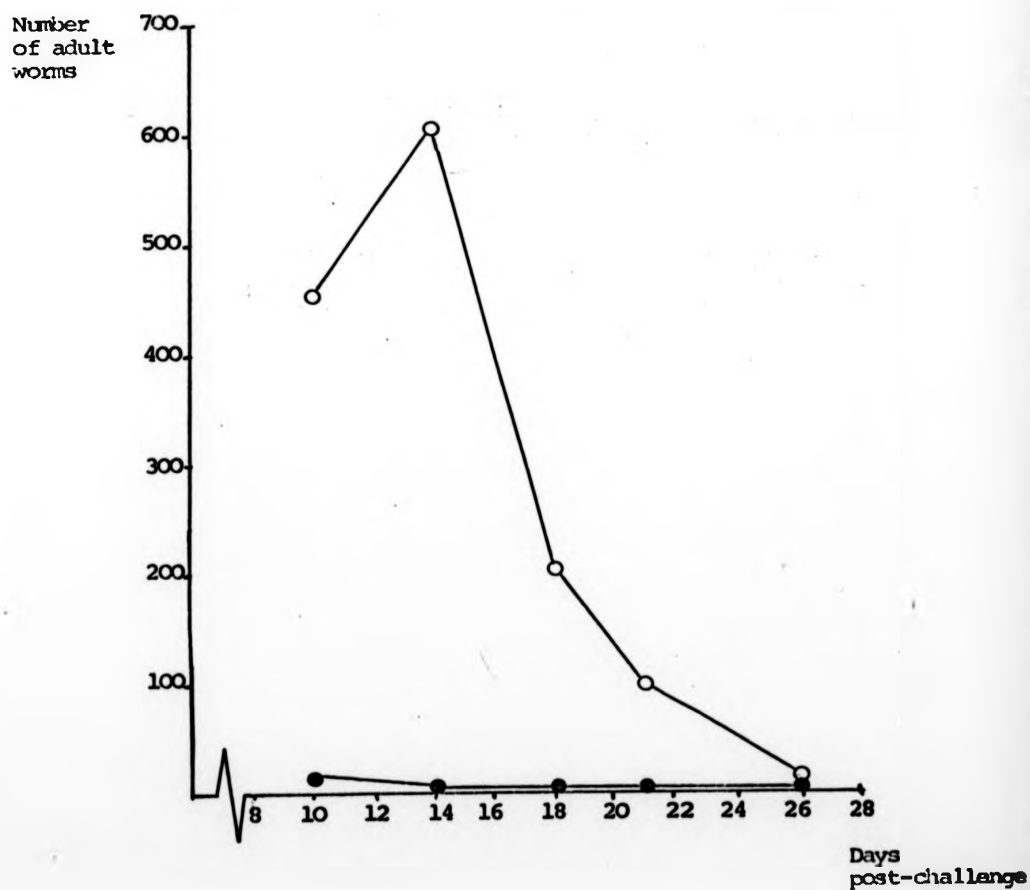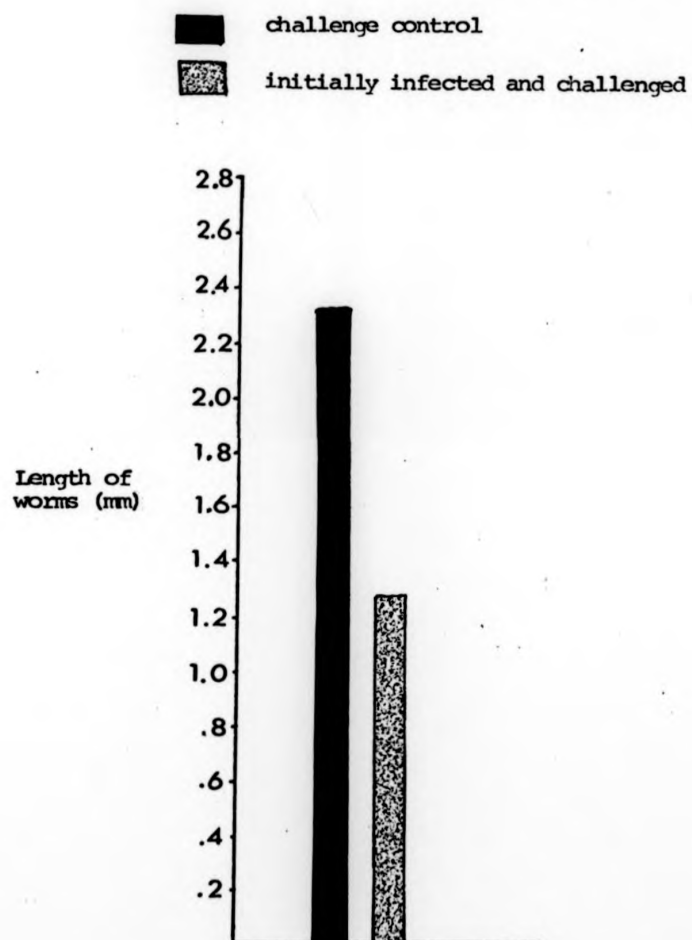   ●——● initially infected and challenged
   o——o challenge control

Figure 6, Exp. 3.1:

Mean length of worms recovered on day 10 post challenge

Length of worms (mm)

■ challenge control

▨ initially infected and challenged

group A killed two days later had no worms in their intestines. Both groups were infected with 1000 ($\pm$ 67.5) L3s per rat on day 34 PI (day 0 post-challenge). The lungs of the rats killed on days 1 and 3 were chopped in saline with a pair of fine scissors, and incubated at 37°C for about 3 hours, and the number of larvae counted. Many live and active larvae were recovered from the lungs in group B while only two dead larvae were recovered from the challenged group. The results are presented in Tables 4 and 5 and Figures 7 and 8.

The speed of migration of larvae from the skin to the gut was not the same in the two groups. The few larvae which succeeded in avoiding the immune reaction in the lungs reached the intestine on day 5 post-challenge. The highest number of worms (87.8) was recovered from the challenged group on day 7 post-challenge but the number decreased sharply as expulsion was faster in this group. Only 2 worms were recovered on day 11 post-challenge, and the rats were worm free one day later. After initial infection, the peak number of adult worms was 463 on day 14 PI and this number decreased to 13.8 on day 26 PI.

The fecundity of the worms was calculated by dividing the mean number of larvae produced by each rat, by the mean number of adult worms obtained from rats of the same group killed on the same day. The fecundity of the worms (Figure 9) in the control group reached a peak of 22.8 larvae/worm on day 7 PI and declined after day 18 to a minimum of 1.5 larvae/worm on day 26 PI. The fecundity of the worms in the challenged group was extremely low being 0.091 and 0.215 L3/worm on days 7 and 9 post-challenge, respectively. The adult worms attained their maximum length (2.81 mm) on day 9 PI and there was little or no change until day 14 PI (Figure 10). A significant decrease in the length was seen on day 18 PI and this coincided with the onset of expulsion. The worms reached their shortest length (0.89 mm) on day 26 PI. There was a slight increase in the length of the worms recovered after a challenge infection from 1.28 to 1.43 mm, between days 5 and 7 post-challenge. However, their length decreased soon after and reached a minimum of 0.59 mm on day 11 post-challenge.

The distribution of lengths of adult worms recovered during the

Table 4   Exp: 3,2   Larval production, number of adult worms calculated and number of larvae

per worm (= fecundity) during primary and secondary infections

Days	Primary infection			Secondary infection		
	mean (SE) larvae / gm faeces	mean (SE) number of adult worms	larvae per worm	mean (SE) larvae / gm faeces	mean (SE) number of adult worms	larvae per worm
7	5261 (+ 446)	159 (+ 15.5)	22.8	9.09	8 7.8 (+ 22.1)	0.09
9	4497 (+ 565)	380 (+ 23.8)	7.1	9.16	30.6 (+ 7.21)	0.21
14	2290 (+ 77.4)	463 (+ 14.6)	11.9	–	–	–
18	520 (+ 44.2)	355 (+ 18)	2.17	–	–	–
21	254 (+ 16)	168 (+ 13.7)	2.34	–	–	–
26	23.6 (+ 0.55)	13.8(+ 8.74)	1.50	–	–	–

Table 5   Exp: 3.2   Worm recovery at autopsy during initial and challenge infections and mean (SE) length of recovered worms

Days		Primary infection				Secondary infection		
	number rats/ group	mean (SE) number of adult worms	number measured	mean (SE) length in mm	number rats/ group	mean (SE) number of adult worms	number measured	mean (SE) length in mm
1	3	-	-	-	3	-	-	-
3	3	-	-	-	3	-	-	-
5	5	80 (+ 8.58)	45	2.36 (+ .02)	4	30.2 (+ 8.8)	40	1.28 (+ .03)
7	5	159 (+ 15.5)	34	2.64 (+ .04)	5	87.8 (+ 22.1)	40	1.43 (+ .04)
9	5	380 (+ 23.8)	42	2.81 (+ .05)	5	30.6 (+ 7.21)	31	1.23 (+ .03)
11	5	419 (+ 16.4)	40	2.80 (+ .05)	5	2.0 (+ 0.31)	10	0.59 (+ .05)
12	N.D.	-	-	-	3	0	-	-
14	5	463 (+ 14.6)	47	2.3 (+ .03)	N.D.	N.D.	N.D.	N.D.
18	5	355 (+ 18)	35	1.8 (+ .05)	"	"	"	"
21	5	168 (+ 13.7)	43	1.23 (+ .03)	"	"	"	"
26	5	13.8 (+ 8.74)	41	0.89 (+ .03)	"	"	"	"

Figure 7, Exp. 3.2:

Larval production in two groups of rats.
●----● previously infected and challenged
○----○ challenge control.

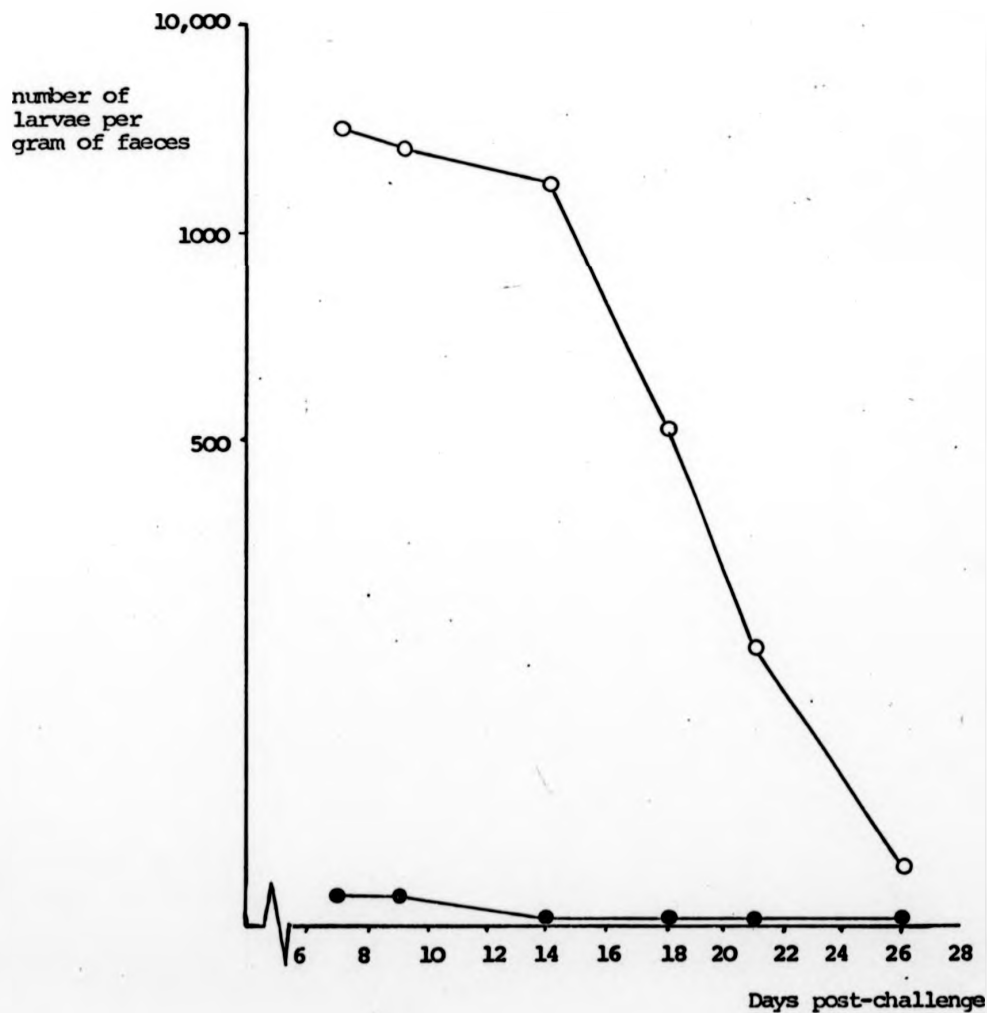number of
larvae per
gram of faeces

Days post-challenge

Figure 8, Exp. 3.2:

      Adult worms recovered from two groups of rats.
      ●———○ initially infected and challenged
      ○———○ challenge control.

Number of adult worms

Figure 9, Exp. 3.2:

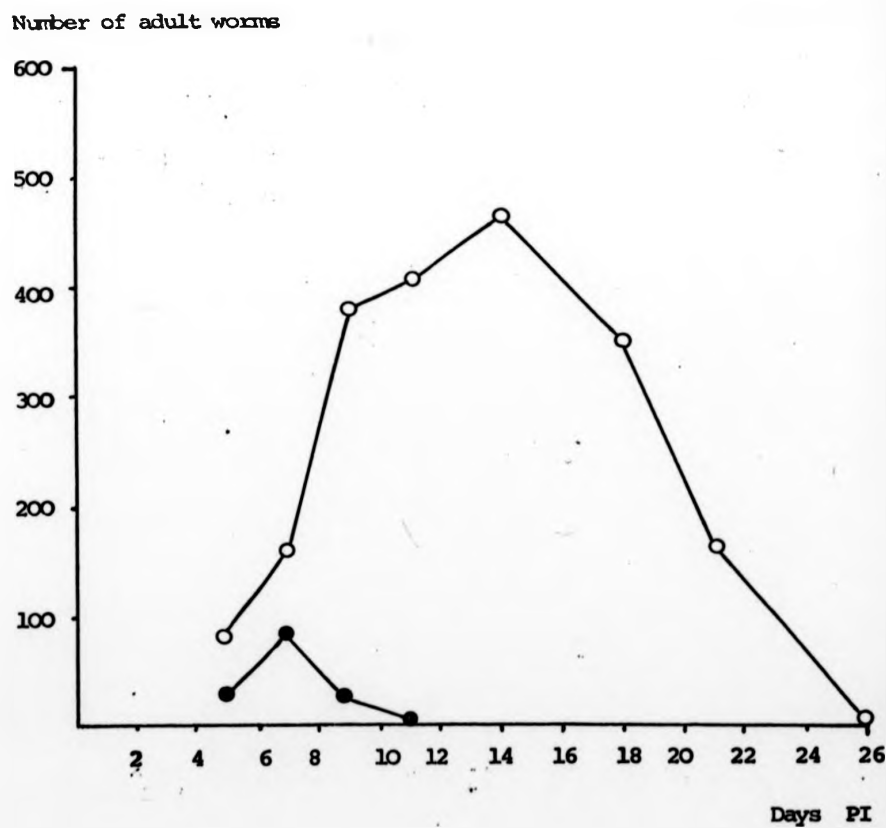Fecundity (number of larvae per worm) in two groups of rats.
●——● initially infected and challenged
○——○ challenge control.
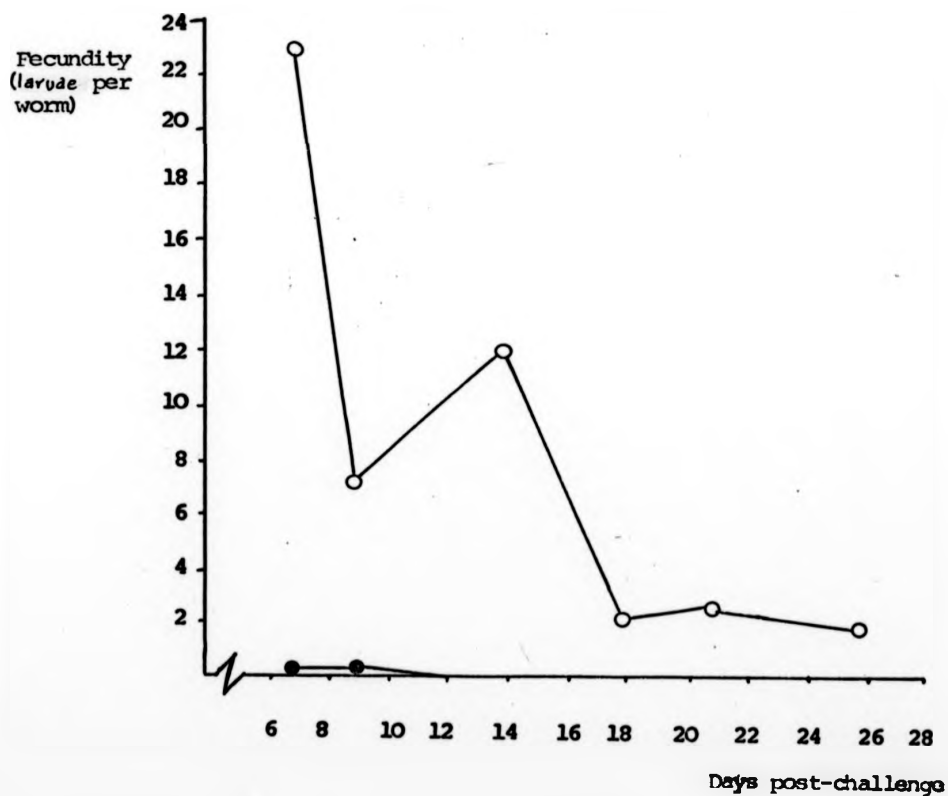
Figure 10, Exp. 3.2:

Mean length of adult worms recovered from two groups of rats.
initially infected and challenged
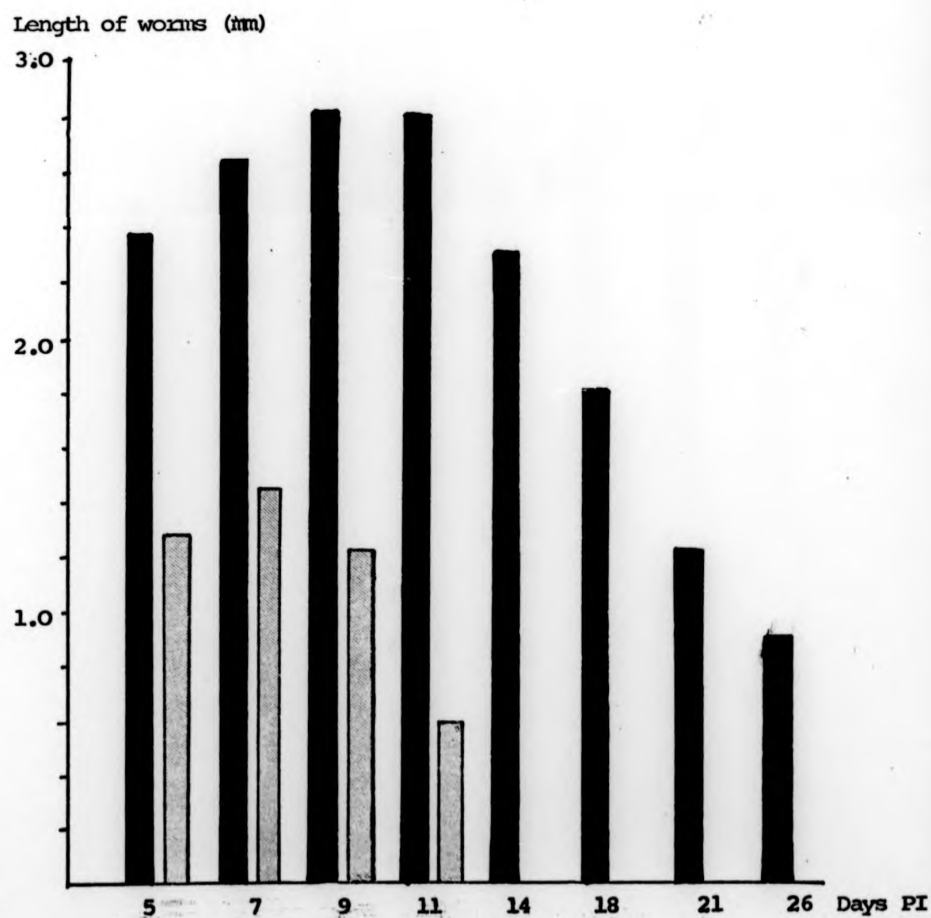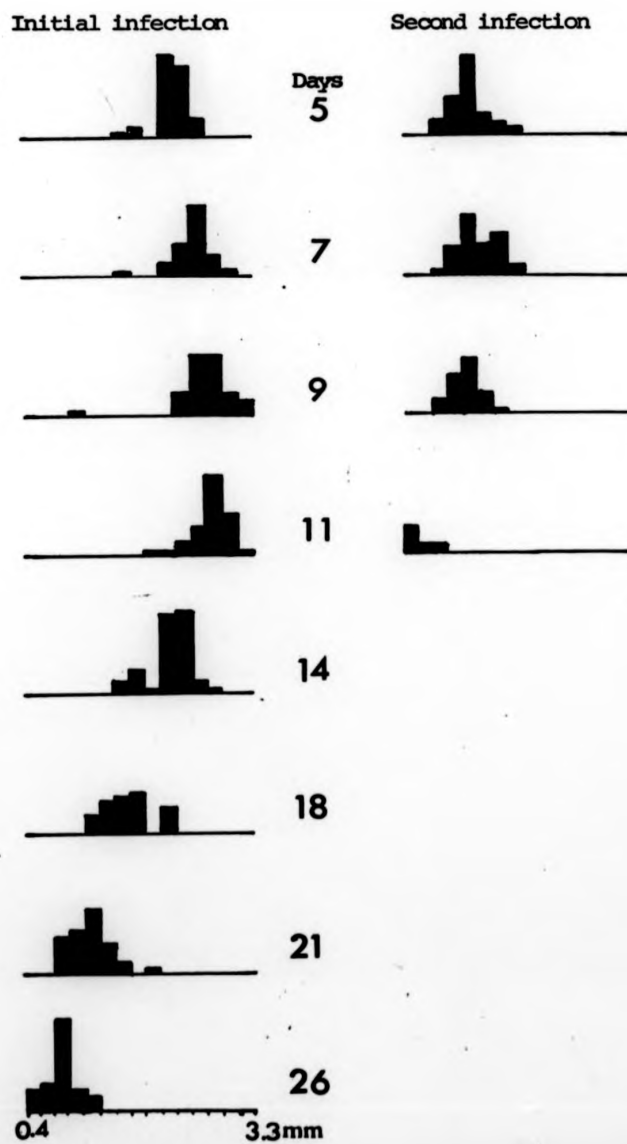challenge control

Length of worms (mm)

Figure 11, Exp. 3.2:

Length distribution of adult worms during the course of initial and challenge infections.

course of initial and second infections (Figure 11) indicates that
the worms shrank rather than that there was a selective loss of a
population of long adult worms first.

Experiment 3.3:

This experiment was carried out to study the distribution of
adult S. ratti in the intestines of infected rats and their migration
during initial and challenge infections.

Thirty seven rats were infected with 1000 ($\pm$ 65) L3s each.
Groups of four rats were killed on days 9, 13, 18, 20, 22 and 25 PI.
The small intestine of each rat was divided into 4 equal segments
and the number of adult worms in each part determined. The results
are summarized in Table 6 and Figure 12. During the first 18 days,
79% of the worms occupied the anterior segment of the small intestine.
By day 13 PI, the worms had begun to move posteriorly. An average of
65% of the worms were recovered from the last segment of the intestine
between days 20 and 25 PI compared to 7.3% in the first segment,
during the same period. The pattern of worm distribution in the two
middle segments, during the course of infection, was similar to the
trend in the first and last segments. This migration to the distal
part of the intestine coincided with the reduction in the number of
adult worms and their shrinkage. No worms were recovered from the
lumen of the segments of the gut suggesting that worms migrated
through the tissue of the small intestine rather than in the lumen.

The remaining 12 rats were treated with 2 x 100 mg/kg of TBZ
and reinfected with 1000 ($\pm$ 81.6) L3 each. The number of worms after
challenge was low, and there was an equal distribution in the anterior
and posterior segments on day 6. 60% of the worms were found in the
last quarter of the intestine on day 8, prior to their expulsion on
day 10, while the anterior segment harboured only 16% of the total
adult population.

This experiment has shown that adult S. ratti undergo a posterior
movement towards the last quarter of the small intestine, prior to
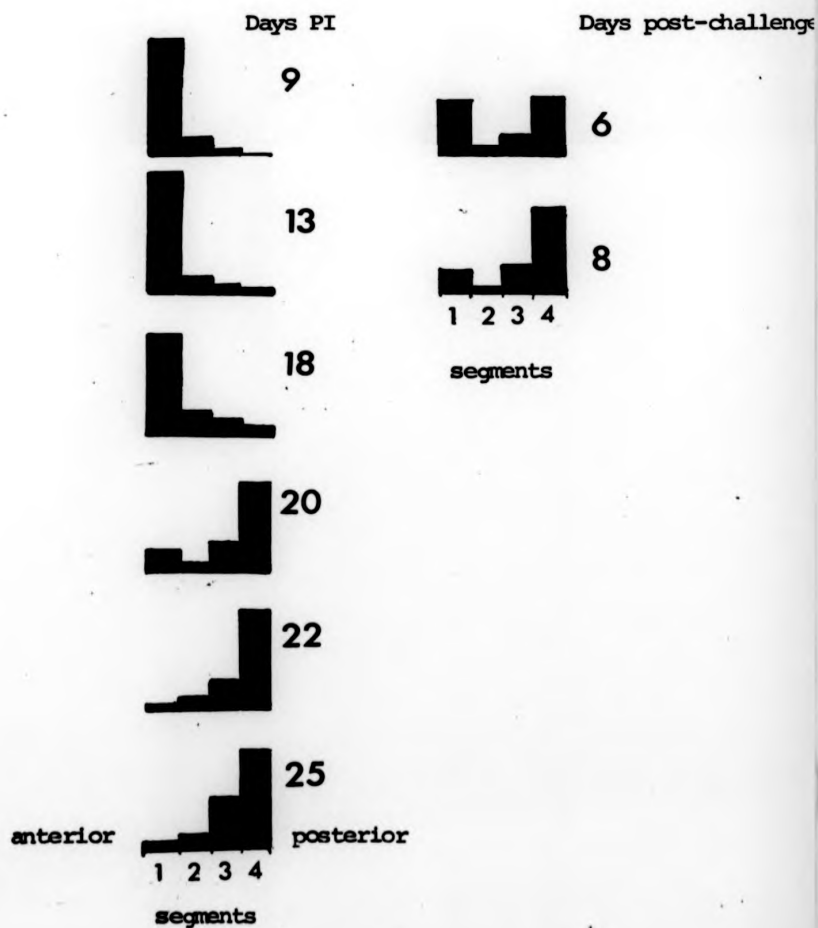their expulsion following initial and challenge infections.

Table 6 Exp. 3.3:

The distribution of adult <u>S. ratti</u> in the small intestine of rats,
during the course of primary and secondary infections

			Mean (SE) number of adult worms		
Days PI	Total	Segment 1	Segment 2	Segment 3	Segment 4
9	400 ($\pm$13.5)	333 ($\pm$25)	53 ($\pm$14.5)	10 ($\pm$1.9)	2 ($\pm$0.85)
13	647 ($\pm$58)	543 ($\pm$48)	74 ($\pm$10)	20 ($\pm$4)	10 ($\pm$1)
18	617 ($\pm$30)	440 ($\pm$25)	89 ($\pm$8.5)	64 ($\pm$3.4)	24 ($\pm$3.4)
20	256 ($\pm$66)	37 ($\pm$29)	10 ($\pm$3)	54 ($\pm$16)	153 ($\pm$31)
22	194 ($\pm$37)	5 ($\pm$2.3)	16 ($\pm$2.2)	39 ($\pm$10)	134 ($\pm$33)
25	83 ($\pm$10)	60 ($\pm$8)	17 ($\pm$2)	5 ($\pm$1)	1 ($\pm$0.5)
Post challenge					
6	36 ($\pm$11)	14 ($\pm$4)	3 ($\pm$1.4)	4.5 ($\pm$2.5)	14.5 ($\pm$7.6)
8	24 ($\pm$8)	4 ($\pm$2.8)	1 ($\pm$0.5)	5 ($\pm$0.8)	14 ($\pm$5)
10	0	0	0	0	0

Figure 12, Exp. 3.3:

The distribution of adult S. ratti in the intestine of rats during primary and secondary infections.

Days PI

Days post-challenge

9

13

18

20

22

25

6

8

1 2 3 4

segments

anterior   posterior

1 2 3 4

segments

Experiment 3.4:

The purpose of this experiment was to study the duration of
the immune response to S. ratti in rats. Rats initially infected
developed very strong resistance to reinfection. However, a few
adult worms persisted for up to 100 days (Sheldon, 1937a).

Fifteen rats were divided into 3 groups of 5 rats each. Ten
of these rats were infected with 1000 ($\pm$ 93.7) L3/rat while the
other 5 were left uninfected to act as challenge controls. After
30 days, 5 infected rats were treated with 2 x 100 mg/kg of TBZ to
remove the residual population, while the other infected group of
5 rats was left untreated. Three months later, all the rats were
challenged with 1000 ($\pm$ 99.4) L3s/rat. Ten days later, all the
rats were autopsied and the number of adult worms in the anterior
and posterior halves of the small intestine was counted. Faecal
cultures were made on days 6, 8 and 10 post-challenge. The results
are presented in Tables 7 and 8 and Figures 13 and 14. There was no
significant difference between larval production in the two
previously infected groups. The mean number of larvae/gm of faeces
was much lower in the two experimental groups than the control group
on days 6, 8 and 10. Only 3.5% and 5% of the worms developed in the
two previously infected groups, while the challenge control group
showed a 53% recovery on day 10 post-challenge and PI, respectively.

Judging from the level of larval production and adult recovery,
it was clear that a substantial degree of resistance was maintained
in rats previously infected with S. ratti for more than 3 months.
The experiment also showed that the residual worm population was not
responsible for maintaining this immunity as its removal with TBZ
did not eliminate the immune response. The distribution of the worms
recovered in the previously infected groups was similar to that
observed in a normal challenge infection prior to worm expulsion.

Experiment 3.5:

This experiment was designed to investigate the fate of infective
larvae and adult worms following a challenge infection. It also

Table 7   Exp: 3.4 Larval production following challenge after an interval of 3 months, in two groups

Days	Mean (SE) number of larvae / gm of faeces		
	Treated with thiabendazole	Not treated with thiabendazole	Challenge control
6	393 ($\pm$ 28.6)	339 ($\pm$ 33.3)	2857 ($\pm$ 182)
8	55.5 ($\pm$ 19.8)	138 ($\pm$ 18.8)	7933 ($\pm$ 416)
10	17.7 ($\pm$ 8.2)	19.4 ($\pm$ 8)	6560 ($\pm$ 165)

Table 8   Exp: 3.4   Mean number of adult worms recovered on day 10 after challenge in

groups reinfected after an interval of 3 months

Mean (SE) number of adult worms

Reinfected after 3 months	anterior half	posterior half	total
treated with thiabendazole	14.6 ($\pm$ 3.7)	21.2 ($\pm$ 1.4)	35.8 ($\pm$ 4.4)
not treated with thiabendazole	17 ($\pm$ 1.87)	34.2 ($\pm$ 3.98)	50.2 ($\pm$ 4.88)
challenge control	450 ($\pm$ 46)	82.8 ($\pm$ 7.3)	533 ($\pm$ 47.8)

Figure 13, Exp. 3.4:

Larval production in three groups of rats.
o——o reinfected after 3 months interval, treated with TBZ
△——△ reinfected after 3 months interval, not treated with TBZ
●——● challenge control

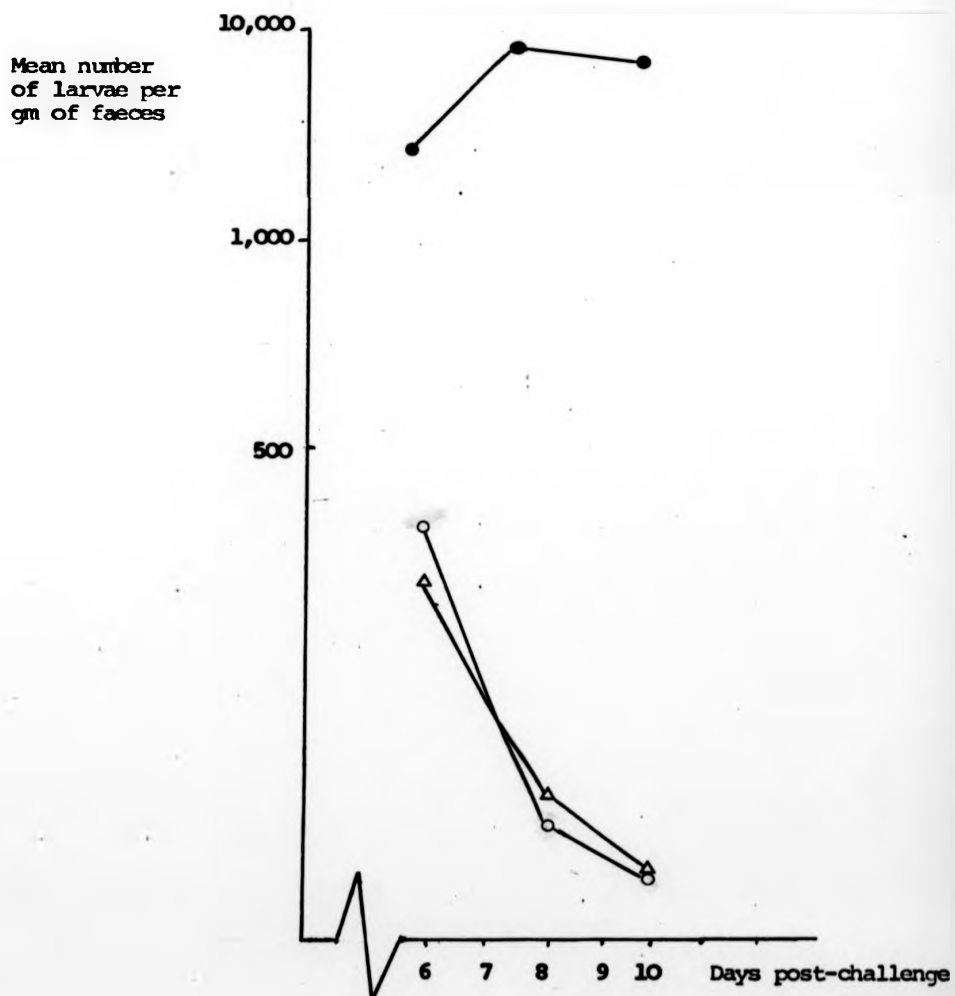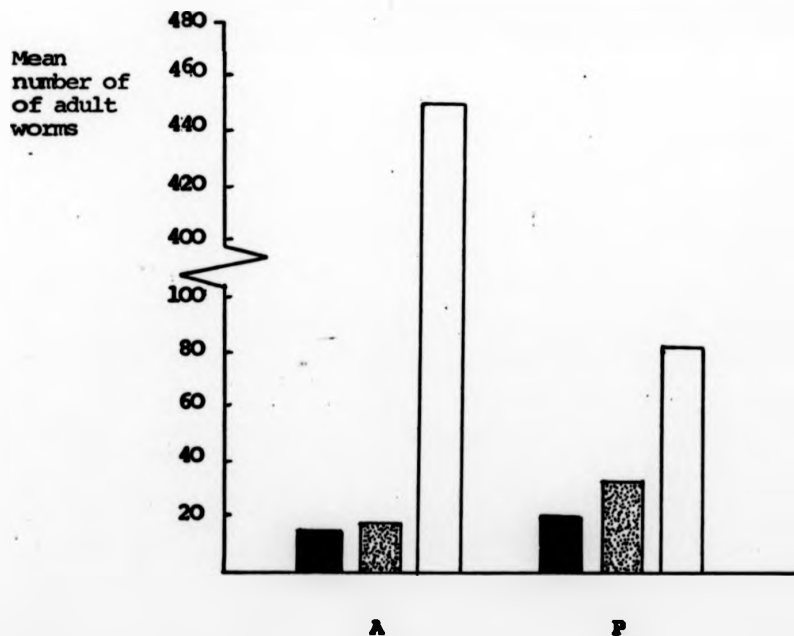

Mean number
of larvae per
gm of faeces

Figure 14, Exp. 3.4:

Mean number of adult worms recovered from the anterior A, and posterior, P, intestine of three groups, on day 10 post challenge.

- ■ challenged after 3 months interval, treated with TBZ
- ▨ challenged after 3 months interval, not treated with TBZ
- ☐ challenge control

studied the effect of the immune response on the larvae during
their skin-lung-intestine migration. Fifteen rats were used in
this experiment and were divided into the following four groups:
Group 1: four rats each received an initial infection followed
by 5 challenges of the same inoculum (1000 L3/rat), at intervals
of 20, 15, 10, 5 and 5 days, respectively. The first 5 infections
were given, percutaneously, on different areas of the back, while
the challenge was given percutaneously on a shaven area of the
abdomen.

Group 2: four rats, each received an initial infection of 1000 L3s
and a challenge of 1000 L3s, percutaneously, on the day the last
challenge was given to group 1.

Group 3: four rats, received a percutaneous infection on the same
day as the last challenge dose was given to group 1.

Group 4: three rats were left uninfected to act as normal controls.

The following histopathological and parasitological changes were
observed:

Skin

Initial percutaneous infections with infective larvae of
S. ratti were very fast. The filariform larvae penetrated the skin
within 30 minutes. Very few changes were observed in the skin soon
after exposure, but there was a slight dilatation of the walls of
both capillaries and lymphatics, with no direct evidence that this
was due to infection (Plate 9). No eosinophils were seen during the
passage of an initial infection through the skin.

Following a second infection, live larvae were demonstrated in
the hypodermis with little or no tissue response around them during
the first 3 hours post-exposure (Plate 10). After this period, the
reaction in the skin changed slightly, with infiltration of lympho-
cytes, mainly in the deeper layers of the subcutaneous tissue. The
reaction was patchy, confined to limited areas of the skin. No
neutrophils or eosinophils were detected (Plate 11). Abundant
macrophages, and lymphocytes were seen between 4-6 hours post-exposure,
with an obvious absence of polymorphs. This indicated that the
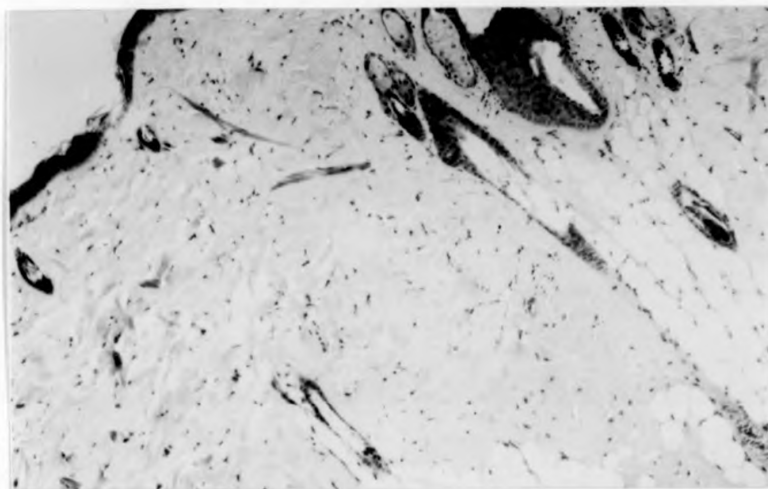reaction had developed into a more pronounced humoral type response.

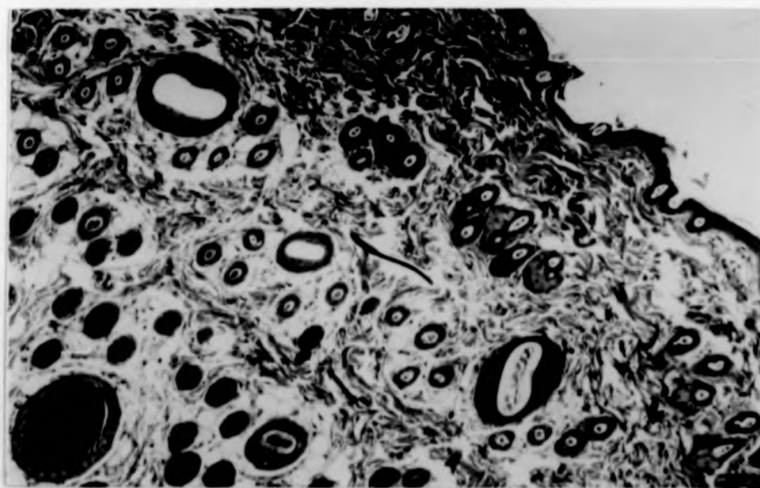Plate 9 : Skin; 30 minutes after primary exposure (H.& E., x 80)

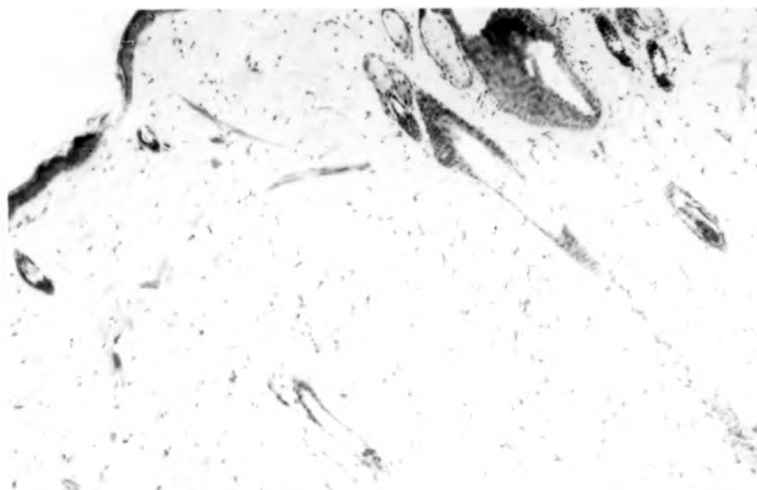Plate 10 : Skin; 30 minutes after second exposure (H.& E., x 80)

Plate 9 : Skin; 30 minutes after primary exposure (H.& E., x 80)

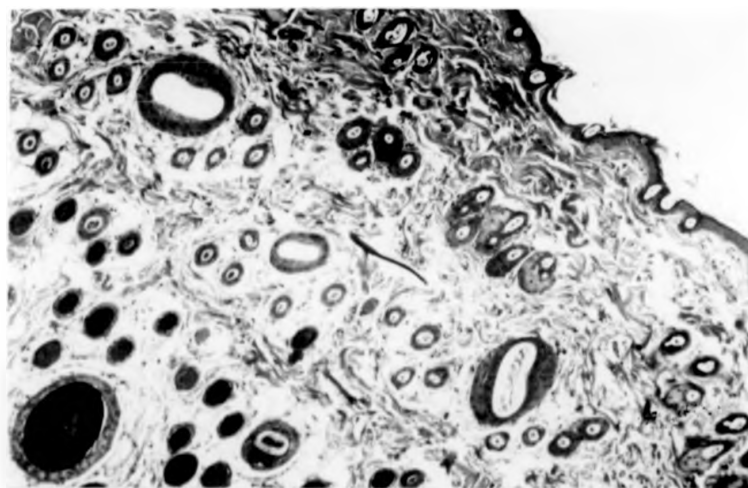
Plate 10 : Skin; 30 minutes after second exposure (H.& E., x 80)

After 24 hours, the reaction was dermal with signs of progressive reaction near hair follicles. It appeared that the larvae were capable of evading the skin stage of the immune reaction early after challenge. However, after 24 hours, there was a response to the passage of the infective larvae which manifested itself in a cellular infiltration of lymphocytes and macrophages and total absence of eosinophils (Plate 12).

The histopathological picture changed entirely following 6 infections. The response to the invading population of filariform larvae was immediate and severe (Plate 13). Within the first 30 minutes, the lymphatics were surrounded by neutrophils, eosinophils and mononuclear macrophages. Dead larvae were seen in the sub-cutaneous tissue, surrounded by many eosinophils, lymphocytes and macrophages (Plate 14). After 24 hours, pronounced oedema and vascular and lymphatic dilatation was evident, as well as a severe infiltration of eosinophils, lymphocytes and macrophages (Plate 15). It was very difficult to quantify the tissue eosinophil response in secondary and multiple infections. However, based on the number of eosinophils per 10 random high power fields, Table 9 was produced to present the difference in this response in the four groups.

Lungs:

Live larvae were frequently seen in histological sections of the lungs of rats infected once (Plate 17) but with little or no reaction surrounding them (Plate 18). A few active larvae were also recovered from chopped lungs after incubation at 37°C in saline. Few lymphocytes, polymorphs and macrophages were seen. Few eosinophils were found in initially infected and normal unexposed rats.

Only dead larvae were recovered from minced lungs during the first day in a secondary infection. Histologically, the lungs developed areas of lymphoid infiltration composed of lymphoblast centres surrounded by small lymphocytes, suggestive of lymphoid hyper-plasia (Plate 19). A dead larva was observed in one of these lymphoid centres with a very strong lymphocytic reaction round it (Plate 20). Many other such granulomas were seen developing around fragments of worms. Vast numbers of eosinophils had infiltrated the lung tissue
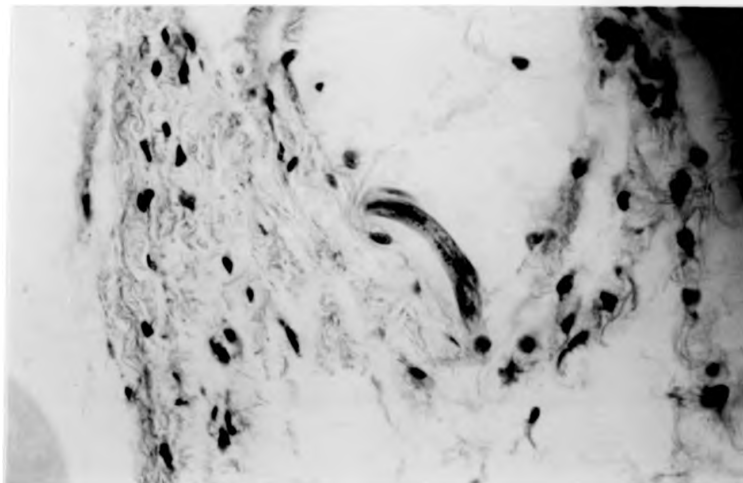
Plate 11 : Skin; 30 minutes after second exposure, no eosinophil
reaction round live larva (Lendrum's, x 250)
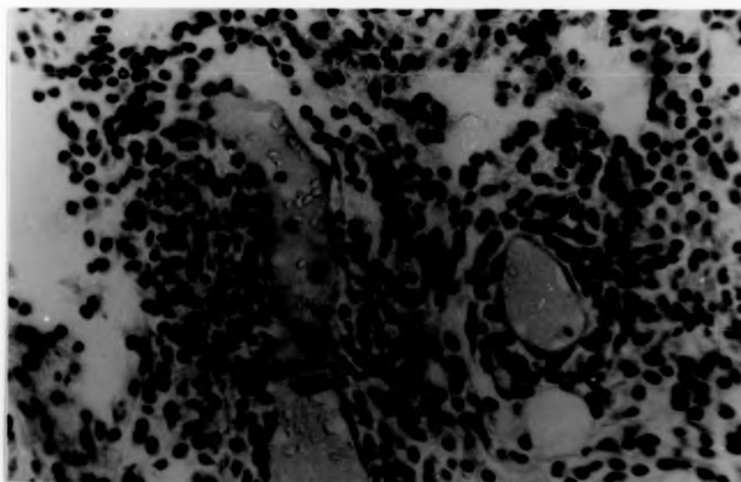
Plate 12 : Skin; 24 hours after second exposure, no eosinophil
reaction (Lendrum's, x 250)
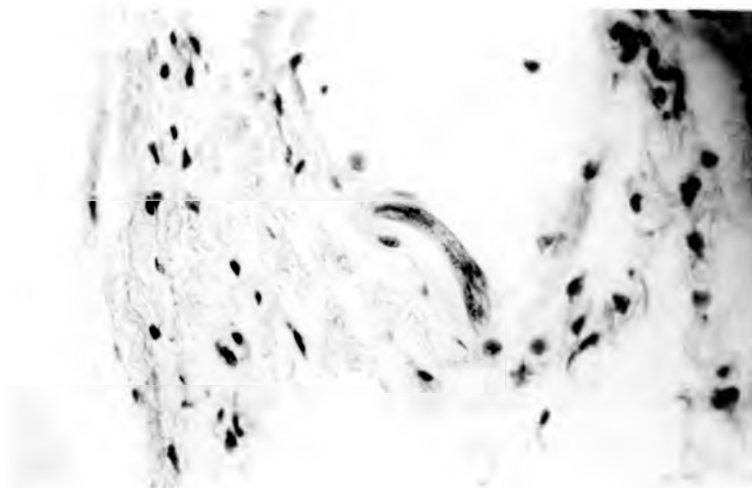
Plate 11 : Skin; 30 minutes after second exposure, no eosinophil reaction round live larva (Lendrum's, x 250)
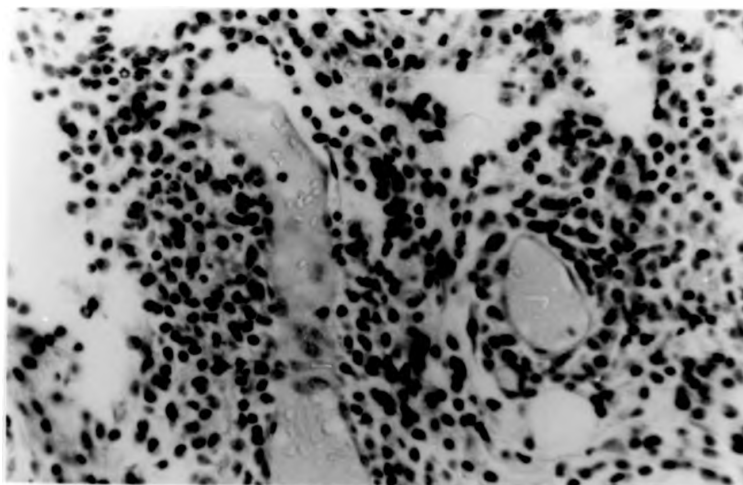
Plate 12 : Skin; 24 hours after second exposure, no eosinophil reaction (Lendrum's, x 250)

Plate 13 : Skin, generalised reaction in the hypodermis, 30 minutes after 6th exposure (H.& E., x 80)

Plate 14 : Skin, eosinophil infiltration around dead larva, following 6 exposures (Landrum's, x 400)

Plate 13 : Skin, generalised reaction in the hypodermis, 30
minutes after 6th exposure (H.& E., x 80)
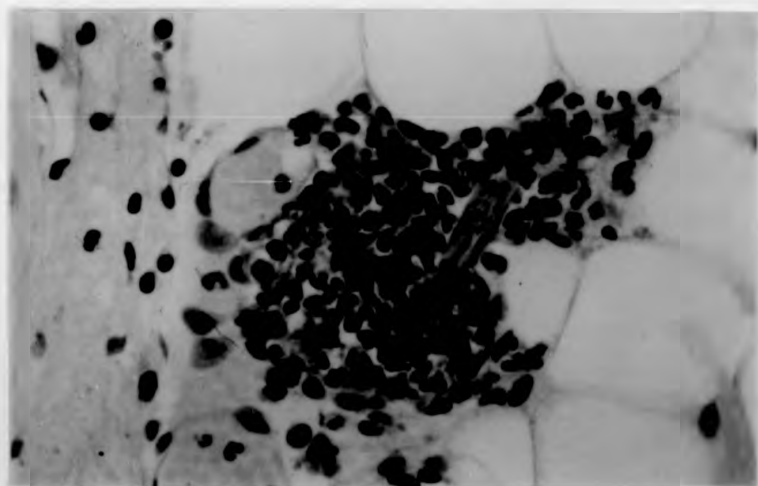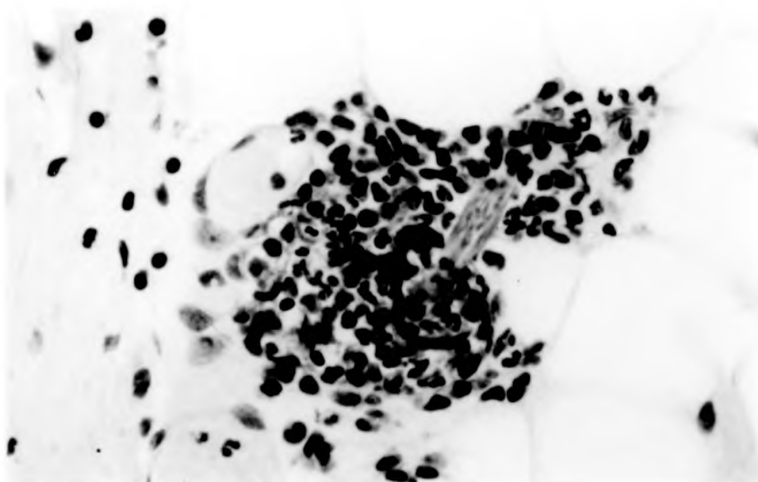
Plate 14 : Skin, eosinophil infiltration around dead larva,
following 6 exposures (Lendrum's, x 400)
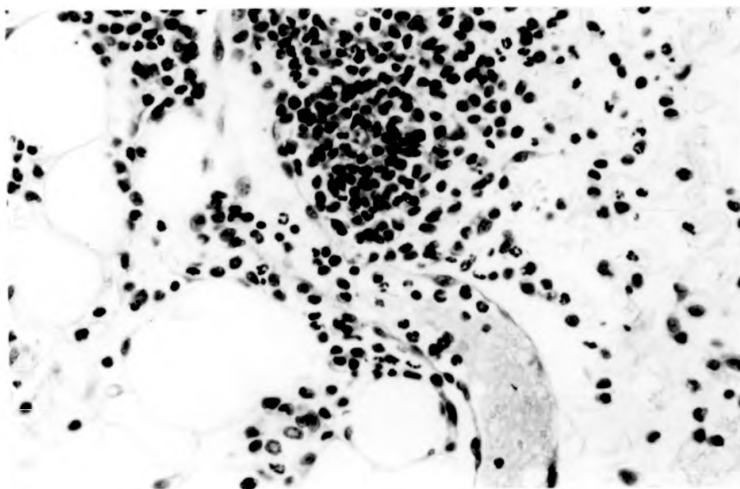
Plate 15 : Skin, extensive eosinophil, lymphocyte and macrophage
infiltration 24 hours after 6th exposure (Lendrum's,
x 400)
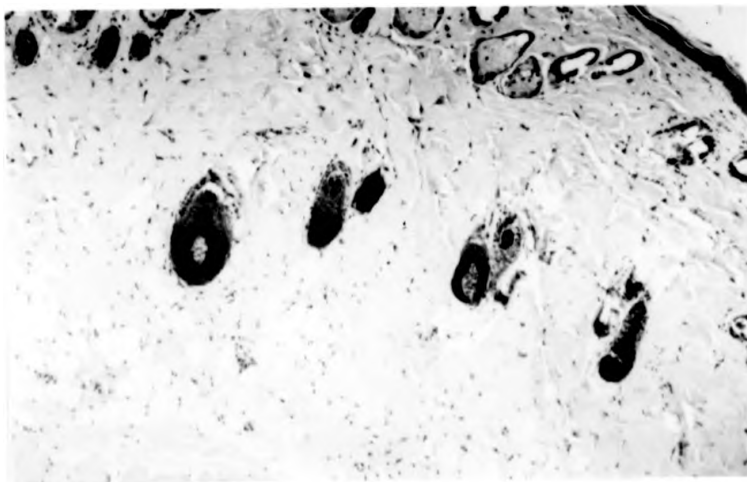
Plate 16 : Normal unexposed rat skin (Lendrum's, x 80)
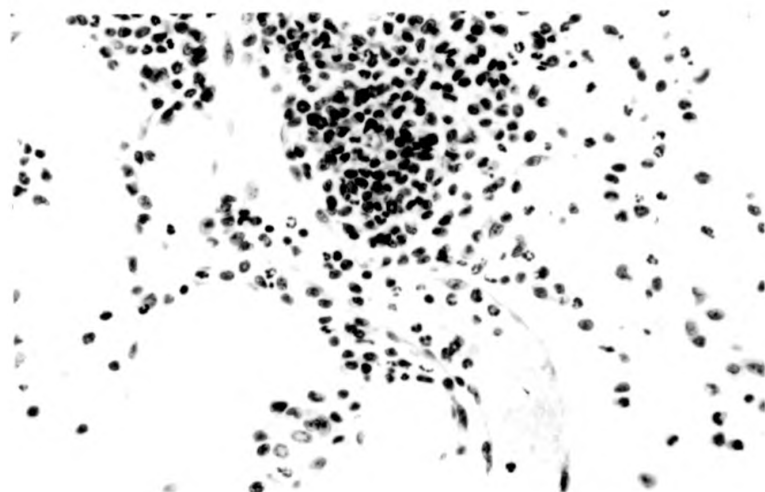
Plate 15 : Skin, extensive eosinophil, lymphocyte and macrophage infiltration 24 hours after 6th exposure (Lendrum's, x 400)

Plate 16 : Normal unexposed rat skin (Lendrum's, x 80)
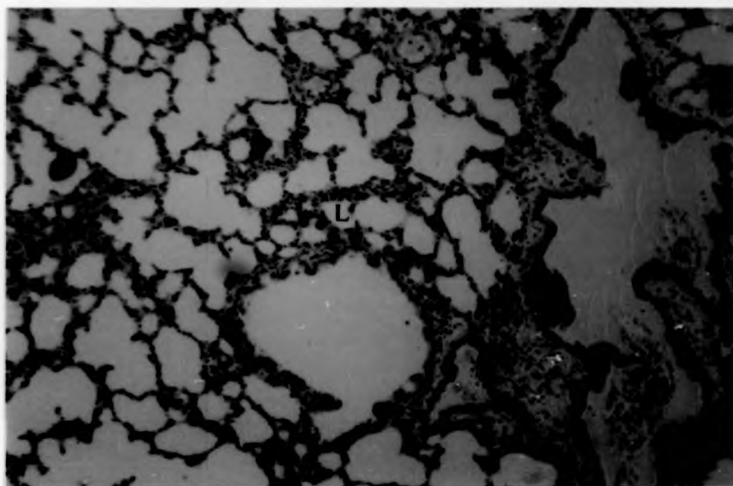
Plate 17 : Lung following initial infection, live larva (L)
within alveoli (H.& E., x 80)
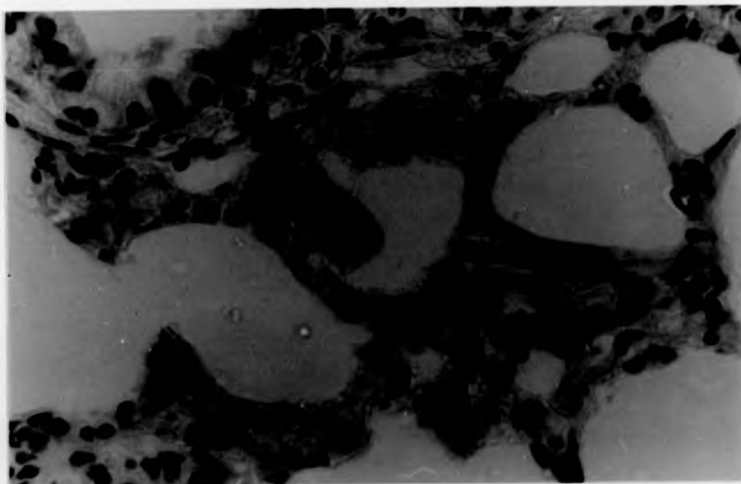
Plate 18 : Lung, live migrating larva in alveoli with very
little reaction (H.& E., x 400)
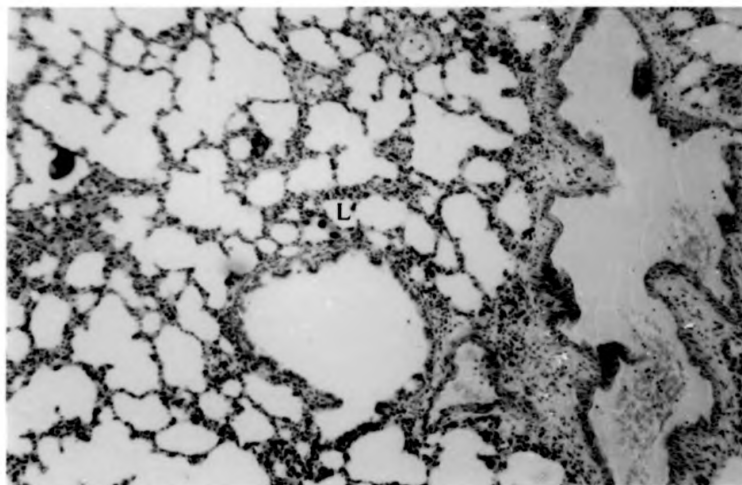
Plate 17 : Lung following initial infection, live larva (L)
within alveoli (H.& E., x 80)
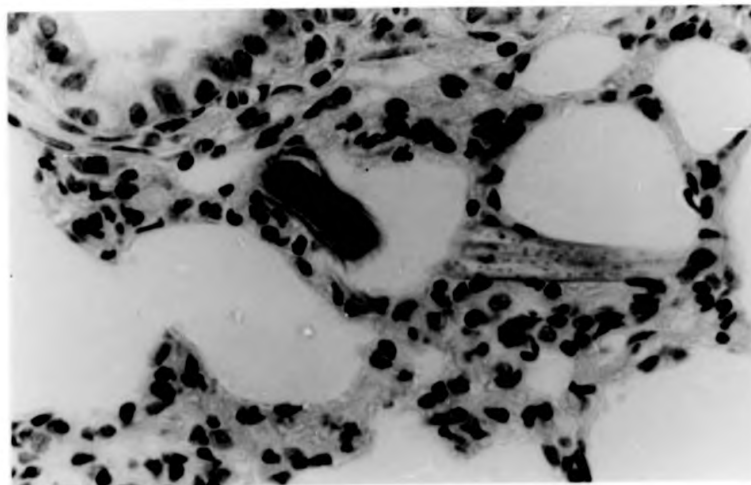
Plate 18 : Lung, live migrating larva in alveoli with very
little reaction (H.& E., x 400)
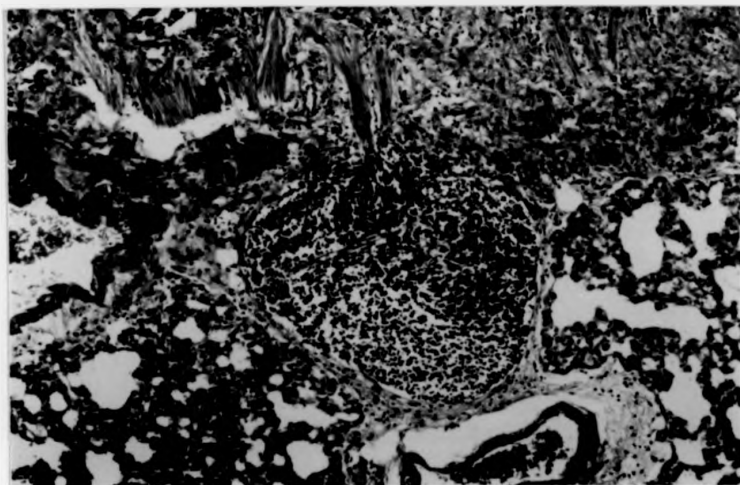
Plate 19 : Lung, lymphoid hyperplasia following second
infection (H.& E., x 80)
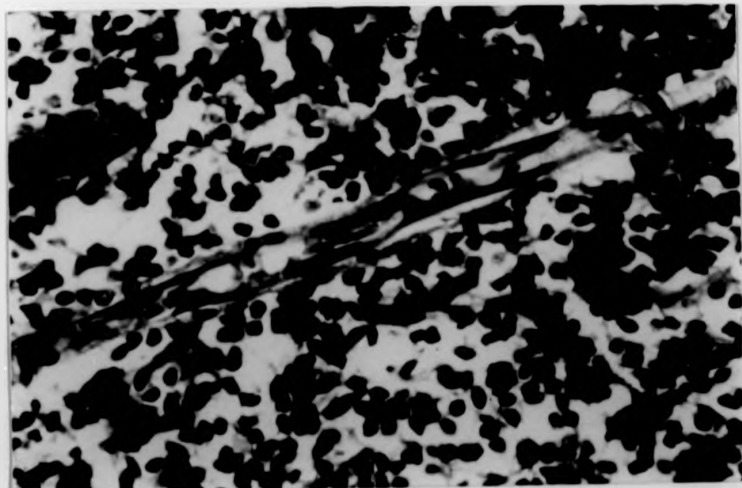
Plate 20 : Lung, dead larva surrounded by small lymphocytes
following second infection (H.& E., x 400)
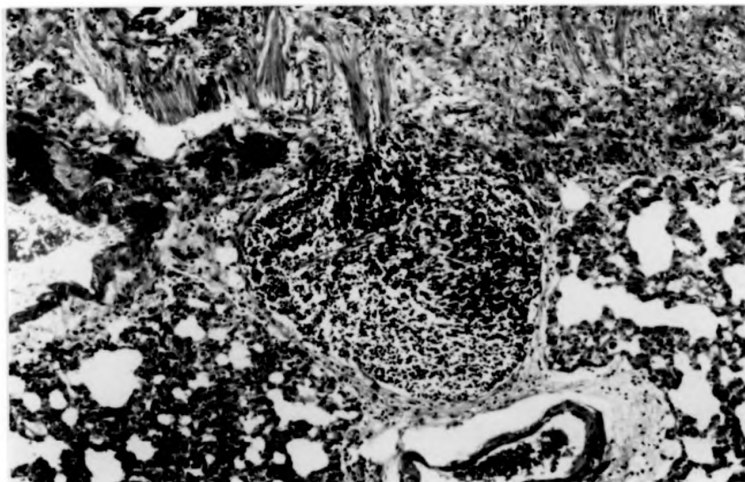
Plate 19 : Lung, lymphoid hyperplasia following second
infection (H.& E., x 80)
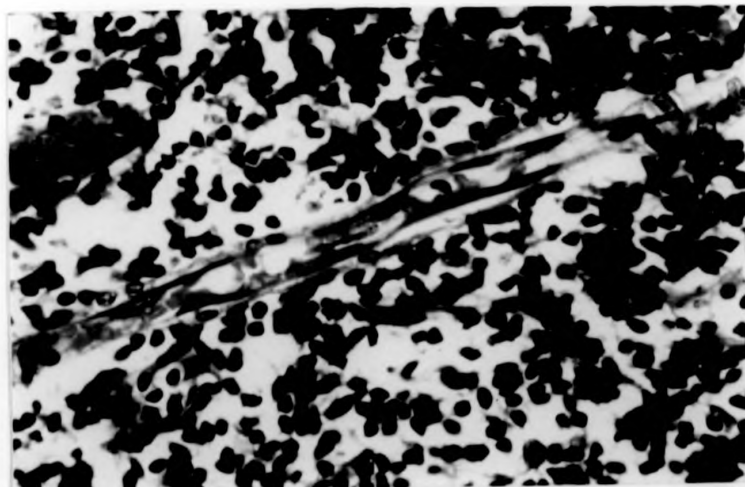
Plate 20 : Lung, dead larva surrounded by small lymphocytes
following second infection (H.& E., x 400)

Table 9, Exp. 3.5:

Tissue eosinophil response in the skin of rats; following 6 infections (group 1); 2 infections (group 2); 1 infection (group 3); and non-infected control (group 4)

Time after last exposure	Group 1	Group 2	Group 3	Group 4
½ hour	++	-	-	-
1 hour	+	-	-	-
1½ hours	+	-	-	-
3 hours	++	-	-	-
4 hours	+++	-	-	-
6 hours	++	+	-	-
24 hours	++++	+	-	-

(Plate 21) and cuffed the blood vessels and the bronchial passages. The multiple granulomas and the infiltration of many granulocytes within the lung tissue was suggestive of pneumonitis.

Following multiple infections no larvae reached the lungs and larvae were killed in the skin. There was a generalized reaction which was manifested by a mild increase in the number of eosinophils and polymorphonuclear cells in the lung tissue.

Small intestine:

There were very few pathological changes in the intestinal tissue following the arrival of worms from the lungs, and during the first two weeks of initial infection. The adult worms embedded their anterior ends in the mucosal crypts and were sometimes seen in the mucosa. The eggs which the adult worms passed were laid in strings of 8-14 eggs inside the submucosa; this was repeatedly seen in squash preparations of the intestinal tissue. Later during an initial infection, infiltration of lymphocytes, plasma cells and eosinophils started, particularly around days 18 PI (Plate 23), when changes in the length and structure of the worms began. Although the Peyer's patches enlarged gradually during the course of infection, few adults or embryonated eggs were seen within the mucosa surrounding the Peyer's patches (Plate 24). This may be due to the gradual movement of the worms from one site to another. There was an increase in the number of eosinophils around the time of worm expulsion (see Chapter 7).

In second infections the few adult worms, which avoided the immune response in the lungs, arrived in the intestine and attached themselves to the mucosa. Egg production was very low, and no egg strings were seen. There was an increase in the number of eosinophils, plasma cells and macrophages in the submucosa of the villi. The Peyer's patches did not increase beyond the size which they reached late during initial infection.

No adult worms were seen in the small intestine following multiple infections.
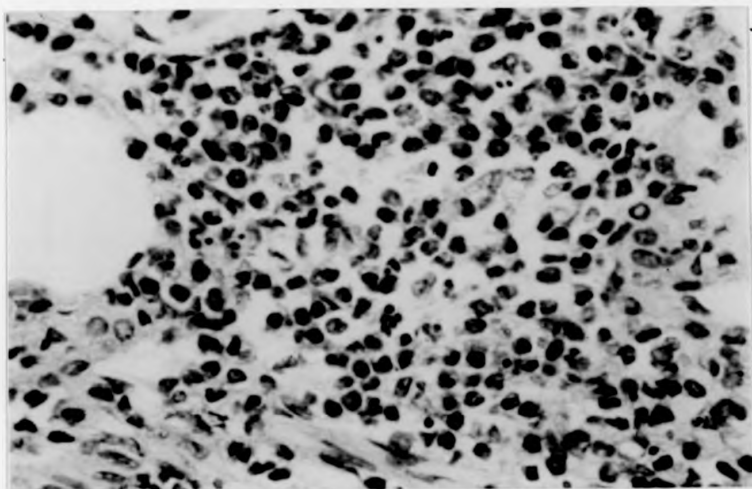
Plate 21 : Lung, extensive infiltration of eosinophils following a second infection (Lendrum's, x 400)
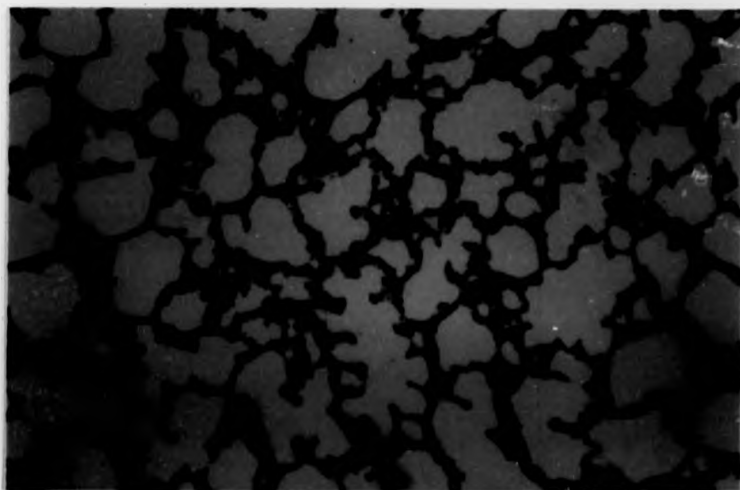
Plate 22 : Normal unexposed lung (H.& E., x 80)
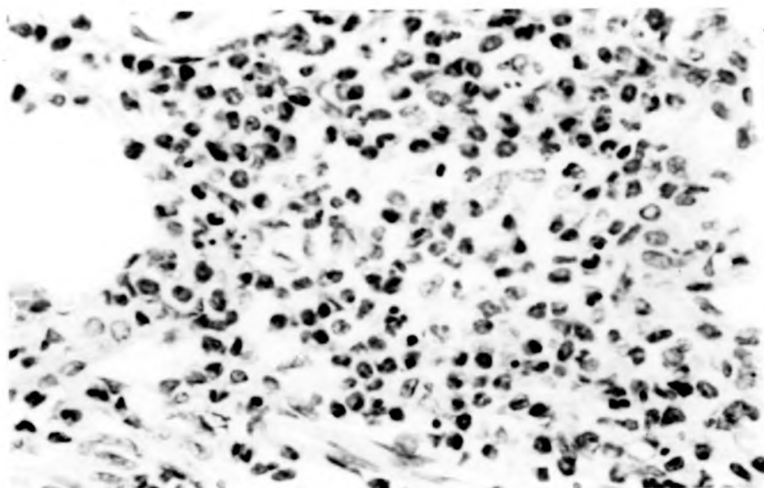
Plate 21 : Lung, extensive infiltration of eosinophils following a second infection (Lendrum's, x 400)
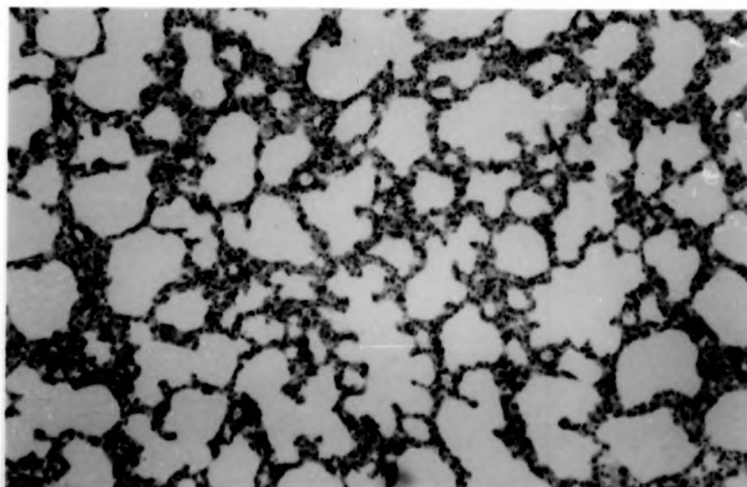
Plate 22 : Normal unexposed lung (H.& E., x 80)
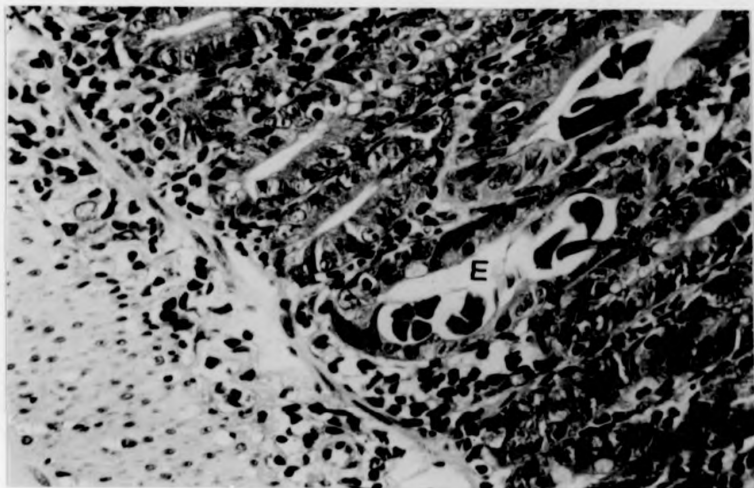
Plate 23 : Small intestine, eosinophil response around
embryonated eggs (E) (Lendrum's, x 250)
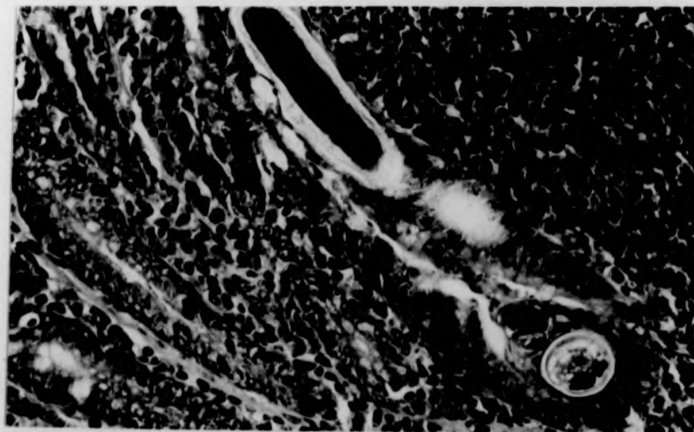
Plate 24 : Small intestine, larvae lying between villi and
a Peyer's patch (H.& E., x 250)
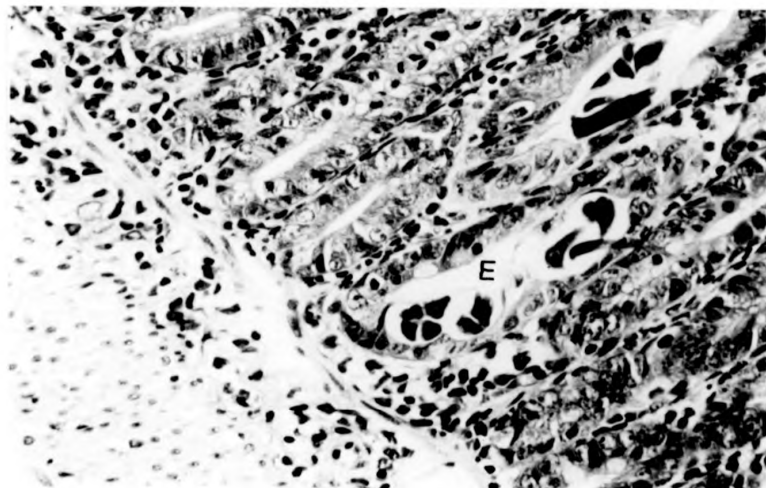
Plate 23 : Small intestine, eosinophil response around
            embryonated eggs (E) (Lendrum's, x 250)
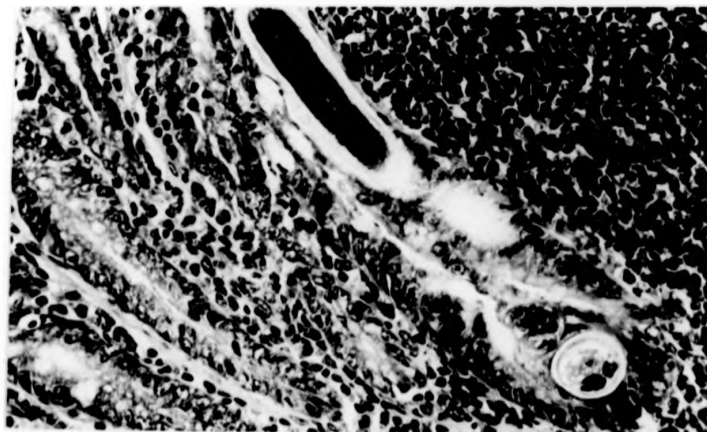
Plate 24 : Small intestine, larvae lying between villi and
            a Peyer's patch (H.& E., x 250)

Experiment 3.6:

In this experiment four different infective doses were given
to groups of rats and particular attention was given to the dis-
tribution of the developing stages in the host's organs and the
percentage of development in the gut.

Twenty two rats were divided into four groups: Group 1,
four rats, received 1000 L3 each; Group 2, six rats, received
4000 L3 per rat; Group 3, six rats, received 6000 L3; Group 4,
six rats, received 8000 L3. All the surviving rats were killed
on day 10 PI, and faecal cultures were prepared from all groups
on days 6, 8 and 10 PI.

The results of this study are summarized in Table 10. The
group which received 1000 L3s harboured a mean of 457 worms on day
10 PI; a percentage development of 45.7%. The rats which received
4000 L3/rat harboured a mean of 1159 worms which represented 28.9%
of the original inoculum.

A similar, proportionately low, recovery rate was observed in
the rats which received 6000 L3s each. Four members of this group
died on day 3 PI and the two which survived were autopsied on day
10 PI. The four rats which died were emaciated, weak and moribund
before their death. They all lost their sight and behaved abnormally.

Live larvae were found in abundance in the lungs and in lesser
numbers in the brain and intestine. Larvae were also seen in histo-
logical sections of the brains (Plates 25 and 26).

Most of the worms recovered from the intestines of the 3 rats
which died on day 3 PI were preadults. A mean of 604 preadults
was recovered from the gut which represented 10% of the initial
inoculum, while the other two rats, which were autopsied on day 10
PI, harboured a mean of 920 adult worms which represented 15.3% of
the original inoculum.

All the rats which received 8000 L3 died within two days fol-
lowing a short period of crisis. There were numerous internal
haemorrhages, particularly in the lungs, where many live larvae
were seen, but no larvae were recovered from the intestine.

This experiment has shown that in heavily infected rats,
larvae disseminated throughout the tissues and the rate of worm

Table 10   Exp: 3.6   Adult worm counts in four groups of rats following initial infection

with 4 different infective doses of S. ratti

	Group 1 Infected with 4000 L3 / rat	Group 2 Infected with 6000 L3 /rat		Group 3 Infected with 8000 L3 / rat	Group 4 Infected with 1000 L3 / rat control
		Died day 3 PI	Killed day 10 PI		
Individual	973	412 (LA)	971	All rats died on	431
counts	1007	743 (LA)	869	day 2 PI	512
	1132	657 (LA)		No larvae were	439
	1731	N.D.		recovered	447
	893				
	1220				
Mean (SE)	1159 (+123)	604 (+99.1)	920 (+51)		457 (+18.5)
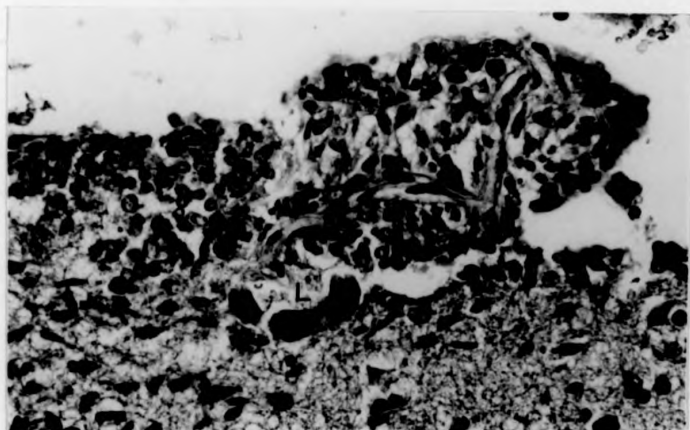
Plate 25 : Cerebrum, live larva within brain tissue with
marked degree of tissue damage (H.& E., x 250)
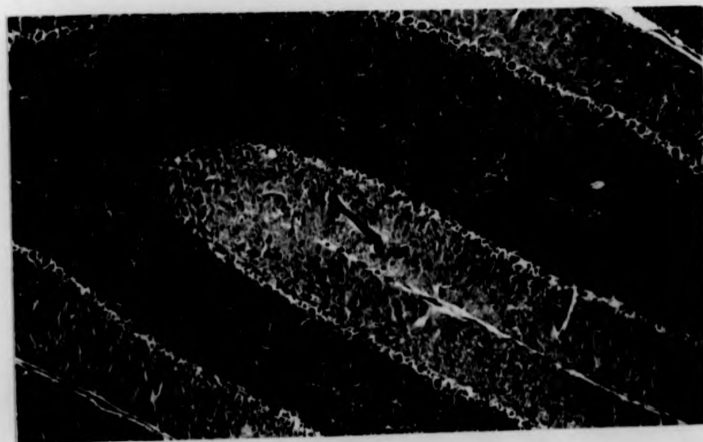
Plate 26 : Cerebellum, live larva within brain tissue with
no reaction (H.& E., x 80)
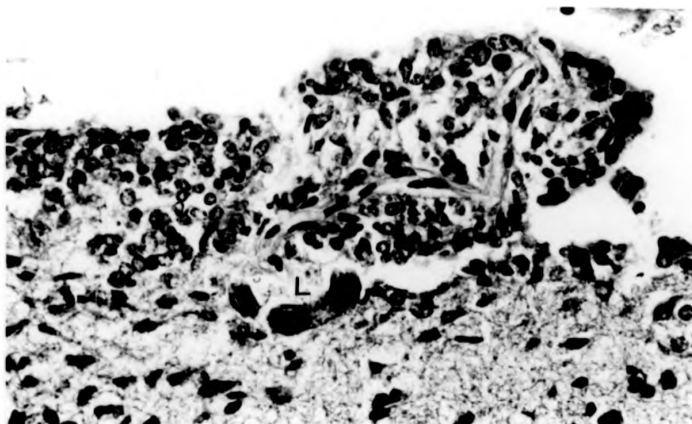
Plate 25 : Cerebrum, live larva within brain tissue with marked degree of tissue damage (H.& E., x 250)
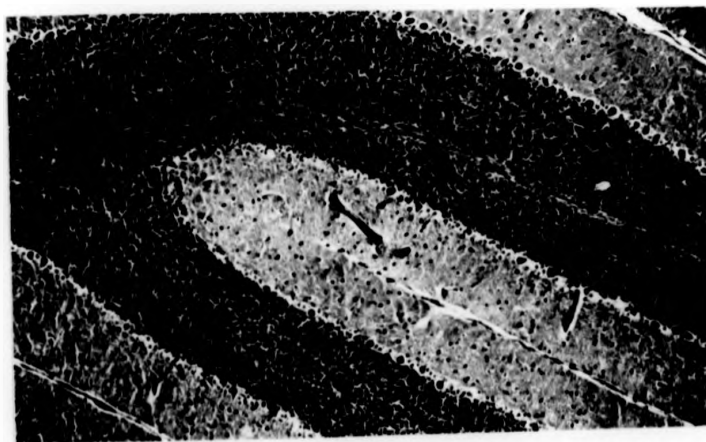
Plate 26 : Cerebellum, live larva within brain tissue with no reaction (H.& E., x 80)

development in the gut was low.

Discussion:

The results of these experiments clearly demonstrate that
rats produce strong resistance to S. ratti and that this becomes
even stronger after multiple infections. Following a primary
infection, very few larvae from a challenge infection manage to
reach the intestine, and the parasitological and histological
study suggests that they are destroyed in the lungs. Their death
leads to extensive lymphoid hyperplasia and formation of granulomas
in the lung. Migrating larvae, presumably, share antigenic
characteristics with adult worms which induce circulating memory
cells to recognize them at the lung stage and initiate the drastic
changes which lead to the death of the majority of the larvae, and
only a few survive and develop into stunted worms in the small
intestine.

After a series of infections, the rat responds much more
rapidly to challenge and larvae are killed in the skin and do not
reach the lungs. Soulsby (1962) suggested that enzymes used by
infective larvae to penetrate the skin, act as functional antigens
and that the immune response causes an inhibition of these enzymes
and this leads to the immobilisation of these larvae. Such inacti-
vation of invading larvae in human strongyloidiasis can result in
creeping eruption. Lee and Lewert (1957) described a factor in
the serum of immune rats which inhibited the action of skin pene-
tration by S. ratti and Schistosoma mansoni. However, the level
of this factor did not increase in hyper-infected rats.

The development of resistance during a primary infection with
S. ratti is slower in comparison with that produced by
N. brasiliensis and T. spiralis infections, but whether this is
due to S. ratti being less immunogenic or not is still undetermined.
The effects of the host's response were seen from day 14 PI. The
reduction in fecundity, migration away from the initial site of
parasitization and expulsion of the adult worms were the most
striking features of this response.

Adult N. brasiliensis migrate anteriorly within the intestine

during the course of infection (Chandler, 1935; Brambell, 1965; Alphey, 1970; Connan, 1974). This movement has been attributed to metabolic and immunologic factors, but there is mounting evidence that the evacuation of the worms from a site of preference during early infection, to another part of the intestine, is mainly due to the immune response of the host, particularly the humoral arm (Alphey, 1970; Connan, 1974). Most adult T. spiralis were found in the anterior half of the small intestine in old mice (Larsh and Hendricks, 1949). Migration of these worms down the small intestine was also observed prior to their expulsion (Larsh et al., 1952). Similar worm movement was noted by Campbell (1967) and Duckett (1970) but was attributed to feeding factors and to host strain. Podhajecky (1962) and Denham (1968) could find no evidence for such a movement.

The capacity of previously infected rats to resist challenge in S. ratti infections was maintained for up to three months after the initial worm expulsion, with residual worms playing no role in maintaining this immunity. Similar duration of immune memory was observed in mice infected with T. muris (Wakelin, 1967). Rats initially infected with N. brasiliensis showed a very strong resistance to reinfection after 2 months. Rats challenged 7 months after an initial infection, expelled their worm burden after a short lag and independent of any residual population in the gut (Ogilvie, 1965a).

Infective larvae disseminated in the different parts of the host, when introduced in very high numbers. The infective larvae in the brain may have been a reason for the moribund state of these rats and may have contributed towards their ultimate deaths. The number of developing adult worms in the gut, following exposure to a very high infective dose, was low, the majority being found in the lungs and brain.

Massive strongyloidiasis in man occurs occasionally (Brown and Perna, 1958; Yoeli et al., 1963; Cahill, 1967). This usually leads to death due to the penetration, by the infective larvae of S. stercoralis, of different body organs (Walker-Smith et al., 1969; Reiff and DeMoraes, 1972; Purtillo et al., 1974; Ali-Khan and Seemayer, 1975). The involvement of the nervous tissue, particularly the brain, in massive disseminated strongyloidiasis was confirmed

histologically (Neefe et al., 1973), and was attributed to auto-infection with S. stercoralis.

CHAPTER 4

The effects of the immune response on
adult S. ratti

Chandler (1938) and Ogilvie (1969) have shown that adult
N. brasiliensis, when transferred to clean rats before exposure
to the developing immune response, i.e. at days 6-9, were able
to continue egg production at the same level as in a primary
infection. Worms which were fully exposed to the immune response,
i.e. at day 10-13, did not resume egg production in new, clean,
hosts and were rapidly expelled. However, a phenomenon which
Ogilvie (1972) called "adaptation" was observed in worms which
had developed in immunised rats. Adult worms, when harvested 6-9
days after a challenge infection and transferred to clean
recipients, persisted much longer than did normal worms (Chandler,
1936; Ogilvie and Hockley, 1968; Ogilvie, 1972). Although these
adapted worms remained stunted, they resumed egg production and
their cytological features were restored to normal (Ogilvie and
Hockley, 1968). Ogilvie (1972) suggested that adaptation to an
immune environment occurred in the lungs. The adaptation of worms
is phenotypic and the progeny of adapted worms were similar to the
progeny of normal worms (Ogilvie, 1972).

No parallel to this phenomenon of worm adaptation has been
recorded outside the rat/N. brasiliensis system, although 10 day-
old adult T. spiralis resumed larval production when transplanted
into clean mice (Mougeot and Lancaster, 1973). When 18 day-old
worms were transplanted, larval production was not resumed suggest-
ing that they were permanently damaged. A considerable degree of
stunting and diminished fecundity of T. spiralis females occurred
prior to expulsion from the intestine (Denham and Martinez, 1970)
and during reinfection (Samrad and Coors, 1951; Rappaport and Wells,
1951).

Nematodes are exposed to a variety of factors produced by the
host, but their cuticle is a very tough barrier (Lee, 1965) and is

impermeable to most substances, including antibodies (Marks et al., 1968; Lee, 1969a). Presumably, the various mediators of the immune response reach the parasite through its orifices.

It is now well established that the humoral components of the immune response act on the worms prior to their expulsion, and cause structural damage particularly to the intestinal cells of the worms (Ogilvie and Jones, 1971, 1973; Love et al., 1976). When these changes occur during the late part of an initial infection with N. brasiliensis, the damage is irreversible (Ogilvie and Hockley, 1968).

The gut of normal N. brasiliensis consists of large cells (Lee, 1969a), the apical surface of which is lined with well-developed microvilli and contain active mitochondria, a large number of ribosomes and Golgi complexes. The gut cells of worms exposed to the immune response are markedly different. The ribosomes are replaced by large vacuoles filled with carbohydrates and lipids (Ogilvie and Hockley, 1968). Similar changes in the structure of gut cells occur in adult Haemonchus placei (Harness et al., 1973).

Within the lumen of the gut of N. brasiliensis, Taliaferro (1940) and Taliaferro and Sarles (1939) repeatedly noted a mass of material which they suggested was an immune precipitate, but this has not been confirmed (Ogilvie and Hockley, 1968; Lee, 1969a). Ogilvie (1964) claimed that these precipitates have no role in the process of worm damage, since similar material was formed round worms incubated in antiserum from rats vaccinated with worm extracts, yet these rats were completely susceptible to a challenge infection.

Externally, precipitates were also seen on worms, in immune hosts, particularly near the mouth (Sarles, 1938; Taliaferro and Sarles, 1939; Oliver-Gonzalez, 1940).

The reproductive system is also adversely affected by the immune response, particularly in females, which results in retardation of their egg production (Lee, 1969b). Female Ostertagia ostertagi develop structural abnormalities of the vulval flap, following multiple infections, due to the host's developing immune response (Michel et al., 1972).

This chapter is concerned with the effects of immunity on S. ratti during primary and secondary infections and the amount of change which is observed in these worms, during initial infection.

Materials and Methods

Transplantation of adult worms:

Adult worms were harvested from infected donor rats using
a Baermann funnel filled with saline at $37^{\circ}C$. The worms were
counted and the required number collected in 0.1 ml volumes of
saline in 1 ml syringes. Recipient rats were anaesthetised with
a mixture of air and Penthrane, and the fur of the ventral side
was clipped and the duodenum exposed by laparotomy. The worms
were injected directly into the duodenum (Plate 27). The
abdominal wall was sutured with patent suture silk No. 00
(Archibald and Turner Co.). The skin was joined together with
12 mm Michel clips. The rats recovered from anaesthesia within
two minutes of the withdrawal of Penthrane. This procedure was
carried out under clean but not aseptic conditions.

Preparation of adult S. ratti for examination under light and electron microscope:

Adult worms were collected in the usual way and washed twice
with sterile saline. For light microscopy, worms were fixed in
Bouin's fluid and left for $\frac{1}{2}$-1 hour and then washed twice for 1
hour in absolute alcohol and cleared twice in Toluene. They were
then embedded in paraffin wax in a small glass tube, the worms
settled in the bottom and were easily accessible for sectioning
when the tube was broken. Sections were cut at 5 um and stained
in haematoxylin and eosin.

For electron microscopy, worms were cut into two using a pair
of fine watch-makers forceps, in 3% glutaraldehyde in 0.1 M caco-
dylate buffer at pH 7.4 and left for two hours, in the presence of
calcium ions. The rear ends were discarded, the suspension was
centrifuged gently and the worm material embedded in agar. The
agar pellet was cut up and the pieces containing the worms were
returned to glutaraldehyde for another 19 hours. They were washed
4 times within 21 hours at equal intervals with cacodylate buffer
to which was added 0.02 M sucrose and postfixed in 1% osmium

Plate 27 : Intra-duodenal transplantation of adult _S. ratti_
into an anaesthetized rat

Plate 27 : Intra-duodenal transplantation of adult S. ratti into an anaesthetized rat

tetroxide in Millonig's buffer (Millonig, 1961) at pH 7.4 for
2 hours at 4°C. After this they were washed 3 times in dis-
tilled water (15 minutes each) and fixed in 0.5% solution of
uranyl acetate containing 45 mg/ml of sucrose (de Harven, 1967)
at pH 5.0 for 1 hour at 4°C.

Cross sections were cut on a Huxley (Cambridge) ultramicrotome,
and were collected on Formvar coated copper grids stained with
lead citrate (Venable and Coggeshall, 1965), before examination
in a Zeiss Em 9A electron microscope.

Experiment 4.1:

This experiment was designed to study the fate of worms of
different ages transplanted into new recipients. A total of 118
female Wistar rats (120 gm in weight) were used for this experiment.
Three sets of donor and recipient rats were prepared. The timing
of the infections was such that all donor rats were killed on the
same day.

Donor group 1: Forty rats were infected with 3000 L3/rat,
reinfected 30 days later with 3000 L3 each, killed 7 days later
and their adult worms collected in a Baermann funnel. Sixteen
groups of 300, 7 day-old, worms from "immune" rats in 0.1 ml of
saline were inoculated, intraduodenally, into 16 rats of the
recipient group A.

Donor group 2: Ten rats were infected with 3000 L3/rat and
killed on day 10 PI. Sixteen groups of 300, 10 day-old, worms
were injected intraduodenally into 16 recipient rats of group B.

Donor group 3: Thirty rats were infected with 3000 L3/rat and
killed on day 22 PI. Sixteen groups of 300, 22 day-old, worms were
injected, intraduodenally, into 16 recipient rats of group C.

Ten worms from each donor group were measured before trans-
plantation. Worms from donor group 1 had a mean length of 1.45
(± 0.05) mm, those of group 2 had a mean length of 2.87 (± 0.03)mm
and those of group 3 a mean length of 1.01 (± 0.06) mm, respectively.

Four rats from each recipient group were killed on days 5, 7,
12 and 15 post-transplantation and faecal cultures were made on the
same days. The worms were counted and measured. The results are

presented in Table 11 and Figures 15 and 16.

The worms transferred from resistant hosts into normal
recipients (group A) were able to re-establish themselves and
commence egg production. However, the larval production was not
very high and was maintained for only 10 days. An average of
22.8 worms was recovered on days 5 and 7 post-transplantation
and decreased to an average of 11.3 worms on days 12 and 15 post-
transplantation. The average length of these worms was 1.33 mm.
The 10 day-old worms transplanted from an initial infection into
recipient rats of group B continued their egg production on a
normal level with the output on day 5 post-transplantation being
3080 larvae/gm of faeces. Larval production dropped on day 12
post-transplantation and ceased on day 15 post-transplantation.
The average length of these worms followed the pattern of a normal
infection and dropped from 2.3 mm on day 5 post-transplantation to
0.91 mm on day 15 post-transplantation. The 22 day-old worms in
recipient rats of group C did not recover after transplantation,
and the majority of the worms were expelled by day 5 post-
transplantation. No larvae were recovered from the faeces of this
group.

This experiment demonstrated that worms, recovered early during
challenge infection, can resume producing larvae without any increase
in their overall length. This might indicate that damage of worms
during challenge infection is reversible. Also the experiment
showed that worms recovered late during initial infection could not
establish themselves in new hosts and hence, the damage was irrever-
sible. Thus worms obtained on days 8 - 16 PI were considered to be
"normal" with 10 day-old worms being typical. Worms recovered
between days 18 - 25 PI were considered "damaged", with 22 day-old
worms being typical. Worms collected early during a challenge
infection were damaged as shown by their reduced size, but were able
to recover.

Table 11   Exp: 4.1   Mean larval production, number and lengths of adult worms during four different periods

A: received 300 worms from a challenge infection

B: received 300 worms from day 10 PI

C: received 300 worms from day 22 PI

Day post transpl- antation	Mean (SE) number of larvae / gm faeces			Mean (SE) number of adult worms			Mean (SE) length of adult worms (mm)		
	A	B	C	A	B	C	A	B	C
5	1866 (± 237)	3074 (± 257)	0	25.2 (± 3.56)	182 (± 10.3)	1.25 (± .62)	1.4 (± .04)	2.3 (± .09)	.88 (± .04)
7	960 (± 88)	650 (± 118)	0	20.2 (± 3.94)	151 (± 26)	0	1.38 (± .03)	1.4 (± .05)	-
12	54.9 (± 12)	26.6 (± 5.5)	0	10.2 (± 1.37)	92.7 (± 8.35)	0	1.30 (± .05)	1.11 (± .04)	-
15	0	0	0	12.5 (± 2.53)	2.5 (± 1.65)	0	1.27 (± .05	0.91 (± .06)	-

Figure 15, Exp. 4.1:

Mean faecal larval production in three groups of rats.
● —— ● A. received 300 worms from challenge infection.
■ —— ■ B. received 300, 10 day-old worms from initial infection
□ —— □ C. received 300, 22 day-old worms from initial infection.

Larvae/gm of faeces
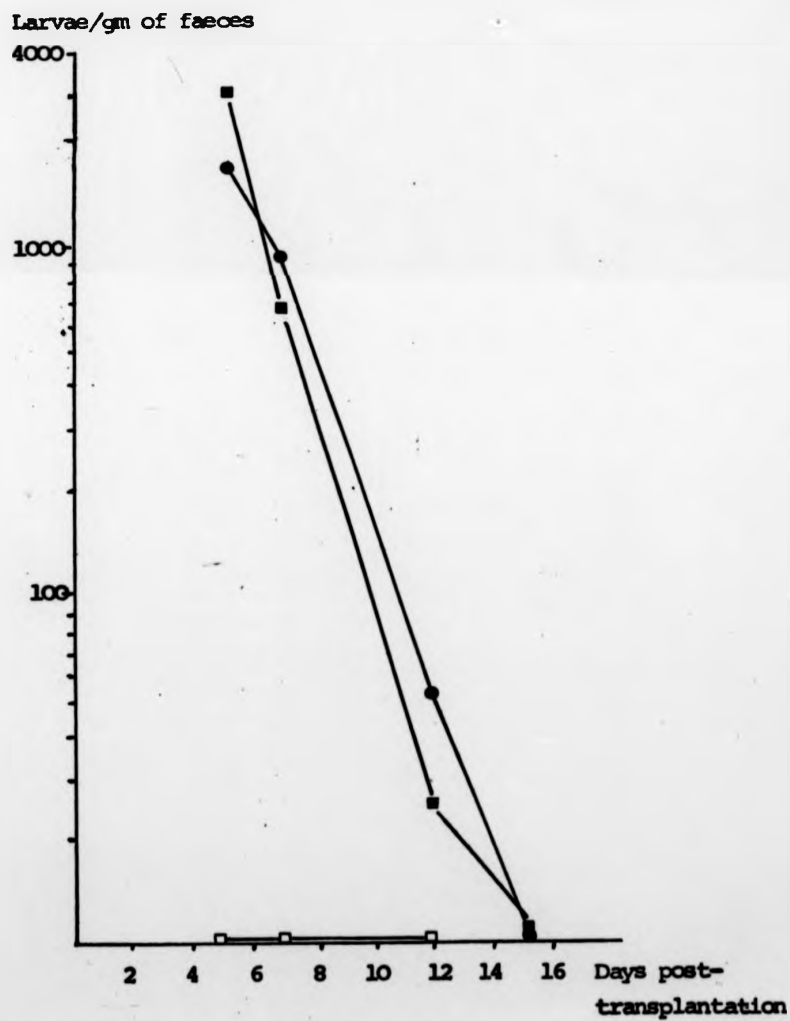
Figure 16, Exp. 4.1:

Mean number of adult <u>S. ratti</u> recovered from three
groups. ▨ Group A received 300 worms from challenge
infection; ☐ Group B received 300, 10 day-old worms
from initial infection; ■ Group C received 300, 22
day-old worms from initial infection.

Number of adult worms

Day 5   Day 7   Day 12   Day 15

Post-transplantation
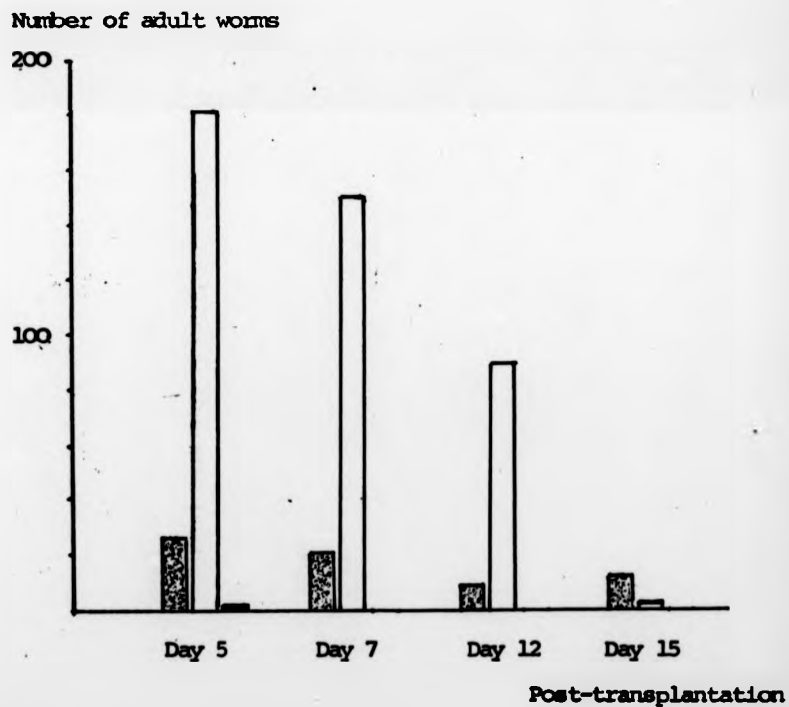
Experiment 4.2:

Studies were made on the morphological changes in S. ratti during the course of an initial infection. Worms were taken from a group of 5 rats on days 10 and 22 PI. The two types of worms were divided into two lots for fixation for light and electron microscopy.

The "normal" worms had a mean length of $2.83 \pm .03$ mm with a range of $2.55 - 3.21$ mm, while the damaged worms had a mean length of $1.1 \pm .06$ mm with a range of $0.55 - 1.9$ mm.

Light microscopy:

The difference in size between "normal" and "damaged" worms under the light microscope was very obvious. The majority of the sections of normal adult worms (Plate 28) had a consistent elliptical shape with most of the internal organs staining densely with H. & E., while the duplicated reproductive tract was usually darkly stained with haematoxylin. The gut showed no vacuolation. The damaged worms (Plate 29) were irregular in shape with all organs staining faintly with H. & E. The reproductive tract was smaller and stained lightly with haematoxylin. The gut showed patches of vacuolation surrounding the lumen which was not patent in most sections. The area under the cuticle and the circular muscles was always vacuolated and unstained.

Electron microscopy:

No studies on the ultrastructure of S. ratti have been reported in the literature. All the observations reported below on the two types of worms, are compared with N. brasiliensis. The observations on the structural and morphological changes in these worms were concentrated on the anterior third and changes in the gut and surrounding tissues were considered. The most obvious difference at EM level, as by light microscopy, between the 10 and 22 day-old
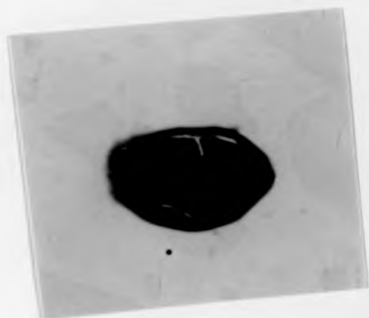
Plate 28 : C.S. Normal adult
S.ratti (H.& E., x 400)
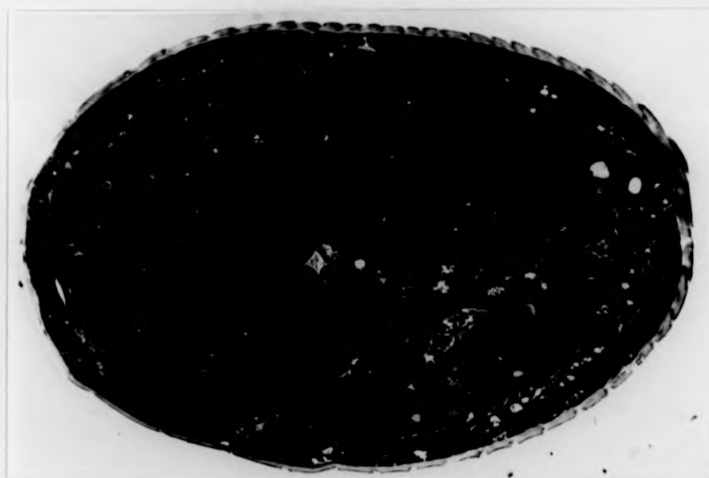
Plate 29 : C.S. Damaged adult
S.ratti (H.& E., x 400)

Plate 30 : Low power electron micrograph of section of a 10 day-old
S. ratti (x 2000)

Plate 31 : Low power electron micrograph of section of a 22 day-old
S. ratti (x 2000)

Plate 28 : C.S. Normal adult
S.ratti (H.& E., x 400)
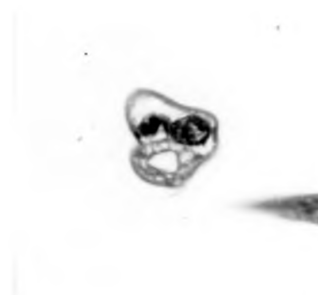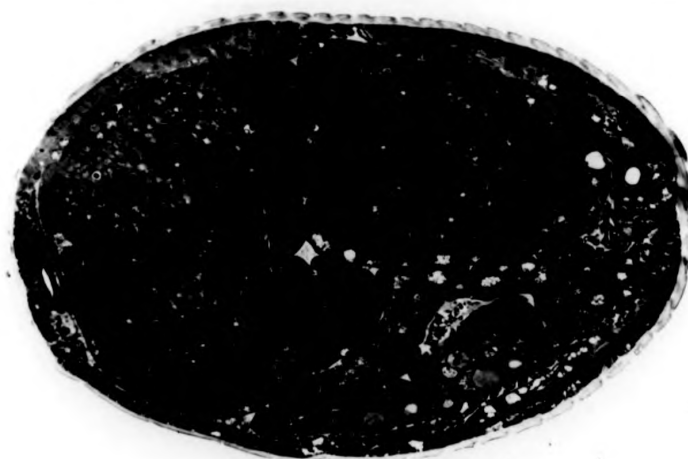
Plate 29 : C.S. Damaged adult
S.ratti (H.& E., x 400)

Plate 30 : Low power electron micrograph of section of a 10 day-old
S. ratti (x 2000)
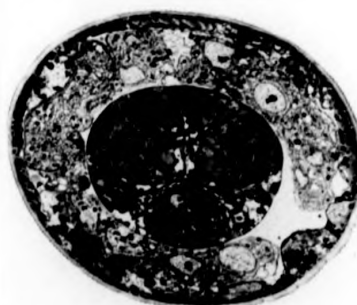
Plate 31 : Low power electron micrograph of section of a 22 day-old
S. ratti (x 2000)

worms, was that of the overall size (Plates 30 and 31). There
were no apparent differences in the cuticle or the underlying
musculature, which was typical of nematodes (Lee, 1969). However,
beneath the muscle layer, the damaged worms often showed
vacuolated spaces or disorganised tissues which were not found in
normal worms (Plates 32 and 33). There were other differences
between the two kinds of worms such as; a tendency for the
damaged worms to contain bigger interstitial spaces (Plates 32
and 33), a reduced amount of rough endoplasmic reticulum in cells
around the gut and an increased number of lysosomes (Plates 34 and
35), many of which would appear to be forming secondary lysosomes
with cell lipids.

It was very difficult to quantify these findings, which were
made on sections cut from over 50 worms. However, there were two
observations that, like the size difference, were invariably noted.
The first was that the gut of young worms was open (Plates 36 and
37) and usually full of a variety of material; while the guts of
the damaged worms were either tightly closed or only just patent.
The second difference was that the contents of the gut of damaged
worms contained peculiar whorls which were persistently seen within
the lumen and could represent lipoproteins, or immune complexes
(Plates 38 and 39). They were never found in the guts of normal
worms.

The microvilli of both 10 and 22 day-old worms were similar
in size and numbers, though the damaged cells beneath them showed
reduced activity (Plates 40 and 41). To sum up, apart from the
general signs within the damaged worms suggestive of malnutrition,
the only invariable findings which occurred in all specimens
examined were an overall difference in size and the presence of
whorls within the closed guts of damaged worms.

Experiment 4.3:

The study below was conducted to identify and endeavour to
define the plugs of precipitates observed, repeatedly, near the
peri-oral area of worms (Plates 42 and 43) during initial and
challenge infections, as well as the peculiar whorls, seen under the EM,

Plate 32 : Electron micrograph of section through anterior third
of a 10 day-old **S. ratti**. C = cuticle; G = Golgi complex;
M = mitochondria (x 29,000)
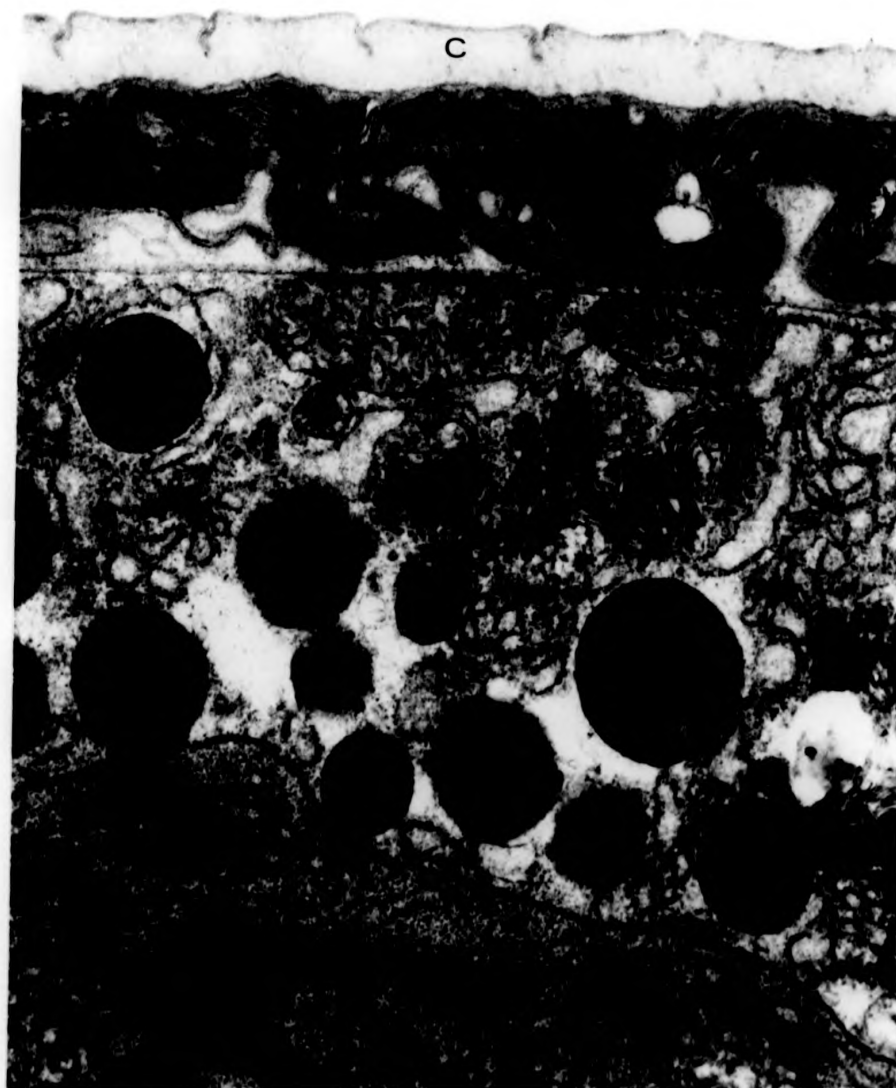
Plate 32 : Electron micrograph of section through anterior third of a 10 day-old _S. ratti_. C = cuticle; G = Golgi complex; M = mitochondria (x 29,000)

Plate 33 : Electron micrograph of section through anterior third of a 22 day-old *S. ratti*. V = vacuolated space (x 30,000)

Plate 33 : Electron micrograph of section through anterior third of a 22 day-old S. ratti. V = vacuolated space (x 30,000)
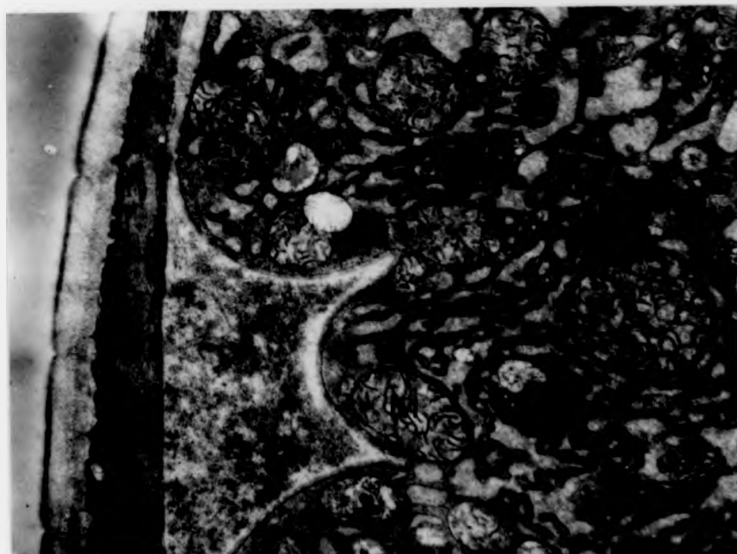
Plate 34 : Electron micrograph of a section through the gut
cells of 10 day-old <u>S. ratti</u>. ER = endoplasmic
reticulum (x 23,000)
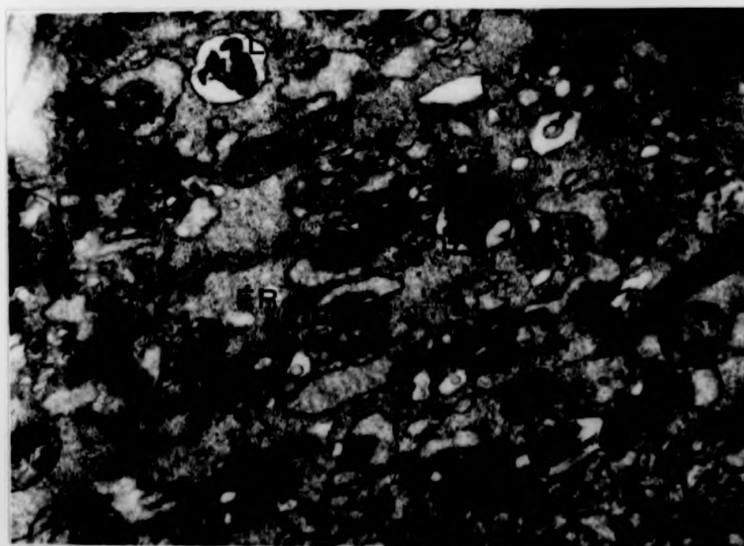
Plate 35 : Electron micrograph of a section through the gut
cells of 22 day-old <u>S. ratti</u>. L = lysosomes
(x 23,000)
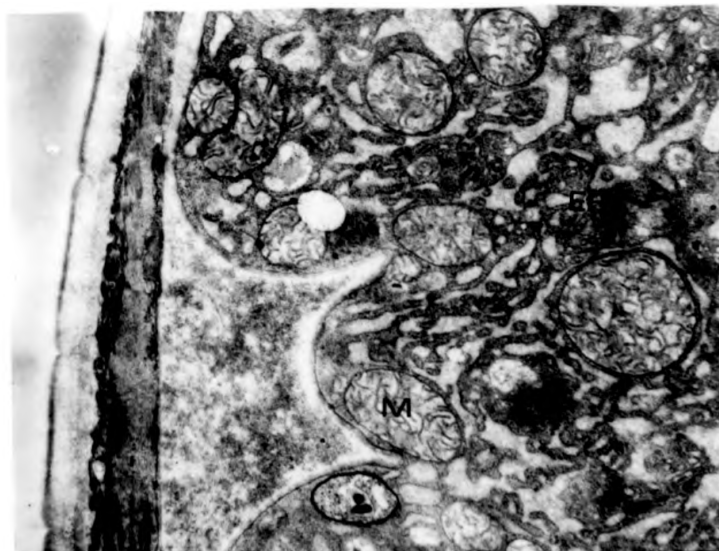
Plate 34 : Electron micrograph of a section through the gut
cells of 10 day-old S. ratti. ER = endoplasmic
reticulum (x 23,000)
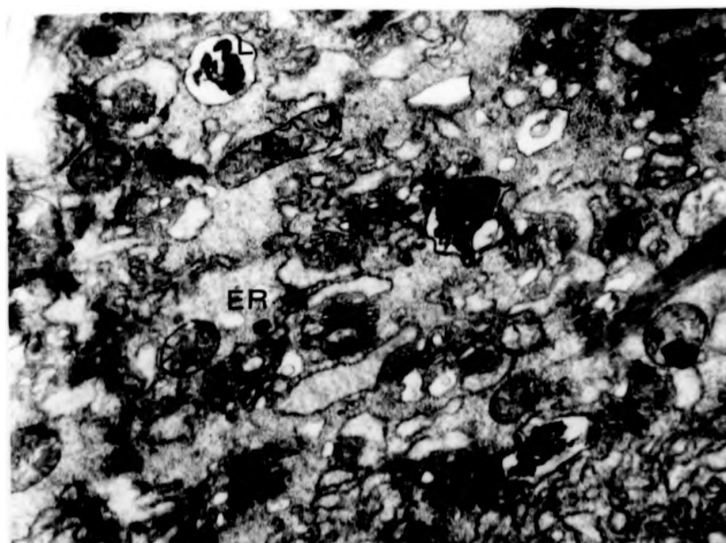
Plate 35 : Electron micrograph of a section through the gut
cells of 22 day-old S. ratti. L = lysosomes
(x 23,000)
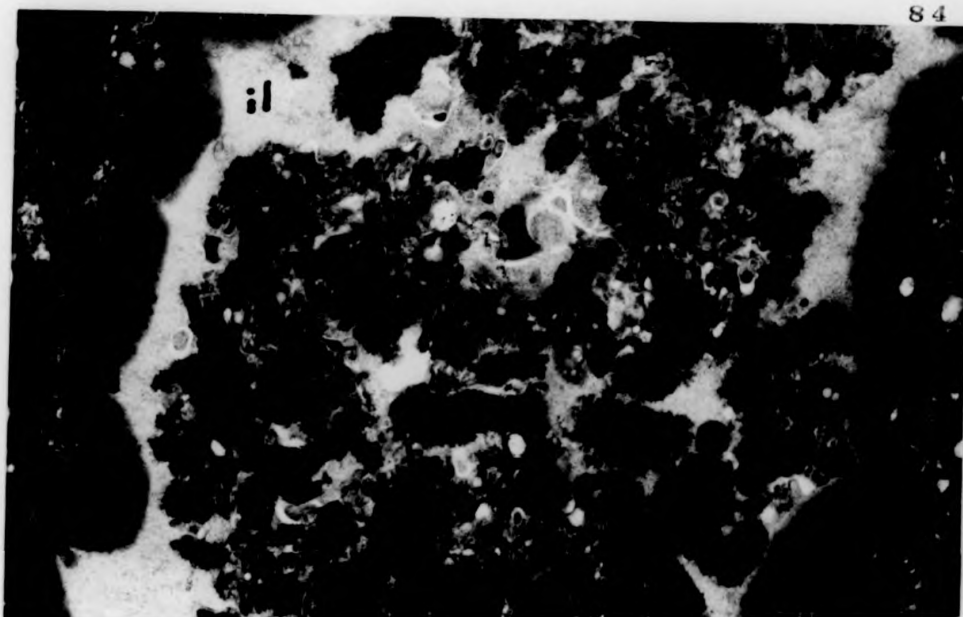
Plate 36 : Electron micrograph of the gut contents of 10 day-old
S. ratti. il = intestinal lumen (x 16,000)

Plate 37 : Electron micrograph of the gut of 22 day-old S. ratti,
note the non-patent lumen (x 16,000)
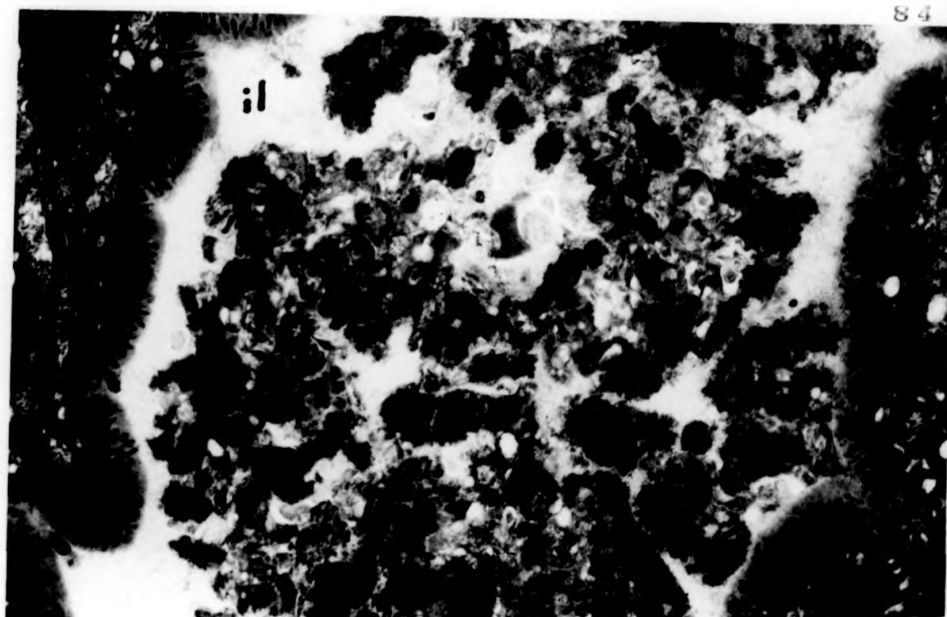
Plate 36 : Electron micrograph of the gut contents of 10 day-old
S. ratti. il = intestinal lumen (x 16,000)
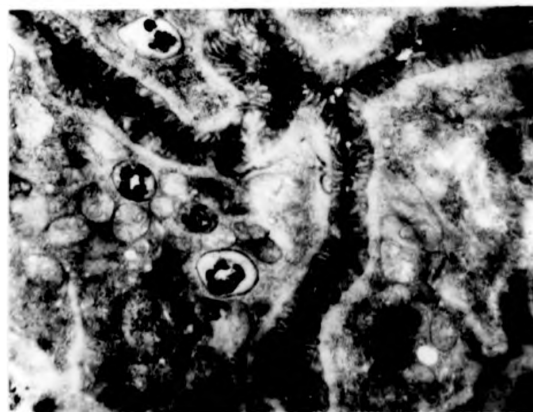
Plate 37 : Electron micrograph of the gut of 22 day-old S. ratti,
note the non-patent lumen (x 16,000)
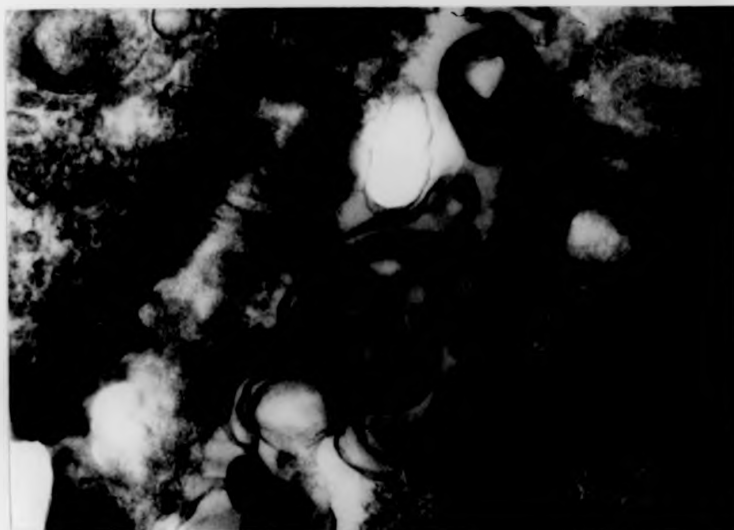
Plate 38 : Electron micrograph of the peculiar whorls observed within the intestinal lumen of 22 day-old S. ratti. Lp = lipoprotein structures; Mv = microvilli (x 75,000)
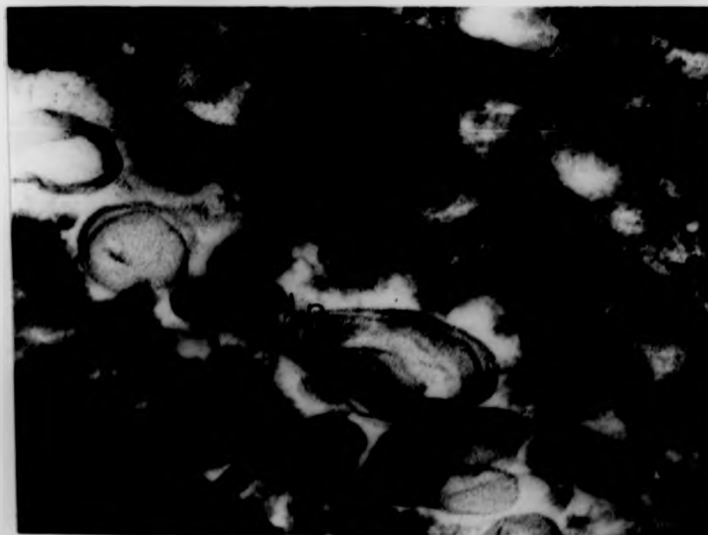
Plate 39 : Electron micrographs of similar whorls (x 75,000)

Plate 38 : Electron micrograph of the peculiar whorls observed within the intestinal lumen of 22 day-old S. ratti. Lp = lipoprotein structures; Mv = microvilli (x 75,000)
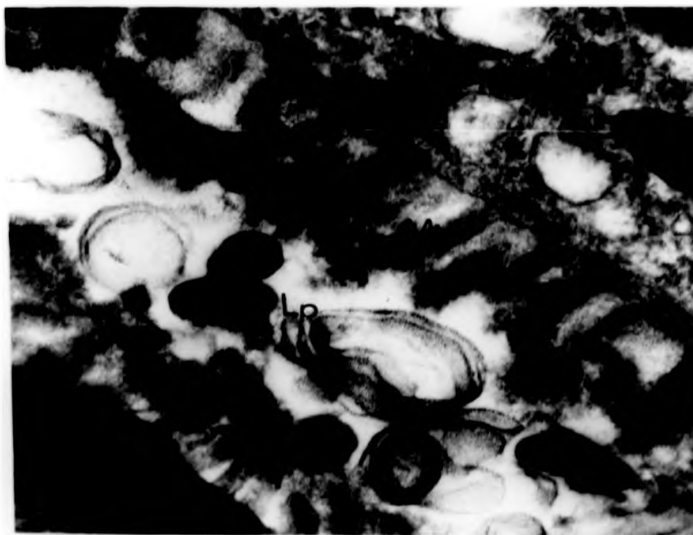
Plate 39 : Electron micrographs of similar whorls (x 75,000)
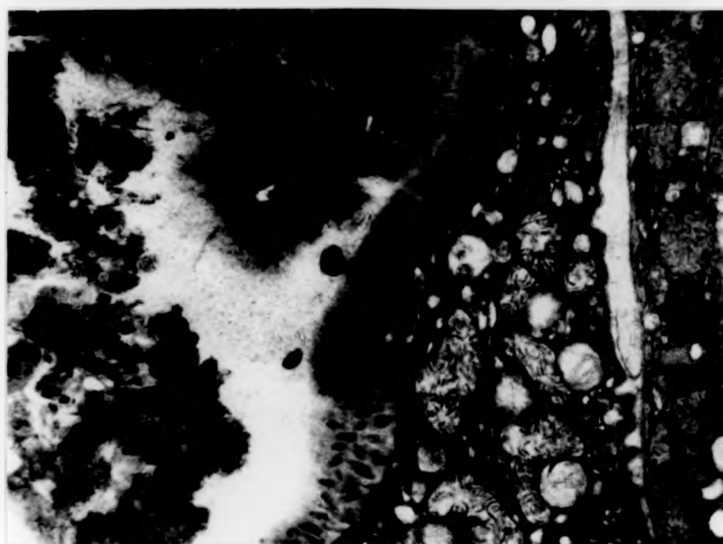
Plate 40 : Electron micrograph of a section through the gut
of 10 day-old S. ratti, to show microvilli.
Mv = microvilli (x 23,000)
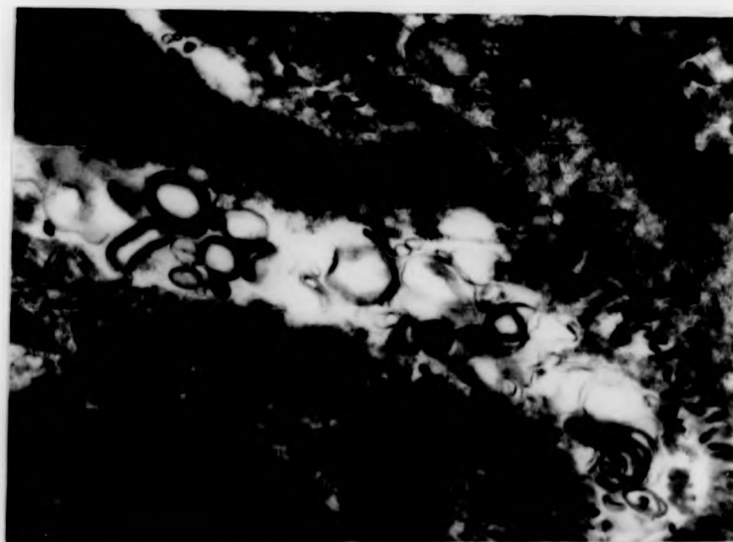
Plate 41 : Electron micrograph of a section through the gut
of 22 day-old S. ratti (x 23,000)
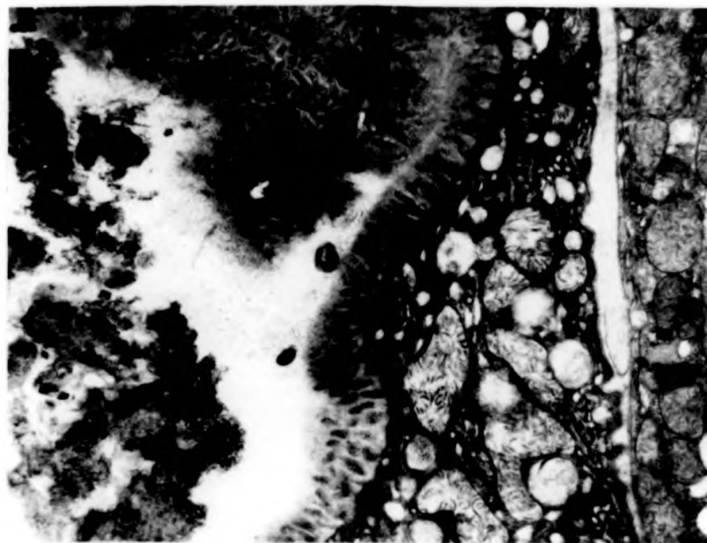
Plate 40 : Electron micrograph of a section through the gut of 10 day-old S. ratti, to show microvilli. Mv = microvilli (x 23,000)
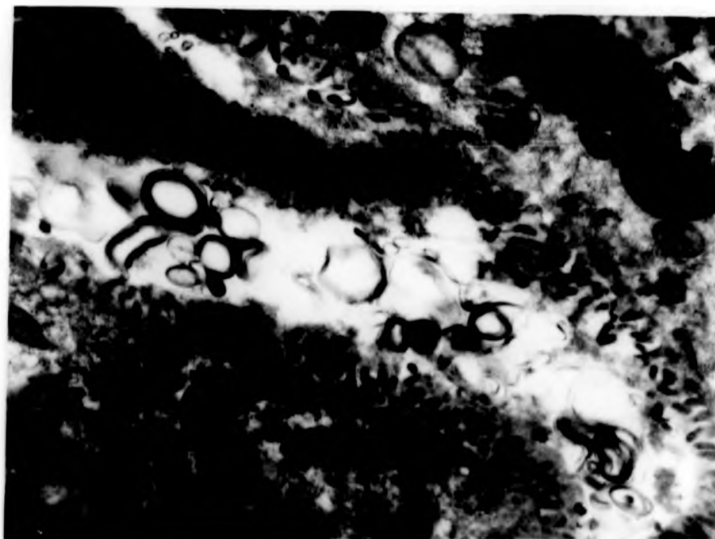
Plate 41 : Electron micrograph of a section through the gut of 22 day-old S. ratti (x 23,000)

to a specific immune reaction against S. ratti, and a non-specific
inflammatory response which lasts for a short period and can expel
and damage other intestinal nematodes when introduced subsequently,
e.g. N. brasiliensis and T. spiralis (Louch, 1962; Kazacos and
Thorson, 1975; Kazacos, 1976; Moqbel and Moloney, 1976, unpublished
observations). The fact that only 5% of the worms transferred
from a challenge infection established does not substantiate the
conclusion that adaptation occurred in this system. On the other
hand, the persistence of these worms in the gut for 15 days after
transplantation might suggest otherwise. This aspect of the
relationship between worms from challenge and clean rats is worth
pursuing. If adaptation was confirmed, S. ratti would be only the
second nematode to manifest this phenomenon, N. brasiliensis being
the first.

The changes observed in the gut cells of damaged worms were
basically similar to those observed in N. brasiliensis and
T. spiralis (Ogilvie and Hockley, 1968; Love et al., 1976),
particularly with regard to the large number of vacuoles and the
distorted endoplasmic reticulum. However, the lipid droplets des-
cribed in the gut cells of N. brasiliensis by Jamuar (1966) and
Ogilvie and Hockley (1968), were not seen in damaged S. ratti
worms.

The cuticle of damaged worms was intact, which indicated that
antibodies which caused internal disorganisation entered the nema-
tode through the body orifices, and most probably, the mouth.

The invariable discovery of peculiar whorls in the lumen of
the gut of damaged worms could be a confirmation of observations
by Sarles (1938) and Taliaferro and Sarles (1939) in N. brasiliensis,
under light microscopy. It is possible that these bodies are
ingested immune complexes (Ab/Ag), but due to the minute size of
the lumen of the gut, no specific fluorescence was detectable under
the UV microscope. It is doubtful whether these bodies, even if
they are immune complexes, have any role in worm damage, since
immune complexes act by activating complement, and through their
chemotactic character, they can be involved in opsonisation. It
is unlikely that this can occur within the worm. These bodies may
have been formed following the ingestion of anti-worm antibodies

Similar changes occur in other parasitic nematode systems
(Soulsby, 1962; Ogilvie and Hockley, 1968; Ogilvie and Jones,
1971, 1973; Ogilvie, 1976; Love et al., 1976).

Normal S. ratti transferred before being fully exposed to
the immune response of rats, were able to continue their egg
production. Worms taken from primary infections, after day 20,
and those from the early stages of a secondary infection, were
the same length. Worms which were transferred after day 22 PI
were unable to resume their egg production and did not survive
in non-immune hosts. This indicated that, as with N. brasiliensis
(Ogilvie and Hockley, 1968), the damage to S. ratti from late
initial infection was irreversible.

However, adult S. ratti collected 7 days after a challenge
survived for 15 days in non-immune rats, three days longer than
normal worms did, and showed preliminary manifestations of
"adaptation" as described by Ogilvie and Hockley (1968) and Ogilvie
(1972). These worms did not regain their original length but
managed to resume egg production, with high fecundity, for a sub-
stantial length of time, similar to the transferred normal worms.

It is possible that adaptation may occur in S. ratti during
a challenge infection, however this, in the light of the results
obtained in experiment 4.1, is still arguable.

As mentioned (see introduction to this chapter), adaptation
of N. brasiliensis was suggested to occur during the L3/L4 moult
in the lungs (Ogilvie, 1972) where there was little response
against the migrating larvae. This evasion of the immune response
led to the establishment of worms which were capable of surviving
and resuming egg production when transferred to naive rats (Ogilvie
and Wilson, 1976).

As far as S. ratti is concerned, it has been established that
no moulting occurs in the lungs of infected rats (Wertheim and
Lengy, 1965). Moreover, there is a severe response against the
pulmonary stages of a challenge infection (see Chapter 3),
resulting in the establishment of very few stunted worms in the gut.
The possibility that adaptation in S. ratti might occur during the
L3/L4 moult in the gut, cannot be excluded, but the few worms which
manage to evade the strong immune response in the lungs may face a
more severe reaction upon their arrival in the gut. This is due

Plate 42 : Anterior end of adult, 10 day-old S. ratti, with no plug of precipitate around the mouth (x 400).
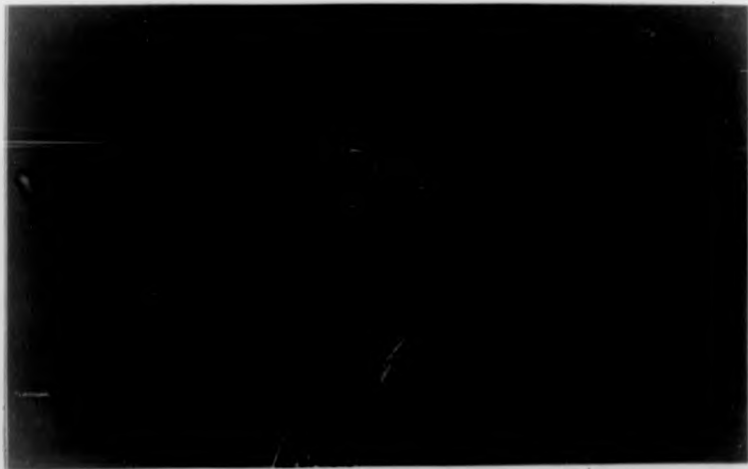
Plate 43 : Anterior end of adult, 22 day-old S. ratti; note the peri-oral precipitate (x 400).
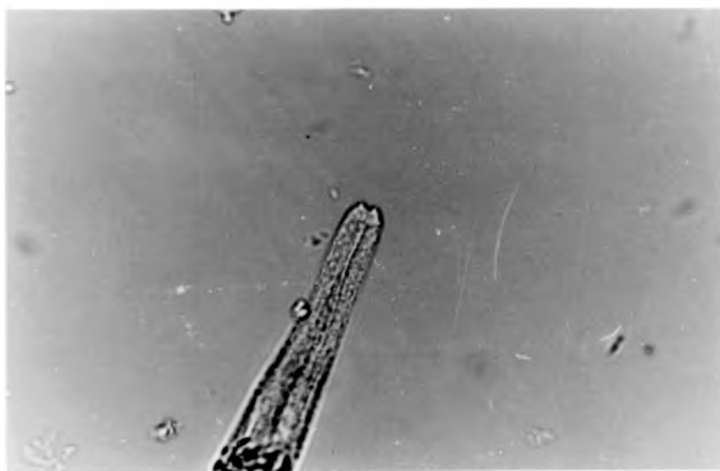
Plate 42 : Anterior end of adult, 10 day-old S. ratti, with no plug of precipitate around the mouth (x 400).

Plate 43 : Anterior end of adult, 22 day-old S. ratti; note the peri-oral precipitate (x 400).

Plate 44 : 10 day-old S. ratti showing fertile embryonated eggs (E) within the uterus (U). V = vulva (x 400)

Plate 45 : 22 day-old S. ratti showing absence of eggs (phase contrast x 400)
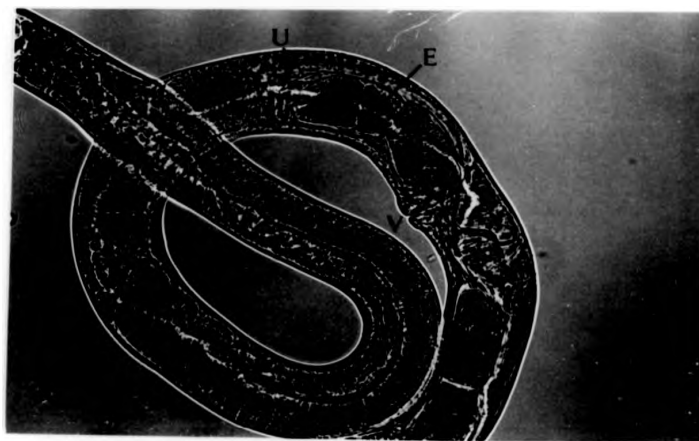
Plate 44 : 10 day-old S. ratti showing fertile embryonated
           eggs (E) within the uterus (U). V = vulva (x 400)

Plate 45 : 22 day-old S. ratti showing absence of eggs
           (phase contrast x 400)

inside the gut lumen of damaged worms.

Normal (10 day-old) and damaged (22 day-old) worms were washed three times in phosphate buffer saline (PBS) with continuous agitation, and then run through a direct fluorescent antibody test (FAT). The washed worms were incubated in fluoroscein isothiocyanate (FITC) conjugated to goat anti-rat immunoglobulin (Wellcome Reagents Ltd.) directly, at a working dilution of 1:20 and 1:40 in $37^{\circ}C$ incubator for 30 minutes. The excess FITC was washed off with PBS and the worms were mounted on slides in buffered glycerine. The slides were examined under an Ultra-violet (UV) microscope (Nikon).

The normal worms had no precipitates near their mouth or any other orifice, and the anterior part showed no fluorescence. Damaged worms had precipitates which varied in shape and size, and they fluoresced strongly in the direct FAT at both dilutions.

For the study of the peculiar bodies within the lumens of damaged worms, normal and damaged worms were washed three times in sterile PBS after being killed by cooling. Sections of adults were prepared by freezing 300 worms of each age in an embedding medium for frozen tissue specimens (Ames O.C.T. Compound) on to a metal block. Sections were cut at 6 μm in a cryostat microtome at $-20^{\circ}C$ and were attached to coated multispot slides (C. A. Hendley & Co., Essex). The sections were left in a dessicator at $4^{\circ}C$ overnight and were subsequently fixed with acetone for 5 seconds immediately before running direct FAT, using FITC conjugated to goat anti-rat immunoglobulin. Even under high magnification, no fluorescence was seen within the very small damaged worms.

Discussion:

The experiments in this chapter have demonstrated that adult S. ratti are affected, at both macro- and micro-levels, by the immune response of rats during the course of primary and secondary infections. The length of adult worms, which decreased with the progress of initial infection (see Chapter 3), was accompanied by internal cyto-pathological, as well as morphological, changes.

which react with internal metabolic secretions and form complexes within the lumen of the gut.

It is likely, however, that these bodies are associated with the plugs of precipitate repeatedly seen attached to the mouth of damaged worms in vivo. The fluorescence of the latter structures in a direct FAT suggests they were immune complexes. Similar plugs were observed in other nematodes particularly in vitro (Sarles, 1938; Taliaferro and Sarles, 1939; Lawler, 1940; Oliver-Gonzalez, 1940; Lukashenko, 1960). However, their role in worm damage is still controversial and worth pursuing, particularly since we know very little about the mechanism(s) of damage and worm deterioration. Ogilvie (1964) suggested that these precipitates were not significant in protection as far as the host was concerned, but they were recognised as being antibody/antigen complexes (Ogilvie and Jones, 1973). It is very likely that worms bring about the immune attack by their own activities. The early production of antibodies to the metabolic presence of the worms controls the production and modulation of the enzymatic activity of the worms, particularly that of acetylcholinesterase (AchE) (Jones and Ogilvie, 1972) which has been shown to be antigenic (Sanderson, 1969; Lee, 1970; Edwards et al., 1971; Jones and Ogilvie, 1972). Soon after AchE is released, specific Ab/Ag complexes develop near the site of production.

It is proposed here that worm damage may be partially instigated by the development of precipitates internally and around the mouth which blocks the worm's alimentary canal and prevents the usual food uptake leading to starvation. The changes within the worms reflect this, as most of the cellular contents were consumed by the worms through the action of lysosomes.

It is essential that, as a follow up to this work, studies should be conducted on the metabolic changes which occur in adult S. ratti during the course of infection similar to those shown in N. brasiliensis by Henney et al. (1971), as well as the activities of AchE in S. ratti which have been shown to be significant in many other nematodes (Rothwell et al., 1973; Ogilvie et al., 1973; Bremner et al., 1973; McLaren et al., 1974).

CHAPTER 5

The role of the different parasitic stages of S. ratti
in stimulating the immune response

Different methods have been employed to determine the immuno-
genic stages of worm infections. These included irradiation of
different stages in order to arrest their growth, particularly to
sterilise infective larvae to prevent them reaching sexual maturity
(Levin and Evans, 1942; Hendricks, 1950, 1952; Gould et al., 1955;
Hartung and Becht, 1960; Jarrett et al., 1960; Zaiman et al., 1961;
Jennings et al., 1963; Miller, 1964, 1966). Using this method,
Kim (1957) concluded that the intestinal phase of T. spiralis had
a significant immunogenic role.

An attempt was made by Chaia and Murta (1967) and followed by
Rego and Kahtalian (1972), to immunise rats against irradiated third
stage infective larvae of S. ratti, with varying degrees of success
(see discussion).

Chemically abbreviated infections have also been used to deter-
mine which stages were immunogenic. This has proved to be a con-
venient technique since anthelminthics are available which affect
one or more stages of different helminths. This method has been
extensively used for the determination of the immunogenic stages of
T. spiralis (reviewed by Zaiman, 1970), T. muris (Wakelin, 1969),
Oesophagostomum radiatum (Roberts et al., 1963) and Haemonchus
placei (Roberts and Keith, 1959a, b).

Sensitization of the host with a specific stage of the infection
by transplantation, into the host, of these stages was also carried
out in this context. This method was used by Ogilvie (1965) to
investigate the role of adult worms in stimulating an immune response
in rats against N. brasiliensis. Muscle larvae of T. spiralis were
put in diffusion chambers and were implanted in rats to investigate
their immunogenic role (Despommier, 1971).

Using each of these methods or a combination of more than one
(Denham, 1966b), it was shown that adult T. spiralis and

N. brasiliensis are important immunogenic stages (Campbell et al., 1963; Campbell, 1965; Denham, 1966   b; Ogilvie, 1965). More protection was obtained by transplanting female N. brasiliensis (Ogilvie, 1965). Both males and females stimulated the same degree of immunity in T. spiralis infection (Anderson and Leonard, 1940) and in Schistosoma mansoni infection in monkeys (Smithers, 1962).

Stage specificity has been demonstrated in T. spiralis infections and a "dual-antibody" hypothesis suggested to support it (Oliver-Gonzalez, 1941). Recently, James and Denham (1975) reiterated this theory and demonstrated a stage specific immune response in T. spiralis in mice.

Immunity elicited by adult N. brasiliensis worms was not stage specific, since larvae developing in rats previously infected with adult worms alone were affected by the immune response (Ogilvie, 1965 a). In normally infected rats, reinfection affected both the larvae during their tissue migration and the adult worms in the gut (Ogilvie, 1965 b).

No stage-specificity was demonstrated in T. muris infections in mice. Using chemically abbreviated infections, the immune reaction was elicited by, and acted against, all stages of infection (Wakelin, 1969).

In the following study the role of both infective larvae and adult S. ratti in the induction of acquired immunity in rats is investigated using chemically abbreviated infections and transplantation of adult worms. Thiabendazole (TBZ) is very effective against the intestinal stages of S. ratti, particularly the parthenogenetic females, but has no effect on the migratory larval stages (Chaia, 1963; Persand and Grant, 1972).

Experiment 5.1:

In this experiment, chemically abbreviated infections were used to assess the role of the migratory stages of infection with S. ratti in inducing protection against reinfection. Fourteen female Wistar rats were divided into two groups: The first group was infected with 1000 (± 94) L3 on day 0. TBZ was administered per os at 100 mg/kg

on days 4, 5, 6 and 7 PI to eliminate intestinal worms as soon
as they arrived in the gut, and to confine the infection to the
migratory stage. The rats of the second group acted as challenge
controls and were treated with TBZ on the same days. On day 8 PI,
two rats from the first group were killed. No worms or larvae
were recovered from their intestines showing that TBZ had eliminated
the infection. On day 9 PI the two groups were challenged with
1000 ($\pm$ 101) L3/rat. All rats of both groups were killed 12 days
later. The results of the worm counts are shown in Table 12.

There was no statistically significant difference between
the number of adult worms recovered from the two groups.

Experiment 5.2:

In this experiment, another approach was used to determine
the role of infective larvae in inducing immunity to reinfection.
Three injections of 2000 freeze-thaw killed L3s were given intra-
venously to a group of five rats at two days intervals. The experi-
mental and the challenge control group (5 rats) were then challenged
with 1000 ($\pm$ 78) viable infective larvae. The rats of both groups
were killed on day 10 PI and the number of adult worms determined.
The results are shown in Table 13. There was no significant
difference between the numbers of adult worms recovered in both
groups.

The two previous experiments suggest that there was no signi-
ficant role of the migratory stages of S. ratti in stimulating
resistance to reinfection.

Experiment 5.3:

The object of this experiment was to determine the role of
transplanted adult S. ratti in inducing a protective immune response.
Ten 120 gm rats were infected with 3000 L3 each. They were killed

Table   12   Exp: 5.1: Adult worm count after a challenge infection
with S. ratti   following chemically
abbreviated sensitization to the migratory
stages.

Sensitized Group	Challenge control
370	682
363	608
658	613
568	521
498	391
376	333
472 ($\pm$ 50.3)	524 ($\pm$ 56)

Sensitized group   vs   Challenge control   N. S.

Table 13   Exp: 5.2: Adult worm count after a challenge infection
                       with S. ratti, following sensitization by
                       serial IV injections with Freeze-thaw
                       killed L3s.

Sensitized Group	Challenge control
631	498
599	531
500	507
612	473
587	510
585 ($\pm$ 22.6)	503 ($\pm$ 9.4)

Sensitized group   vs   Challenge control   N.S.

and the adult worms collected ten days later. 300 adult worms in 0.4 ml volume of saline were transplanted, intraduodenally, into each of 16 rats. A control group of 16 rats was infected with subcutaneous injections of 300 L3/rat on the same day as the donor rats were initially infected. One other group of 4 rats was left uninfected to act as challenge controls. Four rats were killed from both the group given adult worms and the normal infection group on days 5, 10 and 15 post-transplantation and the number of adult worms were counted. On day 15 post-transplantation, the remaining 4 rats from the experimental, normal infection and uninfected control groups were treated with a single dose of TBZ at 200 mg/kg. Three days later all the rats were challenged with 1000 ($\pm$ 107) L3s. Rats of all three groups were killed on day 10 post-challenge and the number of adult worms counted. The results are summarized in Table 14. In this experiment very few transplanted worms (5.1% on day 5, 20% on day 10 and 5% on day 15 post-transplantation) established themselves in clean rats. The normal infection control showed a development rate of 59% on day 15 PI. On day 10 post-challenge, the group of rats which were sensitised to 300 adult worms only harboured 448 worms, while the group which received a subcutaneous infection of 300 infective larvae harboured 79.2 worms. The challenge controls had a mean of 411 worms on the same day.

No protective immunity was stimulated by the transplantation of 300 worms, but a full infection with 300 infective larvae produced a substantial degree of resistance to reinfection.

Experiment 5.4:

The protocol of this experiment was similar to that of the previous one with the exception that the number of transplanted adult worms was increased to 700 per rat in 0.1 ml volume of saline. Ten recipient rats received these worms intraduodenally, while the infection control group was infected with a normal subcutaneous infection of 700 L3s on the day the donors were initially infected. Four rats were left uninfected to act as a challenge

Table 14   Exp: 5.3   Mean adult worm recovery in three groups:

A: received an adult infection only (300 transplanted intraduodenally)

B: normally infected with 300 infective larvae (same day as the donor rats)

C: challenge control group

Days post – transplantation	Days PI	A adult infection only (300)	B normal infection (300 L3)	C challenge control
5	15	15.5 (± 4.19)	177 (± 13.5)	-
10	20	60 (± 22.2)	95.2 (± 7.44)	-
15	25	9.25 (± 4.23)	15.2 (± 3.7)	-
Day 10 post challenge		448 (± 46.6)	79.2 (± 11.7)	411 (± 44.9)

control group. Four rats from the experimental group were sacrificed on day 5 post-transplantation and they harboured a mean of 281 (± 32) worms. Faecal cultures made from the two infected groups showed similar levels of larval production on days 0, 3, 6, 10 and 15 post-transplantation (equivalent to days 10, 13, 16, 20 and 25 PI). The rats in all three groups were given a single dose of TBZ at 200 mg/kg per os 15 days after transplantation. No worms were recovered from two rats killed two days after anthelminthic treatment. Four days after TBZ all rats were challenged with 1000 (± 89.9) L3s. The results are presented in Tables 15 and 16 and Figures 17 and 18. Faecal cultures from all infected groups made on days 6 and 8 post-challenge revealed no larvae in the rats given either a normal infection or transplanted adult worms, whilst the normal control group had an average of 5850 L3/gm of faeces.

All rats were killed 10 days after challenge and the adult worms counted. There was a recovery of 4.5% from the group "immunised" with adult worms and 3.5% from the group previously given a full infection. The challenge control harboured 41.6% of the inoculum and these worms were of normal length, while those of the other previously sensitised groups were stunted.

In this experiment, the transplanted worms established themselves better than in the previous experiment and there was a highly significant difference between the recovery rate in the group which was sensitised with adult worms alone and in the challenge control group. Rats normally infected with a full infection manifested a strong degree of resistance to reinfection.

This experiment suggests that adult S. ratti are a major source of protective antigens, but a full infection was more immunogenic.

Discussion:

Due to the complex nature of the antigens produced by the different stages of nematode infections, it is very difficult to identify the precise immunogenic stage of the life cycle of these worms.

Table 15   Exp: 5.4   Mean larval production in three groups during initial and challenge infections

A: received transplanted adult infection only (700 adults)

B: received normal infection (700 adults)

C: challenge control

Mean (SE) number of larvae

Day	A adult infection only	B normal infection	C challenge control
10	4080 (+ 792)	6693 (+ 478)	0
13	2150 (+ 220)	4165 (+ 143)	0
16	720 (+ 168)	1874 (+ 155)	0
20	204 (+ 39.2)	458 (+ 59.3)	0
25	33.3(+ 22.2)	192 (+ 27)	0

reinfection on day 30

36	0	0	5569 (+ 421)
38	0	0	6122 (+ 282)

Table 16   Exp: 5.4   Adult worm recoveries in rats autopsied 10 days after challenge with 1000 L3 / rat in three groups:

A: initially infected with 700 adult worms only

B: initially infected with 700 L3 (normal course)

C: challenge control

	Group A	Group B	Group C
Individual worm recoveries	31	23	413
	71	28	393
	59	51	473
	21	40	388
Mean (SE) adult recovery	45.5 (± 11.7)	35.5 (± 6.27)	416.75 (± 19.5)
Number of worms measured	39	29	44
Length of worms (mm)	1.39 (± .03)	1.22 (± .02)	2.79 (± .03)

Figure 17, Exp. 5.4:

Larval production in three groups of rats.
●——● infected with 700 transplanted worms only
○——○ infected with 700 infective larvae
■——■ challenge control

Larvae/gm of faeces
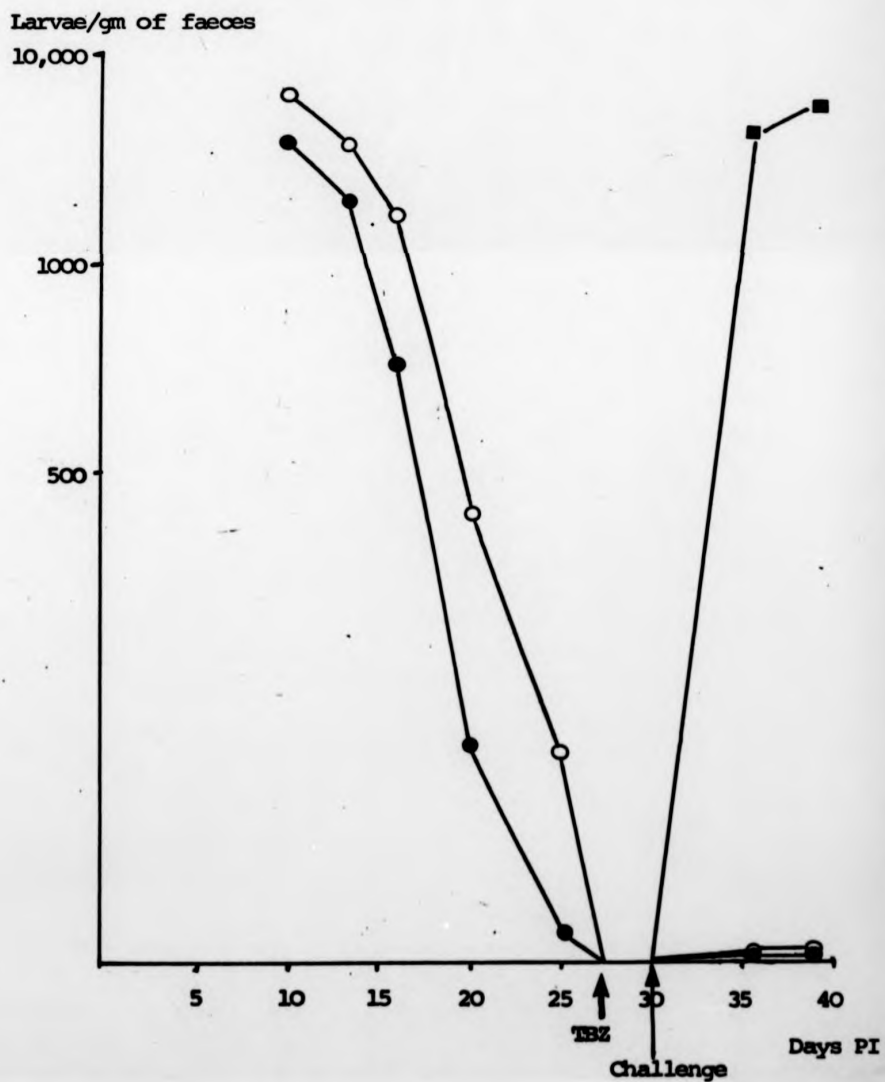
Figure 18, Exp. 5.4:

Worm length and number of adult worms recovered 10 days after challenge in three groups.
initially infected with 700 adult worms only and challenged
initially infected with 700 L3/rat and challenged
challenge control
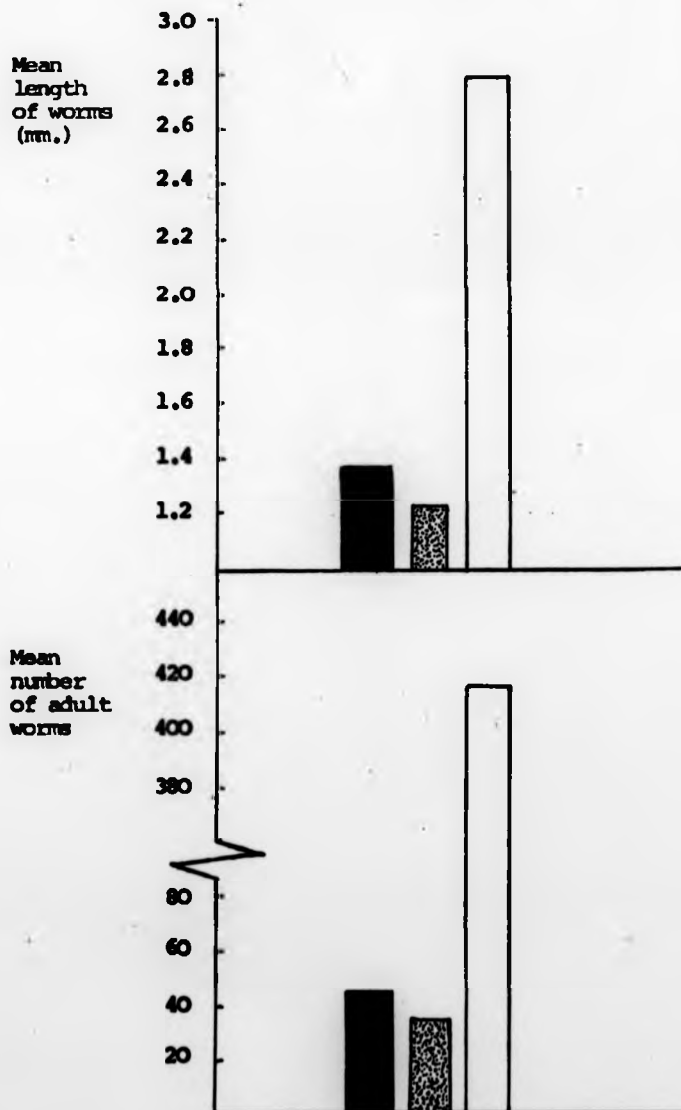
The results of the experiments in this chapter have suggested that adult S. ratti were more immunogenic than infective and migratory larvae.

Soulsby (1958) suggested that L3/L4 moult was the first major source of protective antigens. In S. ratti, this moult occurs in the intestine (Wertheim, 1970). It is possible that TBZ either affected the larvae immediately after their arrival at the intestine, or following their moult to L4 and L5. The time lapse between moulting and evacuation of these worms was, perhaps, not sufficient for the antigens produced to be recognised by the immune system.

Repeated injections of freeze-thaw killed infective larvae of S. ratti conveyed no immunity to reinfection. It was hoped that, since these were injected IV, they would be arrested in the lungs and subsequently produce a response against larvae of a challenge infection. Freeze-thaw killed new born larvae of T. spiralis produced no noticeable immunity whereas viable new born larvae, which were allowed to develop naturally into muscle larvae, produced good immunity (Despommier, 1971).

It seems that sensitisation with non-living nematode material is not effective in protection against a challenge. Live stages and their metabolic products are required to induce a significant immune response. Only Sheldon (1937a) reported successful immunisation of rats against S. ratti using heat-killed infective larvae.

Chaia and Murta (1967) were able to demonstrate a degree of immunisation following 3 injections of 10,000 irradiated larvae of S. ratti/S. venezuelensis at 15 day intervals. On the other hand, 50-54,000 irradiated S. ratti larvae conferred no immunity against challenge in rats (Rego and Kahtalian, 1972). Stankiewicz et al. (1971) successfully immunised rabbits with UV-irradiated infective larvae of S. papillosus and these larvae did not develop to maturity.

In order to assess accurately the role of infective larvae only, in stimulating protective immune response, it is vital that no adult worms reach the intestine. Adult worm antigens, whether they are shared with the larval stages or not, undoubtedly interfere with the development of the immune response against larvae. Sinski and Krzysztofowicz (1971) immunised rabbits against S. papillosus

by exposing them to repeated doses of intact infective larvae at different intervals. It seemed that immunisation was obtained when the challenge was introduced after a period which allowed the development of adult stages in the intestine.

Although a full infection with S. ratti induced the most effective immune response against reinfection (see also Chapter 3), adult worms alone were capable of producing similar resistance. 10 day-old S. ratti transferred from donors into clean recipients survived just as if they were in their original hosts.

The worms transplanted during the first experiment did not convey any protective immunity. This was probably due to the low number used and the relatively large amount of saline used during transfer.

No stage specificity was observed in S. ratti infection since immunity produced by an initial infection acted on all stages of infections (see Chapter 3) and it is possible that transplanted adult worms alone also produced similar effects.

CHAPTER 6

Studies on the mechanism of expulsion of S. ratti
from the intestines of rats

It is now well established that worm expulsion from the host's
intestine is the consequence of an immunological reaction and is
not due to the worm's senescence, as worms can survive for a long
time with a steady level of fecundity when transferred from one
rat to another (Ogilvie and Hockley, 1968) and fertile worms per-
sist in hosts treated with immunosuppressive drugs (Ogilvie, 1965b).

With a classical sense of prediction, Taliaferro and Sarles
(1939) and Taliaferro (1940) concluded that the process of worm
elimination in N. brasiliensis infections in rats was mediated by
a humoral response with subsequent secondary cellular co-operation.
Some 25 years later, it was suggested that local anaphylaxis was
the major event which led to worm damage (Mulligan et al., 1965;
Urquhart et al., 1965; Barth et al., 1966). Since then, this view
has been drastically altered in favour of Taliaferro and Sarles'
hypothesis of a more complicated sequence of events. It is now
generally believed that nematodes are eliminated from the gut, in
rodent parasitic systems, through intricate mechanisms, which
involve both areas of the immune response.

The development of active immunity to N. brasiliensis and its
subsequent expulsion from the host appears to be thymus-dependent
(Wilson et al., 1967; Ogilvie and Jones, 1967). Neonatally
thymectomised and adult thymectomised rats treated with anti-
thymocyte serum (ATS), harboured more adult N. brasiliensis than
sham-operated controls (Kelly, 1972; Keller and Keist, 1972).
Similar results were obtained by treating infected rats with ATS
daily (Kelly, 1972).

Neonatal thymectomy, anti-lymphocyte serum (ALS) and whole-body
irradiation suppressed the expulsion of T. muris in mice, suggesting
that this response was associated with thymus-derived lymphocyte
population (T. cells) (Wakelin and Selby, 1974b).

ATS prevented rats and mice from developing protective

immunity against T. spiralis (Ruitenberg et al., 1974; Walls et al., 1973). On the other hand, antibodies against T. spiralis develop in both thymectomised and intact mice (Ljungström and Ruitenberg, 1976). Ljungström and Ruitenberg (1976) suggested that thymus-dependent and thymus-independent antibodies were formed during infection with T. spiralis, but that the latter were not protective and may have acted as blocking antibodies.

Larsh and his co-workers (Larsh et al., 1952; Larsh and Race, 1954; Larsh et al., 1956) emphasised the role of the inflammatory process before and during T. spiralis expulsion. Cortisone treatment of mice infected with this worm considerably delayed the expulsion of adult worms (Coker, 1956a; Markell, 1958). Coker (1956b) suggested that expulsion required a primary antibody action with secondary cellular co-operation. However, Campbell (1968) demonstrated that non-steroid anti-inflammatory drugs had no effect on expulsion and concluded that intestinal inflammation was not a cardinal part of the mechanism of worm expulsion.

There has been some controversy concerning the role of antibodies in worm expulsion. Passive transfer of immunity with serum was employed early in experimental nematode infections, with a varying degree of success. Although immune serum conferred immunity to N. brasiliensis (Sarles and Taliaferro, 1936; Chandler, 1938b; Taliaferro, 1940), Ogilvie and Jones (1968) showed that only a few of their pools of immune serum were protective against this parasite.

Immunity to T. spiralis was transferred successfully by serum on many occasions resulting in decreased worm burdens (Culberston and Kaplan, 1937, 1938; Oliver-Gonzalez, 1941; Culberston, 1942, 1943; Dorin, 1946; Hendricks, 1953; Mills and Kent, 1965), but this was not always consistent (McCoy and Bond, 1941; Denham, 1969; Larsh et al., 1970).

Antibodies of the class IgG1 have been demonstrated following active T. spiralis infection or immunisation against stichosome granules (Despommier and Muller, 1970; Crandall and Crandall, 1972). Stichocytes contain immunogenic granules and are found in the anterior third of T. spiralis (Despommier and Muller, 1970). Passive immunity from mother to baby, via milk, was also reported in mice infected with T. spiralis (Duckett et al., 1972; Perry, 1974).

It is now generally thought that the major function of
antibodies is to induce worm damage (reviewed by Ogilvie and
Jones, 1971, 1973; see Chapter 4), but there has been consider-
able conflict over the role played by the various types of
cells in worm expulsion.

Transfer of immune spleen cells had little or no effect in
conveying immunity from sensitised to non-sensitised animals
against the intestinal stages of nematodes (Hunter and Leigh,
1961; Ogilvie and Jones, 1968). However, mesenteric lymph node
cells (MLNC), which are closely associated with the infected gut,
instigated the abrupt elimination of an initial infection with
N. brasiliensis (Keller and Keist, 1972; Dineen et al., 1973a, b;
Kelly et al., 1973; Dineen and Kelly, 1973a; Ogilvie and Love, 1974),
T. spiralis (Larsh et al., 1964a, b; Larsh, 1967; Love et al., 1976;
Wakelin and Lloyd, 1976b) and T. muris (Selby and Wakelin, 1973;
Wakelin, 1975a).

Jones and Ogilvie (1971) proposed that expulsion of
N. brasiliensis from rats was achieved in two sequential steps.
The first, antibody-mediated, step damaged the worms structurally
(Ogilvie and Hockley, 1968; Lee, 1969a) and metabolically (Edwards
et al., 1971). The second step involved components of a non-
specific inflammatory process, which correlated with a rise in mast
cell infiltration and amine levels (Barth et al., 1966; Keller,
1970), but it was later shown that inflammation and mast cells do
not play an important role in this step, in N. brasiliensis infec-
tions (Keller, 1971; Keller and Keist, 1972; Kelly and Ogilvie, 1972).

Keller and Keist (1972) showed that functionally intact lympho-
cytes were necessary for the second step of worm expulsion. In
order to assess the overall role of cellular components in immunity
against N. brasiliensis, Dineen et al. (1973b) studied the effects
of transferred sensitised and non-sensitised lymphocytes on trans-
planted, damaged, worms in both irradiated and non-irradiated
recipients. Sensitised cells caused a significant reduction in the
worm burden in both groups of recipients, which demonstrated the
definite role of a committed population of lymphocytes in this
reaction.

Kelly et al. (1973) investigated the effect of sublethal and

lethal whole-body irradiation on the capacity of sensitised MLNC
to expel damaged worms, in syngeneic rats. A substantial degree
of immune competence was demonstrated in sublethally irradiated
rats, as measured by the production of plaque forming cells (PFC)
against sheep red blood cells. This suggested that in non-
irradiated and sublethally irradiated rats, the capacity for
active participation in worm expulsion was present.

Dineen and Kelly (1973b) transferred immune MLNC and normal bone
marrow cells to rats exposed to a lethal dose of irradiation.
These components caused a significant reduction in the number of
adult worms recovered five days later. They concluded from this
that after antibody damage, the expulsion of worms required two
major components. These being sensitised T. cells, which require
the co-operation of a non-specific population of myeloid, radio-
sensitive, bone marrow-derived cells which are able to undergo
rapid regeneration.

In the host-parasite relationship between mice and T. spiralis,
immunity was transferable by sensitised cells from the peritoneal
exudate and lymph nodes (Larsh et al., 1964a, b). From these experi-
ments, Larsh and his group claimed that delayed hypersensitivity
(DH) was involved in the process of worm elimination.

In a recent review, Larsh and Race (1975) have reiterated
their emphasis on the causal relation of DH to the expulsion of
adult T. spiralis, and totally excluded the participation of a
humoral response in this phenomenon. Love et al. (1976) used the
experience derived from their previous work on N. brasiliensis
infections, in both rats and mice to study the expulsion of
T. spiralis from rats. The picture was very similar to that of
N. brasiliensis in that both antibodies and sensitised MLNC were
required to bring about worm expulsion. Structural damage of adult
worms was also observed prior to their expulsion and when the host
received antiserum or cells. Similar results were recently obtained
in mice by Wakelin and Lloyd (1976a, b). In T. colubriformis infec-
tions in the guinea-pigs, cellular transfer of immunity has been
successful (Wagland and Dineen, 1965; Dineen and Wagland, 1966),
leading to the expulsion of these worms before they reach maturity.
Less success was achieved with passive transfer of antibodies

(Connan, 1972). Ogilvie and Jones (1973) proposed that passive or adoptive immunisation against T. colubriformis in the guinea-pig had an enhancing effect on the active immune response which is already developing in this abnormal host. In T. cell-deprived guinea-pigs, the infection was long lived (Dineen and Adams, 1971).

Immunity against T. muris was transfer_able through both antiserum and sensitised cells (Selby and Wakelin, 1973) though the former was more effective. Further work by Wakelin (1975) revealed that a sequential action of antibody-mediated responses and lymphoid cell-mediated immunity was required for the expulsion of T. muris. Although this field requires further elucidation, there is no doubt that work on N. brasiliensis, T. spiralis, T. colubriformis and T. muris has shown that a multi-step mechanism is involved in the process of worm expulsion (Ogilvie and Jones, 1971; Love et al., 1976; Wakelin and Lloyd, 1976; Rothwell and Dineen, 1973; Wakelin, 1975).

The experiments reported in this chapter are an attempt to analyse the role played by the cellular and humoral response in the elimination of S. ratti from rats. This was achieved by passive and adoptive transfer of immunity from competent donors to non-sensitised rats.

Collection of serum:

25 days PI rats were anaesthetized with Nembutal and bled, by cardiac puncture, using 5 ml syringes and $1\frac{1}{2}$ inch x 21 gauge needles. The blood was allowed to clot at room temperature for 1 hour then at $4^{\circ}C$ for three to four hours. The samples were pooled and then stored in 1 ml aliquots at $-70^{\circ}C$. In serum transfer each rat received a total volume of approximately 5 ml of serum. Each experiment had its pool of serum obtained from a group of donors on day 25 PI.

Collection of sensitised immune competent lymphocytes:

Donor rats were infected with 3000 L3 each and killed on
day 25 PI. The enlarged mesenteric lymph nodes were removed and
kept in medium 199 at pH 7.2 on ice and were then gently teased
apart and pushed through a sterile stainless steel tea-strainer
with a 10 ml syringe plunger. The suspension of lymphocytes was
pipetted through a sterile glass wool column to eliminate the
adipose cells and the macrophage population. The MLNC suspension
was collected on ice by washing the column twice with chilled
medium 199. The cells were concentrated by centrifugation at 1000
rpm for 4 minutes at 4°C. The cells were washed 3 times with
medium 199 and the volume of the suspension made up to the required
amount prior to injection. After dilution, lymphocytes were
counted in a "Neubauer" haemocytometer. The viability of the MLNC
was determined by the trypan-blue exclusion test.

Recipient rats received 2×10^9 MLNC each intravenously (IV)
through the lateral caudal vein with a 1 ml graduated disposable
syringe fitted with a ½ inch x 25 gauge needle. When the MLNC
were injected IV, rats were restrained in specially prepared pers-
pex boxes. Three different sizes of boxes were made for different
sizes of rats (Plate 46). The rats' tails were accessible for IV
inoculation (Plate 47).

Experiment 6.1:

This experiment was designed to establish whether sensitised
lymphocytes cause an early expulsion of the worm population,
whether immunised serum causes the expulsion of the worm burden and
to determine whether immunised serum and immunised lymphocytes
cooperate to induce expulsion of adult S. ratti.

50 inbred female Wistar rats were infected with 2000 L3 each
and killed on day 25 PI when serum and MLNC were collected. The
average viability of MLNC was 70% and the concentration of cells
was adjusted so that 2×10^9 viable cells were given to each
recipient rat. The mean worm burden of the donor rats, on day 25,
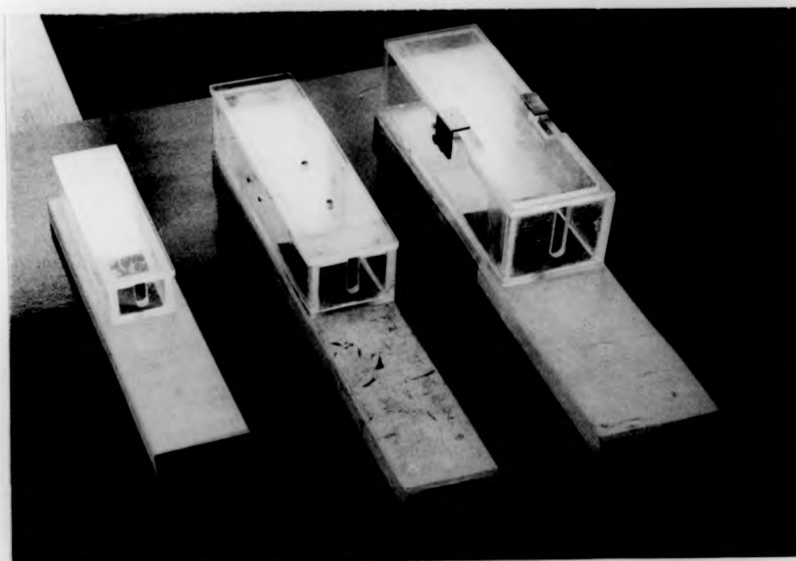
Plate 46 : Three different sizes of restraining boxes for rats

Plate 47 : Intravenous injection of MINC into the caudal vein
            of a rat
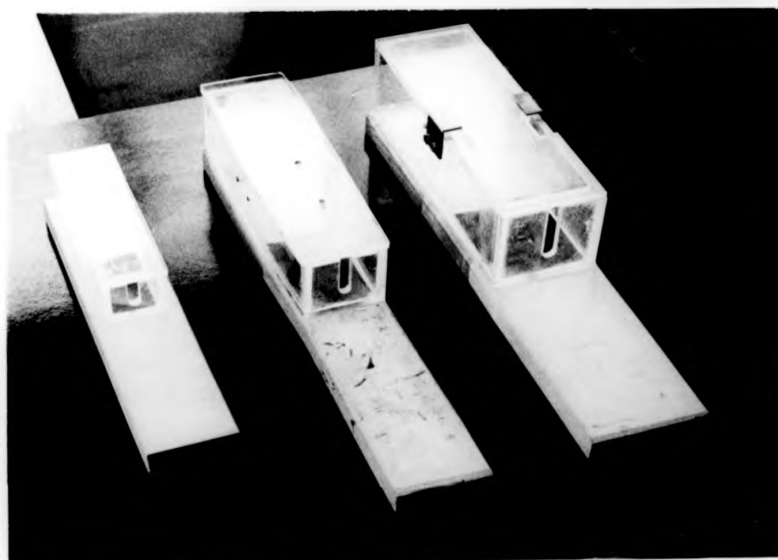
Plate 46 : Three different sizes of restraining boxes for rats

Plate 47 : Intravenous injection of THC into the caudal vein of a rat

counted in a sample of four rats from each donor group was 11.7
(± 6.1). Ten weeks-old inbred female Wistar rats were used as
recipients.

The recipient rats were divided into the following major
groups and each rat infected with 1000 L3 on day 0:

Group 1: This group was divided into 3 sub-groups; and all rats
were infected on day 0 with 1000 (± 39) infective larvae.

A: Received immune serum on days 3, 6, 9 and 12 PI, and immune
   MLNC on day 6 PI (5 rats)
B: Received immune MLNC on day 6 PI (5 rats)
C: Normal infection control (4 rats)

Group 2: This was also divided into three sub-groups of rats which
were infected on day 0 with 1000 (± 86) infective larvae.

A: Received immune serum on days 3, 6, 9 and 12 PI and immune MLNC
   on day 12 PI (5 rats)
B: Received immune MLNC on day 12 PI (5 rats)
C: Normal infection control (4 rats)

Group 3: This contained two sub-groups of rats which were infected
on day 0 with 1000 (± 96) infective larvae.

A: Received immune serum only on days 3, 6, 9 and 12 PI (5 rats)
B: Normal infection control (5 rats)

Faecal cultures were made from all groups on days 8, 12 and 13 PI
and all the recipient rats autopsied on day 16 PI. This day was
chosen because the worms do not show signs of "damage" before day
18 PI and expulsion usually commences after day 22, and thus it was
a good monitor of the effects of the transferred immune components
on the course of infection.

The results of this experiment are presented in Table 17 and
Figures 19 and 20.

Table 17 Exp: 6.1   Effects of the transfer of immune MLNC and serum from sensitised donors to
recipients at different intervals, during the course of infection with S. ratti

| Group | Days of transfer | | Mean (SE) number of larvae/gm faeces on days: | | | Mean (SE) number adult worms on day 16 | Percentage recovery | Number of worms measured | Mean (SE) length of worms (mm) |
	Serum total/ rat 5.3 ml	MLNC viability 70%	8	12	15				
A1	3,6,9,12	6	12692 ($\pm$1293)	2187($\pm$191)	866($\pm$124)	681($\pm$161)	68.1%	50	1.33($\pm$.01)
A2	-	6	8272($\pm$560)	3970($\pm$247)	2146($\pm$238)	166($\pm$64.4)	16.6%	50	2.92($\pm$.05)
A3	-	-	4028($\pm$225)	4596($\pm$430)	2566($\pm$169)	372($\pm$26.5)	37.2%	40	2.85($\pm$.04)
B1	3,6,9,12	12	4714($\pm$522)	2554($\pm$338)	3306($\pm$233)	145($\pm$25.6)	14.5%	50	1.79($\pm$.03)
B2	-	12	2852($\pm$208)	1772($\pm$222)	1976($\pm$298)	96.8($\pm$30.1)	9.6%	34	2.72($\pm$.04)
B3	-	-	2960($\pm$220)	2748($\pm$263)	2821($\pm$225)	424($\pm$26.9)	42.4%	38	2.82($\pm$.04)
C1	3,6,9,12	-	13546($\pm$621)	7414($\pm$378)	755($\pm$171)	1562($\pm$218)	156 %	40	1.66($\pm$.03)
C2	-	-	3087($\pm$231)	5270($\pm$238)	3200($\pm$191)	403($\pm$27.9)	40.3%	35	2.36($\pm$.03)

The effect of immune MINC only:

On day 16 PI, a mean of 166 worms was recovered from the
rats given immune   MINC on day 6, while the control group
harboured a mean of 372 worms, a reduction of 45%. The worms
collected from the recipient group were normal in length (2.92 mm)
and their larval production on days 8, 12 and 15 was similar to
the normal control group.

Group B2 which received immune MINC alone on day 12 PI had
a mean of 97 worms on day 16 compared to the control group mean
of 424 worms. The worms of this recipient group were also of
normal length (2.72 mm), and their larval production was still
relatively high on day 15 PI.

It seems that immune MINC when transferred on day 6 or 12 PI
were very effective in causing a reduction in the number of
intestinal worms.

The effect of immune serum only:

Following passive transfer of immune serum on days 3, 6, 9
and 12 PI, a mean of 1563 adult S. ratti were recovered from rats
of group C1 on day 16 PI. The control group harboured a mean of
403 worms on the same day. The size of the original inoculation
was 1000 ($\pm$ 96) L3 and, thus, it seems that there was the possi-
bility of auto-infection in the recipient rats. The worms col-
lected from group C1 on day 16 PI were small with a mean length
of 1.66 mm while the control worms were 2.36 mm. The number of
larvae/gm in the recipient group showed a maximum number of
13,546 L3/gm on day 8 PI but dropped sharply to 755 L3/gm on day
15 PI when the control group produced a mean of 3200 L3/gm.

Effect of immune MINC and immune serum:

The two groups which received a combination of both immune
MINC and serum, produced different results depending on the time

Figure 19, Exp. 6.1:

The effect of passive and adoptive transfer of immunity on the percentage of worm recovery on day 16 PI.

- received serum and MLNC on day 6 PI
- received serum and MLNC on day 12 PI
- received MLNC alone on day 6 PI
- received MLNC only on day 12 PI
- received serum alone
- normal infection control, no transfer
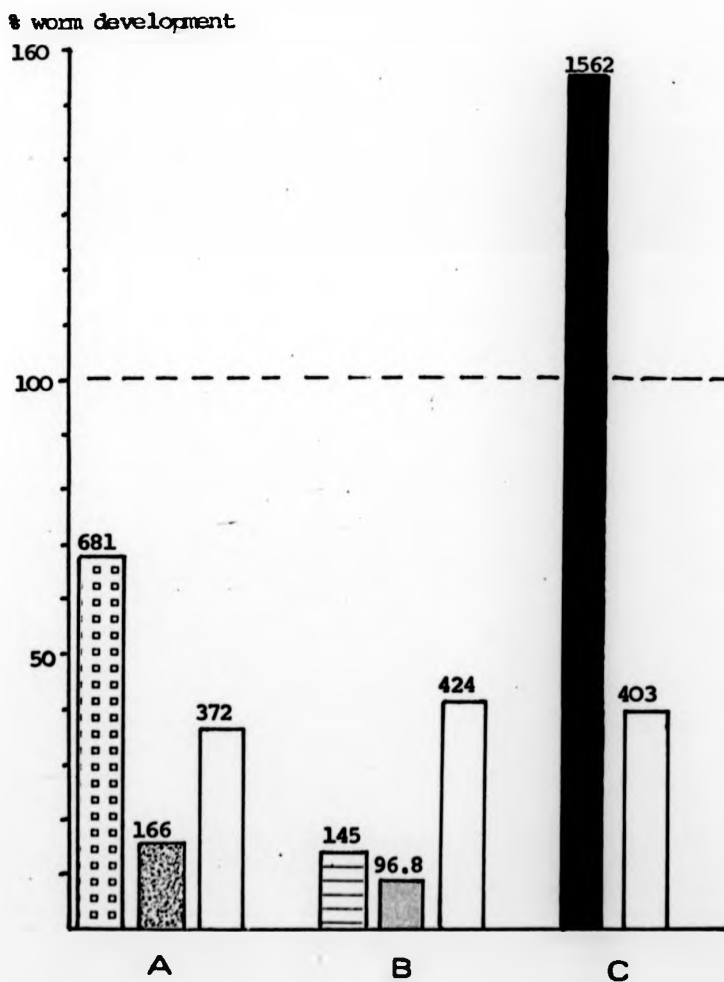
Figure 20, Exp. 6.1:

Larval production in three major groups of rats, A, B. and C. ▲▲A₁ received immune serum and MLNC on day 6 PI; △——△ A₂ received MLNC alone on day 6 PI; ■——■ B₁ received immune serum and MLNC on day 12 PI; □——□ B₂ received MLNC only on day 12 PI; o——o C₁ received immune serum only; ●——● Control groups.
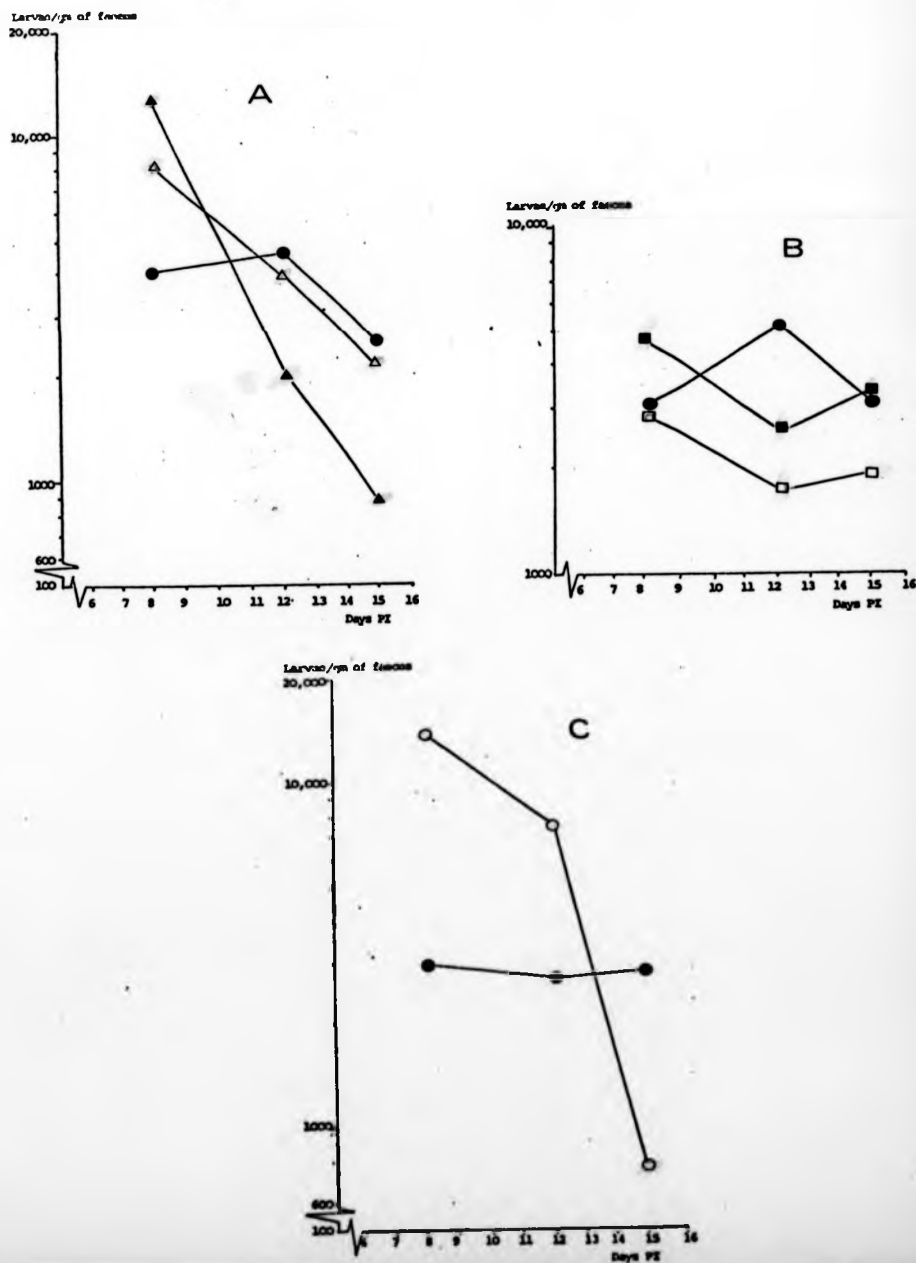
of MLNC transfer. Group A1, which received immune serum on days
3, 6, 9 and 12 PI and immune MLNC on day 6 PI, harboured a mean
of 681 worms on day 16 PI with a range of 1122 - 238 worms. The
control group harboured 372 worms on the same day. The initial
inoculum was 1000 (± 39) L3. This recovery rate also suggested
auto-infection. When immune MLNC were given on day 12 PI in con-
junction with four injections of immune serum in group B1, the
picture was different. A very significant reduction of the worm
burden was encountered on day 16 PI (mean 145 worms) while the
control group harboured 424 worms on the same day. The mean
length of the worms recovered from the recipient group was below
normal (1.79 mm) but larval production on days 8, 12 and 15 PI
was similar to the control group.

    This experiment suggested that immune MLNC alone, whether
given early (day 6 PI), or during the middle of infection (day 12
PI), were capable of causing a reduction in the worm burden and
acclerated the expulsion of normal looking worms. Immune serum
alone appeared to induce auto-infection. Immune MLNC and immune
serum caused expulsion only when immune MLNC were introduced on
day 12 PI. Auto-infection also occurred following transfer of
immune serum and MLNC on day 6 PI.

Experiments 6.2, 6.3, 6.4, 6.5, and 6.6:

    These experiments were repeats of the previous experiment and
the same type of animals and procedures were used in transferring
immune serum and MLNC.

    The protocol of these experiments and their results are sum-
marized in Table 18 and are illustrated collectively in Figure 21.

    Figure 22 summarizes the results of the experiments on adoptive
and passive transfer. In this figure, the normal infection control
group is expressed as 100%. Thus any figure appreciably over 100
indicates auto-infection and any figure appreciably below 50 indi-
cates worm expulsion.

Table 18 Summary of five repeats of serum and cell transfer of immunity to rats infected with S. ratti

Exp:	Groups (no.rats)	Serum	Days of transfer MINC (viability %)	Mean (SE) number of adult worms	Mean (SE) length of worms (mm)	% of development	Statistical significance
6.2	A (4)	–	6 (73%)	181 (±32.2)	2.81 (±.04)	18.1%	A vs B P<.001
	B (4)	–	–	297 (±30.5)	2.83 (±.04)	29.7%	
6.3	A (5)	–	6 (78%)	234 (±47.2)	2.79 (±.03)	23.4%	A vs C P<.01
	B (5)	3,6,9,12	6 (78%)	380 (±104)	2.1 (±.06)	38%	B vs C P<.01
	C (5)	–	–	577 (±71.6)	2.68 (±.04)	57.5%	
6.4	A (4)	–	12 (51%)	401 (±25.3)	2.8 (±.03)	40.1%	A vs C N.S.
	B (4)	3,6,9,12	–	1170 (±100)	1.7 (±.06)	117 %	B vs C P<.001
	C (4)	–	–	681 (±45.7)	2.63 (±.05)	68.1%	
6.5	A (5)	–	12 (81%)	63.2 (±9.8)	2.73 (±.05)	6.3%	A vs C P<.001
	B (5)	3,6,9,12	–	174 (±14.6)	1.6 (±.06)	17.4%	B vs C P<.01
	C (4)	–	–	257 (±27)	2.66 (±.03)	25.7%	
6.6	A (5)	3,6,9,12	12 (71%)	191 (±20.5)	2.65 (±.04)	19.1%	A vs C P<.001
	B (5)	3,6,9,12	–	906 (±230)	1.71 (±.06)	90.6%	
	C (4)	–	–	430 (±46.9)	2.8 (±.03)	43%	

Figure 21

Summary of five repeats of passive and adoptive transfer
of immunity to rats infected with S. ratti.

received MLNC alone on day 6
received serum and MLNC on day 6
received MLNC on day 12
received serum and MLNC on day 12
received serum alone
normal infection control

% worm
development
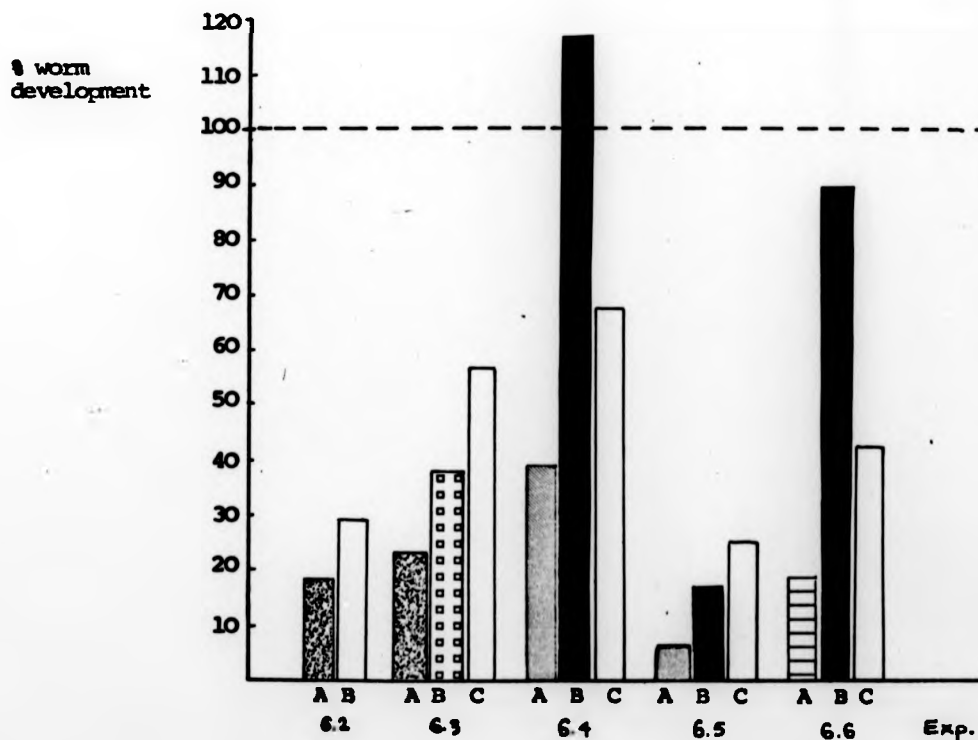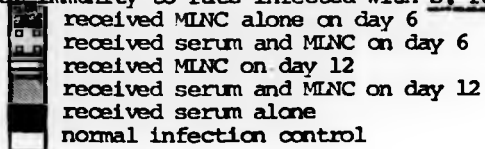
Figure 22

> Pooled results of experiments on adoptive and passive
> transfer of immunity to S. ratti.
>
> A : Normal infection control = 100
> B : Received immune MLNC on day 6 PI = 45
> C : Received immune MLNC on day 12 PI = 43.3
> D : Received immune serum on days 3, 6, 9, 12 PI = 221
> E : Received immune serum and MLNC on day 6 PI = 123
> F : Received immune serum and MLNC on day 12 PI = 39
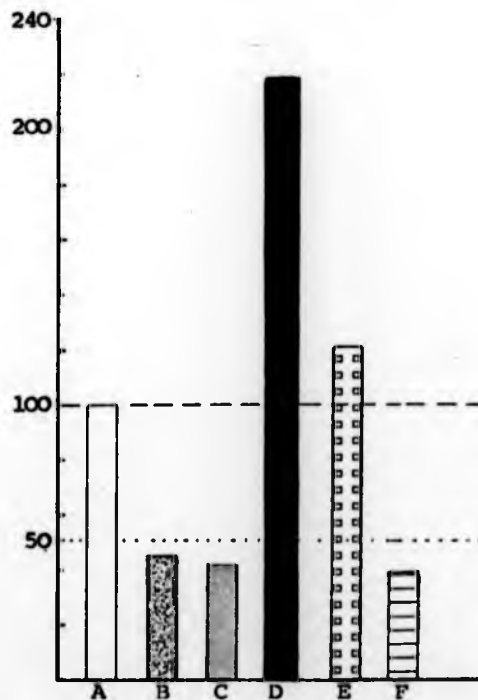
It was evident from experiments 6.1 - 6.6 that transferred sensitised MLNC, whether on day 6 or 12 PI, significantly decreased the number of worms on day 16 PI. On the other hand, immune serum alone induced a significant increase in the number of adult worms. Rats which received immune MLNC on day 6 PI in conjunction with immune serum usually showed signs of auto-infection. However, results of experiment 6.3 indicated a decrease in the number of adult worms. Immune serum and MLNC given on day 12 PI consistently resulted in a significant degree of worm expulsion.

Experiment 6.7:

This experiment was designed to determine whether immune MLNC, injected intraperitoneally 12 days after challenge, could induce immunity. One group of rats received 4×10^8 viable MLNC intraperitoneally from donor rats infected 25 days previously. One group received 2×10^8 MLNC intravenously and another group 2×10^8 MLNC plus immune serum on days 3, 6, 9 and 12 PI. Another group received neither cells nor serum. The results are presented in Table 19 and Figure 23.

The group which received MLNC IP had similar numbers of adult worms to the normal group (354 vs 455). The group given MLNC IV had fewer worms than the controls (94 vs 455). The group given MLNC and serum had rather fewer worms than the controls (247 vs 455).

These results suggest that injecting sensitised MLNC intraperitoneally did not convey immunity to the recipient group.

Experiment 6.8:

This experiment was designed to explore the activity of immune serum and/or cells when transferred to a naive group of rats before or on the day of infection.

Each rat in group A received 2×10^8 immune MLNC, IV,

Table 19　　　Exp: 6.7　　Comparison between two routes of transferring (MLNC) to recipient rats

(IV) and (IP) and effect of serum and lymphocyte transfer on the course of infection

Group	Serum	Days of transfer MLNC (70% viable)	Injection route	Number MLNC	Mean (SE) number of worms on day 16 PI	% development
A	-	12	IV	2×10^8	94.4 ($\pm$ 29)	9.4%
B	-	12	IP	4×10^8	354 ($\pm$ 32.9)	35.4%
C	3,6,9,12	12	IV	2×10^8	246 ($\pm$ 15.3)	24.6%
D	-	-		-	455 ($\pm$ 5.4)	45.5%

A vs D P $<$ .001 :　B vs D　N.S. :　C vs D　P $<$ .01 :　A vs B　P $<$ .001

Figure 23, Exp. 6.7:

Percentage of worm recovery on day 16 PI from four groups of rats. [ ] A received MINC, IV; [ ] B received MINC on day 12 IP; [ ] C received serum and MINC on day 12; [ ] D normal infection control.

% worm development
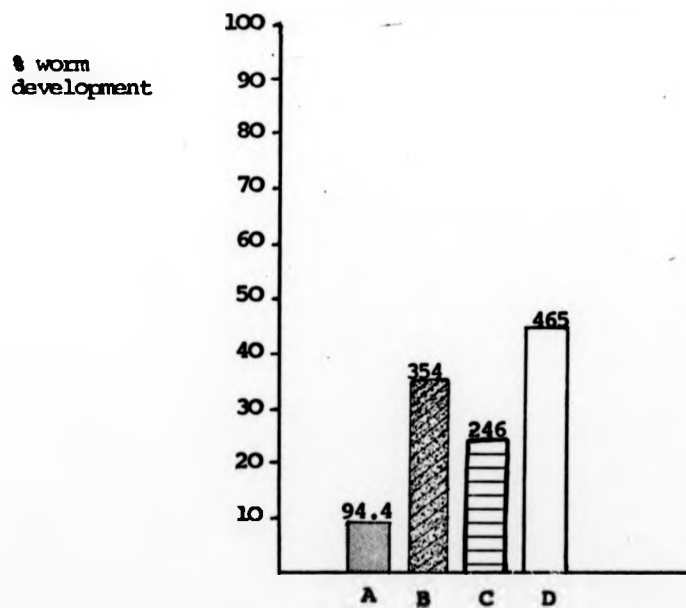
(viability 70%) one day before infection and 1.5 ml of immune
serum on the day of infection. Group (B) received MLNC alone
on day -1, and group (C) received immune serum only on day 0.
The fourth group (D) was infected on the same day but was not
given any MLNC or serum. All the rats in the four groups were
killed on day 8 PI, and the number of adults counted. The
results are summarized in Table 20 and Figure 24. The results
indicated that the best effect of transferred immune components
was obtained when immune MLNC were transferred on day -1 and
immune serum on day 0. The number of worms recovered on day 8
PI was the lowest among the other groups (54 $\pm$ 9). However, MLNC
alone on day -1 were also capable of causing a reduction in the
number of worms following transfer. Immune serum in this experi-
ment did not transfer protective immunity to rats in group (C).
This experiment demonstrated that immune MLNC can sensitise
recipient rats and produce protective immunity against an initial
infection. Immune MLNC and immune serum can co-operate to protect
the recipient rats effectively.

Discussion:

The results of the previous experiments have suggested that
expulsion of S. ratti is due to an immune reaction in rats. It is
inevitable that these results should be compared with results, on
the mechanisms of immune expulsion, obtained in N. brasiliensis,
T. muris and T. spiralis which have been studied extensively. As
mentioned before, a multi-step mechanism has been proposed for
worm expulsion in these parasitic systems. The results in this
chapter have shown that the necessity of worm damage and the effect
of transferred immune serum on the course of S. ratti infection
(see later) did not conform with results obtained in other systems.
New, and sometimes controversial, results have been presented here
which require careful interpretation.
    It seems that MLNC collected on day 25 PI from initially
infected donors contained sufficient committed lymphocytes which
transferred the immune response to recipients.

Table 20   Exp: 6.8   Number of adult worms in four groups following
passive and adoptive transfer of immunity

| | Days of transfer | | | |
Group	Serum	MLNC (viability 70%)	Mean (SE) number of adult worms	Percentage of worm development
A	O	- 1	54 ($\pm$ 9)	5.4%
B	-	- 1	115 ($\pm$ 8.83)	11.5%
C	O	-	268 ($\pm$ 14.5)	26.8%
D	-	-	304 ($\pm$ 10.9)	30.4%

A vs D P $<$ .0005 :   B vs D P $<$ .0005 :   C vs D P $<$ .001

Figure 24, Exp. 6.8:

Percentage of worm development in four groups.
A received immune MLNC on day -1 and immune serum on
day O; B received immune MLNC on day -1 only;
C received immune serum on day O only; D normal
infection control.
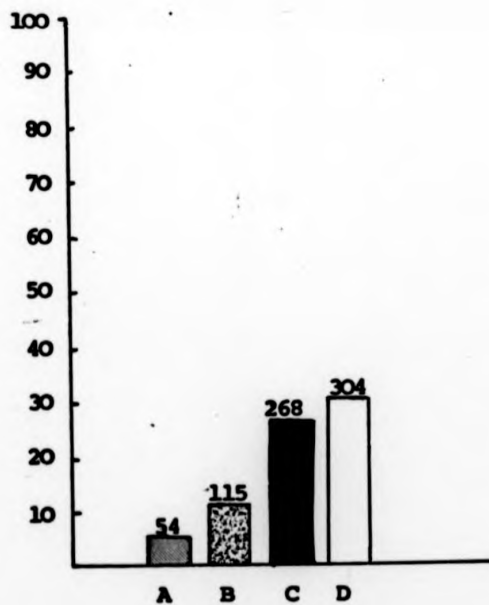
% worm
development

The effect of the transfer of immune MLNC only to syngeneic rats on day 6 PI was intriguing. The worm burden of recipient rats was reduced following this transfer and the worms had lengths similar to those of the control group. It should be remembered that only worm length was used as the criterion for damage. Accordingly, worms were considered to have been expelled when they showed no superficial signs of damage. Since it has been shown that worm damage was a prerequisite for the expulsion of N. brasiliensis and T. spiralis (Ogilvie and Jones, 1973; Love et al., 1976; Wakelin and Lloyd, 1976b) it is possible that worm damage is not required in the process of S. ratti expulsion.

It is important to remember that the amount of worm damage required by the host to induce the second step of worm expulsion, has not been defined as yet. It is possible that there is a minimum amount of damage and that this is sufficient to induce an effective attack by the cellular step. The worms which were expelled following the transfer of MLNC on day 6 might have fulfilled this minimum requirement, which was not detectable on a macro-level. Moreover, it is possible that these expelled worms were shorter than those recovered on the day of autopsy.

The other important fact is that although mesenteric lymph nodes (MLNs) contain a high proportion of T. cells (Raff, 1970), a substantial proportion of these lymphocytes are B. cells. Despite the use of glass wool columns to purify MLNC suspensions from the majority of B. cells and macrophages, some B. cells would have been transferred with the MLNC suspension. Cochrane and Dixon (1962) have demonstrated that the transfer of cells initiates the formation of antibodies in recipients. Thus, such transferred humoral response may have cooperated with the recipient's own response and caused a degree of damage sufficient to allow the second step to act. Crandall and Crandall (1972) demonstrated the presence of IgG1 on cells from MLNs of mice infected with T. spiralis. This is interesting since this immunoglobulin is involved in protective immunity to N. brasiliensis (Jones et al., 1970).

A gap of 10 days between cell transfer and autopsy, in groups which received MLNC on day 6, was sufficient for MLNC to mount a successful attack on the intestinal worm population. The

transferred MLNC possibly acted by producing relevant lymphokines causing the proliferation of specific and by-stander lymphocytes, as well as specific antibody-producing cells, which together cooperated to eliminate the majority of worms.

When immune MLNC were transferred on day 12 PI, leaving a period of 4 days before autopsy, expulsion was evident. The worms had normal lengths and no apparent signs of damage were observed. The argument regarding the validity of damage mentioned earlier applies here as well.

It appears that cooperation between the transferred MLNC and the recipients' own immune system was successful in bringing about a decrease in worm burden within 4 days.

The effects of transfer of immune serum, at 4 intervals, on the course of S. ratti infection was unique. The recovery of worms which exceeded in number that of the original inoculum indicated the development of auto-infection. Auto-infection did not occur during the course of normal infection, but did occur under certain states of immune depletion (see Chapter 8). When immune serum was introduced to infected rats on day 3, 6, 9 and 12 PI, auto-infection occurred repeatedly (with the exception of experiment 6.5) and worms recovered were short and less fecund. The short worms were either damaged through the action of antibodies as described in N. brasiliensis (Ogilvie and Jones, 1973) or they were new young worms which arrived in the intestine as a result of auto-infection.

In human strongyloidiasis, recent evidence indicated that auto-infection occurred in patients with depleted cell mediated immunity (CMI) either due to infections which cause such depletion, e.g. Lepromatous Leprosy and Tuberculosis, or following immunosuppressive therapy (see Chapter 9) (Purtillo et al., 1974). If this was true in rats, then transferred antibodies were causing some state of specific or generalized depletion which led to auto-infection.

It can be suggested that a proportion of these transferred antibodies was not protective and acted as blocking (enhancing) antibodies, possibly similar to those demonstrated in certain tumour-bearing individuals (Hellström and Hellström, 1969, 1970; Hellström

et al., 1969). Since these antibodies, in tumour immunology, may be antibody/antigen complexes (Sjögren et al., 1971), they may operate a feed-back mechanism which inhibits the production and proliferation of active specific lymphocytes.

The mechanisms by which antibodies render the cellular arm of the immune response ineffective are still undetermined. Such depletion must be effective on both the T. cells in the gut and associated lymph nodes, as well as the circulating population, so as to allow the infective stages to re-migrate through the various tissues during auto-infection, without any resistance.

The effects of these antibodies on the course of S. ratti infection may be attributed to mechanisms similar to those sug- gested by Rickard and Outteridge (1974) for the depletion of the CMI response in hyperinfection with Taenia pisiformis in rabbits. Antibodies produced by the infected rabbits, coated the surface of the cysts of this parasite and inhibited the action of CMI on them (Rickard, 1974).

The possibility that a factor, produced by the interaction of worms and antibodies, kills lymphocytes, similar to that suggested in T. spiralis infection (Faubert and Tanner, 1975; Faubert, 1976), also exists.

The blocking effects of these antibodies might be a contributory factor in the tenacity of S. stercoralis in some individuals, which have been attributed to repeated auto-infection. It would be very interesting to study the level of antibody production in those individuals as well as the state of their specific and general CMI response.

Lawler (1940) passively transferred immunity against S. ratti to a group of rats by injecting them with immune plasma from multi- ply infected rats. The degree of protection which he obtained was only partial and was similar in rats given immune plasma before or after the introduction of the infection. This is, obviously, in sharp contrast to the results obtained in my experiments.

It was not possible to assess fully the role of antibodies in inducing worm damage in this system due to the consistent development of

auto-infection. However, when serum did not cause auto-infection, worms were short.

In one experiment (6.5), immune serum alone did not cause auto-infection, but instead produced a significant drop in the number of adult worms. This can be attributed to the different pool of serum used (Ogilvie and Jones, 1968).

Rats which received immune serum and MINC on day 6 PI showed a high rate of worm development, with a few rats showing auto-infection. On the other hand, those which received serum and MINC on day 12 PI showed the best expulsion rate. These two results are difficult to interpret. It is possible that antibodies affected the worms and the MINC which followed on day 12 acted immediately and caused expulsion. It seems that this would be the ideal approach in bringing about worm elimination by these transferred components. Why this serum, which came from the same pool and was given at exactly the same intervals, did not appear to have caused auto-infection on this occasion, is not clear. Antibodies may have inhibited the host's own cells (and probably the transferred MINC on day 6) from recognising the antigens (masking) or possibly produced other factors, as discussed previously.

This aspect of blocking antibodies and their causal effects on auto-infection in this system needs to be pursued. No such immuno-logical enhancement has been demonstrated in other helminthic infection, but preliminary observations suggest that in some para-sitic infections, antibodies are produced in abundance while the CMI response is ineffective (Ogilvie and Wilson, 1976).

MINC, when given through the IP route did not convey immunity compared to the IV route. Undoubtedly, a few of these cells, when put in IP, reach the MINs, but the majority are absorbed and may reach other organs which are not involved in the reaction against the intestinal worms.

MINC given alone one day before infection gave a substantial degree of protection. The action of these lymphoid cells can be aimed against either the migratory larvae, the L4 stage, the adult worms or all three. Love (1975) suggested a quantitative difference between the immunological requirements for the expulsion of larvae and adult N. brasiliensis. The transferred MINC may have lingered

about until L3 of <u>S. ratti</u> moulted to L4 or the subsequent stage, before mounting an attack. On the other hand, it is possible that the cells may have infiltrated to the lungs and affected the migration of the invading larvae by developing lymphoid centres similar to those observed during second infection with <u>S. ratti</u> (see Chapter 3). There was no evidence of larval detention in the lungs in rats infected with <u>N. brasiliensis</u> and given immune serum and MLNC (<u>Love et al.</u>, 1974). Love (1975) suggested that fourth-stage larvae of <u>N. brasiliensis</u> were expelled from the intestine by the action of MLNC before moulting to the fifth-stage.

MLNC transferred one day before infection and serum on the day of infection also gave good protection. Serum alone gave no protection, but produced no auto-infection either. Whether immune serum co-operated in the process of reducing the worm burden on day 8 PI, is still unclear.

In conclusion, it is difficult to propose a multi-step mechanism in the expulsion of <u>S. ratti</u> from rats as described in other systems (Ogilvie and Love, 1974; Wakelin, 1975; Love <u>et al.</u>, 1976; Wakelin and Lloyd, 1976b). However, it is clear that immune MLNC are important in inducing worm expulsion, whether given on day 6 or 12 PI. Co-operation between immune serum and MLNC was evident only when MLNC were given on day 12 and not 6. Immune serum presented a new parameter as far as the relationship between antibodies and worms is concerned.

It is important that studies should be conducted on transfer of immunity to infected, irradiated rats, in order to present conclusive evidence on the role of lymphoid cells in the process of worm expulsion. However, this might prove to be difficult in this system since irradiation causes immune depletion and can subsequently lead to the development of auto-infection.

CHAPTER 7

Studies on the role of other factors in the process
of worm expulsion in S. ratti infections

In many nematode infections of rodents, a variety of addi-
tional factors have been implicated in the humoral and cellular
reactions against the living worms, among them mast cells, baso-
phils and eosinophils.

Although mast cells and basophils have been studied
extensively, there is still uncertainty as to their role in the
expulsion of helminthic infections (Murray et al., 1971a, b, c;
Wells, 1962; Whur, 1966, 1967; Rothwell et al., 1974b). The
trigger for the production of histamine and other substances,
from mast cells and basophils, is the reaction between IgE and
an antigen. This immunoglobulin increased during helminthic
infections, particularly prior to the expulsion of the intestinal
worms (Ogilvie, 1967; Ogilvie and Jones, 1969, 1971). Simulta-
neously with the rise in the amount of IgE, there was an increase
in the infiltration of mast cells and/or basophils near the site
of infection (Murray et al., 1971a, b, c). Analysing the role of
anaphylaxis in worm expulsion in N. brasiliensis, Urquhart et al.
(1965) and Barth et al. (1966) proposed what they called the "leak
lesion" hypothesis. It suggested that the production of amines
by the mast cells of the gut attracted anti-worm antibodies
towards the worms where they caused damage. Murray et al. (1971c)
demonstrated that worm expulsion did not occur when infected rats
were treated with the anti-amine drug, promethazine. However,
mounting evidence against this hypothesis has been presented.
Production of IgE occurred in rats which were not resistant to
infection, and in rats immunized against worm extracts, although
they showed no immunity to re-infection (Ogilvie, 1967). Also,
many pools of antisera had high titres of IgE but were not pro-
tective (Ogilvie and Jones, 1967, 1968). Changes induced by the
immune response in adult N. brasiliensis whether on metabolic or

structural levels occurred before the infiltration of mast cells
into the villi (Henney et al., 1971; Keller, 1971; Murray et al.,
1971c; Whur, 1966). Confirmation of this came from work on neo-
natal and lactating rats in which worm damage was brought about
by a humoral response independent of mast cells (Ogilvie and
Hockley, 1968; Keller, 1971; Kelly and Ogilvie, 1972). Damaged
worms were expelled swiftly in irradiated and T. cell reconstituted
rats long before mast cells appeared (Keller and Keist, 1972).
The anti-amine drugs used by some workers (Campbell et al., 1963;
Murray et al., 1971; Rothwell et al., 1971) to prolong infection
with T. spiralis and N. brasiliensis, were found to be immuno-
suppressive (Henney et al., 1972) which might account for the
delay in expulsion (Kelly and Dineen, 1972; Gudson et al., 1972;
Keller and Ogilvie, 1972). The final argument against the "leak
lesion" hypothesis was based on histopathological evidence, where
expulsion occurred prior to a significant increase in mast cells
in the intestinal tissue (Wells, 1962; Whur, 1966; Keller, 1971;
Kelly and Ogilvie, 1972).

As far as N. brasiliensis is concerned, it appears that amines
are secondary in their role in worm damage and expulsion. However,
a definite role for amines in the process of worm expulsion in
T. colubriformis in guinea-pigs has been firmly established
(Rothwell et al., 1971; Rothwell et al., 1974a, b; Jones et al.,
1974).

Preliminary evidence implicated tissue eosinophils in the
second cell-mediated step of worm expulsion. Tissue eosinophils
have been linked with the time of expulsion (Wells, 1962; Hogarth-
Scott and Bingley, 1971; Kelly and Ogilvie, 1972) and during the
migratory stages of helminthic infections (Opie, 1904; Cornwell,
1962; Zaiman et al., 1963).

The current knowledge of the role of eosinophils in immune
response is still limited. It is well established that the specific
action of eosinophils is thymus-dependent (Walls et al., 1971, 1973).
They act through receptors for immune adherence and possibly the
Fc portion of IgG (Kay, 1974). Eosinophils can be attracted to
the site of infection by a cellular response, through the production,
by sensitized lymphocytes, of an "eosinophil chemotactic factor,

E.C.F.", which attracts these granulocytes towards the site of
antigenic stimulation (Cohen and Ward, 1971). There is some
evidence for the existence of a relationship between the distri-
bution of eosinophils and mast cells. Histamine released by
mast cells is chemotactic for eosinophils (Riley, 1959; Archer,
1963, 1965; Fernex, 1968). Also, antibody-antigen complexes can
be chemotactic for eosinophils which phagocytose these particles
on contact (Archer and Bosworth, 1961; Sabesin, 1963; Litt, 1964).
Eosinophils played a definite role in mediating an antibody-
induced damage to schistosomulae (Butterworth et al., 1974, 1975).
Whether the cells cooperating during the finalization of worm
expulsion in these nematode infections, are eosinophils or macro-
phages, is still under investigation. Macrophages are not
directly involved in this last step (Keller and Keist, 1972), but
have an essential role in the early induction of the immune response.

Although complement is not important during the process of worm
elimination in N. brasiliensis (Jones and Ogilvie, 1971), its direct
role in the final stages of expulsion should not be dismissed.

A new mediator of inflammatory reaction has also been impli-
cated in the final stages of the immunological expulsion of
N. brasiliensis. Kelly et al. (1974) suggested that pharmacolo-
gically active products of different tissue cells might have a role
in the final step of worm expulsion. They suggested that lymphokines
and prostaglandins may play a significant role as non-specific
mediators, since they are widely spread in different tissues of the
body, particularly the intestine. They presented evidence that
prostaglandins from rat semen, which had a smooth muscle stimulating
activity, caused expulsion of N. brasiliensis from rats. Prosta-
glandins are unsaturated hydroxy-fatty acids, found in both sexes
and are present in most body tissues (Smith et al., 1974). The
administration of synthetic prostaglandins, particularly of the class
E, was very effective in causing rapid worm expulsion (Dineen et al.,
1974). Potent inhibitors of prostaglandins, such as aspirin and
dextropropoxyphene hydrochloride, prevented the expulsion of
N. brasiliensis when administered daily during the course of
infection (Kelly et al., 1974).

The experiments in this chapter are preliminary investigations

into the role of eosinophils, mast cells and their derivatives and prostaglandins in the process of S. ratti expulsion from rats.

Method for counting tissue eosinophils in intestine:

Intestinal villi of rats, which are tongue-shaped in longitudinal sections, are separated, at their base, by the gland crypt (Reynolds et al., 1967; Nordstrom et al., 1968). The sections of intestines were stained with Carbol Chromotrope. For counting purposes, the villi were divided into "villus-crypt" units (VCU) and the number of eosinophils lying within each villus, between two gland crypts and its lamina propria, were counted (Jarrett et al., 1968; Kelly and Ogilvie, 1972). Ten VCU were used for each standard count and the mean of six standard counts was determined. Tissue eosinophil counts were expressed as the number of cells/10 VCU.

Mesenteric mast cells (see Chapter 2) were counted in 50 randomly selected microscopic fields (x 16) and the percentage of degranulated mast cells was determined.

Total white blood cell (W.B.C.) count:

The freshly collected blood was sucked up to the 0.5 mark on a white cell haemocytometer pipette. This was diluted 1 in 20 with a fluid consisting of the following:

1 ml of glacial acetic acid

99 ml of distilled water

4 drops of toluidine blue

Cell counts were performed in a "bright-line" improved Neubauer haemocytometer.

Blood eosinophil count:

Fresh blood was diluted 1:10 in cold Discombe's fluid.
This consisted of the following:

5 volumes of 1% aqueous Eosin Y

5 volumes of Acetone

90 volumes of distilled water

In both previous counts, the total number of cells in all 8
corner squares was counted and the number of eosinophils or W.B.C.
calculated as follows:

Total number of cells counted x 25 = Number of cells per
cu.mm. of blood.

Experiment 7.1:

In this experiment, levels of eosinophils, in the peripheral
blood and intestinal lamina propria, were compared and the percen-
tage of mast cell degranulation, during the course of initial and
challenge infections with S. ratti, was determined.

No success was achieved with staining intestinal mast cells
using either Astra-Blue or Acid Toluidine Blue with tissues fixed
with Carnoy's or Formol-saline. However, mesenteric mast cells
were easily stainable with Acid Toluidine Blue and were, thus, used
to assess mast cell activity during the course of infection.

Sixty four female Wistar rats were divided into 2 groups. 28
rats were infected with 1000 (± 43) larvae each and 36 rats were
left uninfected. Four rats from both groups were killed on days 8,
14, 18, 23 and 28 PI and the percentage of degranulating mesenteric
mast cells and the number of tissue eosinophils counted. The intes-
tine was divided into anterior and posterior halves and the number
of adult worms determined. The peripheral blood picture was
examined every three days from day 3.

On day 30 PI, the 8 members of the first group were each
reinfected with 1000 (± 56) larvae, simultaneously with 8 rats from
the control group. This left 8 rats to act as uninfected controls.
4 rats were killed from each of these groups on day 5 and 8 post-

Table **21** Exp: **7**.1 Changes in the tissue eosinophil level in the intestinal mucosa

and the percentage of degrannulation of mesenteric mast cells

during and initial and a challenge infection

Days PI	Mean (SE) total number of worms		% of worm development	Mean (SE) total number of eosinophils in 10 V.C.U./rat		% degrannulation of mesenteric mast cells	
				infected	non-infected	infected	non-infected
8	298	(+21.6)	29.8%	108 (+ 8.04)	94.8 (+ 8.49)	31.6	21.7
14	325	(+36.9)	32.5%	139 (+ 18.2)	125 (+ 6.15)	54.5	19.8
18	349	(+13.6)	34.9%	134 (+ 8.72)	88.5 (+ 1.80)	63.3	27.9
23	187	(+12.2)	18.7%	251 (+ 16.4)	71.8 (+ 6.07)	75.5	26.6
28	46.5	(+16.6)	4.6%	194 (+ 6.63)	72.8 (+ 5.95)	52.8	23.1
Post-challenge							
5	38.5	(+6.17)	3.8%	119 (+ 14.2)	72 (+ 2.89)	62.5	21.9
8	18.5	(+ 4.57)	1.8%	248 (+ 13.4)	73 (+ 3.43)	70	22.6

Table **21**   Exp: **7.1**   Changes in the tissue eosinophil level in the intestinal mucosa

and the percentage of degrannulation of mesenteric mast cells

during and initial and a challenge infection

Days PI	Mean (SE) total number of worms		% of worm development	Mean (SE) total number of eosinophils in 10 V.C.U./rat		% degrannulation of mesenteric mast cells	
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28	46.5	(+16.6)	4.6%	194 (+ 6.63)	72.8 (+ 5.95)	52.8	23.1
Post-challenge							
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8	18.5	(+ 4.57)	1.8%	248 (+ 13.4)	73 (+ 3.43)	70	22.6

Figure 25, Exp. 7.1:

Changes in the levels of tissue eosinophils degranulating
mesenteric mast cells and number of adult worms in two
groups of rats.
●────● percentage of adult development in rats
■────■ percentage of degranulating mesenteric mast cells
in infected group
□────□ percentage of degranulating mesenteric mast cells
in non-infected group
▲────▲ number of tissue eosinophils as mean in 10 VCU in
infected group
△────△ number of tissue eosinophils as mean in 10 VCU in
non-infected group.

% worm development

& Mast cell degranulation
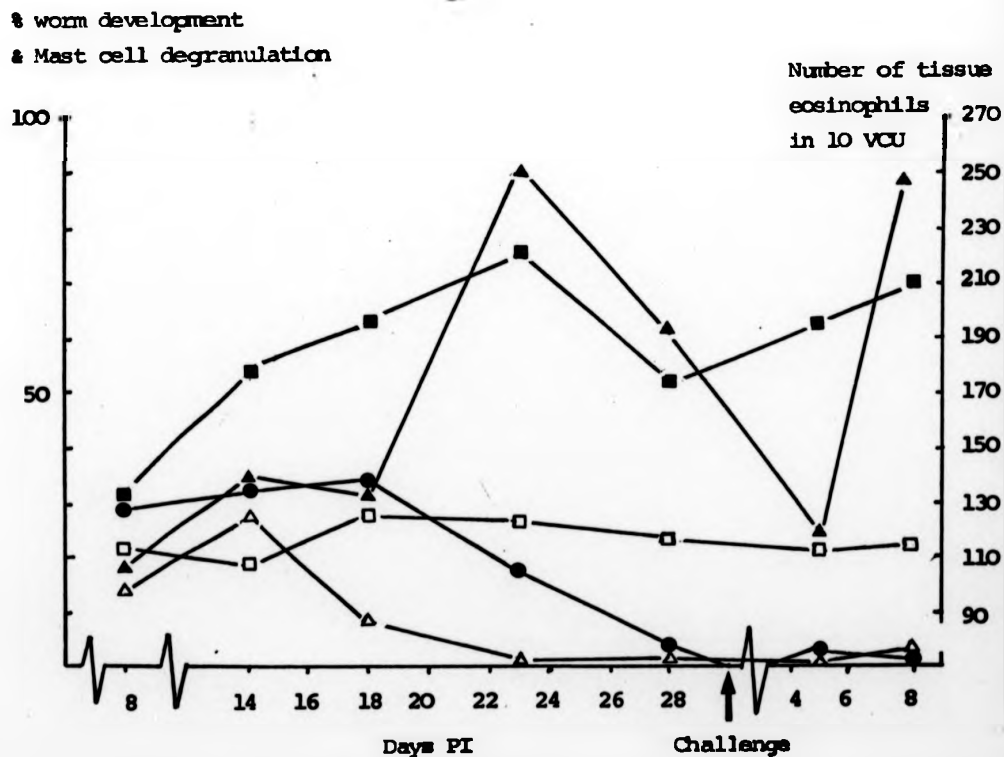
challenge and the levels of peripheral and tissue eosinophils,
mast cell degranulation and adult worms determined.

The results are summarized in Table 21 and Figure 25. There
was an increase in the level of tissue eosinophils from 134, on
day 18 PI, to 251 cells/10 VCU on day 23 PI, which coincided with
the period immediately before worm expulsion. The infiltration
of eosinophils was extensive in both the mucosa and the Peyer's
patches (Plates 48 and 49). Tissue eosinophils showed another
sharp increase to 248 cell/10 VCU on day 8 post-challenge (Plate
50) while the uninfected control (Plate 51) maintained an average
of 85 cells/10 VCU throughout the experiment.

The percentage of degranulating mesenteric mast cells (Plates
52 and 53) increased from day 14 PI and reached a maximum on day 23
PI, after which it dropped slightly. A similar increase was
observed on day 8 post-challenge.

In the initially infected group, there was a sharp rise in the
total W.B.C. count (Figure 26) to 20,000 cells/mm^3 between days 21-
28 PI. The levels of blood eosinophils demonstrated peaks on days
6-12 and 28-34 PI. Following challenge, the total W.B.C. count
rose sharply to 18,000 cells/mm^3, but this was not paralleled by
any rise in the level of blood eosinophils.

Experiment 7.2:

In this experiment, the role of a histamine derivative, on
the course of S. ratti infection, was investigated.

Ten rats were each infected with 1000 ($\pm$ 93) larvae. Histamine
diphosphate (Koch-Light Laboratories Ltd.) was administered by
intubation, twice daily, to 5 rats on days 12, 13, 14 and 15 at
100 mg/kg of body weight, while the other 5 rats were given equal
volumes of saline. All the rats were killed on day 16 PI and the
number of adult worms, in the anterior and posterior intestine,
and larvae/gm of faeces determined.

The results are presented in Table 22 and Figure 27. Rats
which received histamine harboured a mean of 317 worms on day 16,
compared to 575 worms in the untreated group. This difference was

Figure 26, Exp. 7.1:

Blood eosinophilia and total white blood cell count in two groups of rats.

o———o   eosinophil count in infected group.
●———●   eosinophil count in normal rats.
△———△   total W.B.C. count in infected group.
▲———▲   total W.B.C. count in normal rats.

Plate 48 : Section through villus/crypt units showing infiltration
of eosinophils prior to S. ratti expulsion (Lendrum's,
x 250)

Plate 49 : Infiltration of eosinophils within a Peyer's patch
at the end of an initial S. ratti infection
(Lendrum's, x 250)

Plate 48 : Section through villus/crypt units showing infiltration of eosinophils prior to S. ratti expulsion (Lendrum's, x 250)

Plate 49 : Infiltration of eosinophils within a Peyer's patch at the end of an initial S. ratti infection (Lendrum's, x 250)

Plate 48 : Section through villus/crypt units showing infiltration of eosinophils prior to S. ratti expulsion (Lendrum's, x 250)

Plate 49 : Infiltration of eosinophils within a Peyer's patch at the end of an initial S. ratti infection (Lendrum's, x 250)

Plate 50 : Tissue eosinophils in villi of rats challenged with S. ratti (Lendrum's, x 250)

Plate 51 : Eosinophils in a normal uninfected rat (Lendrum's, x 250)

Plate 50 : Tissue eosinophils in villi of rats challenged with
S. ratti (Lendrum's, x 250)

Plate 51 : Eosinophils in a normal uninfected rat (Lendrum's,
x 250)

Plate 52 : Intact mesenteric mast cells (Acid Toluidine Blue, x 400)

Plate 53 : Degranulated mesenteric mast cells (Acid Toluidine Blue, x 400)
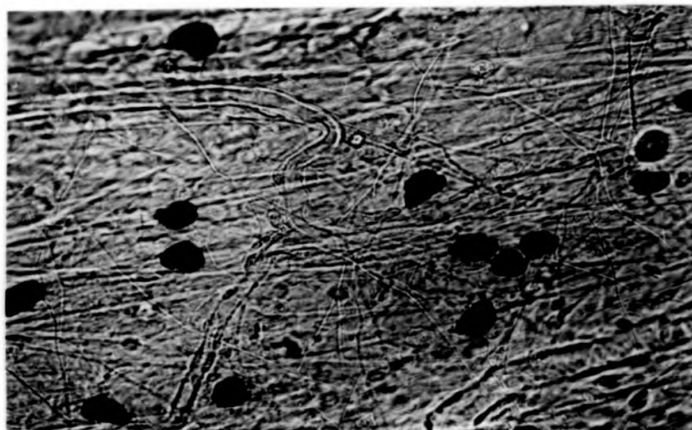
Plate 52 : Intact mesenteric mast cells (Acid Toluidine Blue, x 400)
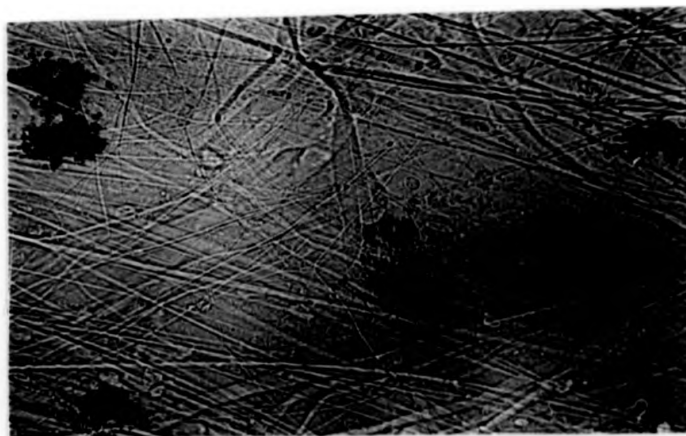
Plate 53 : Degranulated mesenteric mast cells (Acid Toluidine Blue, x 400)

Table **22**   Exp : **7.2:**   Result of autopsy on day 16PI of two

groups: one treated with histamine and

the other a normal infection.

	Infected treated	Infected non-treated
Number of rats	5	4
Total number of worms	317 ($\pm$ 69)	575 ($\pm$ 33)
Mean (SE) number worms in anterior intestine	287 ($\pm$ 61)	535 ($\pm$ 34)
Mean (SE) number worms in posterior intestine	29.6 ($\pm$ 61)	40 ($\pm$ 4.3)
Larvae / gm of faeces	1077 ($\pm$ 64.6)	15,300 ($\pm$ 437)
Fecundity larvae/worm	4.07($\pm$ 0.24)	26.6 ($\pm$ 1.4)
Mean overall length of adult worms	1.81($\pm$ 0.03)	2.77 ($\pm$ 0.02)

Infected treated   vs   Infected non-treated   P $<$ .01

Figure 27, Exp. 7.2:

Effect of histamine treatment on the recovery of adult worms on day 15 PI and their fecundity

■ infected, histamine treated

□ normal infection
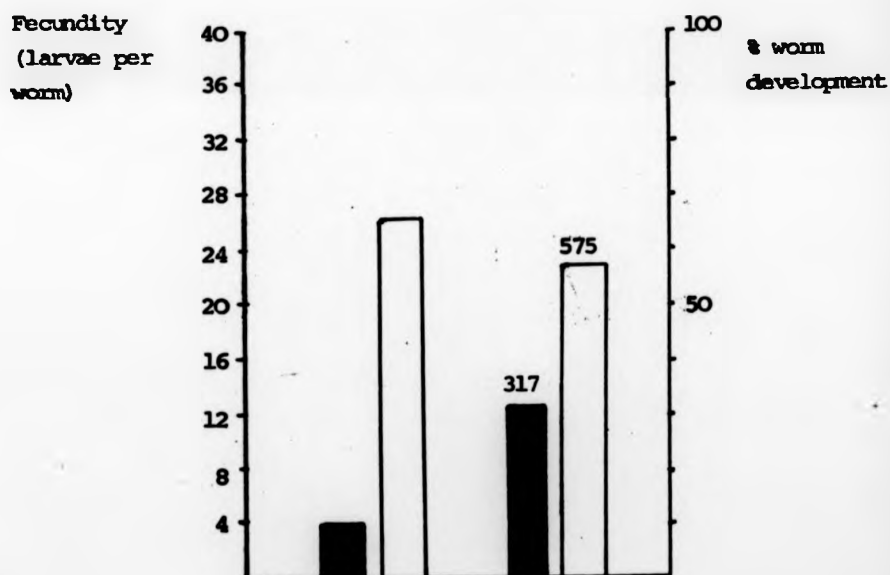
statistically significant. There was no difference in the distri-
bution of adult worms between the two groups. Worms recovered
from histamine treated rats, were stunted and showed diminished
fecundity (4.07 vs 26.6 larvae/worm).

This experiment suggests that histamine may play a part in
the elimination of S. ratti infection from rats, but has no effect
on the distribution of adult worms in the intestine.

Experiment 7.3:

In this experiment, the effect of the prostaglandin inhibitor,
aspirin, on the expulsion of S. ratti, was noted.

Sixteen rats were infected with 1000 (± 69) larvae each.
Aspirin powder B.P. (Acetylsalicylic acid, John Bell and Croydon)
was administered to 8 rats twice daily at 3 mg/kg, per os, for the
entire period of experiment. 8 rats were treated with equal vol-
umes of saline alone, on the same occasions. Faecal cultures were
made on days 14, 22 and 24 PI. Four rats from each group were
autopsied on days 28 and 30 PI and the number of adult worms, in the
anterior and posterior intestine, counted. The results are presented
in Table 23 and Figures 28 and 29.

The aspirin-treated group harboured more adult worms, on day 28
and 30 PI, than the saline-treated group. This indicated that a
relative, but significant, delay in worm expulsion occurred in rats
treated with aspirin. Aspirin also had no effect on the distribu-
tion of worms in the intestine. There were more larvae per gram of
faeces produced by the rats treated with aspirin, on day 24 PI,
than the control group.

This experiment suggests a possible role for prostaglandins
in the final step of S. ratti expulsion, as indicated by the effect
of a potent inhibitor.

Table 23, Exp. 7.3:

Result of autopsies on two different days of two groups:

A: infected and treated twice daily with Aspirin

B: normal, non-treated group

Day	Group	Mean (SE) total number of worms	Mean (SE) number adult worms recovered anterior intestine	posterior intestine
28 PI	A	114 ($\pm$ 52.2)	4 ($\pm$ 1.08)	110 ($\pm$ 52.1)
	B	24.7 ($\pm$ 3.49)	2 ($\pm$ .40)	22.5 ($\pm$ 3.17)
30 PI	A	38.7 ($\pm$ 7.25)	1 ($\pm$ .70)	37.7 ($\pm$ 6.62)
	B	0.25 ($\pm$0.25)	0	0.25 ($\pm$0.25)

Figure 28, Exp. 7.3:

Effect of aspirin on the number of adult worms
■ anterior intestine; ☐ posterior intestine.
A = treated with aspirin, B = treated with saline.

Number of
adult worms
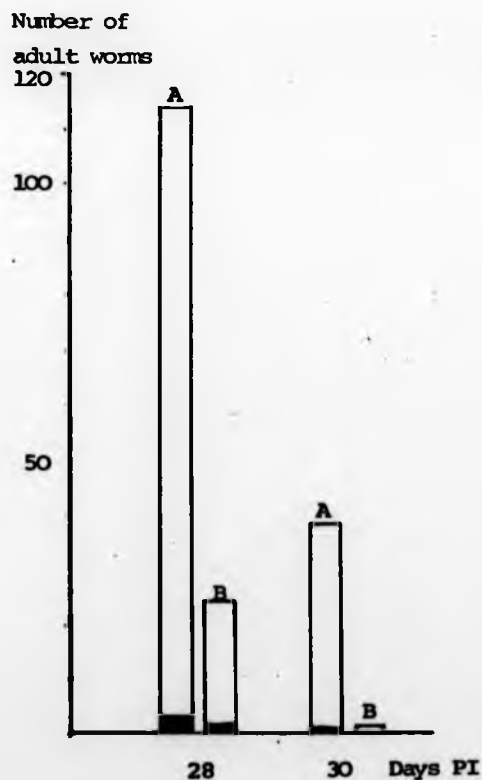
Figure 29, Exp. 7.3:

Effect of aspirin on the level of larval production.
o——o treated with aspirin; ●——● normal control.

Number of
larvae/gm of faeces
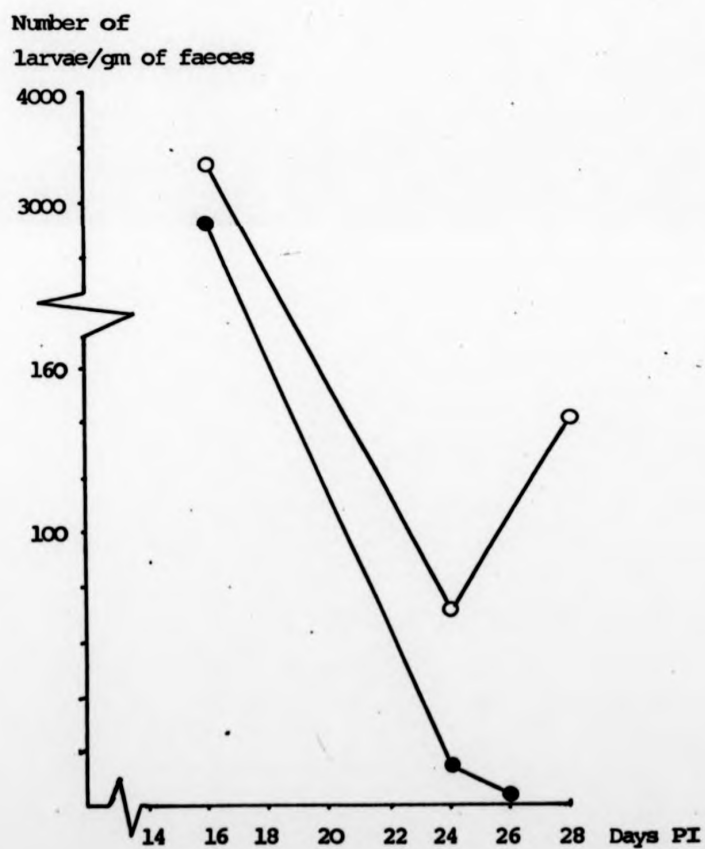
Discussion:

The results of the previous experiments suggest that factors, other than antibodies and cells, might be involved in the expulsion of S. ratti.

The rise in the level of eosinophils in the lamina propria of infected intestines, coincided with the onset of worm expulsion. This also occurred in rats infected with N. brasiliensis (Wells, 1962; Hogarth-Scott and Bingley, 1971; Kelly and Ogilvie, 1972).

Blood eosinophils, on the other hand, increased in two separate peaks before and after the expulsion. This result was in agreement with those obtained by Bailenger and Carcenac (1974) in rats infected with S. ratti. The decline in the number of blood eosinophils can be correlated with the rise in tissue eosinophils.

The activities and properties of eosinophils and their involvement in immune reactions are not fully elucidated (Review by Archer, 1970). Eosinophils are associated with cellular immune reactions and, thus, can be expected to appear in the intestinal tissue during the second step of worm expulsion.

Eosinophils are attracted to the site of inflammation by a variety of factors (see introduction, this chapter). The duration of life of blood eosinophils is brief and they can leave the circulation very rapidly (Archer, 1970) and it is generally known that there are 4-5,000 tissue eosinophils to each circulating one (Delanie and Garraty, 1975).

The presence of eosinophils near the site of infection, particularly close to the time of expulsion, might indicate that the cooperation between antibodies and cells can be substantiated by the activities of eosinophils. Drastic alteration of the intestinal environment, through the production of pharmacologically active substances by eosinophils or other myeloid cells (e.g. mast cells) can lead to the evacuation of worms. However, the chronological order of this reaction is still not determined.

Direct reaction between eosinophils and worms in vivo has not been demonstrated. However, sensitised eosinophils to N. brasiliensis or T. spiralis readily adhere to these worms in vitro, particularly to their anterior ends (McKenzie, 1976,

personal communication). The significance of this reaction in
vivo is yet to be evaluated.

The rise in the W.B.C. count during the period of worm
expulsion was interesting, but no definite interpretation can be
offered except for the possible involvement of other types of
leucocytes in this response. Normal rats have a high percentage
of lymphocytes in their peripheral blood (about 75-80%) (Cameron
and Watson, 1949; also see Table 26 in Chapter 8). The signifi-
cance of this, as far as worm expulsion is concerned, is still
unclear.

No explanation can be offered regarding the lack of success
in staining the intestinal mast cells, which was unfortunate, since
this might have shed direct light on their role during worm expul-
sion. The level of degranulation of the perivascular mesenteric
mast cells might be of relevance to the activities of these cells
during the course of infection. These cells are close to the site
o f infection and are supplied and drained by the same vascular net-
work as the intestine. Mesenteric mast cells showed an increased
level of degranulation at the onset of worm expulsion and following
a challenge infection. This degranulation might be due to the
sensitization of these cells through the circulation of antibodies
and worm antigens within the mesentries and the production of mast
cell degranulating factor (Uvnas and Wold, 1967) by the worms.

The administration of histamine, intragastrically, to rats
infected with S. ratti, suggested that this amine might be involved
in the process of worm elimination. The worms, which were recovered
from histamine-treated rats, developed signs of early damage.
Whether histamine attracted homocytotropic antibodies (Hogarth-Scott
and Bingley, 1971), which in turn caused morphological changes and
diminished fecundity, is still doubtful.

The role played by histamine, in worm expulsion, in vivo, has
been shown in guinea-pigs infected with T. colubriformis. Infusion
of histamine and 5-hydroxytryptamine directly in the intestine caused
rapid expulsion of these worms (Rothwell et al., 1974a). Injection
of reserpine, an antagonist of 5-HT, caused a delay in the expulsion
of T. colubriformis (Rothwell et al., 1974b). Bailenger and Faraggi
(1975b), using reserpine, prevented the expulsion of S. ratti from
rats and maintained the infection at a high level, but they attributed

this delay to a state of hypercorticosteronaemia (Bailenger and Faraggi, 1975a).

When infected rats were treated with the analgesic drug, aspirin, the expulsion of S. ratti, which usually occurs on day 25 PI, was delayed for 5 days. The faecal larval count was higher in the treated group than in the controls.

Aspirin, which is a potent inhibitor of prostaglandins, both in vivo and in vitro (Vane, 1971; Aiken, 1972; Smith and Temple, 1973), caused a delay in the expulsion of N. brasiliensis and prevented the drop in the egg production (Dineen et al., 1974).

Results obtained by the use of antagonists and inhibitors to determine the role of pharmacologically active substances, have been treated with reservations (Ogilvie and Jones, 1973). Some amine antagonists, which caused delay in worm expulsion, had anti-lymphocytic activities (Henney et al., 1972; Kelly and Dineen, 1972b). However, Dineen et al. (1974) showed that administration of aspirin to rats infected with N. brasiliensis had no suppressive effects on their lymphocytic activity.

Recent studies on the role of prostaglandins in the expulsion of N. brasiliensis suggested that they were capable of causing rapid expulsion associated with a reduction in the egg/gm counts (Dineen et al., 1974; Kelly et al., 1974).

Prostaglandins interfere with both humoral and cellular reactions (Piper and Vane, 1969; Søndergaard and Greaves, 1970). In the intestine, where they are found in the tissue and fluids, they affect peristalsis (Bennet, 1972). It was suggested that prosta-glandins also contribute towards environmental changes in the gut which makes it difficult for the worms to survive (Dineen et al., 1974).

The trigger for the increase in the level of prostaglandins in the intestine, during expulsion, is still not known. However, it has been suggested, recently, that eosinophils might be involved in the production of these substances (Kay, 1976). If this was ascertained in nematode infections, the interaction between these two factors, during the period of expulsion, could be better understood.

The clinical significance of aspirin treatment to individuals infected with S. stercoralis, and possibly other nematode infections, should be investigated.

The present study presented a preliminary evidence that tissue eosinophils, histamine and prostaglandins might play some role in the process of S. ratti expulsion from rats.

CHAPTER 8

Studies on the effect of Betamethasone on the course
of S. ratti infections in rats

Corticosteroids have a remarkable, and as yet ill-defined,
capacity to immunosuppress and act as anti-inflammatory agents
(Allison, 1965). Their inhibitory action on the nonspecific
inflammatory response in a wide range of diseases is often bene-
ficial. They are also useful in assisting with the control of
allografts. However, they can be very harmful due to their non-
specific action on the adoptive immunological mechanisms which
are necessary for the natural defence against viral, bacterial
and parasitic infections and which lead to opportunistic infections.

Corticosteroids have a variety of effects on delayed hyper-
sensitivity (DH) (Medawar, 1969). These include their action on
the number of circulating lymphocytes and monocytes (Boggs et al.,
1964; Thompson and van Furth, 1970), their effect on vascular
activity, which causes the inhibition of diapedesis of the dif-
ferent cellular components of the inflammatory reaction (Ashton
and Cook, 1952; Ebert, 1952), their inhibitory action on lympho-
cytopoiesis through their involutionary and cytotoxic effect
(Gyllenstein, 1962; Humphrey and White, 1970) and their inhibitory
action on the activities of macrophages (Gabrielson and Good, 1966),
mast cells (Cavallero and Braccini, 1950, 1951) and eosinophils
(Archer, 1956). Casey and McCall (1971) working with BCG, demon-
strated the suppressive effects of corticosteroids on DH, both in
vivo and in vitro. They concluded that their effects were on the
afferent (adaptation) stage of DH rather than the efferent
(expression) stage. Corticosteroids also affect antibody response
during the presence of antigen (Darrach, 1959; Berglund, 1962).

Corticosteroids have been widely used in a variety of studies
on resistance to helminthic infections, e.g. to study the effects
of immunosuppression on initial and subsequent infections in those
host-parasite relationships characterized by a strong immune

reaction against the parasite (Stoner and Godwin, 1953, 1954; Weinstein, 1953, 1955; Coker, 1954, 1955, 1956a, b; Markel and Lewis, 1957; Markel, 1958; Campbell and Collette, 1962; Campbell, 1963; Ogilvie, 1965; Tewari, 1970; Wakelin, 1970; Hopkins et al., 1972; Moss, 1972). Also to introduce parasites into naturally resistant hosts (Roman, 1956; Bezubik, 1960; Cross, 1960, 1963; Harley and Gallicchio, 1970) to inhibit the action of the immune response of hosts immunized with antigens from worms (Avlavidou, 1970) and to induce a state of immunological tolerance (Wakelin and Selby, 1974; Behnke, 1975). Corticosteroids depress the immune response to parasites, allowing them to persist in the host beyond the normal period.

The persistence of adult worms in animals treated with cortisone has been reported in many different nematode (Weinstein, 1953, 1955; Ogilvie, 1965; Stoner and Goodwin, 1953, 1954; Coker, 1955, 1956; Markell and Lewis, 1957; Markell, 1958; Cross, 1960; Campbell and Collette, 1962; Campbell, 1963, 1968; Wakelin, 1970, 1974; Avlavidou, 1970), trematode (Tewari, 1970) and cestode infections (Hopkins et al., 1972; Moss, 1972), and this was usually accompanied by a rise in the egg or larval production.

Corticosteroid treatment suppressed acquired immunity following an initial infection with N. brasiliensis and a second infection was successfully established (Ogilvie, 1965). Mice treated with cortisone acetate during a primary infection with T. muris did not expel the developing larvae within the usual 3 weeks (Campbell, 1963; Wakelin, 1967) and the immune response against a challenge infection was suppressed (Wakelin, 1970). Prolonged cortisone treatment of rats infected with T. spiralis suppressed the acquisition of immunity to a primary infection and rendered immunised animals non-immune to a challenge infection (Coker, 1956a; Markell and Lewis, 1957).

Wakelin and Selby (1974) presented the only available purpose-designed experiments to induce a state of immunological tolerance in mice to the nematode worm T. muris. Mice which were given cortisone acetate during the second week of T. muris infection were rendered tolerant for a period of up to 7 weeks. They concluded that this was not due to small doses of the antigen but rather to

reaction against the parasite (Stoner and Godwin, 1953, 1954;
Weinstein, 1953, 1955; Coker, 1954, 1955, 1956a, b; Markel and
Lewis, 1957; Markel, 1958; Campbell and Collette, 1962; Campbell,
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that this was not due to small doses of the antigen but rather to

the immunosuppressive action of the drug during strategic points
of infection when the immune system is about to recognise the
antigenic materials present. This treatment stopped the usual
process of worm elimination even when the drug was only used 4
times during the course of infection. However, Olivier (1962)
working with Taenia taeniaformis in mice obtained a long-lasting
tolerance to this parasite following a few cortisone injections.

Human strongyloidiasis has always been thought of as innocuous,
but incidences of overwhelming infections have been reported (Brown
and Perna, 1958; Wilson and Thompson, 1964; Toh and Chow, 1969;
Rivera et al., 1970; Behar, 1970, 1971; Huggins, 1971a, b; Adam
et al., 1973). These infections produced enteritis (De Paolo et
al., 1962; Louisy and Barton, 1971; Berkmen and Rabinowitz, 1972),
necrotic jejunitis (Craven et al., 1971), peritonitis (Olurin,
1970), ulcerative colitis (de Campos, 1964), paralytic ileus
(Nolasco and Africa, 1936; Frengley and Trewby, 1973) and occasional
pulmonary (Poltra, 1972) and skin involvements (Thomas et al., 1971).
Many reports have shown that corticosteroid therapy had grave reper-
cussions on people infected with S. stercoralis. The most common
cases of overwhelming human infection occur when patients with a
non-patent or a very mild S. stercoralis infection are treated with
corticosteroids. They develop severe and often fatal infection
(Rogers and Nelson, 1966; Cruz et al., 1966; Civantos and Robinson,
1969; Yim et al., 1970; Fagundes et al., 1971; Cookson et al., 1972;
Bitoun et al., 1972; Fowles, 1973; Neefe et al., 1973). Willis and
Nwokolo (1966) recognised the dangers associated with steroid
therapy unless patients were S. stercoralis-free and warned medical
workers to be aware of this phenomenon. Such treatment, probably
causes a shift in the equilibrium between the parasite and the host
in favour of the parasite which leads to auto-infection and increased
worm burden. They may penetrate and damage many organs of the body,
and ultimately lead to the death of the host. This was also true
in individuals inflicted with diseases characterised by depressed
cell-mediated immunity, e.g. Leprosy and Tuberculosis (Purtillo et
al., 1974).

The effects of corticosteroids on experimental S. ratti infec-
tions were, therefore, relevant to the clinical problem and to the

understanding of the role of the immune system in preventing auto-infection. The effects of Betamethasone on the course of initial and subsequent infections are reported in this chapter.

Administration of corticosteroids:

Betsolan (Betamethasone sodium phosphate, Glaxo) was administered intraperitoneally. Betsolan is a veterinary form of Betnesol used for the treatment of human conditions such as asthma, hay fever, rheumatoid arthritis, inflammatory skin disorders and other allergies (Mims, 1975). The rats were injected with an initial dose of 2 mg of Betamethasone (20 mg/kg body weight) which was followed by 1 mg (10 mg/kg) every two days for the whole period of the experiment, unless otherwise stated.

Administration of antibiotics:

In all experiments where corticosteroids were employed, 1000 units of penicillin (Triplopen, Glaxo) in 0.1 ml plus 2.5 mg of streptomycin in similar volume, both diluted in sterile distilled water, were administered intramuscularly on the same days as steroid treatment to avoid any opportunistic bacterial infections.

Experiment 8.1:

In this experiment, the effect of Betamethasone on the course of an initial infection was investigated. Eighty 100 gm rats were infected with 1000 ($\pm$ 90) L3 each and then divided into 4 groups of 20. Group 1 received an initial dose of 2 mg of Betamethasone two days before infection, followed by 1 mg every two days for the duration of the experiment. Group 2 was first treated with Betamethasone 9 days PI followed by 1 mg every two days until the end of the experiment. Group 3 was first treated 25 days PI after

which time it received similar treatment to groups 1 and 2. Group 4 was not given steroid treatment. Faecal cultures were prepared on days 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, and 50 PI. Four rats from each group were autopsied on days 10, 17, 24 and 31 PI and the number of worms counted and their lengths measured. The weight of the rats in all groups was monitored during the course of the experiment.

The results obtained are presented in Table 24. Larval production (Figure 30) in the groups which were treated with Betamethasone, from 2 days before and 9 after infection, was very low on day 8 PI. However, the number increased after day 22 PI and persisted with occasional fluctuations. The remaining rats in group 1 died on day 43 PI, with infection in the lungs possibly due to the effects of the drug. The larval production in group 2 increased to a very high peak on day 46 PI (15,800 L3/gm of faeces) and they died on days 51 and 52 PI.

Throughout the experiment, the number of adults recovered (Figure 31) from rats treated with Betamethasone on days -2 and 9 PI was lower than the untreated control. The mean number of adult worms was 98 in group 1 on day 10 PI and 147 in group 2, while the control rats harboured a mean of 430 on the same day. Group 3 before Betamethasone treatment also had a mean number of 404 worms on day 10. On day 24 PI, the control untreated group 4 had only 11 worms in the gut and group 3 had a mean of 12 worms while the corticosteroid-treated rats of groups 1 and 2 harboured 93 and 87 worms per rat, respectively.

When rats in group 3 were treated with Betamethasone on day 25 PI, they showed an immediate rise in the larval output on day 29 PI from 133 to 1970 L3s/gm of faeces. The larval production continued until day 43 PI, though on a lower level.

The number of adult worms in group 3 increased after the administration of Betamethasone on day 25 from a mean of 12 on day 24 to 77.7 on day 31.

The length of worms recovered (Figure 32) from groups 1 and 2 did not alter throughout the period of treatment while those of the untreated control group 4 followed the trend observed previously (see Chapter 3) and decreased in length after day 17 PI. The length of the worms recovered from group 3 after treatment increased from

Table 24 Exp. 8.1:

Effects of Betamethasone treatment on the course of initial infection

with S. ratti. A: Mean (SE) number of adult worms, B: Mean (SE) worm length (in mm.).

Days PI	Group 1		Group 2		Group 3		Group 4	
	A	B	A	B	A	B	A	B
10	98 (±10)	2.83 (±0.03)	147 (±23.7)	2.88 (±0.02)	404 (±11.6)	2.79 (±0.03)	430 (±19.3)	2.86 (±0.03)
17	77.7 (±9.55)	2.81 (±0.03)	78 (±9.6)	2.79 (±0.04)	254 (±20.8)	2.2 (±0.04)	311 (±15.1)	2.03 (±0.05)
24	93 (±19.5)	2.77 (±0.03)	87 (±14.8)	2.71 (±0.04)	12 (±1.8)	0.9 (±0.06)	11 (±1.03)	1.01 (±0.05)
31	93.5 (±7.3)	2.63 (±0.04)	57.7 (±15)	2.78 (±0.03)	77.7 (±13)	2.81 (±0.02)	1.5 (±0.64)	0.8 (±0.1)

Figure 30, Exp. 8.1:

Effect of Betamethasone on the level of larval production during an initial infection with S. ratti. ■——■ treated from day -2; □----□ treated from day 9 PI; o——o treated from day 25 PI; ●——● infected, not treated.

Larvae/gm of faeces

Larvae/gm of faeces

Larvae/gm of faeces

Days PI
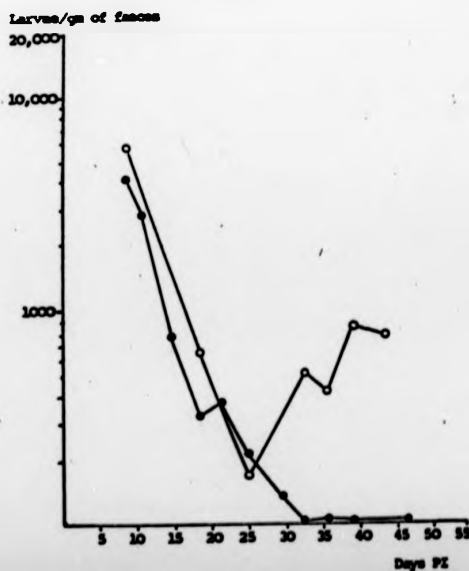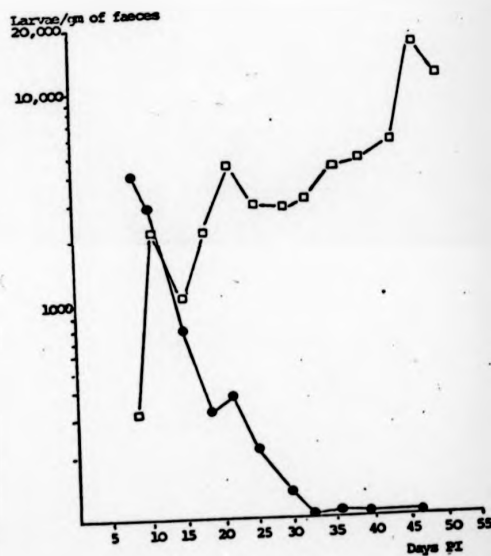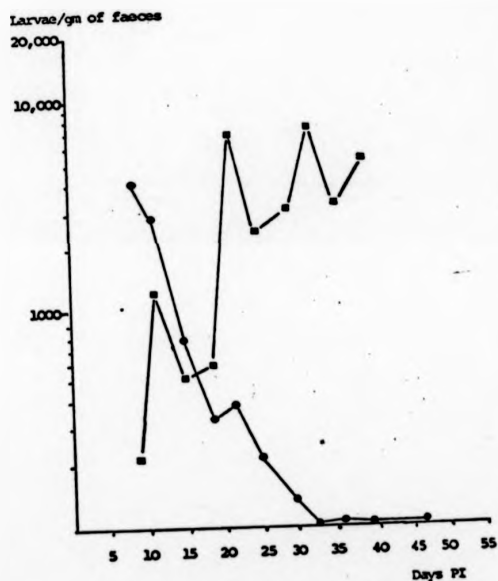
Figure 31, Exp. 8.1:

Number of adult S. ratti in four groups of rats:
■——■ treated with Betamethasone from day -2; □——□
treated from day 9 PI;   o----o   treated from day 23 PI;
●——● infected, not treated.

Mean number of
adult worms

Days PI
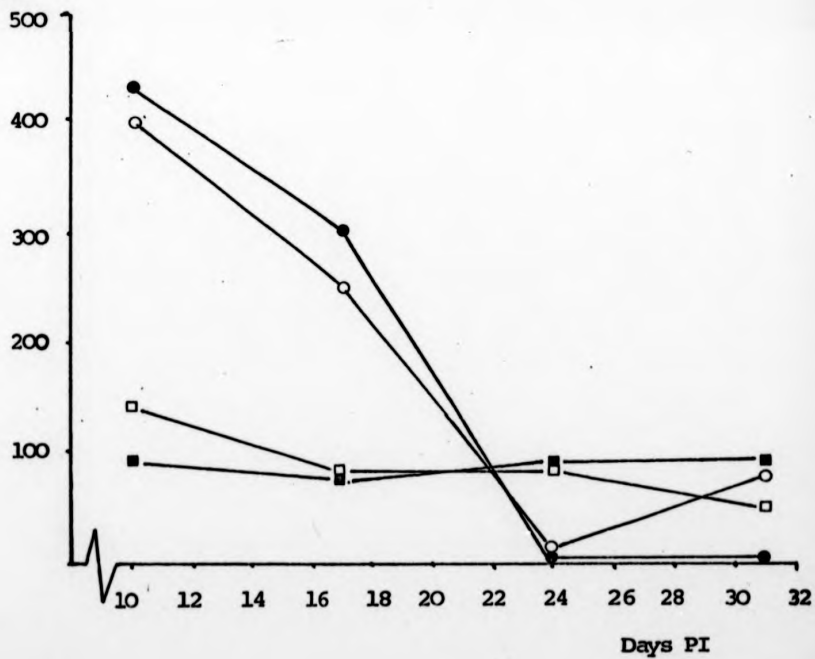
Figure 32, Exp. 8.1:

Changes in the length of adult worms recovered from four groups of rats. ■—■ treated from day -2; □——□ treated with Betamethasone from day 9 PI; o——o treated from day 23 PI; ●——● infected, not treated.
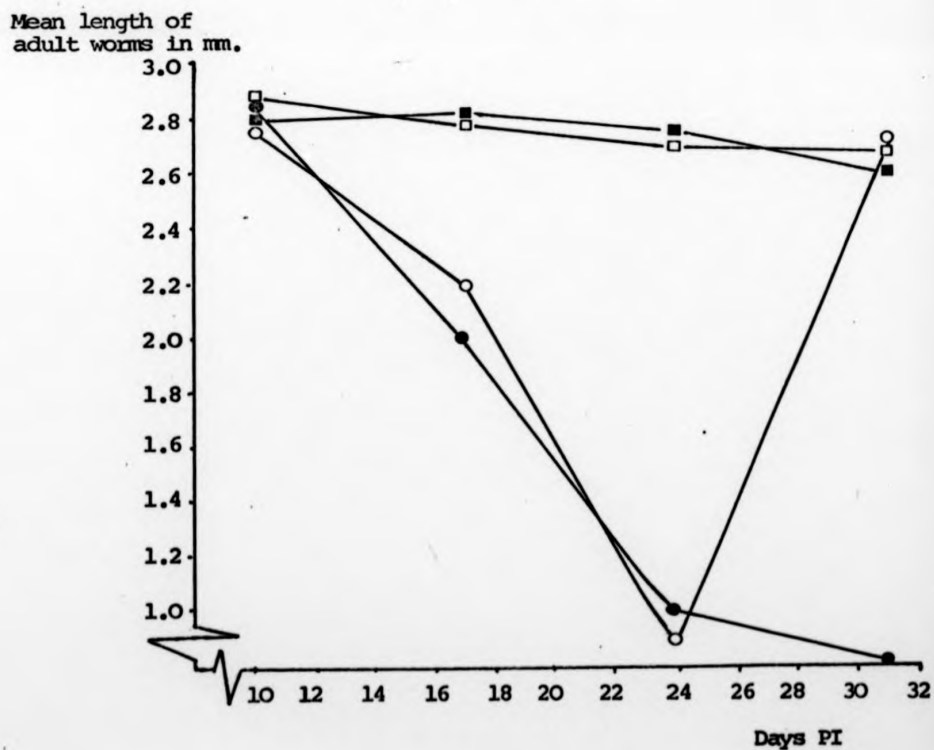
Figure 33, Exp. 8.1:

Changes in the body weight observed in 4 groups of rats. ■,□,○ treated with Betamethasone; ●—● no treatment control; ↓ day Betamethasone treatment commenced.
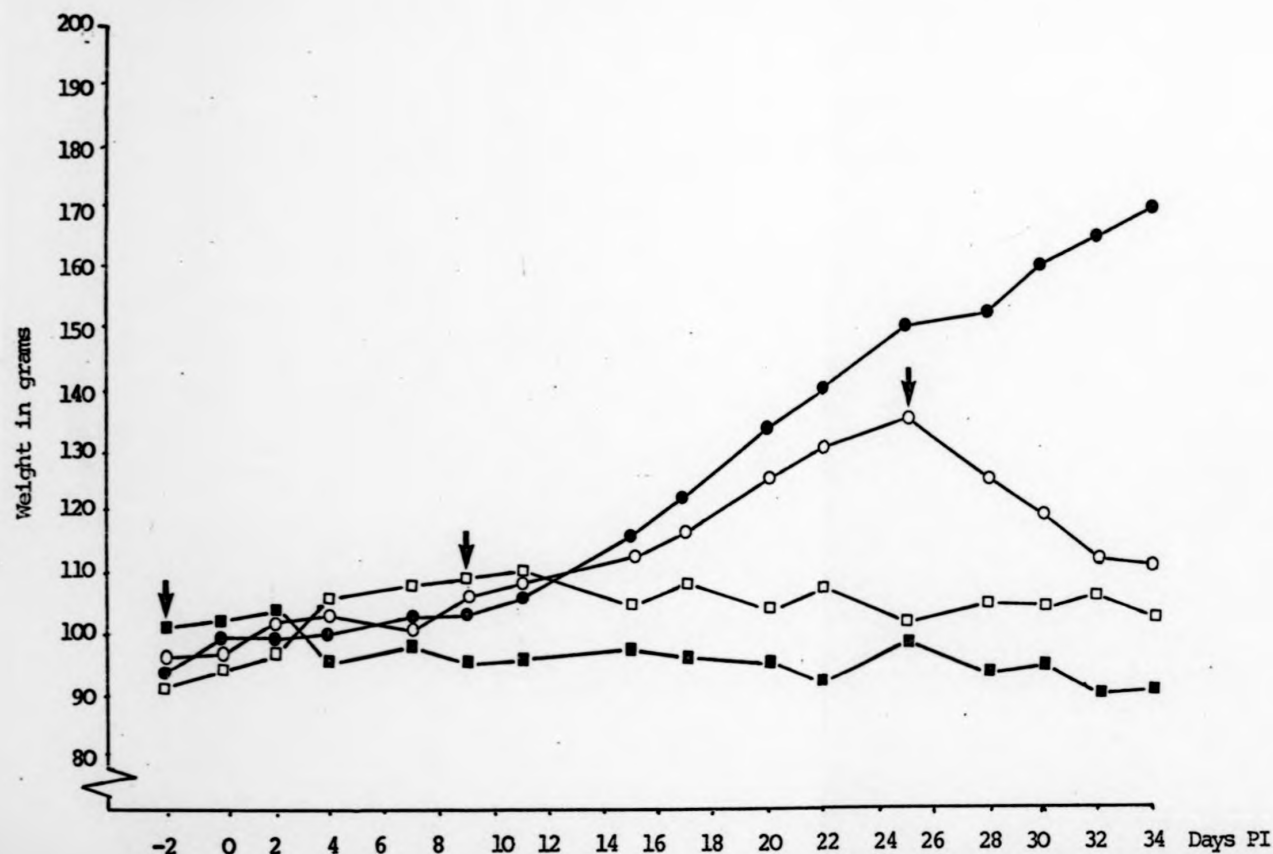
0.9 mm on day 24 PI to 2.81 mm on day 31.

Corticosteroid treatment resulted in weight loss in all the treated groups (Figure 33). The spleens and mesenteric lymph nodes were also reduced in size. The walls of the small intestine in the treated animals became more translucent and less active and no Peyer's patches were seen. Most of the treated rats developed a diarrhoea and when they died they had patches of pus in their lungs.

Experiment 8.2:

This experiment was designed to repeat the previous experiment with special attention being paid to the distribution of the worms in the intestine in groups treated with corticosteroids before, early and late during infection, as well as the changes in the blood picture during such treatment.

Sixty four rats were infected on the same day with 1000 ($\pm$ 78) L3/rat and were separated into 4 equal groups. Group 1 was treated with corticosteroids two days prior to infection and every two days until the end of experiment. Group 2 commenced similar treatment on day 9 and group 3 on day 23 PI. Faecal cultures were made from all groups on days 5, 7, 10, 17, 21, 25, 28, 31, 35 and 74. Autopsies of four rats from each group were performed on days 15, 23 and 33 PI. The intestine of these rats was divided into anterior and posterior halves to examine the distribution of these worms. In group 1, the larval production (Figure 34) was steady for the first 31 days but increased to over 4000 L3s/gm on day 35 PI and persisted up to 74 days when it reached a maximum of 5800 L3/gm. In group 2, larval output was also steady, although it stayed higher than group 1. The larval production also extended until day 74 as long as the treatment with the immunosuppressant was continued. The larval production in group 3 after being treated with Betamethasone on day 23 PI, increased gradually from 330 L3/gm on day 21 to 840 on day 25 PI and reached a maximum of 3200 on day 74 PI. The number of adult worms (Table 25 and Figure 35) in the three treated groups did not correspond to the increase in the larval

Figure 34, Exp. 8.2:

Effect of Betamethasone on the level of larval production during an initial infection with S. ratti. ■——■ treated from day -2; □——□ treated from day 9 PI; o——o treated from day 23 PI; ●——● infected not treated.
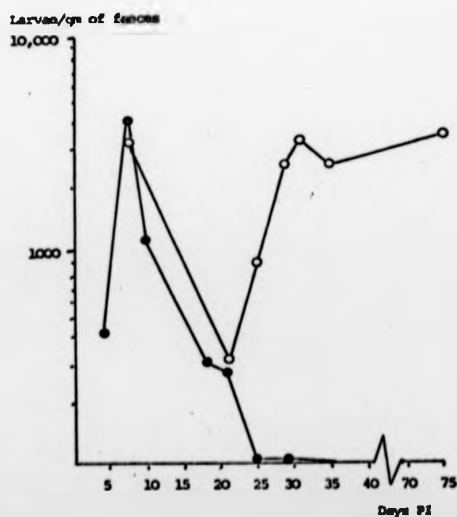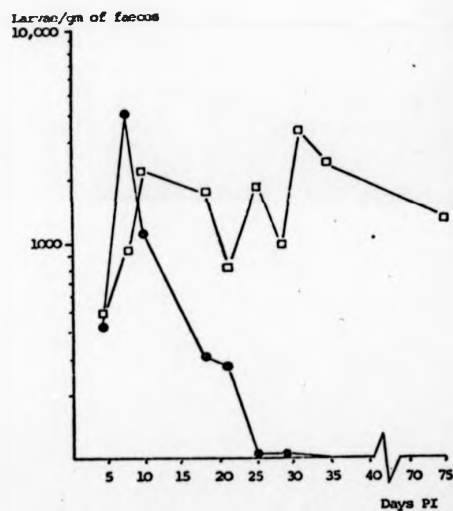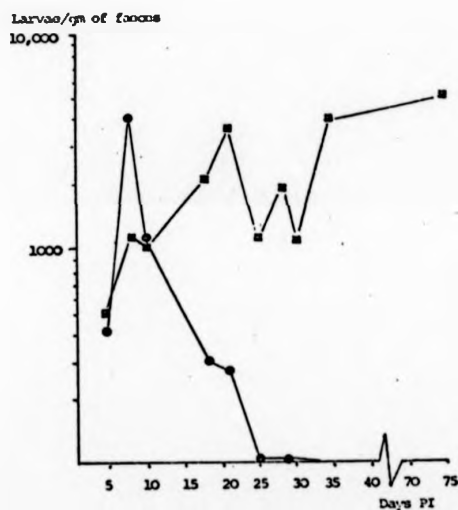
Table 25 Exp. 8.2:

Effect of Betamethasone on the recovery and distribution of adult worms

in four groups of rats infected with S. ratti

	Group 1			Group 2			Group 3			Group 4		
	Day 15	Day 23	Day 33	Day 15	Day 23	Day 33	Day 15	Day 23	Day 33	Day 15	Day 23	Day 33
Mean (SE) total number of adult worms	127 (±32)	94.7 (±27)	141 (±30)	103 (±22)	105 (±11)	130 (±36)	322 (±13)	116 (±19)	275 (±79)	355 (±25)	100 (±6.6)	0.25 (±0.25)
Mean (SE) number of worms in anterior intestine	122 (±20)	91.7 (±26)	134 (±28)	99 (±20)	102 (±10)	122 (±32)	274 (±19)	1.75 (±0.8)	3.5 (±1.5)	342 (±26)	2.5 (±0.8)	0 –
Mean (SE) number of worms in posterior intestine	4.75 (±1.9)	3 (±1.1)	6.75 (±2.7)	4.25 (±2.3)	3.5 (±1.3)	7.25 (±4)	22.7 (±2.8)	114 (±18)	271 (±78)	13 (±2.1)	97.5 (±6.7)	0.25 (±0.25)
Mean (SE) length of adult worms (mm)	2.81 (±0.03)	2.77 (±0.05)	2.63 (±0.05)	2.75 (±0.04)	2.51 (±0.04)	2.53 (±0.06)	2.5 (±0.04)	1.28 (±0.05)	2.81 (±0.03)	2.48 (±0.05)	1.33 (±0.05)	–

Figure 35, Exp. 8.2:

Recovery of adult S. ratti from the anterior ▮ and posterior ☐ intestines of rats treated with betamethasone: Group 1: commenced treatment on day -2; Group 2: commenced treatment on day 9 PI; Group 3: on day 23 PI; Group 4: not treated.

Number of
adult worms
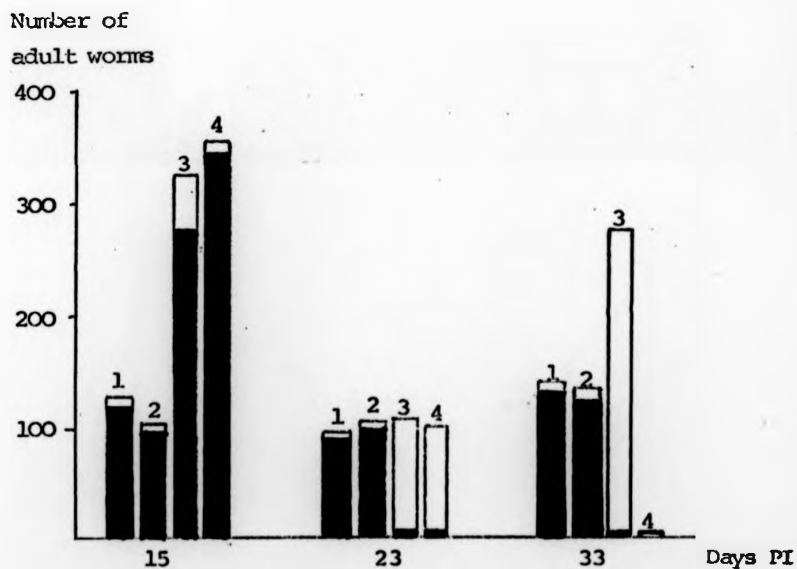
production. The number of adult worms in groups 1 and 2 were 127 on day 15, 94.7 on day 23 and 141 on day 33 PI. Rats in group 2 harboured a mean of 103, 105 and 130 worms on days 15, 23 and 33 PI, respectively. The distribution of the worms was constant in both groups 1 and 2, concentrating in the anterior half of the intestine throughout the period of corticosteroid treatment. The worms migrated from the anterior segment of the intestine (where they were mainly found on day 15 PI) in groups 3 and 4 to the posterior half as shown on day 23 PI. However, after group 3 was treated with Betamethasone on day 23 PI, the number of adult worms increased from 116 on day 23 to 275 on day 33 PI when the normal infection control group harboured a mean of 0.25 worms. The majority of the adult worms in group 3, on day 33, were found to occupy the last half of the intestine unlike the worm population in groups 1 and 2 on the same day. The distribution of the strings of eggs, which are laid by the females in the mucosa of the intestine (see Chapter 1) followed that of the adult worms particularly in the Betamethasone-treated groups. The worms in groups 1 and 2 did not shrink during the entire period of treatment, while those in groups 3 and 4 showed signs of such morphological damage on day 23 PI. However, the worms obtained on day 33 PI from group 3 had normal lengths (mean 2.81 mm). This again indicated that an auto-infection cycle might have been the reason for the rise in the number of worms and the change in their lengths following treatment with Betamethasone on day 23 PI.

The total white blood cell counts (Figure 36) were very low in groups 1 and 2 throughout the corticosteroid treatment. The WBC count in group 3 and 4 increased sharply on day 23 PI (prior to worm expulsion). However, the count dropped in group 3 after treatment with Betamethasone on day 23, and in group 4 on day 33 PI following the termination of the initial infection.

The differential white blood cell count (Table 26) on day 15 PI in all the groups, revealed that Betamethasone had a suppressive effect on lymphocytes and eosinophils, but there was no significant difference in the percentage of neutrophils and monocytes in all the four groups.

This experiment demonstrated that treatment with corticosteroids caused a delay in expulsion of S. ratti worms for as long as 49 days

Figure 36, Exp. 8.2:

Effect of Betamethasone treatment on the W.B.C. count in four groups of infected rats. ■——■ treatment commenced on day -2; □——□ treatment commenced on day 9 PI; o——o treatment commenced on day 23 PI; ●——● no Betamethasone treatment.
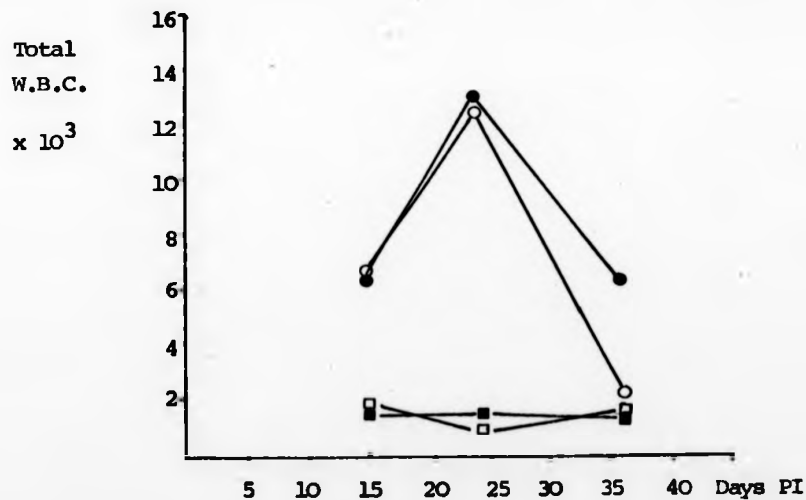
Table 26, Exp. 8.2:

Effect of Betamethasone, on day 15 of an initial S. ratti infection,

on the differential white blood cell (W.B.C.) count

Groups: (day treatment commenced)	% lymphocytes	% monocytes	% neutrophils	% eosinophils
1 (-2)	0	0	16	0
2 (9 PI)	0	0	18	0
3 (25 PI)	73	1	17	1
4 (No treatment)	75	1	16	1

when administered before or after infection. It also demon-
strated that Betamethasone can cause a rise in the number of
worms suggestive of auto-infection. The movement of worms from
the anterior to the posterior half of the small intestine during
the course of infection was inhibited by Betamethasone.

Experiment 8.3:

This experiment was designed to study the effect of ceasing
immunosuppressive treatment on the restoration of the immune
reaction in infected rats. It also investigated the effects of
Betamethasone on the level of tissue eosinophils and on the degree
of mesenteric mastocytosis. Fourty eight rats were divided into
three groups of 16 rats each, and infected with 1000 ($\pm$ 77) L3 each.
Another four rats of the same age were left as normal uninfected
controls. Group 1 was treated with Betamethasone every two days
commencing on day 0 until the end of the experiment while group 2
had similar treatment which was terminated on day 24 PI. Group 3
was left untreated. Four rats were killed from groups 1, 2 and 3
on days 10, 20, 25 and 32 PI and the number of adult worms counted.
The number of tissue eosinophils and the level of mastocytosis was
determined on day 25 PI when the four normal uninfected rats were
killed. The mean number of adult worms (Table 27 and Figure 37)
in both groups 1 and 2, during the period of Betamethasone treatment
was 208 and 85 on day 10 and 134 and 138 on day 20 PI. The control
group harboured 422 and 236 worms on the same days. One day after
the withdrawal of the treatment from group 2, the worm burden was
not different from that of group 1 (128 in group 2 and 101 in
group 1), while the control group had a mean of 10 worms. However,
the rats in group 2 harboured only 20 worms by day 32 PI, while
group 1, which was receiving Betamethasone continuously, maintained
the number of worms at 117. The normal infected, untreated, con-
trol group 3 had a mean of 0.25 worms on the same day. The number
of tissue eosinophils (Figure 38) was quantified in a similar manner
to that described in Chapter 8, i.e., dividing the intestinal villi
into villus-crypt units and counting the number of eosinophils

Table 27 Exp. 8.3:

Effect of the withdrawal of Betamethasone on the course
of initial infection with S. ratti

Mean (SE) number of adult worms

Groups	Day 10	Day 20	Day 25	Day 32
1	208 ($\pm$38)	134 ($\pm$31)	101 ($\pm$11)	117 ($\pm$24)
2	85 ($\pm$17)	138 ($\pm$31)	128 ($\pm$19)	19.5 ($\pm$8)
3	422 ($\pm$21)	236 ($\pm$25)	10 ($\pm$1.9)	0.25 ($\pm$0.25)

Figure 37, Exp. 8.3:

Number of adult worms in three groups of infected rats.
■——■ treated with Betamethasone until day 32 PI; o——o
treated until day 24 PI; ●——● untreated control.
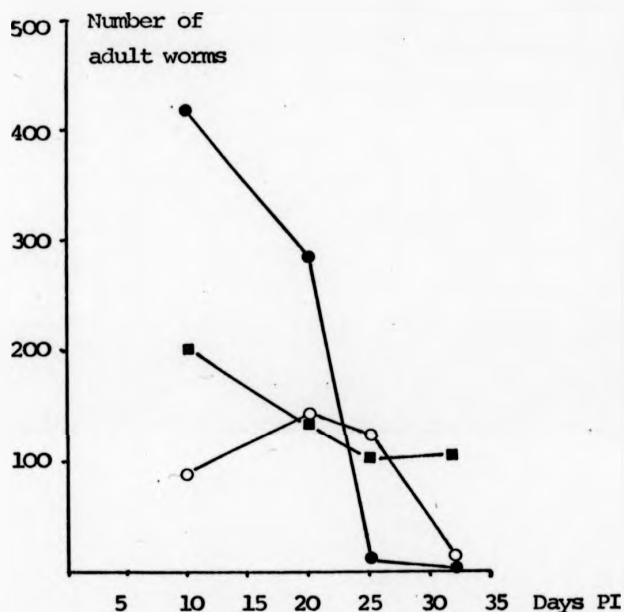
Figure 38, Exp. 8.3:

Effect of Betamethasone on the number of tissue eosinophils and
percentage of mesenteric mast cell degranulation, on day 25 PI.
■ infected, treated with Betamethasone; ▓ infected non-
treated; ☐ normal uninfected, untreated rats.
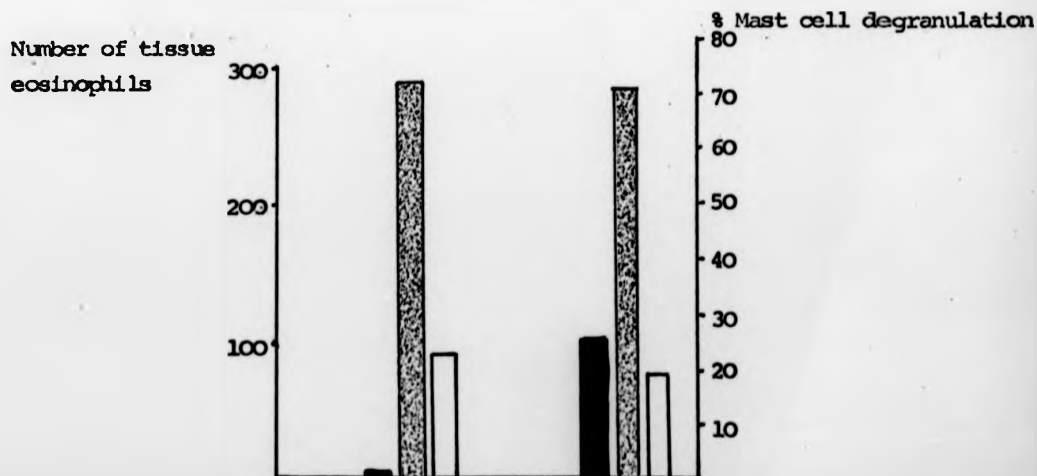
within 10 units. The rats of group 1 which were treated with Betamethasone and examined on day 25 PI, showed a mean of 0.88 tissue eosinophil per 10 VCU (Plate 54). The normal infection controls had a mean of 290 eosinophils/10 VCU and the normal uninfected rats had a mean of 92 eosinophils/10 VCU on the same day (see Plates 50 and 51, Chapter 8). No immune reaction was observed around sections of adults, larvae or embryonated eggs in the intestines of treated rats (Plate 55).

The rats of group 1 had a mean percentage of 26% degranulating mesenteric mast cells, while rats in group 3 showed a higher degree of degranulation on the same day. The normal rats of group 4 had a mean of 19% degranulating mast cells in the mesenteries on the same day (Figure 37). Macroscopically, the mesenteric lymph nodes of rats treated with Betamethasone (Plates 56 and 57) were capable of restoring their shape and size after the termination of treatment and sections of such nodes were similar to those of the untreated controls and contained numerous lymphocytes and precursor cells (Plates 58 and 59).

Experiment 8.4:

This experiment investigated the effect of corticosteroid treatment on a challenge infection into previously infected rats which had received Betamethasone after they had expelled their worm burden.

Thirty two rats were each infected with 1000 ($\pm$ 85) L3 each and then divided into 4 groups of 8. Group 1 received 2 mg Betamethasone every two days commencing on the day of infection. Group 2 received 4 mg of Betamethasone on days 28, 29, and 30 PI. Group 3 received 4 mg of Betamethasone on days 29 and 30 PI and on day 3 post-challenge (see later). Group 4 received no Betamethasone. A fifth group of 4 rats was left uninfected and untreated to act as challenge control. Four rats from the first four groups were killed on day 13 PI. All five groups were treated with a single dose of 200 mg/kg of TBZ on day 26 PI and challenged with 1000 ($\pm$ 97) L3 each on day 30. The rest of the rats in all
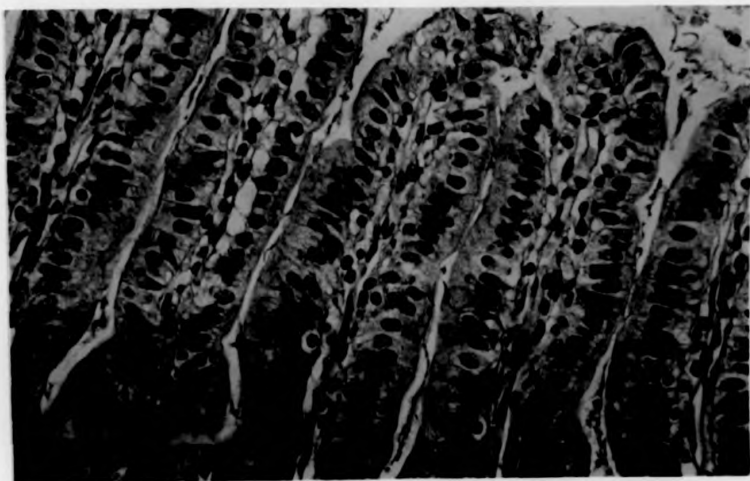
Plate 54 : Section through villi of rats treated with
Betamethasone; note the absence of eosinophils
(Lendrum's, x 250)
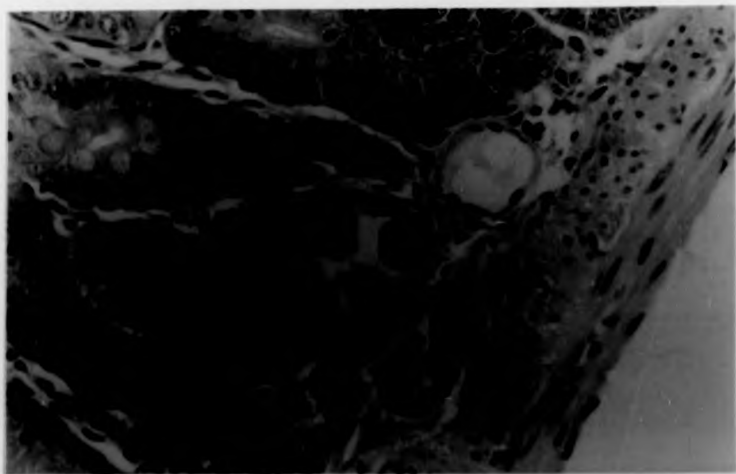
Plate 55 : Embryonated eggs embedded in the crypts of intestinal
mucosa; note lack of reaction (Lendrum's, x 250)
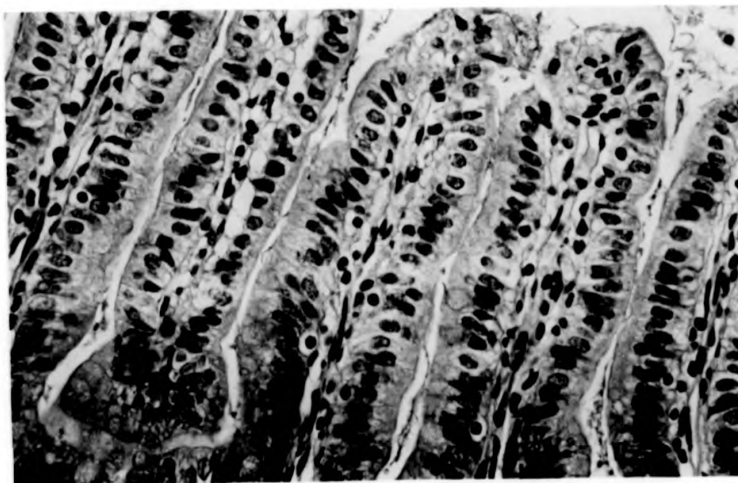
Plate 54 : Section through villi of rats treated with
Betamethasone; note the absence of eosinophils
(Lendrum's, x 250)
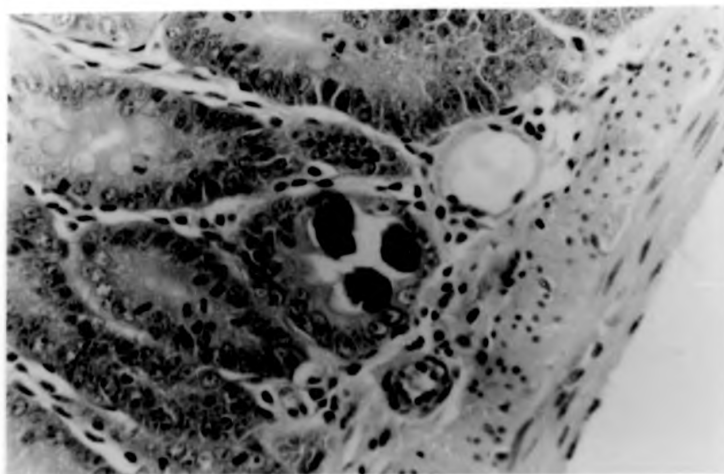
Plate 55 : Embryonated eggs embedded in the crypts of intestinal
mucosa; note lack of reaction (Lendrum's, x 250)
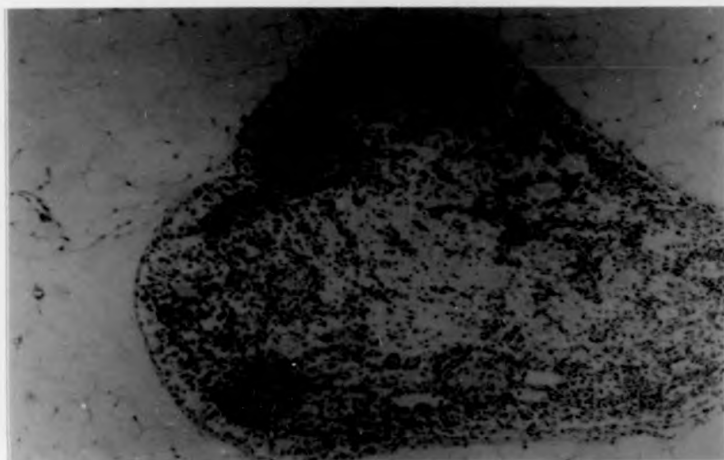
Plate 56 : Mesenteric lymph node treated with Betamethasone
(H.& E., x 80)
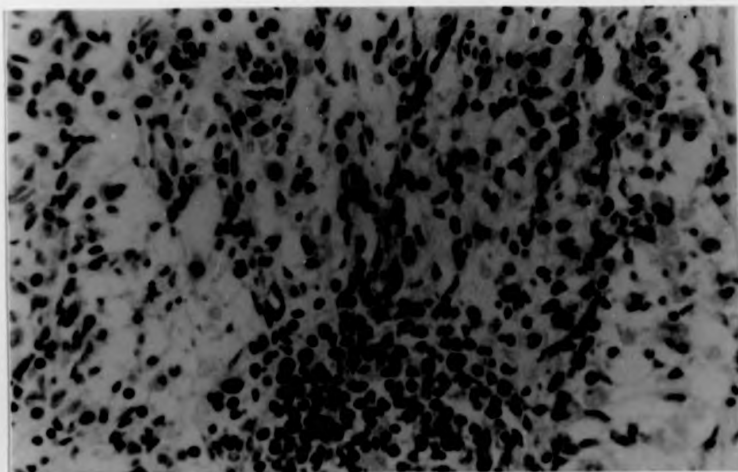
Plate 57 : Types of cells present in MLN treated with
Betamethasone (H.& E., x 250)
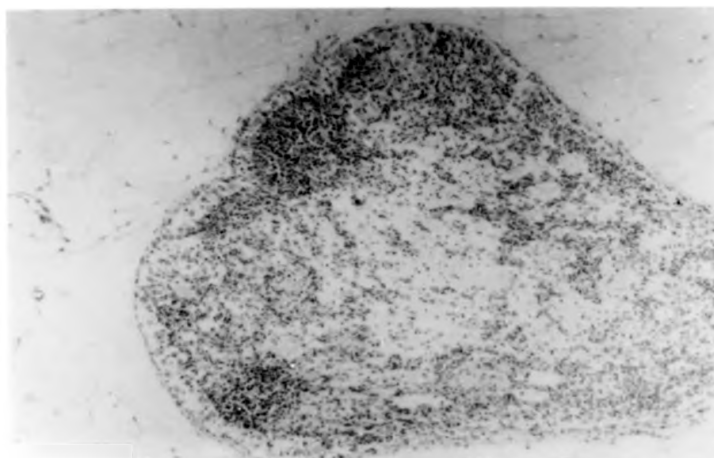
Plate 56 : Mesenteric lymph node treated with Betamethasone (H.& E., x 80)
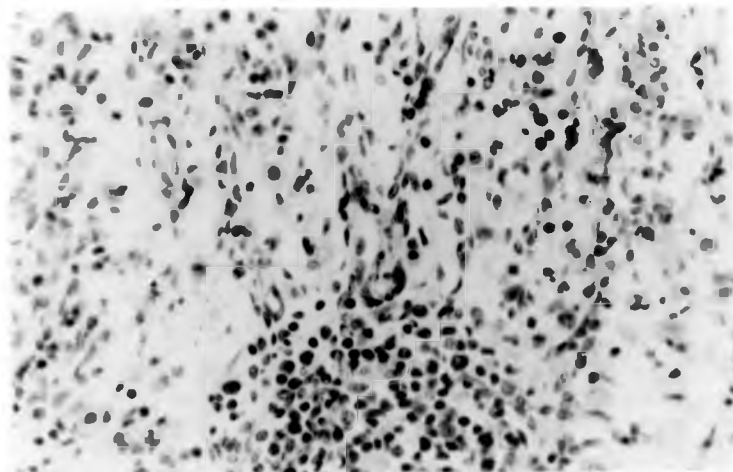
Plate 57 : Types of cells present in MLN treated with Betamethasone (H.& E., x 250)
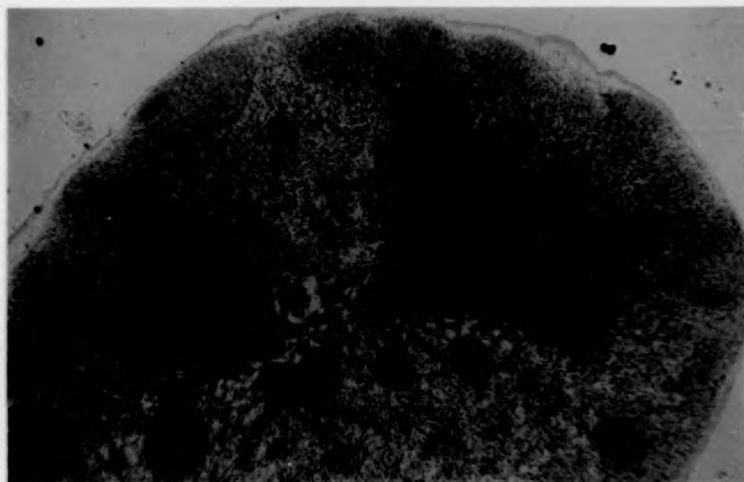
Plate 58 : MLN from a non-treated control rat (H.& E., x 30)
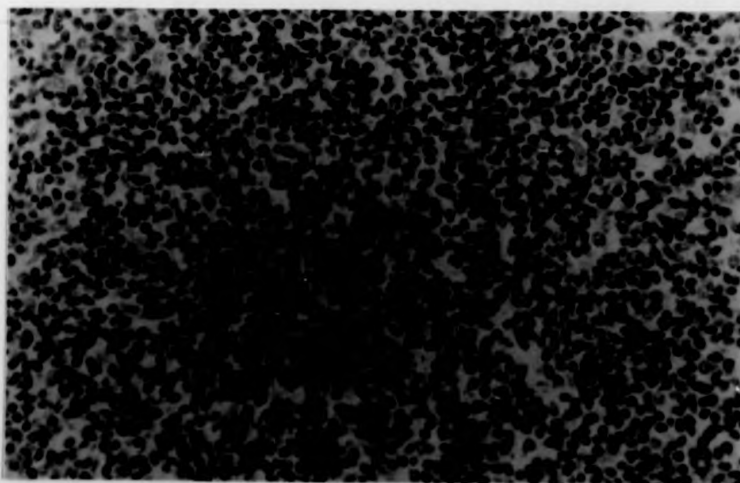
Plate 59 : Types of cells present in normal MLN (H.& E., x 250)
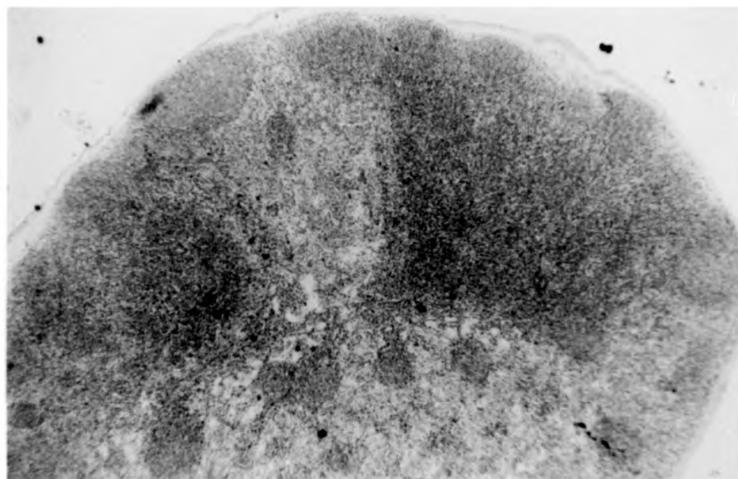
Plate 58 : MLN from a non-treated control rat (H.& E., x 30)
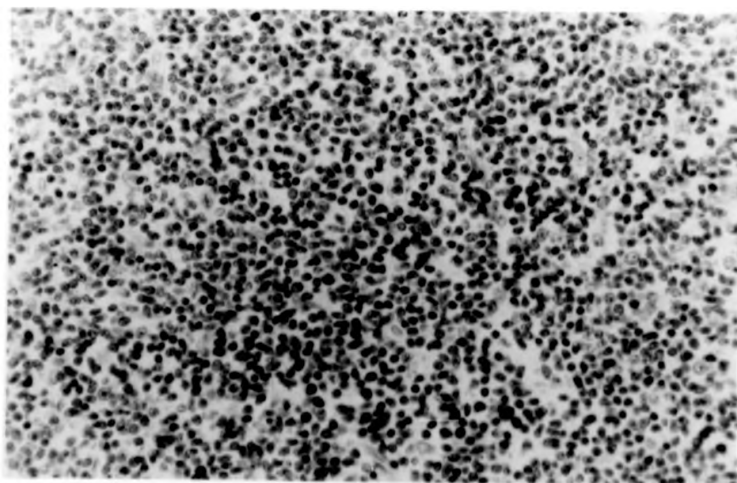
Plate 59 : Types of cells present in normal MLN (H.& E., x 250)

groups were autopsied on day 13 post-challenge. In both autopsies, the number of adult worms in the anterior and posterior small intestine was counted and their lengths measured. Faecal cultures from all groups were made on day 13 post-challenge. The results are presented in Table 28).

On day 13 PI, the rats of group 1 (i.e. continuous Betamethasone treated) had the lowest adult count (mean 128) while groups 2, 3 and 4 had mean totals of 406, 410 and 433, respectively. Worms in all groups were concentrated in the anterior small intestine.

Following challenge, 145 worms were recovered from group 1 and these were distributed anteriorly. The mean length of the adult worms was 2.9 mm, and their fecundity was very high at 26.3 larvae/worm. The rats of group 2 had a mean total of 19.5 worms, the majority of which were occupying the posterior half of the gut. The worms were stunted with a mean length of 1.8 mm and showed a very low fecundity rate (2.6 larvae/worm). The rats in group 3, which received corticosteroids on days 29, 30 PI and day 3 post-challenge harboured a mean of 388 worms of normal (2.8 mm) length. Their larval production was high with a fecundity rate of 19.7 larvae/worm. No adults were recovered from group 4 on day 13 post-challenge while 373 worms were collected from the challenge control group 5, mostly in the anterior intestine.

This experiment demonstrated that continuous treatment of Betamethasone during initial infection completely suppressed the development of any acquired resistance to reinfection. Corticosteroid treatment when introduced on three consecutive days prior to the introduction of the challenge dose was unable to destroy the immune memory against an initial infection with the parasite. However, the drug was effective in suppressing the manifestation of acquired immunity when introduced on two consecutive days before the secondary infection followed by another dose after the challenge.

Experiment 8.5:

This experiment attempted to induce the delay of expulsion of S. ratti worms by treatment with corticosteroids at different times

Table 28, Exp. 8.4: Effect of Betamethasone treatment on the course of initial
and second infections with <u>S. ratti</u>, on days 13 PI (A) and
13 post-challenge (B)

	Group 1		Group 2		Group 3		Group 4		Group 5	
	A	B	A	B	A	B	A	B	A	B
Mean (SE) total number of adults	128 (±27.6)	144 (±13.4)	406 (±26)	19.5 (±8.4)	410 (±18.6)	388 (±60.2)	433 (±19.5)	O	O	373 (±20)
Mean (SE) number in anterior half	122 (±26)	141 (±13)	398 (±26)	2.25 (±2)	401 (±18)	301 (±35.6)	429 (±20.8)	O	O	363 (±19)
Mean (SE) number in posterior half	5 (±1.8)	3 (±0.7)	8 (±1.5)	17.2 (±6.5)	9 (±2)	87.2 (±60.3)	9.5 (±2.1)	O	O	10 (±2.3)
Mean (SE) length of adults (mm)	N.D.	2.9 (±0.03)	N.D.	1.8 (±0.04)	N.D.	2.8 (±0.03)	N.D.	O	O	2.8 (±0.03)
Mean (SE) number of larvae/gm	N.D.	3821 (±231)	N.D.	51 (±8)	N.D.	4380 (±371)	N.D.	O	O	3906 (±216)
Fecundity (larvae/worm)	N.D.	26.3	N.D.	2.6	N.D.	19.7	N.D.	O	O	21

during the course of an initial infection. Thirty eight rats were infected on the same day with 1000 ($\pm$ 53) L3/rat and were divided into four groups:

  Group 1: Ten rats received 2 mg Betamethasone every two days from the day of infection until day 23 PI. Group 2: Ten rats received 4 mg of Betamethasone on days 4, 6, 8 and 10 PI. Group 3: Ten rats received 4 mg Betamethasone on days 6, 10, 14 and 18 PI. Group 4: Eight rats were left untreated as controls.

  Half of each group was killed on days 30 and 35 PI and the number of adult worms was counted. The results are presented in Table 29.

  Very few worms were recovered from the control group 4, on day 30 PI, and no worms were recovered from this group on day 35. Rats in group 1 had a mean worm burden of 50 on day 30 and this number had dropped to 7.8 worms on day 35 PI. The rats in group 2, which were treated with Betamethasone on days 4, 6, 8 and 10 harboured a mean of 597 on day 30 PI and 537 on day 35. The members of group 3 harboured a statistically significant mean of 225 worms on day 30 PI, but the number dropped sharply to 16 on day 35.

  This experiment demonstrated that treatment with Betamethasone on days 4, 6, 8 and 10 delayed the action of expulsion of adult S. ratti worms up to day 35 PI. Betamethasone injected every two days for a period of 23 days did not induce similar delays in worm expulsion, and when introduced on days 6, 10, 14 and 18 only resulted in a partial delay of worm elimination.

Discussion:

  The experiments in this chapter have demonstrated that cortico-steroids have an immunodepressive effect on the course of S. ratti infection. If the drug was used early during infection there was no increase in the number of the adult females in the gut, in fact, worm recovery was usually very low. This might be attributed to different factors: The action of Betamethasone on the mucosa of the gut was severe, whether introduced IP or IM. The villi of

Table 29   Exp: 8.5   Effect of betamethasone treatment when administered at specific times during primary infection

	Group 1		Group 2		Group 3		Group 4	
	Day 30 PI	Day 35 PI	Day 30	Day 35	Day 30	Day 35	Day 30	Day 35
	91	3	470	551	169	14	4	0
	78	1	721	671	351	7	0	0
Number of	17	15	660	451	200	23	31	0
adult worms	23	12	501	533	215	20	12	0
in individual	41	8	636	481	191	16	-	-
rats								
Mean (SE)	50($\pm$14.7)	7.8($\pm$ 2.6)	597($\pm$48)	537($\pm$37.8)	225($\pm$32.3)	16($\pm$2.7)	11.7($\pm$6.8)	0

treated rats were much smaller than those of normal controls.
It is also possible that the immunodepressive action of
Betamethasone allowed multiplication of bacteria in the gut and
that they adversely affected S. ratti. Treated rats had con-
tinuous diarrhoea or loose faeces. It was hoped that infected
rats treated with immunodepressive drugs, would allow the parasite
to undergo auto-infection and, thus, harbour more adult worms than
the original inoculum, as seen in human cases of infected immuno-
depressed individuals (Rogers and Nelson, 1966; Cruz et al., 1966;
Willis and Nwokolo, 1966; Civantos and Robinson, 1969; Yim et al.,
1970; Fagundes et al., 1971; Bitoun et al., 1972; Cookson et al.,
1972; Neefe et al., 1973; Purtillo et al., 1974). This did not
occur when treatment was begun early during infection. However,
there was an increase in the number of adult worms when rats were
treated around the time of worm expulsion. These worms were fertile
and not stunted. Since it was shown that the changes in the morpho-
logy of adult S. ratti during the final stages of an initial infec-
tion were irreversible (see Chapter 4), this increase in number and
size of adult worms indicated that corticosteroid treatment caused
auto-infection. It was not clear whether the same treatment in
groups 1 and 2 had caused the same cycle, due to the low number of
worms recovered.

Adult S. ratti persisted in rats treated with Betamethasone for
as long as the treatment continued. Enhancement of worm survival in
animals was obtained following cortisone treatment in other nematode
(Weinstein, 1953, 1955; Ogilvie, 1965; Stoner and Goodwin, 1953,
1954; Coker, 1955, 1956; Markell and Lewis, 1957; Markell, 1958;
Cross, 1960; Campbell and Collette, 1962; Campbell, 1963, 1968;
Wakelin, 1970, 1974; Avlavidou, 1970; Behnke, 1975   ), trematode
(Tewari, 1970) and cestode infections (Hopkins et al., 1972; Moss,
1972). Similar results were obtained using more specific methods of
immunosuppression such as neonatal thymectomy, irradiation, anti-
lymphocyte serum and thoracic duct drainage (Ogilvie and Jones, 1967;
Dineen and Adams, 1970; Hopkins et al., 1972; Di Netta et al., 1972).

The precise mechanisms of such immune suppression are still
undetermined, but it seems that both arms of the immune response
are affected. It has been suggested that T. cells, which have been
shown to be involved in worm expulsion (see Chapter 6), are resistant

to cortisone (Cohen et al., 1970; Cohen and Claman, 1971). However, North (1972) suggested that a population of T. cells were sensitive to the suppressive effects of cortisone. The action of Betamethasone on S. ratti may be aimed at such a population and possibly at the extra factors e.g. eosinophils and pharmacollogically active substances (see Chapter 7), which might play some role in worm expulsion.

Betamethasone treatment inhibited the posterior movement of the worms which occurs normally during the course of an initial infection (see Chapter 3) and most adult worms in treated rats were found in the anterior intestine. In group 3 of experiment 8.2, the recovery of a majority of normal, fertile, adult worms from the posterior small intestine following treatment on day 23 might be attributed to the fact that auto-infection took place within the intestine and not by migration to the lung as classically known (Faust and De Groat, 1940). It is possible that when the immune status of the host is low, intestinal rhabditiform larvae moult twice in the lumen and penetrate the gut mucosa, where they undergo two more moults and develop into adult females. It is known that in S. ratti no moulting occurs in the lung (Wertheim, 1965), which suggests that the pulmonary stage is not critical. This hypothesis does not reject the possibility of pulmonary migration during auto-infection but offers an explanation for the unexpected recovery of new, normal , adult worms in the pre-expulsion site normally occupied by damaged worms. These experiments have also shown that structural and morphological damage of worms was not genotypic but phenotypic since all the worms which developed following treatment with Betsolan at the terminal period of an infection, were fertile, and normal in size.

The recovery of rats after the withdrawal of corticosteroid treatment was fast. Within 8 days of the cessation of treatment, rats were able to expel the majority of the worms from their guts and restore the shape and size of their lymph nodes. Restoration of immunity and abrupt expulsion of worms was also observed in other helminthic infections, following the withdrawal of cortisone treatment (Coker, 1955; Ogilvie, 1965; Hopkins et al., 1972).

Continuous treatment with Betamethasone during an initial

infection with S. ratti suppressed all acquired resistance to reinfection. However, treatment was necessary prior to and after the introduction of the challenging dose, in rats untreated previously, to eliminate the immunological memory and suppress the immune response against a second infection. Wakelin (1970) showed that immunological unresponsiveness to T. muris could be achieved if cortisone was given at the time where the immune system was set to recognize the antigenic stimulus produced by the worms. It was later possible to achieve immune tolerance by subjecting infected mice to four injections of cortisone acetate on days 8, 10, 12 and 14 PI, or by giving a single large dose on day 11 PI (Wakelin and Selby, 1974).

Behnke (1975b) obtained long-term survival of Aspicularis tetraptera in mice given a short term hydrocortisone treatment. He suggested that this treatment brought about a state of tolerance, due to the suppression of the action of recognition, by the host, of the antigens released during the cryptic-phase (Behnke, 1975a) of infection. Olivier (1962) working with Taenia taeniformis in mice obtained a long-lasting tolerance to this parasite following few cortisone injections.

In experiment 8.5, the number of adult worms on day 35 was still high in the group treated with Betamethasone on days 4, 6, 8 and 10 PI rather than days 6, 10, 14 and 18 PI. This is not a final indication of immune tolerance in this group but could give a pro-visional indication that corticosteroid treatment on days 4, 6, 8 and 10 only can paralyse the normal immune response against S. ratti and render the rats unresponsive for a period of 35 days, at least.

There is still a wide field of research to be explored concern-ing the effects of immune suppression in this host-parasite system, particularly as concerns the mechanisms involved in the cortisone-induced auto-infection. Apart from chemical suppression, the role of the specific suppression of T. and B. cells using thymectomy and whole body irradiation, as well as anti-lymphocyte or anti-thymocyte serum, requires investigation.

CHAPTER 9

Summary and Conclusions

The experiments carried out during this project can be classified under four major headings:

1. Those which studied parasitological aspects of acquired immunity during primary, secondary or multiple infections.
2. Those which studied the effects of the immune response on the adult worm population.
3. Those which considered some of the mechanisms of worm expulsion.
4. Those which investigated the effects of immunosuppressive treatment on the course of primary and secondary infections.

Following a primary infection, rats developed strong resistance to S. ratti which manifested itself by a sharp drop in larval production after day 14 PI and a mass expulsion of the parthenogenetic adult females, although a few adult worms persisted for a period as a residual population. The worms also migrated towards the distal half of the small intestine, before expulsion.

Following a challenge infection, S. ratti was expelled from the gut within 10 days and immediately before expulsion, the worms occupied the posterior half of the intestine.

Resistance to reinfection with S. ratti was maintained in rats for a period of 3 months. The residual population of worms had no apparent role in this.

The host response during an initial infection was aimed at the adult worms in the gut. However, during a second infection, there was a severe cellular reaction around the migratory stages in the lungs, where larvae were trapped and killed. Only few larvae avoided the serious response in the lungs, and developed in the gut into stunted and almost sterile worms.

Following multiple exposures, the immune response killed the infective larvae in the skin and there was a generalized dermal

reaction with infiltration of eosinophils and lymphocytes.

A full infection with S. ratti gave complete protection to the rats against reinfection. 10 day-old adult females also conferred a substantial degree of protection. The migratory stages did not stimulate immunity.

Worms which were obtained from infected rats 8-16 days I.P. were 2.88 mm in length and their uteri were full of embryonated eggs. When these worms were transplanted into clean rats, they continued their egg production as if they were in the original host.

Worms which were harvested from infected rats, between days 18-25 PI, were much shorter in length averaging 0.9 mm and contained no eggs. When these worms were transplanted, into new rats, they did not recover and were expelled immediately, indicating that they had been irreversibly damaged.

These two types of worms were studied with light and electron microscopy. This revealed that the damaged worms had a disorganised endoplasmic reticulum with increased numbers of lysosomes and vacuoles. No fat droplets were seen but whorls of lipoprotein were spotted, invariably, within the lumen of damaged worms. These were suggested to be immune complexes, which had been either formed internally, or swallowed. Precipitates around the mouth of the worms fluoresced in a direct F.A.T., showing them to be immune complexes, but it was not possible to demonstrate specific fluorescence of the lumen contents of the guts in sections of damaged worms.

When short and stunted worms, obtained early during a secondary infection, were transferred to new rats, they were able to establish themselves. They renewed egg production, which had ceased in the original hosts. Worm adaptation, similar to that observed in N. brasiliensis (Ogilvie, 1972), was discussed as a possibility.

Experiments were carried out to investigate the mechanisms of worm expulsion. It was concluded that worm expulsion was an immunological event, since immune MLNC were capable of inducing a reduction in the worm burden. MLNC transferred on day 12 PI were more effective than those transferred on day 6. Immune serum had a blocking effect and, in 3 out of 4 experiments, resulted in

auto-infection in the recipient rats. It is suggested that antibodies may paralyse the cellular arm of the immune response by either masking the worms, and possibly larvae, or through producing specific suppressive factors, which inhibit the function of the specific population of lymphocytes. However, immune serum caused a reduction in the size of the adult worms given early during infection.

Auto-infection also occurred in rats which received immune serum and MLNC on day 6 PI, but the best rate of worm expulsion was recorded when serum and MLNC were transferred on day 12 PI.

Immune MLNC were capable of conferring a substantial degree of protection when given 1 day before infection, but better protection was obtained when immune serum was added on day 0. However, immune serum alone given on day 0 did not confer any significant degree of protection. It was demonstrated that tissue eosinophilia increased during the period of worm expulsion. No success was achieved in repeated endeavours to stain and demonstrate intestinal mast cells during the period of expulsion, but studies on the level of degranulation of mesenteric mast cells revealed that there was a very significant rise in the percentage of degranulating mast cells in the adjoining tissues during worm expulsion in initial and second infection. There was a parallel rise in the total white blood cell count, but blood eosinophils did not follow this pattern. The number of peripheral lymphocytes was very high in infected rats.

Histamine injected directly into the stomach of infected rats caused a slight acceleration of both worm damage and expulsion. Aspirin, a potent inhibitor of prostaglandins, when introduced daily, per os, delayed worm expulsion by 5 days. These two results suggest a role for both histamine and prostaglandins in the elimination of S. ratti from the rats.

The final sets of experiments dealt with the effects of immunosuppressive corticosteroid treatment on initial and subsequent infections with S. ratti. Corticosteroid treatment caused a long persistance of the worm burden during an initial infection, for as long as the treatment was continued. The withdrawal of the drug caused the rats to recover and expel their infection in a

short period. No auto-infection was demonstrable when cortico-
steroids were introduced early during infection and continuously
every two days. In fact the number of adult worms in these rats
was very low compared to the untreated controls. However, when
corticosteroids were introduced 2 days before worm expulsion
(day 23 PI), auto-infection occurred and there was an increase
in the number of adult worms, these being normal in their measure-
ments and anatomy. This indicated that worm damage was phenotypic
and due to an immune phenomenon rather than senescence. Larval
production of worms in rats treated with corticosteroids was main-
tained for the duration of continuous treatment. A period of
unresponsiveness was induced when rats were treated with cortico-
steroids at strategic points during infection. Adult worms
persisted up to day 35 PI when rats were treated with 4 large
doses of Betamethasone on days 4, 6, 8 and 10 PI.

S. ratti infections are like many nematode infections of
rodents in that they are expelled from the intestine of the host
within 2 or 3 weeks of arriving in the gut. The major complication
of the host-parasite relationship is the possibility of auto-
infection which does not occur in other nematode systems.

The parasite S. ratti has proved a very good laboratory model
for the study of aspects of acquired immunity to nematode infections.

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