IMMUNOLOGICAL AND INFLAMMATORY RESPONSES IN THE INTESTINES OF MICE INFECTED WITH THE PARASITIC NEMATODE TRICHINELLA SPIRALIS

THESIS

for the

Degree of Doctor of Philosophy

by

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To my Parents

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SUMMARY

The work presented in this thesis has shown that in both NIH mice and Wistar rats the expulsion of a primary infection with Trichinella spiralis occurs towards the second week of infection. In mice the expulsion is preceded by a movement of the worms from their initial position in the anterior to the posterior region of the small intestine.

Challenge infections given shortly after the expulsion of a primary infection (day 14) are expelled rapidly, within 24 hours. In mice rapid expulsion of a challenge infection was a short-lived phenomenon (expressed only until day 16), but in rats the response was expressed for at least 7 weeks after the primary infection. Mice and rats showed differences in the conditions (e.g. size and duration) of infection necessary to prime for rapid expulsion, and also in their ability to respond to priming with irradiated larvae. The expression of the response, however, appeared to be similar in both species. Rapid expulsion could also be induced by drug abbreviated infections (3 days duration). Two or more drug abbreviated infections with normal larvae were necessary to induce rapid expulsion in mice, but the response then persisted for a prolonged period of time. One drug abbreviated infection was sufficient to induce rapid expulsion in rats.

The stage specificity of the induction and expression of rapid expulsion was examined and it was found that both larvae and adult worms were susceptible to rapid expulsion induced by complete infection.

Complete infections of T. spiralis in both NIH mice and Wistar rats resulted in increased levels of mucosal mast cells and goblet cells. In mice the numbers of both cell types rose sharply before worm expulsion and remained at an increased level for a short period of time. In contrast, in rats, the number of goblet cells and mast cells increased during worm expulsion and remained above control levels for a prolonged period.

In mice given one drug abbreviated infection, the numbers of intestinal mast cells were similar to that of control (uninfected) mice, but after two or more drug abbreviated infections, the numbers were increased considerably. Exposure to a 7-day infection resulted in an increase in mast cells, but their numbers then declined very quickly, i.e. within a few days after drug treatment. The mast cell response to a secondary (challenge) infection was accelerated both in onset and magnitude.

In mice it was shown that rapid expulsion of T. spiralis was a response evoked specifically by prior infection with this species, priming provided by Nematospiroides dubius and Nippostrongylus brasiliensis

failed to induce rapid expulsion. Likewise priming with T. spiralis did not induce rapid expulsion of these species, although it was seen to affect a partial expulsion of the related trichuroid nematode Trichuris muris. It was concluded that either specific inflammatory changes generated during a primary infection result in environmental changes which are unsuitable for the establishment of subsequent infections or that challenge infections provide a stimulus that can provoke an almost instantaneous response in the primed intestine. The relationship of the observed cellular changes to such mechanisms is discussed.

Challenge infections were prevented from establishing in immune mice and had no effect on homing characteristics of injected immune MLNC. However, blast cell activity appeared to increase in the MLN 1 day after a challenge infection in immune mice. Subsequent (tertiary) challenge infections given 1, 2 and 3 days after an initial challenge (secondary) infection were expelled rapidly from immune mice, but those given 7 days after the initial challenge were not subject to rapid expulsion.

Rapid expulsion was partially prevented by prior irradiation or cortisone treatment and was restored in irradiated mice by the adoptive transfer of immune MLNC. Immune MLNC and a T cell enriched population (but not a B cell enriched population) were

able to transfer an accelerated mast cell response in normal or irradiated mice and it was concluded that T cells or their products may regulate the generation of the mast cell response. The relationship between transferred immune MLNC, the mast cell response and the subsequent rapid expulsion response in irradiated mice has been discussed. Slow responder mice (B10.G, C57/B10, B10.BR, B10.D2) with the same genetic background but carrying different H-2 haplotypes showed a delay in the mast cell response when compared with rapid responder mice (NIH and DBA1). Immune MLNC from rapid (NIH) or slow (B10.G) responder mice accelerated the mast cell response in normal or irradiated recipient NIH mice but had less effect in B10.G recipient mice. The role played by H-2-linked and non H-2-linked genes in generating the mast cell response is discussed. Further lines of research are suggested to investigate the origin and control of mast cells and in particular, the vasoactive amines which are released from these cells during the infection of mice with T. spiralis.

ABBREVIATIONS

Ag	Antigen
B	Thymus independent; antibody producing
c.p.m.	Count per minute
FCS	Foetal calf serum
Fig.	Figure
H-2	Genetic region concerned with histocompatibility in mice
HBSS	Modified Hanks' balanced salt solution
%ID	Radioactivity in an organ as percentage of injected dose of isotope
Ig	Immunoglobulin
ip	Intraperitoneal(ly)
^{125}I-UdR	(^{125}I)-iodo-deoxyuridine
i/v	Intravenous(ly)
IMLNC	Immune mesenteric lymph node cells
K.rad(s)	Kilorad(s)
MHC	Major histocompatibility complex
MLN	Mesenteric lymph node
P	Probability
PHA	Phytohaemagglutinin
p.i.	Post infection
S.D.	Standard deviation
T	Thymus dependent
%TR	Radioactivity in an organ as percentage of the total isotope recovered from the small intestine, mesenteric lymph, liver, spleen and large intestine

ABBREVIATIONS

continued

V.C.U.	Villus, crypt unit
v/v	Volume/volume ratio
w/v	Weight/volume ratio

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Interest in the immunity of mammals to nematode parasites is widespread and has focussed particularly on pathogenic species of economic and medical importance to man. Nematode parasites are responsible for a considerable proportion of human helminth diseases such as bancroftian filariasis, onchocerciasis, trichinellosis, trichuriasis, ascariasis and strongyloidiasis which often have enormous pathological effects on the host.

Trichinella spiralis in particular has had a considerable impact because of its medical and economic importance as a parasite of man and his domestic animals, as well as affecting wild animal populations. This parasite has also been established as a convenient laboratory model for the study of immunity to gastro-intestinal helminth infections in various laboratory rodents (mice, rats, guinea pigs).

This parasite was first noted in man by Paget in 1835, but the first description in the literature, in the same year, was by Owen (see Campbell, 1979).

T. spiralis is an unusual nematode parasite in that all the larval and adult stages, which are both enteral and parenteral, occur within the same host. Because of the extremely rapid development of the parasite from infective larva to the adult stage, it is difficult

to define the development in the intestine in terms of larval stages. However, Ali-Khan (1966) has described four larval stages and adult worms in the intestine. It is known that infective larvae establish themselves in the intestinal mucosa shortly after infection (Wright, 1979; Despommier, Sukhdeo and Meerovitch, 1978).

According to Despommier (1977) the infective larvae moult four times within 36 hours and transform into immature adult worms. The females are viviparous and larvae (the so-called "newborn larvae") are released into the intestine 5 or 6 days after infection. These newborn larvae migrate to the mucosal lymphatic system and are carried by the blood stream until they eventually lodge in striated skeletal muscle tissue where they become encapsulated. The life span of these encapsulated larvae is limited and depends upon the host species. The migrating newborn larvae and the larvae in the muscle tissue are collectively known as the parenteral stages and although these stages unquestionably stimulate host responses to their presence, these responses are considered beyond the scope of the present study which deals exclusively with the immune responses of the host species to the enteral stages of the parasite.

During the intestinal stages the host is exposed to a variety of antigens released by the parasites. The antigens which induce the protective response have not been characterized and although recent work (Phillipp, Parkhouse

and Ogilvie, 1980) has suggested that cuticular antigens may be involved, earlier work (Despommier, 1977) implicated antigens from stichocyte granules as being the functional antigens. The latter supposition has more support in the literature dealing with other nematode parasites, as Jenkins and Wakelin (1977) have found that stichocyte secretions were effective at engendering immunity against Trichuris muris in mice, and other workers (Jones and Ogilvie, 1972; Rothwell and Merrit, 1974) have suggested that the functional antigens of Nippostrongylus brasiliensis and Trichostrongylus colubriformis are also associated with ensyme secretions from particular glands.

The effective recognition of these antigens stimulates a host response which eventually results in worm expulsion within 2-3 weeks of infection (depending on the species). Some of the recorded effects on T. spiralis in infected rats or mice include drastic changes in morphology (accumulation of lipid droplets in the gut cells and tissues), decrease in egg production by the ovary, reduction of fecundity and reduction of worm size (Love, Ogilvie and McLaren, 1976; Fatunmbi, 1978; Kennedy and Bruce, 1981).

A considerable amount of work has been done to identify the mechanisms responsible for this protective immunity, with conflicting results. There is evidence, for example, that antibodies make a significant contribution to the response. In rats the transfer of immune serum has

resulted in passive protection against T. spiralis (Love et al., 1976) and it has been suggested that IgA plays an important role in worm expulsion (Despommier, McGregor, Crum and Carter, 1977). In mice there is some evidence of antibody linked passive transfer of immunity to suckling young (Ducket, Denham and Nelson, 1972; Perry, 1974) and, more recently, it has been reported that a combination of IgG_1 and IgE effectively transfers protection against T. spiralis (Gabriel and Justus, 1979).

The role of antibodies in the immune expulsion of T. spiralis is, however, controversial. In some parasitic infections it has been found that antibodies alone are not capable of inducing worm expulsion and that for this to occur other components are necessary (see Ogilvie and Love, 1974; Wakelin, 1978a). The evidence suggests that antiworm antibodies do not cause worm expulsion directly, but that they participate by interfering with worm metabolism, thus rendering the worms susceptible to subsequent effects of other components (cells) of the protective response. In vitro studies have shown, for example, that when larvae and adult worms of T. spiralis are incubated in specific anti-Trichinella serum for 18 hours precipitates are found at the parasite orifices and the cuticular surface (Kim and Ledbetter, 1981). Newborn larvae are susceptible to attack by eosinophils and their destruction required the presence of antibodies (Kazura and Grove, 1978; Mackenzie, Preston and Ogilvie, 1978).

However, the effects of the antiserum (presumably containing T-dependent antibodies) may be mediated through the stimulation of other components of the protective response. In the N. brasiliensis/rat model, it was shown that an increase in mucosal mast cell numbers occurs in recipients of immune serum (Miller, 1979; Befus and Bienenstock, 1979).

It is possible that antibodies at the mucosal surface participate in the protective response. Crandall and Crandall (1972), for example, reported that the immunocyte population of the intestinal mucosa of mice showed a relative increase in IgM and IgG_1 containing cells during the second week after infection with T. spiralis. Such increases in immunocyte numbers in the intestinal mucosa have been reported in other host parasite systems (see review by Ogilvie and Jones, 1973). A separation of direct, antiworm immune responses (i.e. reduction in fecumdity and growth) from actual worm expulsion in T. spiralis infected mice has been suggested by Wakelin and Wilson (1980). They concluded that the former can be mediated through antiworm antibodies as well as inflammatory responses and possibly physicochemical changes in the intestinal environment. Further evidence has shown that direct antiworm immunity is not the cause of the worm expulsion. For example, using transplantation techniques it has been shown that even worms taken during the process of expulsion, were able to survive and resume production in naive recipients. This finding suggests that extensive damage to the worms is not the major cause of expulsion.

The role of sensitised lymphocytes in transferring immunity is well documented. In the T. spiralis/mouse model, the transfer of immune MLNC results in reduced worm growth and fecundity and subsequently, results in worms expulsion. It has been shown that T cells are more effective than B cells in passively transferring immunity, but that bone-marrow components are necessary for an effective expulsive response (see Wakelin, 1978a).

The observable host response to infection with T. spiralis consists of a variety of components. For example, MLN blast cell activity increases between days 4 and 8 post infection, transferred MLNC and particularly T lymphoblasts preferentially localise in the small intestine early after infection and inflammatory cellular infiltration and physicochemical changes occur in the intestinal environment (see also chapters 3 and 5). There is evidence to suggest that worm expulsion is associated with these latter changes. It has been shown that the number of mast cells, goblet cells and eosinophils increases dramatically during nematode infection and that T cells or their products are involved in the regulation of these cells. The possible involvement of mast cells and their products and other local intestinal cellular changes in the response to nematode infection, is discussed in chapter 3.

One of the consequences of the mucosal immune response is the rapid expulsion of a challenge infection.

Recently, more attention has been paid to the factors and components which are involved in this response because of its importance in the study of how worm expulsion is brought about.

The primary objectives of the work presented in this thesis were to examine: 1) the conditions necessary for the induction and expression of rapid expulsion by complete or drug-abbreviated infections, and 2) the stage-specificity of the induction and expression of the rapid expulsion response.

In addition, the intestinal mast cell and goblet cell responses during T. spiralis infection in mice and rats have been characterised (chapter 3) and comparisons made of the rapid expulsion responses in mice and rats.

The specificity of the rapid expulsion response to T. spiralis infection is examined in chapter 4. Chapter 5 provides evidence for the mechanisms of rapid expulsion and the components involved in this response in mice. Special attention has been paid to the effect upon the mast cell response of transferring immune MLNC or separated T and B cell populations into irradiated and unirradiated mice.

The strain characteristics of the mast cell responses to T. spiralis infection and the underlying genetic control of the generation of these cells are examined in chapter 6.

GENERAL MATERIALS AND METHODS

1. Animals

Mice and rats.

Male and female mice were used, usually males unless otherwise stated. For the majority of experiments, helminth-free, inbred NIH mice were purchased from Hacking and Churchill Ltd. Rats used were outbred Wistar rats (bred at the Wellcome Laboratories).

In most experiments, mice were caged in groups of six in polypropylene cages .48 cm x 15 cm x 13 cm. Rats were caged, usually in groups of eight, in polypropylene cages 56 cm x 38 cm x 18 cm (North Kent Plastics Ltd.). Sawdust or wood shavings was provided as bedding and replaced twice per week. Animals rooms were maintained on a 12 hour light-dark cycle in winter, but followed normal day length in summer. Tap water and pelleted food (Grain Harvesters Ltd.) was available to the animals ad libitum.

2. Parasite

The strain of Trichinella spiralis used in the experiments was obtained from the London School of Hygiene and Tropical Medicine and maintained by passage through outbred CFLP mice. Infective larvae were obtained by digestion of stock mice which had been infected for at

least 2 months. The animals were killed, skinned, cut into pieces and then minced. The material was digested for 2-2.5 hours in a solution of 0.5% pepsin in 0.5% HCl in tap water (500 ml/mouse) at 37°C with continuous agitation. Undigested sediment was filtered off on a coarse sieve (mesh size 1 mm) and the larvae collected by repeated washing and sedimentations in 0.9% NaCl. The larvae were finally suspended in 0.2% agar and the total volume adjusted to give the required inoculum in 0.2 ml. Mice or rats were infected by injection (per os) into the stomach using a syringe and a blunted needle. For irradiation of T. spiralis larvae, infective larvae were suspended in 0.2% agar and placed in a glass bijou bottle which was exposed to 4 Krad irradiation from a 500 Ci ^{60}Cobalt Source (dose rate 1.05 Krad/min). At this level of irradiation, worms are rendered completely sterile.

3. Adult worm recovery

Adult worms were recovered from the intestines of animals using a modified Baermann technique (Wakelin and Lloyd, 1976a). Animals were killed by an overdose of chloroform vapour and the entire small intestine removed and cut open along its length. It was then placed on a piece of nylon gauze and submerged in 40 ml of preheated (40°C) Hanks' Balanced Salt Solution (HBSS) in a 50 ml beaker. Incubation was then continued for a further 2.5-3 hours, during which time worms migrated through the

gauze. As confirmed by examination of the intestinal mucosa, the majority of worms left the intestine and collected in the beaker. The worms were transferred to a Petri dish and were counted using a binocular dissecting microscope. If not counted immediately, beakers were stored overnight at 4°C.

4. Anthelmintic

Methyridine (Mintic and Promintic, I.C.I.) was used to remove T. spiralis. Mintic was given orally to mice at a dose level of 1000 mg/kg body weight; Promintic was injected subcutaneously into rats at a dose of 300 mg/kg. The efficiency of the drug was determined in infected mice given anthelmintic and killed 1 day later; no worms were observed in the gut at this time. Neither of these drug treatments has any effect upon subsequent infection with T. spiralis.

5. Antibiotic

Oxytetracycline hydrochloride (Terramycin, Pfizer Ltd.) at a concentration of 300 mg/litre was given in drinking water to mice which had undergone surgery or immunosuppression as well as to their controls.

6. Statistics

The statistical significance of differences between mean worm recoveries from experimental and control

groups was determined using students' 't' test. A probability greater than p = 0.05 was considered to be non-significant.

7. Histology

Mice or rats were autopsied and the small intestine removed entirely. In some experiments the entire gut was divided into four segments, but in the majority of the experiments only one segment (5 cm) from the middle of the small intestine was taken. Each segment was opened longitudinally and rolled on a syringe plunger with the mucosal surface out (swiss roll technique of Reilly and Kirsner , 1965). Tissues were fixed in Carnoy's or Bouin Hollande fixative, embedded in polywax (Difco Laboratories) and sectioned at 5 um. (Humoson, 1972).

For examination of mast cells, sections which were fixed in Carnoy's were stained with Alcian blue (1% W/V in 0.7N HCl; Alcian blue 8 Gx, (Searle Diagnostic, High Wycombe) and Safranin "O" (0.5% W/V in 0.125N HCl, michrome No. 405, (Searle Diagnostic, High Wycombe) by modification of the method of Enerback (1966a).

For quantitative purposes, all Alcian blue positive cells were recorded, whether in the lamina propria or intraepithelial in position and as the precise identity of cells in this location is presently disputed (Askenase, 1980) they are referred to collectively as mucosal mast

cells (see also chapter 3). In each intestinal segment 20 villus crypt units (V.C.U.) were counted. A V.C.U. represents that portion of intestinal mucosa lying between two gland crypts and the lamina propria of the villus above (Miller and Jarrett, 1971).

For the staining of goblet cells, segments of the small intestine were fixed in Bouin Hollande's fixative for 24 hours (or in Carnoy's - as above) and processed as previously. Sections were stained with periodic acid/ Schiff (PAS) and Alcian blue by the methods described by Mowry (1963) and were counterstained with haematoxylin. For quantitative purposes, goblet cells were taken as those mucus producing cells located in the epithelium of the villi and crypts and 20 villus crypt units were counted in each intestinal segment (see also chapter 3).

Fixatives and stains for light microscopy

A - Fixatives:

i Carnoy's fixative - tissues fixed for 3-6 hours

Absolute ethanol	60 ml
Chloroform	30 ml
Glacial acetic acid	10 ml

ii Bouin Hollande's fixative - tissues fixed for 24 hours to 3 days

Copper acetate	2.5 g
Picric acid crystals	4.0 g

Formalin, concentrated (100%)	10.0 ml
Distilled water	100.0 ml
Glacial acetic acid	1.5 ml

Copper acetate was dissolved in water without heat; picric acid was added slowly while stirring. When dissolved the solution was filtered and formalin and acetic acid were added.

B - Staining solutions

Dominici's method for staining eosinophils

Fixation - Bouin Hollande.

i Phosphate buffer

a) Na_2HPO_4 14.196 g made up in 1000 ml of distilled H_2O

b) KH_2PO_4 13.609 g made up in 1000 ml of distilled H_2O

200 ml of solution a) were added to 800 of solution b).

Final pH 6.2

ii Orange G and eosin y

Orange G	0.5 g
Eosin y	0.5 g
Phosphate buffer	200 ml

iii Toluidine blue

Toluidine blue	0.3 g
Phosphate buffer	100 ml

Staining method

1 - Sections were taken through xylene and graded ethanol to water

2 -	Orange G and eosin y	5 mins
3 -	Phosphate buffer	Rinse
4 -	Toluidine blue	1 min
5 -	Phosphate buffer	Rinse
6 -	Dehydrated in graded ethanol	3-5 mins each
7 -	Xylene	5 mins
8 -	Mounted in DPX	

Prolonged staining with the acid dyes or prolonged rinsing resulted in a shift from orange toward increased red staining. When properly stained eosinophils had bright red or orange granules.

Alcian blue/Safranin "O" for mast cells

Fixation - Carnoy's

i 1% Alcian blue 8GX pH = 0.3

Alcian blue	1 g
0.7N HCl	100 ml

ii 0.5% Safranin "O" pH = 1

Safranin O	0.5 g
0.125N HCl	100 ml

Staining

1 - Sections were taken through xylene and graded ethanol to water

2 -	1% Alcian blue	30-45 mins
3 -	0.7N HCl	10 mins
4 -	0.5% Safranin	5 mins
5 -	Distilled water	Rinse
6 -	70% ethanol	1 min
7 -	90% ethanol	1 min
8 -	Absolute ethanol	1 min
9 -	Xylene	5 mins
10 -	Mounted in DPX	

PAS and Alcian blue for staining of goblet cells

Fixation - Carnoy's or Bouin Hollande's

(i) Schiff reagent (B.D.H. Chemicals Ltd., Poole, England).

(ii) Periodic acid solution

Stock solution (B.D.H. Chemicals Ltd., Poole, England).

Working solution

Periodic acid solution	1 ml
Distilled water	99 ml

(iii) 1% Alcian blue

Alcian blue	1 g
Glacial acetic acid	3 ml
Distilled water	97 ml

iv Mayer's Haematoxylin

1 g haematoxylin was added to 1 litre of distilled water and heated gently, 0.2 g sodium iodate and 50 g potassium alum ($KAl(SO_4)_2.12H_2O$) were added and the

solution heated until dissolved; 1 g citric acid and 50 g chloral hydrate were then added. The stain was allowed to ripen preferably for 6-8 weeks, although it can be used within 1-2 weeks (see Humason, 1972).

v 0.5% sodium metabisulphite

Sodium metabisulphite	0.5 g
Distilled water	100 ml

Staining method

1 -	Sections were taken through xylene and graded ethanol to water	
2 -	1% Alcian blue	30 mins
3 -	Washed in water	5 mins
4 -	Oxidised in 1% periodic acid	5 mins
5 -	Washed in running water	5 mins
6 -	Schiff reagent	10-20 mins
7 -	Three changes of 0.5% sodium metabisulphite	Rinse
8 -	Washed in running water	10 mins
9 -	Haematoxylin	2 mins
10 -	Dehydrated in graded ethanol	3-5 mins each
11 -	Xylene	5 mins
12 -	Mounted in DPX	

Balanced salt solution

The modified Hanks' Balanced Salt Solution (HBSS) used was as described by Hopkins and Stallard (1974), i.e.

Hanks' saline was modified by excluding glucose and $NaHCO_3$ and by increasing remaining salts to an osmotic pressure of 300 m-osmols.

Solution 1

NaCl	168 g
KCl	8 g
KH_2PO_4	2 g
Na_2HPO_4	4 g
0.2% Phenol red	200 ml

made up to 2 litres with deionised water.

Solution 2

$CaCl_2.2H_2O$	3.92 g
$MgCl_2.6H_2O$	2.00 g

made up to 2 litres with deionised water.

105 ml each of Solutions 1 and 2 were mixed and made up to 1 litre with deionised water, giving a final pH of 7.2.

CHAPTER 1

RAPID EXPULSION: EXPRESSION AND INDUCTION BY COMPLETE INFECTION IN MICE AND RATS

INTRODUCTION

The enteral and parenteral stages of *Trichinella spiralis* induce a complex immune response in the host (Wakelin, 1978a). Bell and McGregor (1979a) have identified four distinct responses which operate in the intestine of *Trichinella* infected rats, namely, (i) rapid expulsion, (ii) anti-preadult immunity, (iii) anti-adult immunity and (iv) anti-fecundity response.

The first of these responses was described by McCoy as early as 1940 when he noted that a secondary infection was expelled within 12 hours from immune Wistar albino rats. Love, Ogilvie and McLaren (1976) have shown that infective larvae are expelled rapidly from immune PVG/c rats; Wakelin and Lloyd (1976a) and Alizadeh and Wakelin (1980) have noted a similar response in NIH mice.

This phenomenon has received little experimental attention until recently when the work of Bell and McGregor (1979a, b) confirmed that rapid expulsion operates as a major protective response against *T. spiralis* in rats.

The mechanisms that underlie this response are not yet clear, but Russell and Castro (1979) have reported that rapid expulsion was not associated with the acute inflammatory cell infiltrate or with the patho-physiology that characterizes a primary infection in T. spiralis infected Sprague Dawley rats. Recently Castro, Hessel and Whalen (1979) have shown that there is a rapid onset of intraluminal fluid accumulation soon after secondary contact with T. spiralis in the immunized challenged host and that this is associated temporárily with prevention of worm establishment. Lee and Ogilvie (1981) have suggested that the failure of challenge larvae to become established is due to their being trapped in the intestinal mucus layer of immunized rats. At present rather more is known of the mechanisms of immunity which operate during primary infection of T. spiralis in mice than rats and therefore study of rapid expulsion in this host may provide a better understanding of mechanisms involved.

It is well known that the kinetics of infection of T. spiralis in different strains of host species can vary very considerably and also, the strain characteristics in this respect can alter over a period of time. It is therefore necessary at the start of such a programme of work to define precisely the response characteristics of the animals available. In this chapter, the course of primary infections and the conditions necessary for induction or expression of

rapid expulsion in NIH mice and Wistar rats have been defined. The occurrence of rapid expulsion of challenge infections given immediately and up to 5 and 7 weeks following the complete expulsion of a primary infection of T. spiralis, is examined in both NIH mice and Wistar rats respectively.

An attempt is made to examine the level of primary infections with normal or irradiated larvae necessary to evoke the rapid expulsion response. Comparisons are made between mice and rats concerning some of the aforementioned characteristics of rapid expulsion.

MATERIALS AND METHODS

The strain of mice or rats and the methods used for maintenance, infection and worm recovery have been described in the general materials and methods.

RESULTS

1. Kinetics of a primary infection in mice

An initial experiment was carried out to define the course of a primary infection in NIH mice. Forty-eight 7-week-old male NIH mice were infected with 300 T. spiralis larvae on day 0. Groups of four mice were killed daily from days 1 to 21 after infection, to assess the total number and distribution of worms in the anterior and posterior halves of the small intestine.

The kinetics of primary infection (shown in Fig. 1.1) were as follows.

a) Establishment

Over 55% of the infecting dose established and developed to maturity in the small intestine.

b) Constant period

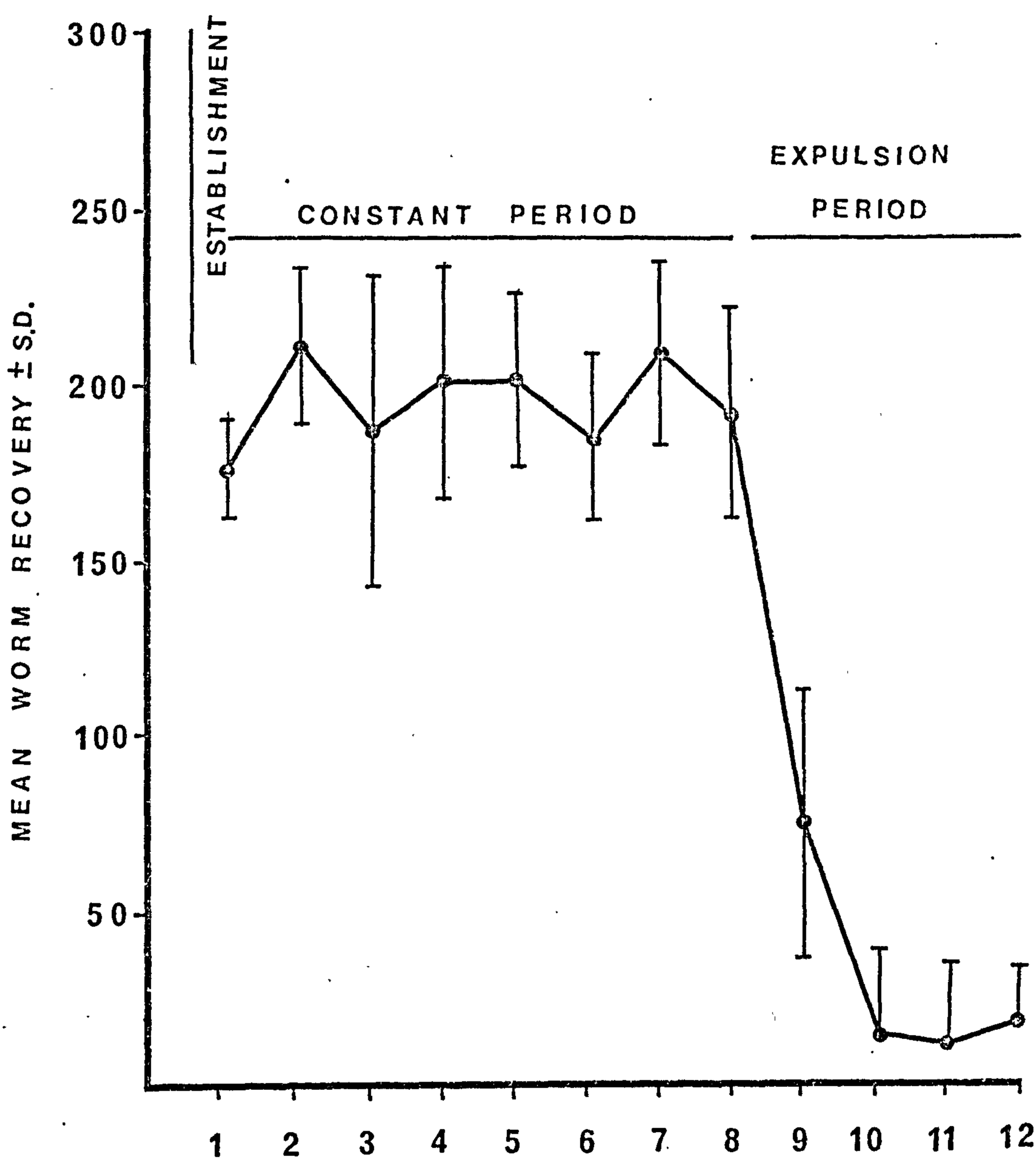
After establishment the number of worms in the small intestine remained relatively constant between days 1 and 8 after infection. A few worms were observed in the large intestine on days 7 or 8, but not previous to these days.

c) Expulsion period

Total worm numbers were markedly decreased after day 8. A sharp decline occurred within 2 days (between day 8 and day 10) and worm

Fig. 1.1

The kinetics of a primary infection of T. spiralis in NIH mice.


ESTABLISHMENT
CONSTANT PERIOD
EXPULSION PERIOD
MEAN WORM RECOVERY ± S.D.
300
250
200
150
100
50
1 2 3 4 5 6 7 8 9 10 11 12
DAYS POST INFECTION

expulsion was complete by day 12 post infection (p.i.). It was noted that there was an increase in worm numbers in the large intestine during this period.

d) Distribution of adult T. spiralis in the small intestine of mice

Total worm recoveries from the anterior and posterior halves of the small intestine are shown in Fig. 1.2. During the constant period (days 1-8), between 70% and 90% of the worm population which established were located in the anterior half of the small intestine. During the expulsion period (between days 8 and 10), total worm numbers decreased markedly in both segments.

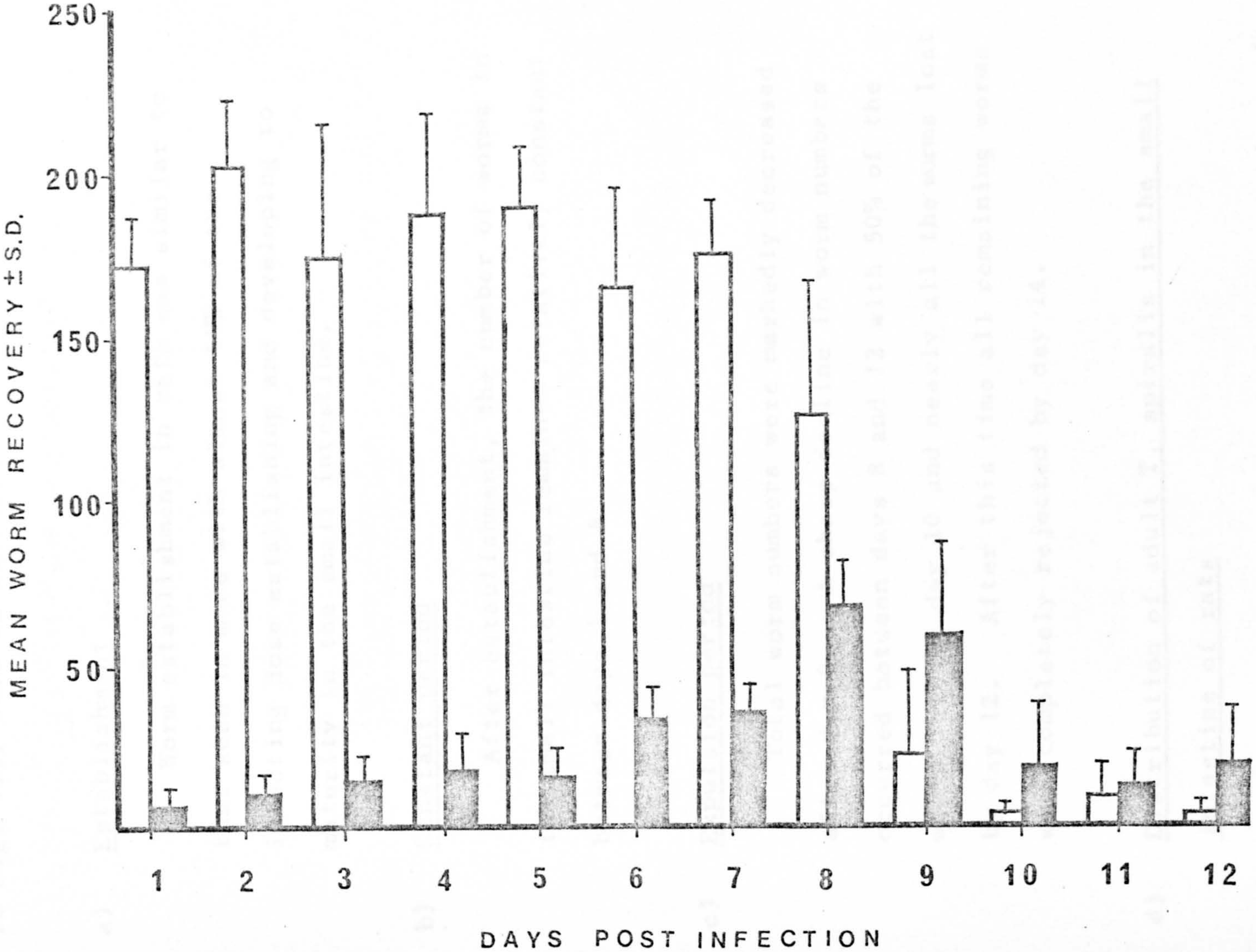
It was apparent that worms were being lost from both segments of the small intestine with the majority of the worms being recovered from the posterior half during the period of rejection.

2. Kinetics of a primary infection in rats

An initial experiment was designed to determine the course of a primary infection in rats. A total of twenty-one 8-9-week-old male Wistar rats were given an infection of 2000 T. spiralis larvae on day 0. Groups of 3 rats were killed on days 2, 4, 6, 8, 10, 12 and 14 after infection to assess the total number and distribution of worms in the anterior and posterior halvesof the small intestine.

Fig. 1.2

Distribution of adult T. spiralis in the anterior ▭ and posterior ▬ halves of the small intestine of NIH mice during a primary infection.


250
200
150
100
50
MEAN WORM RECOVERY ± S.D.
1
2
3
4
5
6
7
8
9
10
11
12
DAYS POST INFECTION

The kinetics of a primary infection (shown in Fig. 1.3) were as follows.

a) Establishment

Worm establishment in rats was similar to that seen in mice with almost 57% of the infecting dose establishing and developing to maturity in the small intestine.

b) Constant period

After establishment, the number of worms in the small intestine remained relatively constant between days 1 and 8.

c) Expulsion period

Total worm numbers were markedly decreased after day 8. A sharp decline in worm numbers occurred between days 8 and 12 with 50% of the worms lost by day 10 and nearly all the worms lost by day 12. After this time all remaining worms were completely rejected by day 14.

d) Distribution of adult T. spiralis in the small intestine of rats

The total worm recovery in the anterior and posterior halves of the small intestine is shown in Fig. 1.4. During the constant period almost

Fig. 1.3

The kinetics of a primary infection of *T. spiralis* in Wistar rats.

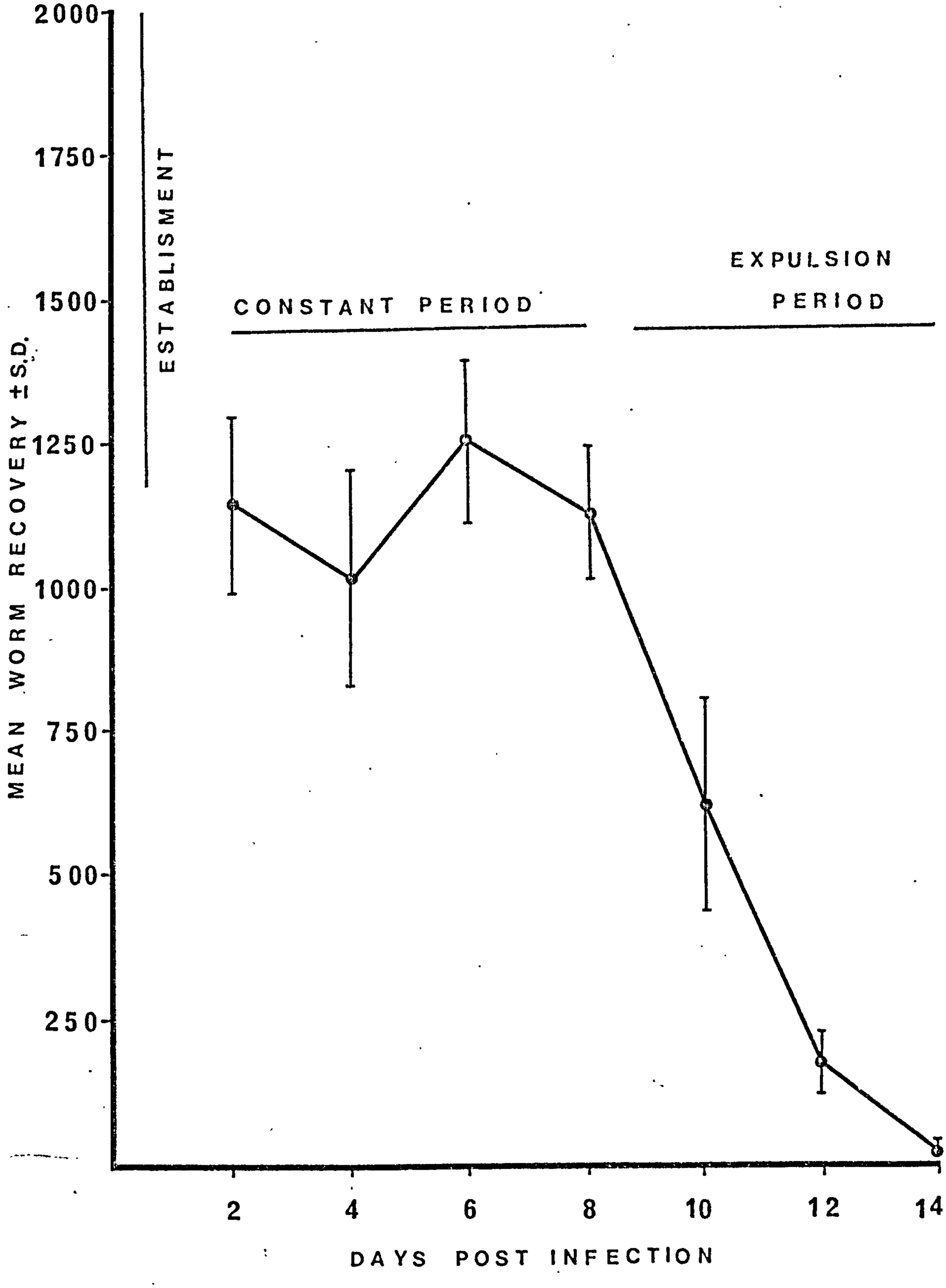
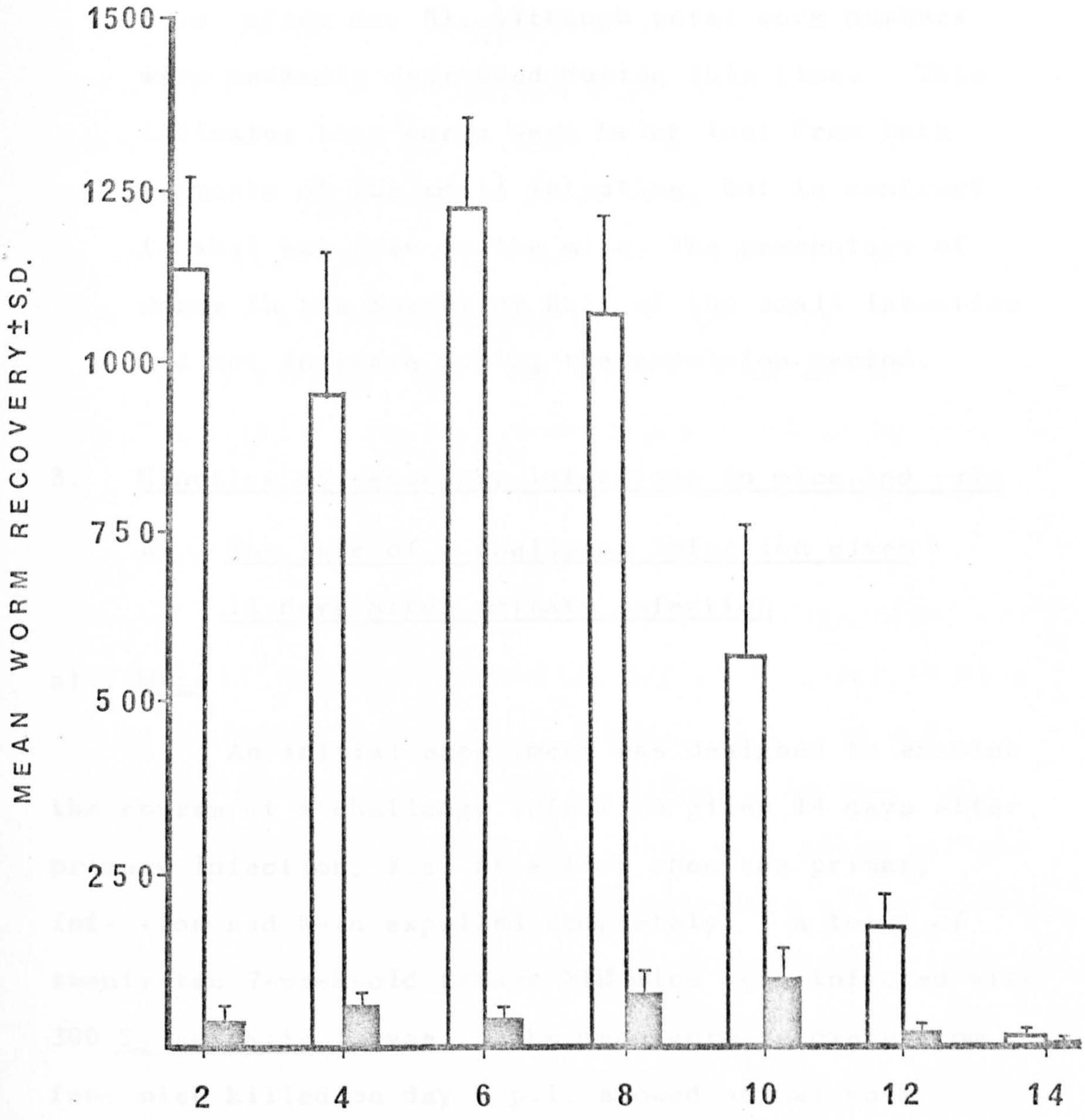

ESTABLISHMENT
CONSTANT PERIOD
EXPULSION PERIOD
MEAN WORM RECOVERY ±S.D.
2000
1750
1500
1250
1000
750
500
250
2
4
6
8
10
12
14
DAYS POST INFECTION

Fig. 1.4

Distribution of adult T. spiralis in the anterior ▭ and posterior ▬ halves of the small intestine of Wistar rats during primary infection.


1500
1250
1000
750
500
250
MEAN WORM RECOVERY ± S.D.
2
4
6
8
10
12
14
DAYS POST INFECTION

95% of the worm population was located in the anterior half. The percentages of worms recovered in both halves of the small intestine remained constant even during the expulsion period (i.e. after day 8), although total worm numbers were markedly decreased during this time. This indicates that worms were being lost from both segments of the small intestine, but in contrast to what was seen in the mice, the percentage of worms in the posterior half of the small intestine did not increase during the expulsion period.

3. Kinetics of secondary infections in mice and rats

A. The fate of a challenge infection given 14 days after primary infection

a) Mice

An initial experiment was designed to examine the course of a challenge infection given 14 days after primary infection, i.e. at a time when the primary infection had been expelled completely. A total of twenty-two 7-week-old female NIH mice were infected with 300 T. spiralis larvae. The mean worm recovery from four mice killed on day 7 p.i. showed normal worm establishment (202.0±33.5). On day 14 after the primary infection, 18 infected mice and 18 control uninfected age-matched mice were given a challenge infection of 300 T. spiralis larvae. Six mice from each group were

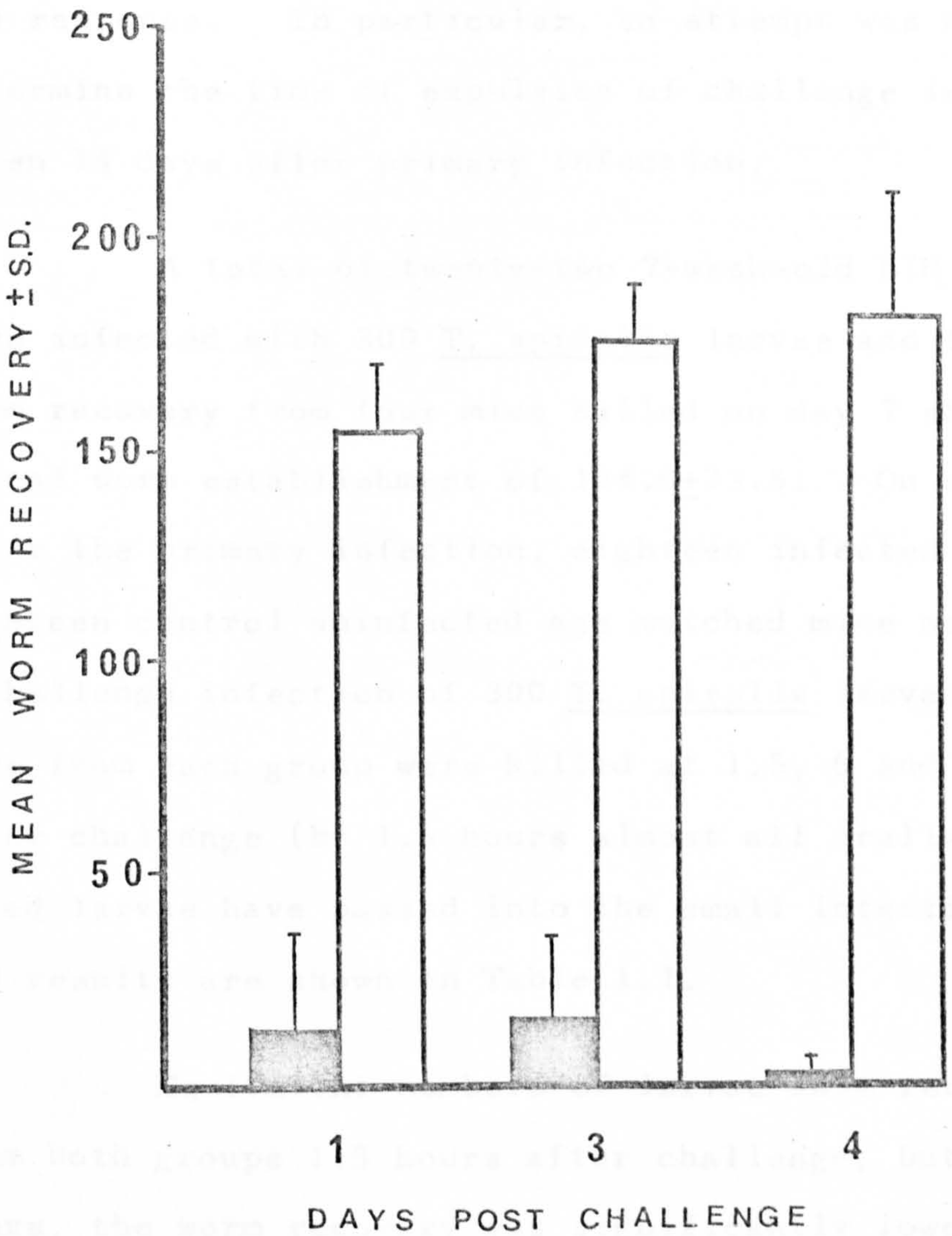
killed on days 1, 3 and 4 after the challenge infection. Worm recoveries from the intestines of immune mice were compared with those from non-immune mice 24 hours after challenge infection. The results of this experiment are shown in Fig. 1.5. Differences in the worm recoveries between the groups were statistically significant 24 hours after infection, indicating the majority of the larvae had been expelled rapidly from immune mice. In this experiment a few worms were still present in the immune mice 3 days after the challenge infection. In repeat experiments, however, it was observed that challenge infections were completely expelled by 24 hours.

b) Rats

A preliminary experiment was designed to examine the course of secondary infection given 14 days after a primary infection. A group of six male Wistar rats were infected with 2000 T. spiralis larvae. On day 12 after primary infection, they were given anthelmintic (Methyridine, see general materials and methods), to remove any residual worms. These previously infected (immune) rats, together with six control (uninfected) rats, were then challenged with 500 T. spiralis larvae. To assess rapid expulsion all the rats were killed 24 hours later and worm recoveries from the immune rats were compared with those from non-immune (control) rats. The challenge infection was expelled rapidly within 24 hours. The numbers of worms recovered from immune and

Fig. 1.5

Number of worms (mean ± SD) recovered from the small intestine of NIH mice given a challenge infection with Trichinella spiralis on day 14 of primary infection. Immune ▓▓▓ and non-immune (control) mice ▭ .


250
200
150
100
50
MEAN WORM RECOVERY ± S.D.
1
3
4
DAYS POST CHALLENGE

control rats were 4.6±2.5 and 140±12.6 respectively.

B. Time of expulsion of challenge infection given on day 14 after primary infection in NIH mice

Having established some of the basic parameters, attention was focussed on the rapidity of the response. In particular, an attempt was made to determine the time of expulsion of challenge infection given 14 days after primary infection.

A total of twenty-two 7-week-old NIH mice were infected with 300 T. spiralis larvae and the mean worm recovery from four mice killed on day 7 showed a normal worm establishment of 104.0±23.5. On day 14 after the primary infection, eighteen infected mice and eighteen control uninfected age matched mice were given a challenge infection of 300 T. spiralis larvae. Six mice from each group were killed at 1.5, 6 and 24 hours after challenge (by 1.5 hours almost all orally administered larvae have passed into the small intestine). The results are shown in Table 1.1.

Equivalent numbers of larvae were recovered from both groups 1.5 hours after challenge, but by 6 hours, the worm recovery was significantly lower in previously infected mice and by 24 hours very few worms were recovered. A similar result was obtained in a replicate experiment.

TABLE 1.1

Time course of loss of T. spiralis from NIH mice given a challenge on day 14 after primary infection.

Hours after challenge infection	No. of worms			
	Control mice		Challenged mice	
	Mean	S.D.	Mean	S.D.
1.5	137.0	31.8	105.0	29.7
6	139.0	40.6	46.0*	52.8
24	157.2	7.7	17.7*	18.9

* Mean significantly lower than control.

$p < 0.05$

In the above experiment worms were recovered from the intestine by the normal Baermann technique. To confirm that the absence of worms at 6 and 24 hours post challenge (p.c.) in previously infected mice reflected expulsion from the intestine and not the failure to recover larvae from the mucosa, a further experiment was carried out. Mice given a primary infection were then challenged on day 14 and larvae recovered by the Baermann technique after 6 and 24 hours. After each recovery period, the intestines were cut into pieces and digested for 2 hours in pepsin-HCl (see general materials and methods). The digest was filtered through a layer of nylon gauze, with a sufficient mesh size to allow any worms to pass through and the filtrate was examined for worms. Very few worms (less than twelve) were recovered in the mucosa at 6 hours and expulsion was complete by 24 hours after challenge.

C. The fate of challenge infections given at intervals after primary infection

a) Mice

Thirty 7-week-old male NIH mice were given a primary infection of 300 T. spiralis larvae and divided into six groups of five mice. Groups were given a challenge infection of 300 larvae on days 14, 16, 18, 21, 28 or 35 p.i. Groups of five control (uninfected) age-matched mice were given challenge infections at the same

times as the experimental groups and mice of each group were killed 24 hours after challenge.

Rapid expulsion of challenge larvae occurred only in mice challenged 14 and 16 days after primary infection (Table 1.2). The establishment of challenge infections given on days 18, 21, 28 or 35 after primary infection, was similar to the control groups.

An attempt was made to induce rapid expulsion on day 21 of a primary infection by increasing the initial infection dose to 500 T. spiralis larvae. The infected mice, together with age-matched controls, were challenged with 300 larvae, but again the immune mice did not show rapid expulsion 24 hours after challenge, the mean worm recoveries from the immune and control groups being 106.0±7.6 and 116.0±11.0 respectively. The immune status of mice exposed to challenge after the sixteenth day of a primary infection was examined in a further experiment.

Two groups of five mice received primary infections of 300 T. spiralis larvae and together with control (uninfected) age-matched mice were then given a challenge infection of 300 T. spiralis larvae 20 days later. Mice were killed on either day 4 or 7, post challenge. The mean worm recoveries from the challenge and control mice are shown in Fig. 1.6. The results indicate the mice were substantially immune and that worm expulsion began by day 4 and was completed by day 7.

TABLE 1.2

Rapid expulsion of challenge infections given at intervals after a primary infection of T. spiralis in NIH mice.

Group of mice		Challenge infection Day	No. of worms recovered 24 hours after challenge Mean	S.D.
1	Challenged	14	3.2*	6.6
	Control		151.8	18.5
2	Challenged	16	8.0*	5.2
	Control		154.8	21.0
3	Challenged	18	153.0	37.4
	Control		182.2	48.7
4	Challenged	21	176.4	22.3
	Control		164.8	65.0
5	Challenged	28	135.5	25.9
	Control		142.6	36.3
6	Challenged	35	131.6	14.3
	Control		110.4	28.5

* Mean significantly lower than control

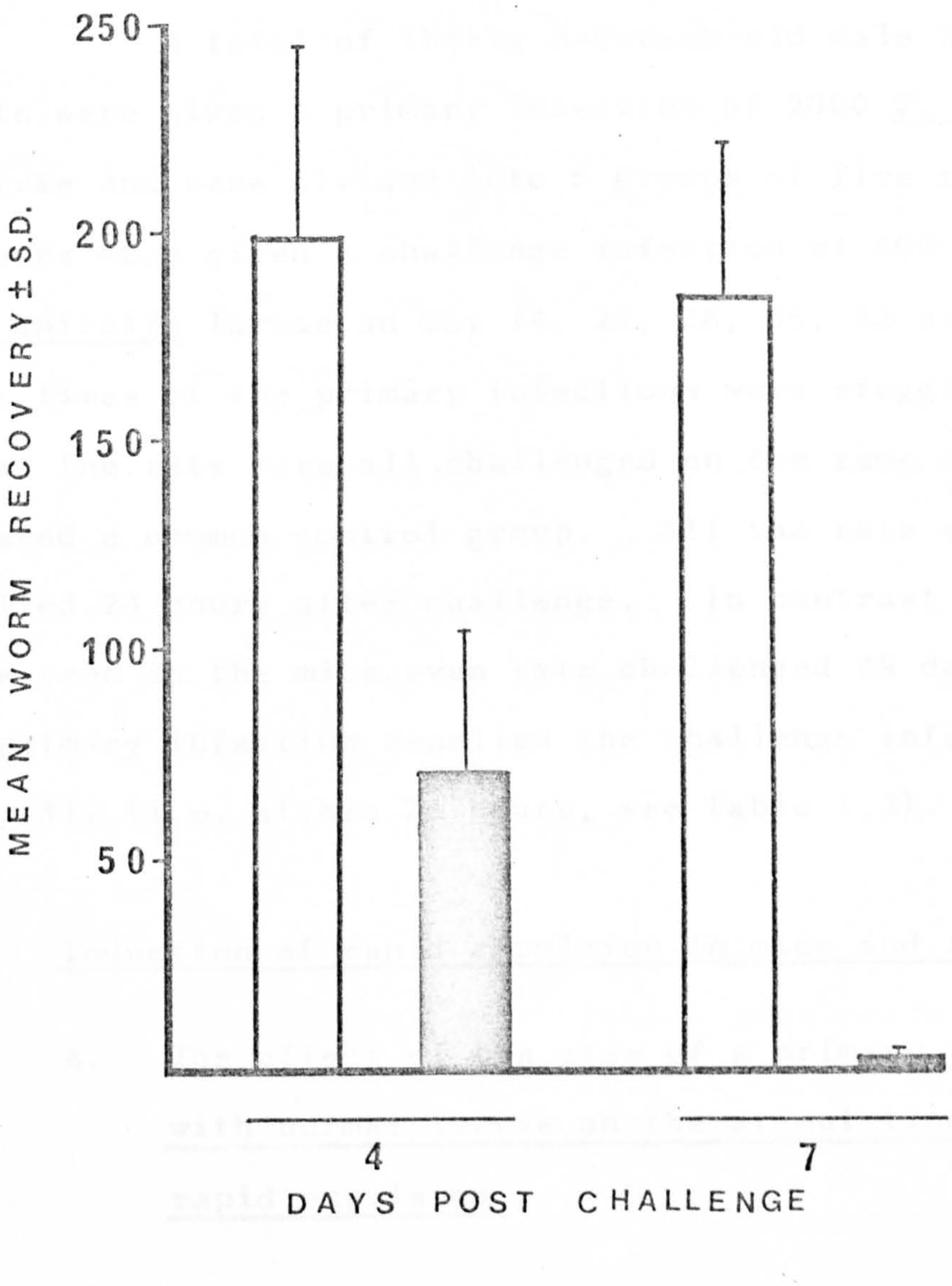
$p < 0.05$

Fig. 1.6

Number of worms (mean±SD) recovered from the small intestine of NIH mice following challenge infection with T. spiralis on day 20 of primary infection.

▬ immune

▭ non-immune (control)


MEAN WORM RECOVERY ± S.D.
250
200
150
100
50
4
7
DAYS POST CHALLENGE

Hence the failure to expel worms rapidly within 24 hours was not associated with any general reduction in protective immunity.

b) Rats

A total of thirty 8-9-week-old male Wistar rats were given a primary infection of 2000 T. spiralis larvae and were divided into 6 groups of five rats. Groups were given a challenge infection of 500 T. spiralis larvae on day 14, 21, 28, 35, 42 or 49 p.i. The times of the primary infections were staggered so that the rats were all challenged on the same day and shared a common control group. All the rats were killed 24 hours after challenge. In contrast to what was seen in the mice, even rats challenged 49 days after a primary infection expelled the challenge infection rapidly (i.e. within 24 hours, see Table 1.3).

4. Induction of rapid expulsion in mice and rats

A. The effect of the size of a primary infection with normal larvae on the stimulation of rapid expulsion

a) Mice

An experiment was carried out to characterize some of the conditions necessary for the induction of rapid expulsion in mice. Fifty-six 7-week-old male

NIH mice were divided into four groups (1-4, 14 mice per group). These mice received primary infections of 50, 150, 300 or 500 normal T. spiralis larvae respectively. Four mice from each group were killed on day 7 and another four on day 14 p.i. to determine the establishment and survival of the various infections. The remaining six mice from each group and a control group of six uninfected age-matched mice, were challenged on day 14 after primary infection with 300 T. spiralis larvae and then killed 24 hours later. The results are shown in Table 1.4.

In all groups of mice, primary worm establishment and expulsion were at the expected level (see Chapter 1). Rapid expulsion did not occur in mice given a primary infection of 50 larvae, but when higher levels of primary infection were used, the challenge infections were expelled rapidly.

b) Rats

For comparative purposes, twenty-six 8-9-week-old Wistar rats were divided into three groups, six rats each in groups 1 and 2 and fourteen rats in group 3. These rats were given a primary infection of 200, 500 or 2000 T. spiralis larvae respectively. (Infections were similar in terms of larvae per gram body weight to those used in mice). Four of the rats which had been infected with 2000 larvae (group 3) were killed on day 7 and another four on day 14 of the primary infection.

TABLE 1.3

Rapid expulsion of challenge infections given at intervals after a primary infection of T. spiralis in Wistar rats.

Group of rats	Challenge infection	No. of worms recovered 24 hours after challenge	
	Day	Mean	S.D.
1	14	3.8*	5.7
2	21	4.8*	5.2
3	28	26.3*	2.0
4	35	2.4*	2.5
5	42	3.4*	3.2
6	49	5.8*	5.1
Challenge infection only	none	188.0	24.3

* Mean significantly lower than control

$p < 0.05$

TABLE 1.4

Rapid expulsion of T. spiralis from NIH mice given different levels of primary infections of normal larvae. (Challenge infection given 14 days after primary infection).

Size of primary infection	Primary infection				Challenge infection	
	Day 7		Day 14		24 hours after challenge	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Normal larvae						
50	31.2	8.8	0.0	0.0	165.0	39.9
150	95.7	12.6	0.0	0.0	8.8*	20.2
300	191.2	24.2	0.0	0.0	0.6*	1.3
500	363.2	11.7	0.0	0.0	0.0*	0.0
none	-	-	-	-	151.5	19.7

* = Mean significantly lower than control

$p < 0.05$

Mean worm recoveries were 967.0±206 and 140±170 respectively. Three of the rats killed on day 14 had very few worms present, one rat still had 385 worms. Groups 1 and 2 and six rats from group 3, were given anthelmintic (methyridine) on day 13 of the primary infection to remove residual worms. These previously infected rats, together with six control (uninfected) age-matched rats, were given a challenge infection of 500 T. spiralis larvae on day 14 p.i. All the rats were killed 24 hours later (Table 1.5). Rapid expulsion of challenge larvae occurred in all the previously infected rats within 24 hours. Even the lowest level of primary infection (200 larvae) was effective in priming for rapid expulsion.

B. Ability of primary infections with irradiated larvae to stimulate rapid expulsion

a) Mice

Twenty 7-week-old male NIH mice were divided into two groups of 10 (groups 1 and 2). These mice received primary infections of 300 and 500 irradiated (4 Krad) T. spiralis larvae respectively. This level of irradiation produces sterility in the developing adult worms, (Larsh, 1963), so that any immunizing properties are those related to preadults and sterile adults, without any contribution from migrating and encysting larvae. Four mice from each group were killed on day 7 to determine the establishment of the

TABLE 1.5

Rapid expulsion of T. spiralis from Wistar rats given different levels of primary infection of normal larvae. (Challenge infection given 14 days after primary infection).

Size of primary infection	No. of worms recovered 24 hours after challenge	
	Mean	S.D.
Normal larvae		
200	16.8*	15.0
500	2.6*	1.7
2000	3.0*	3.5
none	141.2	11.0

* Mean significantly lower than control

$p < 0.05$

infection. The remaining six mice from each group and a control group of six uninfected age-matched mice were challenged 14 days after primary infection with 300 normal T. spiralis larvae and were killed 24 hours later. The results are shown in Table 1.6. Mice given a primary infection of 300 or 500 irradiated larvae did not show rapid expulsion of the challenge infection of normal larvae.

The immune status of mice infected with irradiated larvae in terms of their ability to reject worms over a 7 day period, was then examined. Mice were infected with 300 irradiated (4 Krad) T. spiralis larvae, challenged with normal larvae (together with control uninfected mice) on day 21 p.i. and were killed on day 7 post challenge. Immune mice showed significant loss of worms by day 7 post challenge, worm recoveries from the immune and control groups being 46±7.4 and 144.2±36.8 respectively, thus indicating that the irradiated larvae render mice immune to challenge infection.

b) Rats

Six 8-9-week-old Wistar rats were infected with 2000 irradiated larvae (4 Krad) T. spiralis larvae. Fourteen days after the primary infection, these rats, together with six control uninfected rats, were given a challenge infection of 500 T. spiralis normal

TABLE 1.6

Rapid expulsion of T. spiralis from NIH mice given different levels of primary infection of irradiated larvae. (Challenge infection of normal larvae given 14 days after primary infection).

Size of primary infection	Primary infection		Challenge infection	
	Day 7		24 hours after challenge	
	Mean	S.D.	Mean	S.D.
Irradiated larvae				
300	12.4	23.5	130.2	26.8
500	180.2	26.7	162.4	32.4
none	-	-	149.8	33.1

Table 1.7

Rapid expulsion of T. spiralis from Wistar rats infected with irradiated larvae. (Challenge infection of normal larvae given 14 days after primary infection).

Size of primary infection	No. of worms recovered 24 hours after challenge	
	Mean	S.D.
Irradiated larvae		
2000	7.8*	4.0
none	141.2	11.0

* Mean significantly lower than control

$p < 0.05$

larvae and were killed 24 hours later. The results (Table 1.7) indicate that in rats, in contrast to the above experiment in mice, an infection with irradiated larvae was effective in priming for rapid expulsion.

5. Effect of size of challenge infection upon expression of rapid expulsion in mice

In rats, rapid expulsion is known to be effective against both small and large challenge infections (Bell and McGregor, 1979a). To find whether this held true for mice, a group of eighteen 7-week-old male NIH mice was infected with approximately 300 T. spiralis larvae and divided into three groups of six mice (groups 1, 2 and 3). Fourteen days after the primary infection, groups 1, 2 and 3, together with the three similar groups of non-infected age-matched control mice, were challenged with 30, 300 and 600 T. spiralis larvae, respectively. All of the groups were killed 24 hours after the challenge infection. The results are shown in Table 1.8. The results indicate that expulsion of the lowest challenge infection (30 larvae) was as complete as that of the highest level of challenge infection (600 larvae).

TABLE 1.8

Rapid expulsion of T. spiralis from low- and high-level challenge infections given to NIH mice on day 14 of a primary infection.

Size of challenge infection	No. of worms recovered 24 hours after challenge			
	Control mice		Challenged mice	
	Mean	S.D.	Mean	S.D.
30	13.8	3.3	0.1*	0.4
300	139.5	45.2	1.0*	2.3
600	371.0	36.7	12.0*	15.1

* Mean significantly lower than the corresponding controls

$p < 0.05$

DISCUSSION

The course of a primary infection with T. spiralis in NIH mice has been studied by a number of workers (Wakelin and Lloyd, 1976a; Manson-Smith, Bruce, Rose and Parrott, 1979b; Kennedy, 1980). The results of the initial experiment described here, confirm their results, in that primary infections were expelled between days 8-12 and indicate that the response characteristics of NIH mice had not altered significantly over a 5 year period.

A primary infection is characterized by three stages. Following infection, almost 55% of the infecting dose establish and develop to the maturity in the small intestine. A proportion (45%) of the larvae do not establish. As in the case of Nippostrongylus brasiliensis (Jarrett, Jarrett and Urquhart, 1968), it is doubtful if this initial failure to establish is associated with the acquired immune response, but its cause remains unknown. The number of worms present in the gut remained relatively constant between days 1 and 8 and the majority of the worms were located in the anterior half of the small intestine during this time. Expulsion was preceded by a movement of worms from their initial position in the anterior of the small intestine to the posterior region. Larsh, Gilchrist and Greenberg (1952) and Campbell (1967) found a similar pattern of distribution of T. spiralis in

young mice. However, other patterns of distribution can occur as demonstrated by Denham (1968) and Manson-Smith et al. (1979b), who found that most of the adult worms were recovered from the posterior half of the small intestine in ICl albino mice and BALB/c mice respectively. Although the factors that control worm distribution and site selection are not fully understood, there is good evidence that some helminths are capable of moving into preferred sites after introduction into the intestine (Alphey, 1970; Bråten and Hopkins, 1969). However, in the case of T. spiralis this does not seem to be so and worms injected directly into the intestine will remain at the site of injection (Kennedy, 1980; Sukhdeo and Croll, 1981). Thus, the distribution of T. spiralis in hosts infected orally presumably reflects the pattern of initial establishment and this will be determined by a variety of host factors, such as age, diet and intestinal motility (Campbell, 1967). A change in pattern of distribution of T. spiralis in NIH mice presumably reflects the onset of intestinal changes associated with the development of the immune response as has also been suggested for a number of other nematode parasites, (e.g. Nippostrongylus brasiliensis, Alphey (1970) and Strongyloides ratti, Moqbel and Denham (1977).

In NIH mice the loss of worms which accompanies the changes in distribution pattern is very sudden in onset and proceeds to completion very rapidly. The

intestine at this time shows gross inflammatory changes which appear initially in the anterior half of the small intestine, but then spread to involve the whole gut (see chapter 3).

It is known that the inflammation is mediated by thymus-dependent immune responses (Wakelin, 1978a) and is associated with cellular infiltration in the mucosa (Larsh, 1963); at the same time there are profound physico-chemical changes in the intestinal environment (Castro, 1976). Larsh and Race (1975) suggested that worm loss may result directly from such environmental changes, which produce unfavourable conditions for worm survival and only indirectly from the immune response.

A similar pattern of worm expulsion during primary infection was observed in rats, with worm expulsion occurring after day 8 and being almost complete by day 14. These results confirm those that have been reported by Gursch (1949), Gore, Burger and Sadun (1974), Ruitenberg (1974) and Love, Ogilvie and McLaren (1976), but in rats the worm distribution was not similar to that seen in NIH mice. In rats the majority of the worm population was located in the anterior half of the small intestine throughout the course of the primary infection.

The ability of primary infections of T. spiralis to engender immunity to challenge, has been

reported by several workers with the expression of this immunity being measured in terms of an accelerated reduction in number of adult worms. For example, Rappaport and Wells (1951) reported that when mice were challenged after a single infection with T. spiralis, accelerated worm expulsion occurred after 8 days compared with 17 days in the primary. In the experiments described here it was evident that challenge infections could be expelled much more rapidly.

Initial experiments showed that challenge infections given shortly after expulsion of a primary infection when the gross inflammatory changes had disappeared, were expelled rapidly within 24 hours (rapid expulsion) in mice. Detailed observation showed that rapid expulsion occurred between 1.5 and 6 hours after challenge. The rapidity with which challenge infections are expelled suggests that either the intestinal environment is so altered by the primary infections that conditions are unsuitable for subsequent parasite establishment, or that the intestine is primed in such a way that the stimulus provided by the challenge evokes an almost instantaneous response. The recovery at 1.5 hours after challenge of an equivalent number of larvae from both immune and control mice, might imply normal establishment as it is known (Despommier, Sukhdeo and Meerovitch, 1978) that larval penetration into the mucosa is extremely rapid, but there are insufficient experimental data to justify this

conclusion at present and it is equally possible that the larvae recovered were in the lumen rather than in the mucosa. Thus both mechanisms of rapid expulsion must be considered. The temporal relation between the intestinal changes and rapid expulsion and also the factors that generate such a condition will be discussed later (see chapter 3 in this section). A similar time course for rapid expulsion has been reported by Russell and Castro (1979) who noted that rapid expulsion occurred within 15 minutes after contact of the parasite with intestinal mucosa in immune rats. In mice this response to challenge was short-lived, challenge infections given more than 16 days after primary infection were not subjected to rapid expulsion, in this case worms were expelled more slowly (between days 4 and 7 after challenge). In rats, however, the rapid expulsion response was persistent and was shown to operate against challenges given up to 49 days after primary infection, confirming the earlier observation of Bell and McGregor (1979a).

The loss of the rapid expulsion response after day 16 of a primary infection in immune mice might be linked with a shorter duration of the cellular changes associated with intestinal inflammation. However, many other factors may be involved and the possibility of there being immune suppression induced by the muscle larvae present at that time cannot be excluded as it is

well established that infection with T. spiralis depresses both humoral (Faubert and Tanner, 1971) and cellular (Ljungstrom and Huldt, 1977) immune responses to unrelated antigens.

The persistence of the response in rats may be due to long-term persistence of cellular or physico-chemical changes in the intestinal mucosa, or it may be that the capacity of rats to express rapid expulsion is different from that of NIH mice and may involve quite distinct mechanisms. In the rat system it has been shown by Russell and Castro (1979) that resistance to the actual establishment of larvae is the major factor responsible for the reduced worm burden found after challenge infection in immune rats.

Primary infections with more than 50 larvae were necessary to induce rapid expulsion in mice, indicating that the factors required for the response are dependent upon the size of the primary infection. However, infection with irradiated larvae did not prime for rapid expulsion even when relatively large infections were given. It is possible that irradiated larvae do not survive as well as normal larvae or that they cause less changes in the intestinal environment, but it may also be that such larvae are less immunogenic, at least as far as rapid expulsion is concerned, because of irradiation induced damage to their development processes (Kim, 1957). Failure of irradiated larvae

to induce rapid expulsion was not associated with any loss of immune capacity, because challenge infections given on day 21 after primary infection with irradiated larvae were expelled in an accelerated fashion. Here again, rats differed markedly from mice, with even low level primary infections (200 normal larvae) or infections with irradiated larvae stimulating a strong rapid expulsion response.

In contrast to the species differences in priming for the response, the expression of rapid expulsion seems to be similar in both rats and mice. Rapid expulsion was effective against both small and large challenge infections. Bell and McGregor (1979a) have reported similar results in rats.

CHAPTER 2

STAGE-SPECIFICITY OF INDUCTION AND EXPRESSION OF RAPID EXPULSION

INTRODUCTION

It has been shown (Chapter 1) that in NIH mice, a complete infection with T. spiralis is required to stimulate rapid expulsion of a challenge infection given immediately after the primary infection has been expelled.

The immunity engendered by a complete infection of T. spiralis in the host is considered to be due to all stages of the parasite (Despommier, 1977) and the response induced by the intestinal (enteral) phase of infection, is extremely complex. In a complete infection of T. spiralis, three separate stages are involved: 1 - preadult - the development from infective larvae to the final moult to the adult (this takes approximately 48 hours), 2 - adult - the stage after the final moult (this stage includes both sexually immature and mature adults), 3 - migrating larvae - larvae released from adult females into the small intestine from day 5. These migrate and encyst in skeletal muscles (see review of these stages by Larsh, 1963 and Bell, McGregor and Despommier, 1979).

In complete infections, it is difficult to study the contribution of the intestinal stages in isolation from the newborn and migrating larvae. Many techniques have therefore been devised to study the immunity against particular stages of T. spiralis.

Studies have been undertaken on the induction of immunity by exposure to intestinal infections that are limited in duration or that are restricted to a particular developmental stage by the use of irradiated larvae or drug abbreviated infections (see Campbell, Hartman and Cuckler, 1963a; Kim, 1957; Denham, 1966b). The development of transplantation techniques has allowed investigation of the expression of immunity against adult worms.

One of the objectives of the studies in this chapter, was to elucidate some of the conditions necessary for the induction of rapid expulsion against T. spiralis larvae by the use of an anthelmintic to limit the duration of primary infections. Comparisons were made between the duration of primary infections needed to stimulate rapid expulsion in mice and rats. In some cases, infections with normal or irradiated larvae were limited to 3 days, i.e. to the intestinal stages only without any immunizing contribution from migrating larvae. In others, multiple, 3 day infections were used to examine their effects on the stimulation of the rapid expulsion response with normal and irradiated larvae.

A second objective was to investigate the susceptibility of adult T. spiralis to the rapid expulsion response induced by a complete primary infection with normal larvae. Transplantation

techniques were used to obtain adult worm infections without prior exposure to infective larvae. Finally, the susceptibility of normal and irradiated larvae to the rapid expulsion response, induced by complete infections with normal or irradiated larvae, was examined.

MATERIALS AND METHODS

The strain of mice or rats and the methods used for maintenance, infection and worm recovery have been described in the general materials and methods.

Drug abbreviated infections

To expose animals to the intestinal phase of T. spiralis, mice or lightly anæsthetised rats were infected orally with normal or irradiated larvae. Three days after infection animals were given an anthelmintic (see general materials and methods) to eliminate adult worms before larval production (i.e. infections were limited to 3 days). The animals were given one or several such infections. Methods used for monitoring the efficiency of the drug and for irradiating larvae have been described in the general materials and methods.

Methods for transplantation of adults or T. spiralis larvae

Infective T. spiralis larvae were obtained by the methods described previously. Five-day-old adult worms were recovered by the modified Baermann technique, but incubation was limited to 45-60 minutes, instead of 2 hours. Worms were selected randomly and no attempt was made to differentiate between male and female worms.

The required number of adult worms was removed into a small test tube (70 x 10mm) and allowed to sediment at room temperature. The volume of HBSS (Hanks balanced salt solution) was then reduced to approximately 0.2 ml. Worms were normally transplanted not more than 2 hours after the donors had been killed. (It was found that worms used more than 2 hours after the death of the donors established less readily).

Recipient mice were anaesthetised with sagatal (sodium pentobarbitone 60 mg/ml, May and Baker Ltd., Dagenham), in a solution of 95% ethanol and HBSS in the ratio of 1:2½ :9 by volume. This solution was injected intraperitoneally (I/P) at a dose of 0.01 ml/g body weight. At this dosage, anaesthesia is induced within a few minutes and persists for 2-3 hours. Operations were performed within 30-60 minutes of administration of anaesthetic. The mice were fixed to the operating surface using plastic tape and the skin over the upper abdomen shaved and sterilised with 70% ethanol. A small incision (approximately 1 cm long) was made through the skin near the mid-line. The body wall was pierced with a scalpel and the incision was extended using blunt forceps. The proximal duodenum was pulled out through the incision from under the liver using a small metal hook. A hole was made through the duodenal wall with a hypodermic needle and the adult worms or larvae were injected from a drawn out glass pipette. Parasites were expelled into the duodenum in

0.2 ml HBSS (adult) or 0.2% agar (larvae) in a direction away from the stomach. After removal of the pipette the hole was sutured (0.7 Mer Silk Mersuture, Ethicon Ltd.,Edinburgh). The peritoneal cavity and intestine were sprayed with an antibiotic mixture of bacitracin, neomycin and polymixin (Rikospray, Riker Laboratories Ltd., Loughborough). The intestine was replaced in the body cavity and the body wall closed with sutures and sprayed again with Rikospray. The skin was sutured, sprayed with Rikospray and sealed with aerosol plastic skin (Nobecutane, Astra Chemicals Ltd., Watford). Blood loss was negligible and deaths following laparatomy were rare (usually due to anaesthesia). Operated animals were provided with antibiotic (terramycin) in the drinking water (see general materials and methods).

RESULTS

1. Induction of rapid expulsion in mice and rats - duration of infection

Large primary infections were necessary to evoke a rapid expulsion response in mice (see Chapter 1). To examine the duration of infection necessary to stimulate rapid expulsion, animals were immunized by primary infections which were terminated at various times post infection by the use of anthelmintic.

a) Mice

Twenty 7-week-old male NIH mice received primary infections of 300 T. spiralis larvae and were divided into four groups (1-4) of five mice. Three groups of these mice (1, 2 and 3) received Methyridine on days 7, 9 or 13 respectively, group 4 was untreated.

On day 14 post infection, these previously infected mice, together with a control group of six uninfected age-matched mice, were given a challenge infection of 300 T. spiralis larvae and were killed 24 hours later. The results are shown in Table 2.1. Rapid expulsion did not occur in mice given anthelmintic treatment on day 7 (group 1). The majority of challenge larvae were expelled from previously infected mice given anthelmintic treatment on day 9 (group 2) and challenge larvae were expelled completely from

Table 2.1

The effect of duration of primary infection in NIH mice on the rapid expulsion of a challenge infection given on day 14.

Groups	Anthelmintic drug, primary infection	No. of worms recovered 24 hours after challenge Mean	S.D.
1	Day 7	128.0*	20.5
2	Day 9	40.0	16.4
3	Day 13	2.6	2.0
4	none	4.2	3.4
5	none-challenge control	138.0	8.4

* Mean was not significantly lower than control.

$p > 0.05$

infected mice given anthelmintic on day 13 (group 3) and from the untreated group (group 4).

b) Rats

Six, 8-9-week-old female Wistar rats were given a primary infection of 2000 T. spiralis larvae and received Methyridine on day 7 post infection. Fourteen days after this primary infection these previously infected rats, together with six control, uninfected age-matched rats, received a challenge infection of 500 T. spiralis larvae and were killed 24 hours later. The mean worm recoveries from experimental and control rats were 27.3±39 and 188±24 respectively. The results indicate that in contrast to the above experiment in mice, in rats even 7 days exposure to T. spiralis infection stimulated a strong rapid expulsion response to challenge larvae.

2. Induction of rapid expulsion by single or multiple abbreviated infections

It has been shown that rapid expulsion of T. spiralis in rats is phase specific in induction and expression, for example, exposure to preadult infection lasting only 48 hours will elicit a rapid expulsion response on challenge with infective larvae (Bell and McGregor, 1979b). Experiments were designed to see whether this also held true for mice.

A. Abbreviated infections with normal T. spiralis larvae

For these experiments, mice were exposed to single or repeated infections (with normal larvae) which were limited to 3 days duration (i.e. before the production of migrating larvae by the adult females). Any immunization that resulted could then be assumed to be due to the intestinal phase of T. spiralis (preadult and immature adult) without any contribution of migrating and muscle larval stages.

a) Mice

Forty-eight, 7-week-old male NIH mice were divided into four groups of 12 mice (1-4) and half of each group exposed to primary infections of 3 days duration. Mice of group 1 were given one infection, those of groups 2, 3 and 4 received two, three and four such infections, each infection being given 7 days after the previous infection. (During the anthelmintic treatment one mouse died from each of groups 2, 3 and 4). All infections were with 300 larvae. The experimental design is shown on page 70.

Mice in groups 1 and 2 were challenged, together with control groups on day 14. Mice in group 3 were challenged together with a control group on day 21 and mice in group 4 were challenged together with a control group on day 35. All the groups were killed 24 hours after challenge. The results are shown in

Table 2.2. Mice given one abbreviated infection and challenged on day 14 did not show rapid expulsion (group 1). Rapid expulsion of challenge larvae occurred only in the groups of mice given two or more drug-abbreviated infections (groups 2, 3 and 4).

b) Rats

To confirm the results obtained by Bell and McGregor (1979b) with 48 hour immunizations, and also to compare the induction of rapid expulsion by abbreviated infections in rats and mice similar experiments were designed for rats in which thirty-six, 8-9-week-old male Wistar rats were divided into 6 groups of six rats (1-6). These rats were infected every 7 days with 2000 T. spiralis larvae and the infections were limited to 3 days duration by anthelmintic. The experimental design shown on page 72 was essentially similar to that used with mice except that all the challenge infections were given at the same time.

Rats in groups 1, 2, 3, 4, 5 and 6 received two, three, four, five, six and seven drug-abbreviated infections respectively and challenge infections of 500 larvae were given on days 14, 21, 28, 35, 42 and 49 respectively. All the groups were killed 24 hours after challenge. The results are shown in Table 2.3.

All the immune rats which had been given two or more repeated infections showed rapid expulsion of

Abbreviated infections with normal T. spiralis larvae in mice

Experimental design

	Days treatment										
	0	3	7	10	14	17	21	24	28	31	35
Group 1	inf	m	-	-	chall	-	-	-	-	-	-
Control	-	-	-	-	chall	-	-	-	-	-	-
Group 2	inf	m	inf	m	chall	-	-	-	-	-	-
Control	-	-	-	-	chall	-	-	-	-	-	-
Group 3	inf	m	inf	m	inf	m	chall	-	-	-	-
Control	-	-	-	-	-	-	chall				
Group 4	inf	m	inf	m	inf	m	inf	m	inf	m	chall
Control	-	-	-	-	-	-	-	-	-	-	chall

inf = infection

m = Methyridine

chall = challenge infection

Table 2.2

Rapid expulsion of challenge infections of *T. spiralis* from NIH mice given single or repeated drug abbreviated infections (3 day duration).

Groups		No. of drug abbreviated infections	Challenge infection Day	No. of worms recovered 24 hours after challenge Mean	S.D.
1	infected	1	14	176.2	28.6
	control	-	14	152.8	18.4
2	infected	2	14	8.6*	7.4
	control	-	14	136.4	62.5
3	infected	3	21	4.9*	2.3
	control	-	21	158.5	48.2
4	infected	5	35	22.5*	12.6
	control	-	35	126.3	16.5

* Mean significantly lower than corresponding controls

$p < 0.05$

Abbreviated infections with normal T. spiralis larvae in rats

Experimental design

	Days treatment														
	0	3	7	10	14	17	21	24	28	31	35	38	42	45	49
Group 6	inf	m	inf	m	inf	m	inf	m	inf	m	inf	m	inf	m	chall
Group 5	-	-	inf	m	inf	m	inf	m	inf	m	inf	m	inf	m	chall
Group 4	-	-	-	-	inf	m	inf	m	inf	m	inf	m	inf	m	chall
Group 3	-	-	-	-	-	-	inf	m	inf	m	inf	m	inf	m	chall
Group 2	-	-	-	-	-	-	-	-	inf	m	inf	m	inf	m	chall
Group 1	-	-	-	-	-	-	-	-	-	-	inf	m	inf	m	chall
Challenge infection only	-	-	-	-	-	-	-	-	-	-	-	-	-	-	chall

All the groups killed one day after challenge

inf = infection

m = Methyridine

chall = challenge infection

Table 2.3

Rapid expulsion of challenge infections of *T. spiralis* larvae from Wistar rats given repeated drug-abbreviated infections (3 days duration).

Groups	No. of drug abbreviated infections	Challenge infection Day	No. of worms recovered 24 hours after challenge Mean	S.D.
1	2	14	8.6*	7.1
2	3	21	2.0*	1.7
3	4	28	1.6*	0.8
4	5	35	4.6*	4.2
5	6	42	0.8*	1.3
6	7	49	0.8*	1.2
7	none	challenge infection only	188.4	24.3

* mean significantly lower than control

$p < 0.05$

challenge larvae whether given on day 14 or up to 7 weeks after the first immunizing infection.

B. Abbreviated infection with irradiated larvae in mice

The above experiments showed that two or more drug abbreviated infections with normal larvae were necessary to induce rapid expulsion and that with continued repeated infections, it was possible to elicit the response for at least 5 weeks after the first immunizing infection. Although complete infections with irradiated larvae did not stimulate rapid expulsion (see chapter 1), it was decided to study the ability of repeated abbreviated infections with irradiated larvae to stimulate the response in order to determine whether an increased total exposure would be effective. As before, infections were limited to 3 days.

Fourteen 7-week-old male NIH mice were divided into two groups of 7 mice (1, 2). Mice from group one were given two infections of 300 irradiated T. spiralis larvae on day 0 and day 7 and each infection was terminated with Methyridine three days later. Mice from group 2 were similarly given three infections of 300 irradiated larvae on days 0, 7 and 14. Groups 1 and 2 were given challenge infections of 300 normal larvae on days 14 and 21 respectively. The times of the infections were staggered so that experimental groups were challenged on the same day and shared

Table 2.4

Rapid expulsion of challenge infections of normal T. spiralis larvae from NIH mice given repeated drug-abbreviated infections (3 days duration) with irradiated larvae.

Groups	No. of drug abbreviated infections	Challenge infection Day	No. of worms recovered 24 hours after challenge Mean	S.D.
1	2	14	132.5	31.4
2	3	21	161.3	26.3
3	none	challenge infection only	182.5	16.4

a common control. All the groups were killed 24 hours after challenge. The results are shown in Table 2.4. Mice given two or three drug-abbreviated infections with irradiated larvae did not show rapid expulsion.

3. Expression of the rapid expulsion response as measured by the susceptibility of different stages of T. spiralis to this response

The previous experiments showed that infective larvae given orally were susceptible to rapid expulsion generated both by complete infections and by two or more repeated intestinal infections with preadult and immature adult stages. It has been shown in rats that adult worms are resistant to rapid expulsion and that administration of a superimposed challenge infection of infective larvae, which is itself rapidly expelled, does not affect an existing adult worm population (Bell and McGregor, 1979a).

Experiments were designed to test a) whether laparatomy itself had any effect on the ability of subsequent oral infections or of transplanted worms to establish in the intestine; b) whether surgically implanted infective larvae or adult worms would initiate and be susceptible to rapid expulsion in mice primed by a complete infection; c) whether orally administered challenge larvae have any effect on an existing adult population present at the time of challenge.

a) Effect of laparatomy on subsequent infection:
Twelve 7-week-old male NIH mice were infected orally with 300 T. spiralis larvae on day 0 and they were divided into two groups of six mice (1-2). On day 14 group one, together with six control uninfected age-matched mice, was given a sham transplant operation, i.e. instead of worms, 0.2 ml of HBSS was injected into the small intestine. Three hours later (the time necessary for recovery from anaesthesia) the mice were challenged orally with 300 T. spiralis larvae. Mice from group 2, together with control uninfected mice, were also challenged and all the mice were killed one day later. The numbers of worms recovered from the sham operated immune and control mice were 21±19 and 122.5±18 respectively. The numbers of worms recovered from immune and control mice not given the sham operation were 3.6±1.8 and 154.8±43 respectively. The results suggest that sham operation had no major effect on subsequent infection, as far as establishment in control mice, or as far as rapid expulsion in immune mice was concerned.

b) Susceptibility of transplanted infective larvae or adult worms to rapid expulsion induced by complete infection:
Six 7-week-old male NIH mice were infected orally with approximately 300 T. spiralis larvae. On day 14 of a primary infection, these infected mice together with six control uninfected mice were given 300 infective larvae

by direct injection into the duodenum. All the mice were killed 24 hours after transplantation. The results are shown in Table 2.5 (groups 3 and 4). In this experiment, the majority of the transplanted larvae were expelled rapidly from immune mice, but transplanted larvae established normally in the control uninfected mice.

This experiment showed that transplanted infective larvae were susceptible to rapid expulsion. The next experiment examined whether this would also be true of transplanted adult worms. Six 7-week-old male NIH mice were infected orally with approximately 300 T. spiralis larvae. On day 14 of the primary infection, these previously infected mice, together with six uninfected age-matched control mice, were given 100 5-day-old adult worms by direct injection into the duodenum. Receipient mice (immune and control) were killed one day after transplantation of adult worms. The results are shown in Table 2.5 (groups 1 and 2). Transplanted adult worms were expelled rapidly from immune mice, but they had the ability to establish normally in relation to control mice.

In order to examine whether challenge larvae given orally have any effect on an existing adult population present at a time of challenge, a further experiment was designed.

Six 7-week-old male NIH mice were infected orally with 300 T. spiralis larvae. On day 14 of the primary infection, those mice, together with six uninfected, age-matched control mice, were given 100 5 day adult worms by direct injection into the duodenum. Three hours after transplantation of adult worms (i.e. the time necessary for recovery from anaesthesia) recipient mice were infected orally with 300 larvae and were killed 24 hours later. The results are shown in Table 2.5 (groups 5 and 6). Both the 5 day-old adult worms and the larvae were expelled rapidly from immune mice and both established normally in control uninfected mice.

4. Susceptibility of normal or irradiated larvae to rapid expulsion induced after primary infection with irradiated or normal larvae

In repeated experiments (Chapter 1) it has been observed that stimulation with irradiated (4 Krad) T. spiralis larvae, did not prime mice to express rapid expulsion when a challenge infection of normal larvae was given on day 14 of a primary infection. A series of experiments was carried out to, a) confirm this finding, b) examine the susceptibility of irradiated larvae to rapid expulsion induced by prior infection with irradiated larvae and c) examine the susceptibility of irradiated larvae to rapid expulsion induced by prior infection with normal larvae.

Table 2.5

Rapid expulsion of 5-day-old adult worms or infective larvae of T. spiralis transplanted into, or given orally, to NIH mice on day 14 of a primary infection.

Group	Infection Day 0	Challenge Day 14	Worm recoveries 24 hours after challenge Mean	S.D.
1	300 larvae	100 adult+	1.6	1.7
2	none	100 adult+	30.0	9.4
3	300 larvae	300 larvae+	20.5	16.2
4	none	300 larvae+	127.2	13.4
5	300 larvae	100 adult+	2.6	3.4
		300 larvae (orally)	12.2	5.5
6	none	100 adult+	36.0	9.7
		300 larvae (orally)	102.6	30.2

+ Transplanted worms

The design of the first experiment followed previous patterns (Chapter 1). In the second experiment, six mice were infected with 300 irradiated larvae and 14 days after primary infection these mice, together with six control uninfected mice, were given 300 irradiated larvae and killed 24 hours later.

In the third experiment, twelve 7-week-old male NIH mice were infected orally with 300 T. spiralis larvae on day 0 and divided into two groups of six mice (groups 2a and 2b). On day 14 of the primary infection, group 2b mice, together with control uninfected mice, were given a challenge infection of 300 irradiated larvae and the other group (2a), together with six uninfected control mice, were given a challenge infection of 300 normal larvae. All the mice were killed 24 hours later. The results of all these experiments are shown in Fig. 2.1.

(N.B. The controls were common to both sets of experimental groups).

Fig. 2.1

The effect of normal of irradiated larvae on induction and expression of the rapid expulsion of challenge, given on day 14 of a primary infection.

Group 1a infected and challenged with irradiated larvae

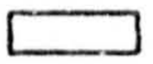
Group 1b infected with irradiated larvae, challenged with normal larvae

Group 2a infected with normal larvae challenged with irradiated larvae

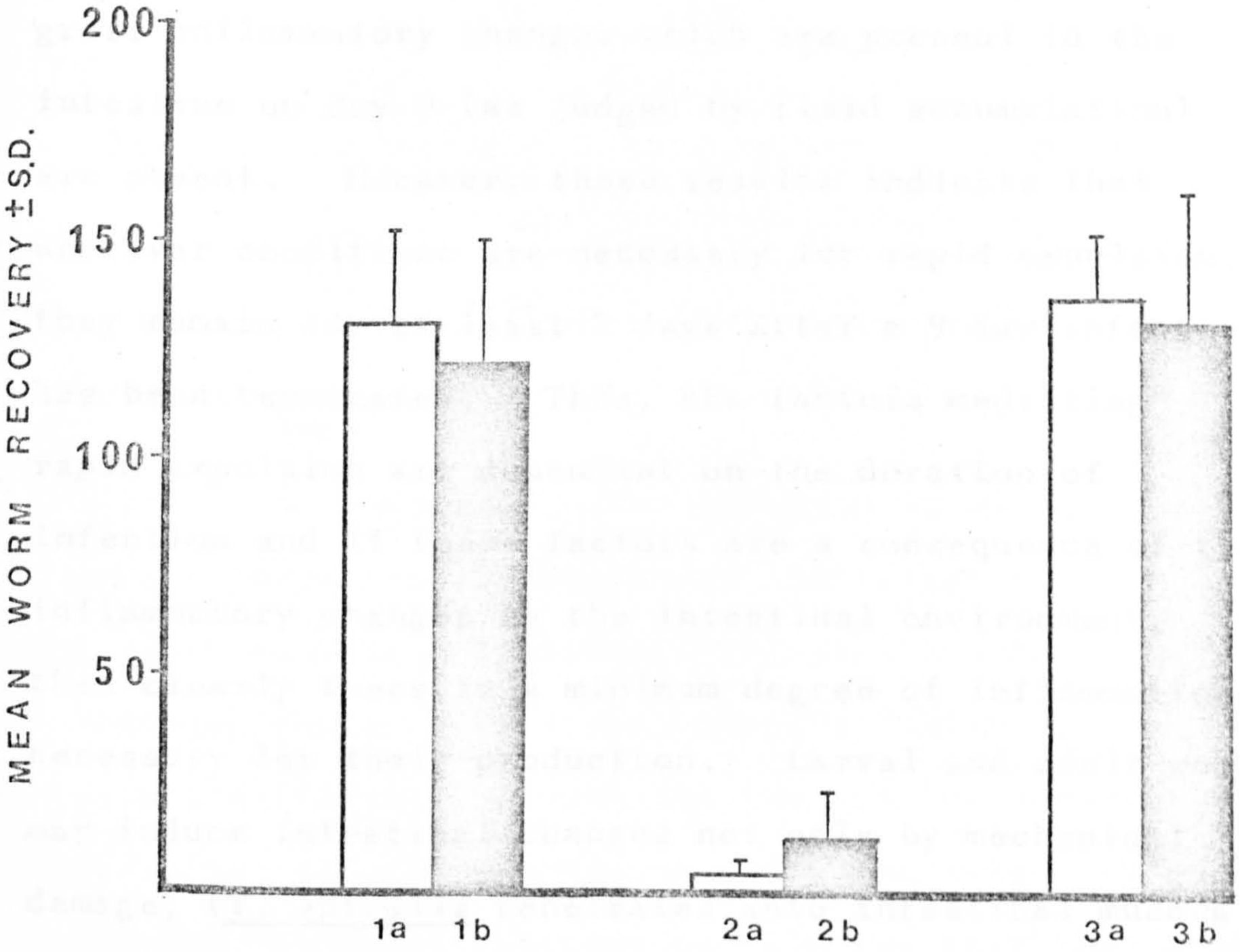
Group 2b infected and challenged with normal larvae

Group 3a non-infected control, challenged with irradiated larvae

Group 3b non-infected control, challenged with normal larvae

 challenged with irradiated larvae

 challenged with normal larvae


MEAN WORM RECOVERY ±S.D.
200
150
100
50
1a
1b
2a
2b
3a
3b

DISCUSSION

The results from the first experiment described in this chapter showed that a 7-day intestinal exposure to T. spiralis did not prime mice for a rapid expulsion response when they were challenged on day 14. When the period of infection was increased to 9 days, rapid expulsion did occur. By day 14 the gross inflammatory changes which are present in the intestine on day 9 (as judged by fluid accumulation) are absent. However, these results indicate that whatever conditions are necessary for rapid expulsion, they remain for at least 5 days after a 9 day infection has been terminated. Thus, the factors mediating rapid expulsion are dependent on the duration of infection and if these factors are a consequence of the inflammatory changes in the intestinal environment, then clearly there is a minimum degree of inflammation necessary for their production. Larval and adult worms may induce intestinal changes not only by mechanical damage, (T. spiralis penetrates into intestinal mucosa), but also through mechanisms which are stimulated by the release of antigenic material. Nine day infections would involve greater direct damage than 7 day infections and would expose the host to a greater total antigenic stimulus and therefore, would presumably induce more severe changes in the intestinal environment. In addition, the release of new born larvae would continue for a longer period of time and these migrating larvae

should also be considered as a factor in the induction of intestinal changes.

It is probable that the longer persistence of the above stimulatory factors brings about more pronounced cellular changes in the intestine. Indeed, when infections were terminated on day 7 post infection, the level of the cellular response, as measured histologically, declined sharply over the next few days. This indicates that if the intestinal stimulation is interrupted early in the infection, the cellular response will not reach the maximum level seen in complete infections. The relation of these changes to rapid expulsion are discussed further in Chapter 3.

In contrast to the situation in mice, in rats 7 days exposure to infection was sufficient to stimulate the rapid expulsion response, the results obtained here confirming those of Bell and McGregor (1979b). This distinct difference between the two species suggests that in the rat, the mechanism which generates rapid expulsion, is developed very quickly and that the innate capacity of rats to mount the response is different from that of mice. One reason for this may be that the factors which are involved in rapid expulsion are normally present at higher levels in rats than in mice (also see histology, Chapter 3).

Thus, it appears that there is a minimum duration of infection necessary for priming the rapid

expulsion response and this threshold is greater in mice than in rats. This hypothesis has been tested by the use of drug-abbreviated infections. One drug-abbreviated infection lasting for 3 days did not prime mice for rapid expulsion. Therefore, the duration of infection and presumably also the total amount of antigenic material released from the parasite, are factors which are important to the generation of the response. These result amplify the observations made from 7 day infections in mice. Again, the minimum duration of infection necessary to elicit rapid expulsion is quite different in mice and rats. Indeed, as Bell and McGregor (1979b) have shown, a single exposure to preadults lasting only 48 hours provokes rapid expulsion in rats, whereas an infection of 3 days duration was quite ineffective in inducing this response in NIH mice.

Interestingly, two drug-abbreviated infections, each lasting 3 days, were sufficient immunization to give rise to a rapid expulsion response in mice challenged 14 days after the first infection. It is unlikely that the effectiveness of this protocol was related simply to the total duration of infection (i.e. 6 days), and it seems more likely that the effects are due to the fact that the second infection, given on day 7, caused a much more rapid development of cellular changes in the intestinal mucosa. Three and five abbreviated infections (i.e. given between days 0 and 14 or days 0 and 28,

respectively), induced rapid expulsion to challenge infection given on day 21 or 35. These findings raise an interesting point. After two immunizing infections, the duration of subsequent immunizing infections would be short, as they would themselves be subjected to rapid expulsion. However, unlike the situation that follows a single complete infection, multiple immunizations apparently brought about a more persistent capacity for expressing rapid expulsion. The mechanisms which may be involved in this phenomenon can be considered in the following order.

1. Small numbers of worms may remain in the gut after the third or subsequent infections and may generate inflammatory changes making the intestinal environment unsuitable for the next infection to establish. In this case, this condition would have to remain for at least 4 days after the drug treatment.

2. The challenge infection may trigger a new response in which the primed intestine, upon contact with the parasites, gives an immediate response leading to the rapid expulsion of the challenge larvae. In this case, this sensitized condition would have to persist for at least 7 days after the infection. If all infections given after day 14 were subjected to rapid expulsion, this condition would have to remain for one or three weeks when challenge infections were given on day 21 or 35. An attempt was made to increase the time between the last abbreviated immunization and the

challenge infection to determine whether in fact, sensitization would persist for this time. In this case, mice were given three drug-abbreviated infections and challenged either on day 28 or 35 (i.e. two or three week gap respectively). Unfortunately, because of technical problems, the results were variable and therefore unsatisfactory. (These have not been included in the text).

3. The persistence of rapid expulsion after two or more drug-abbreviated infections may suggest that by terminating the infection before larval production, mice were not subjected to suppression induced by encysted muscle larvae. It has been shown, for example, that after a complete infection (in which muscle larvae also were involved) rapid expulsion persists for a rather shorter period of time (until day 16 post infection, see Chapter 1). In addition, it is known that muscle larvae suppress both humoral and cellular responses to unrelated antigens in mice (Faubert and Tanner, 1971; Ljungstrom and Huldt, 1977). It is, of course, possible that these three hypotheses are not exclusive and that they may work in combination to affect the induction and/or the suppression of the rapid expulsion response in mice.

In rats the rapid expulsion response induced with either single complete or repeated drug-abbreviated infections, persists for a longer period of time (up to 7 weeks). Therefore, it is likely that of the three

possibilities suggested above for the mechanism of rapid expulsion in mice, probably the first and second also apply to rats, but there is no justification for the third hypothesis, since at present there is no evidence to show that muscle larvae are involved in the suppression of immunity in rats; indeed, this could be an interesting subject for further investigation.

Although a single drug-abbreviated infection does not give rise to a rapid expulsion response in mice, there is evidence to show that it does immunize against a subsequent challenge infection. Denham (1966a) for example, reported that intestinal infections of 3 or 4 days duration, terminated by means of Methyridine treatment, produced strong immunity against challenge infection as measured by the number of larvae in the muscle. Even infections limited to one day duration can stimulate strong immunity to challenge infection, as measured both by the number of adults in the intestine and of larvae in the muscles (Campbell et al., 1963a).

It has been shown that complete infections with irradiated larvae did not prime mice for rapid expulsion, therefore an attempt was made to induce rapid expulsion by repeated intestinal infection with such larvae. The results showed that even repeated intestinal infections (3 days duration) did not induce a rapid expulsion response. Although it is

known that a dose of 4 Krad irradiation given to infective larvae will prevent sexual maturation (Levin and Evans, 1942; Kim, 1957), this does not necessarily suggest that migrating or encysted muscle larvae are necessary for induction of rapid expulsion, since the experiment using drug-abbreviated infections with normal larvae has excluded this possibility. The failure of repeated (3) abbreviated infections with irradiated larvae to induce rapid expulsion, may suggest that such larvae are not capable of generating the factors necessary for the induction of this response. By contrast, two drug-abbreviated infections with normal larvae generate this response effectively. Gould, Gomberg, Villella and Hertz (1957) and Kim (1957) noted that irradiated larvae are less immunogenic and smaller than normal larvae and it is possible that irradiated larvae cause less inflammatory changes in the intestinal environment. In support of this, Denham (1966b) has reported that the immunity induced by irradiated larvae to challenge infection (as assessed by the number of larvae in the muscles) was weaker than that from normal larvae. In contrast to the failure of irradiated larvae to induce rapid expulsion, the expression of the response against normal or irradiated larvae seems to be the same and therefore, in this situation, the larvae must be capable of stimulating the response to an equivalent degree.

The rapid expulsion of transplanted adult worms from the mouse intestine suggests several interesting possibilities. Firstly, the minimal environmental changes present on day 14 after primary infection could result in conditions unsuitable for adult worm establishment, i.e. rapid expulsion is not a de novo secondary response. Secondly, and alternatively, both the larvae and adult worms could be antigenically related and each able to trigger rapid expulsion as a secondary response. Thirdly, because of the large size of the adult worms and their greater locomotory capability, it is possible that establishment could occur before triggering and ultimate expression of the rapid expulsion response.

The expulsion of adult worms does not seem to be related to the time spent in the donors. For example, Kennedy, Wakelin and Wilson (1979) have shown that adult worms taken from NIH donors at different times after infection were capable for surviving after transplantation into naive mice.

The effect of inflammation on worm rejection has been examined by Wakelin and Wilson (1979b) who noted that the worms transplanted into infected recipient mice (at the time at which the intestine was actually inflamed) were expelled rapidly. In this study, however, the adult worms were transplanted when the gross inflammatory changes had disappeared, but rapid

expulsion still occurred. At present, all of the aforementioned possibilities for expression of rapid expulsion against adult worms have to be considered. Bell and McGregor (1979a, b) reported that although rapid expulsion of larval stages occurred in rats that were immunized with large numbers of adult worms, 4-day-old adult worms were not susceptible to the rapid expulsion response produced by either 48 hours or 7 days exposure to intestinal infection. Again in this respect, rapid expulsion differs in mice from what has been shown in rats. Bell and McGregor (loc. cit.), therefore concluded that the induction and expression of rapid expulsion was stage-specific. However, the mechanism through which this stage-specificity operates is not clear and its involvement in induction and expression of rapid expulsion has still to be proven. Indeed, stage-specificity may not occur with the enteral phases of development. It is possible that their results with adult worms reflect the fact that restricted intestinal infections may stimulate weaker responses than do complete infections. In addition, it is not possible to evaluate accurately the role of separate intestinal stages in priming for rapid expulsion, because the use of drug treatment cannot restrict the infection wholly to one particular stage, as development in the intestine is so rapid.

If rapid expulsion depends on antigenic presentation by specific stages, it is possible that

all of the intestinal stages, larvae preadult and adult of T. spiralis, are immunogenic and antigenically related. In fact, there is some evidence to suggest that both larvae and adults have common functional antigens (possibly the stichocyte antigens - Despommier, 1974). The present results support this view and confirm that both adults and larvae are susceptible to rapid expulsion.

Although it appears that the enteral and parenteral stages of the parasite have various basic similarities, it is likely that they may also exhibit a number of differences. For example, Philipp, Parkhouse and Ogilvie (1980) have demonstrated that infective larvae and adults share a number of surface (cuticular) proteins, but those on the newborn larvae are qualitatively different from those on enteral stages. Complement activation is triggered by the cuticle of the enteral stages, but not by that of newborn larvae MacKenzie, Jungery, Taylor and Ogilvie (1980). Therefore, it seems plausible that the host may generate separate, possibly different, immune responses to the enteral and parenteral stages of this parasite. Indeed, Oliver, Gonzalez and Levine (1962) have proposed the existence of a dual antibody response to T. spiralis infection and suggested that these antibodies are specific to either the enteral or parenteral stages. Subsequent studies by James and Denham (1974) reported that the migrating and encysting muscle larvae provoke

a response against challenge only with these stages. The effector mechanisms (antibody and/or cells) which contribute to this stage-specificity, may be important in the expression of the response against the enteral and parenteral stages of the parasite. In fact, there is an interesting *in vitro* study which has shown that the serum of animals infected with *T. spiralis* contains particular antibodies, each specific to the surface antigens of particular stages in the life cycle (new born larvae, infective larvae and adult worms) and capable of mediating attachment by inflammatory cells to the parasite surface (MacKenzie, Preston and Ogilvie, 1978).

In conclusion, the results suggest that the parenteral stages of *T. spiralis* are not required to produce rapid expulsion and that stimulation by the intestinal stages alone is effective in bringing about rapid expulsion. However, the results do not rule out the possibility that intestinal changes caused by migrating larvae (released from adult worms into the intestine) may contribute to the response. This phenomenon (rapid expulsion) can be considered as a specific response to the enteral stages and the results also suggest that in some way the muscle larvae may exert some suppressive effect upon the rapid expulsion response in mice.

CHAPTER .3

A. INTRODUCTION TO CELLULAR AND PATHO-PHYSIOLOGICAL CHANGES IN THE SMALL INTESTINE INDUCED BY PARASITIC INFECTION

The intestinal immune response generated during a primary infection with T. spiralis may operate in a variety of ways to reduce any pathogenic effects of parasite origin. The outcome of this immune response includes structural damage to the parasites, reduction of worm fecundity and growth and, most importantly, the expulsion of the worms from the host intestine (see Despommier, 1977; Wakelin, 1978a). It has been suggested that in T. spiralis infections, the mechanisms which bring about worm expulsion are generated by a complex interaction between immunological components (i.e. antibodies and lymphocytes) and factors which are nonspecific in action (Wakelin, 1978a). Rejection of T. spiralis appears to be caused directly by the effects of inflammatory changes rather than by the direct action of the immune response (Larsh and Race, 1975). This mechanism however, does not necessarily operate in other parasite systems, as in some helminth infections expulsion of the parasite is not characterised by a severe inflammation of the gut (for example, Trichuris muris in mice - Jenkins, 1977 and Lee, personal communication; Hymenolepis diminuta in mice - Andreassen, Hindsbo and Ruitenberg, 1978).

Castro (1976) has defined the inflammatory responses found in T. spiralis infections as a chain of events leading from mucosal injury to gross inflammation. Initiation of these events may arise from traumatic or chemical stimuli, provided by the parasite upon contact

with the host and by additional immunological stimuli. The characteristics of inflammation may be different in various tissues and much depends on the nature and the site of stimulus.

To gain an insight into the complex mechanisms involved in inflammation, it is necessary to be aware of some of the components of the inflammatory process. The following brief review will examine the available data on the inflammatory processes and the cellular and physico-chemical factors generated in response to nematode infections, particularly infections with T. spiralis.

It is difficult to separate functionally the components or mediators which contribute to the inflammatory process but these factors will be discussed in the following order:

1. cellular changes
2. mediators
3. patho-physiological changes

1. Cellular changes

A. Mast cells

Mast cells are mononuclear cells of between 10-30 microns in diameter. They are present in the extravascular tissues throughout the body (Askenase, 1980). Mast cell size increases steadily as animals grow older and the size may vary between species (Padawer, 1979). Mast cells possess in their cytoplasm several meta-

chromatic granules which stain with basic dyes. These granules contain a variety of substances such as mucopolysaccharides, which are responsible for the characteristic metachromasia and vasoactive amines such as histamine and 5-hydroxy-tryptamine (5.HT). These vasoactive amines can be present singly or together depending on the species. The other biological substances which are present and which are released on degranulation of the mast cell are fully reviewed in the literature (see Askenase, 1980; Leid and Williams, 1979).

Mast cells have been shown to possess receptors for the Fc portion of homocytotropic antibodies (Wasserman, 1979). It has been reported that mast cells in the intestinal mucosa and the lungs differ from those in connective tissues in their morphology, histochemistry and mucopolysaccharide contents (Enerback, 1966a, b and c; Miller, 1969). They are also different in their responses to degranulating agents such as 48/80 (Enerback, 1966b; Miller, 1969; Befus, Pearce, Gauldie, Horsewood, Goodacre, Cole, Heatley and Bienenstock, 1979a). In contrast to connective tissue mast cells, which have receptors for IgE only on their surfaces, it has been demonstrated that IgE is present not only on the surface of mucosal mast cells, but also in the cytoplasm (Mayrhofer, 1977; Mayrhofer, Bazin and Gowans, 1976). Most of these studies have been done in rats and little is known of these cells in mice. The origin of the mucosal mast cell is not known, but it has been reported that it is

thymus dependent and appears to be under thymus regulation at least in response to parasite infection (Ruitenberg and Elgersma , 1976; Mayrhofer, 1979b and Mayrhofer and Fisher, 1979). Intestinal mastocytosis can be adoptively transferred by injecting immune MLNC (mesenteric lymph node cells) or immune TDL (thoracic duct lymphocytes) into animals infected with N. brasiliensis or T. spiralis (Befus and Bienenstock, 1979; Nawa and Miller, 1979; Alizadeh and Wakelin, 1981a). This aspect will be more fully discussed in chapter 5. By contrast, it has been proposed that connective tissue mast cells originate from fetal liver, blood, spleen or bone marrow precursors (see Kitamura, Yokoyama, Matsuda, Ohno and Mori, 1981). Other more minor differences between connective tissue mast cells and mucosal mast cells have also been reported (see Askenase, 1980).

It has been suggested that intestinal mast cells can be divided into two sub-groups, those which are located in the lamina propria and the so-called globule leukocytes which are found in the intra-epithelial position (Ruitenberg and Elgersma, 1979; Murray, Miller and Jarrett, 1968; Whur and Gracie, 1967; Miller and Jarrett, 1971). The origin and function of both cell types is controversial. Murray et al. (1968) considered globule leukocytes amine-containing cells which were derived from subepithelial mast cells. However, Whur and Gracie (1967) considered

them unrelated to the subepithelial mast cells. Extensive studies in mice and rats (Ruitenberg, Elgersma and Kruizinga, 1979a; Ruitenberg, Elgersma and Lamers, 1979b; Ruitenberg and Elgersma, 1979, 1980), have suggested that the globule leukocytes are an independent cell population and that the degree of the globule leukocyte response to T. spiralis is thymus dependent.

Function of mast cells

In vivo mast cells mediators are released when antigen combines with homocytotropic antibodies attached to the mast cell membrane. Although there is some evidence (Askenase, 1980) that the release of mast cell mediators can occur without the contribution of antibody, the weight of evidence suggests that antibody is usually implicated in mast cell degranulation.

Two distinct antibodies, namely IgE and a sub-class of IgG have been found to be responsible for triggering this degranulation. These have in the past been referred to as "homocytotropic antibody" (Mota, 1964), "reagin like antibody" (Ogilvie, 1967) or loosely as 'IgE". These terms simply refer to specialised immunoglobulins capable of sensitizing target cells such as mast cells for the release of pharmacological agents on reaction with antigen. The major functional difference between the two aforementioned antibodies is simply

the length of time that they persist on the cell surface and thus allow the release of the mast cell mediators. In addition IgE is thermo-labile.

These mediators, two of the most important of which have been found to be histamine and 5HT, possess the ability to induce immediate tissue reactions such as weal and flare and anaphylaxis and also may mediate more prolonged inflammatory events (Wasserman, 1979).

Although reaginic antibody is commonly found in animals infected with helminth parasites, e.g. in rats infected with N. brasiliensis (see Ogilvie, 1967; Jarrett, 1973) and in mice and rabbits infected with T. spiralis (see Sadun, Mota and Gore, 1968), its role in the immune response to parasites is not well understood. In most helminth infections it has been reported that the circulating levels of this antibody increased after worm expulsion (Murray, 1972; Katz, 1980) and in addition, Rivera-Ortiz and Nussenzweig (1976) have reported that no correlation could be demonstrated between worm numbers and circulating reaginic antibody titers in various strains of mice infected with T. spiralis. The site of sensitization of mast cells by reaginic antibody is not known. Recently it has been suggested that sensitization of mast cell percursors may occur in the MLN and that sensitized mast cells may then home to the intestine (Gillon, 1981). This local sensitization could explain

the coexistence of sensitization with low titers of circulating antibody. It also may be that IgE is, in fact, produced locally, since it is known that IgE is present in the cytoplasm of mucosal mast cells and it is possible that upon degranulation IgE is released into the intestinal environment.

The role of mast cells in parasite infections

Nematode parasites which live in the gut often cause the accumulation of mast cells in the intestinal mucosa. Such an increase has been demonstrated in rats infected with N. brasiliensis (Taliaferro and Sarles, 1939; Wells, 1962; Miller and Jarrett, 1971; Befus and Bienenstock, 1979) and in mice and rats infected with T. spiralis (Karmanska, Kozar, Seniuta and Czajkowska, 1971; Ruitenberg and Elgersma, 1976 and 1979). In both NIH and B10LP mice, the peak mast cell response has been found to coincide with the expulsion of T. spiralis (see Alizadeh and Wakelin, 1981c and Ruitenberg and Elgersma, 1976 respectively), but in other strains of mice, such as CBA, the peak response does not occur until after worm expulsion (Tronchin, Dutoit, Vernes and Biguet, 1979). These differences in the rise of mast cells have also been reported in strains of rats infected with N. brasiliensis (Nawa and Miller, 1979). Apart from such differences in the time of peak response, host strain differences have been found in the time of the

initiation of the response, for example, to T. spiralis and T. muris in mice (Alizadeh and Wakelin, 1981b; Lee, personal communication, see also chapter 6).

The role of mast cells and their function in immunopathological responses to nematode parasites, have been studied for many years and several hypotheses have been suggested. It is possible that mucosal mast cells participate in inflammatory responses via an anaphylactic reaction mediated by reaginic antibodies. The release of mediators such as vasoactive amines would increase both vascular and mucosal permeability and these changes in permeability may then cause local changes in the intestinal environment, making it unsuitable for parasite survival (Urquhart, Mulligan, Eadie and Jennings, 1965; Murray, Jarrett and Jennings, 1971). Barth, Jarrett and Urquhart (1966) suggested that an increased gut permeability would allow anti-worm antibodies to reach worms present in the lumen. The possibility that biogenic amines affect worms directly has been proposed by Jones and Ogilvie (1971) and is supported by some in vitro studies (Rothwell, Prichard and Love, 1974). There are, however, many arguments against the direct involvement of mast cells in parasitic infection.

Kelly and Ogilvie (1972), for example, reported that while an increase of mast cells occurred in lactating rats infected with N. brasiliensis, this did not result in normal worm expulsion (the failure of lacting rats to

reject the worms, however may be due to a deficiency in lymphocyte function). The lack of correlation between mast cell response and expulsion of T. spiralis in some strains of mice has already been discussed.

It is possible that mast cells are involved more directly in the secondary response to infection. This concept is suggested by the fact that the numbers of mast cells remain high for many days after the parasite has been expelled and the host is frequently capable of a rapid response to reinfection. A number of authors have noted, for example, that in rats infected with N. brasiliensis the number of mast cells remained higher than control levels for up to 56 days after infection (see Miller, 1969; Mayrhofer, 1979a; Macdonald, Murray and Ferguson, 1980). In addition, the mucosal mast cell response develops more quickly in a secondary infection and it has been suggested that there is a mast cell "memory" in rats after infection with N. brasiliensis (Mayrhofer, 1979a). More recently it has been suggested that mast cells may be involved in repair mechanisms following parasitic infection (Ferguson and Miller, 1979), protease secreted by these cells contributing to the processes involved in re-organization of the intestinal structure.

The implication of all the above hypotheses may well be that mast cells are, in fact, in some way,

directly or indirectly, involved in the regulation of the overall response to parasite infection.

B. Goblet cells

It has been reported that two types of mucus secreting cells are found in the small intestine of rats, namely goblet cells, which are located in the epithelial lining of the lumen and cuboidal cells, forming the acini of Brunner's glands (Wells, 1963).

The role of mucus in the protective response against bacterial infection has been shown by Florey (1955) and Dixon (1960) who noted that mucus has properties enabling it to trap bacteria. More recently, attention has been focussed on the role of goblet cells and the intestinal mucus layer in protective responses to helminth infection. Some time ago, Frick and Ackert (1948) showed that mucus from chickens had an inhibitory effect on the growth of Ascaridia galli in vitro. Wells (1963) has reported that the number and output of mucus secreting cells increases during the expulsion of N. brasiliensis in rats and Miller and Nawa (1979a and b), as well as confirming this earlier finding, noted that the adoptive transfer of TDL also resulted in an increased proportion of goblet cells in the small intestine. In her discussion of the function of mucus in N. brasiliensis infected rats, Wells (1963) suggested that it may have a mechanical role in trapping the worms and facilitating

their intestinal passage and expulsion. In fact, both mast cells and goblet cells are present in large numbers during the expulsion of the parasite and this may suggest that goblet cells also participate in the environmental changes in the small intestine and may directly or indirectly, influence intestinal pe ristalsis thus facilitating parasite expulsion. Several mechanisms have been suggested for the release of mucus from goblet cells. It has been shown, for example, that mast cells or mediators released from them can increase the production of mucus by goblet cells and that immune complexes and anaphylatoxin have some influence on the release of mucus from goblet cells (Lake, Bloch, Neutra and Walker, 1979; Lake, Bloch, Sinclair and Walker, 1980; Weir, 1974). It is possible that the environmental changes induced as a result of a primary infection and also the continued influence of mast cells and mediators on mucus production after the primary infection has been expelled, may have some effect on a secondary infection. In fact, the role of the mucus layer on the prevention and elimination of a secondary infection in immune rats infected with T. spiralis or N. brasiliensis has been explored by Lee and Ogilvie (1981) and Miller, Huntley and Wallace (1981) respectively. The factors associated with mucus which result in the prevention of parasite establishment, are not fully understood and should be considered for further investigation.

C. Polymorphonuclear cells

Many other cellular components are associated with the inflammatory response (plasma cells, lymphocytes, intraepithelial lymphocytes and basophils), however, polymorphonuclear cells (neutrophils, eosinophils) are particularly important. Much attention has been focussed on the role and function of these cells, especially eosinophils. However, there is much conflicting evidence on the role played by these cells and the timing of their infiltration. For example, Castro, Olson and Baker (1967) reported a marked infiltration of eosinophils and plasma cells in the intestinal mucosa of guinea pigs, infected with T. spiralis, prior to expulsion. However, the number of both of these cell types decreased during expulsion, with the number of plasma cells rapidly returning to normal levels. In contrast, in CBA mice infected with T. spiralis in which worm expulsion is rapid, Walls, Carter, Leuchars and Davies (1973) noted only a mild inflammatory infiltrate (consisting of eosinophils, lymphocytes and macrophages) in the submucosa between days 3 and 10 p.i. Various authors have also described the presence of an inflammatory infiltrate during helminth infections, but their work has been mainly restricted to a quantitative rather than qualitative analysis of the cellular infiltration. The in depth histological study by Taliaferro and Sarles in 1939 of

the cellular infiltrate associated with N. brasiliensis infection led them to suggest that the cells concerned with this inflammation are associated with the protective response during the infection.

Increased numbers of eosinophils are found in the blood and other tissues during allergic reactions and in response to helminth infections as well as in inflammatory responses. Their accumulation in the tissues is presumably due to a positive chemotaxis and it has been reported that the chemotactic substances which are released from mast cells can attract eosinophils to the site of infection (Weller and Goetzl, 1979). An important function of eosinophils at this site may be to modulate inflammation. Mediators such as histaminase released from eosinophils, can suppress the inflammatory response directly (MacKenzie, 1980). There is more evidence from studies of both rats and mice which suggests that the eosinophil response is mediated by thymus dependent immune reactions (see Basten, Boyer and Beeson, 1970; Basten and Beeson, 1970; Walls et al., 1973; Kay, 1977) and it is known that the inflammatory response can be inhibited by immune suppression. This concept of immune dependence has been examined in mice given irradiation (Larsh and Race, 1975) and/or cortisone treatment (Coker, 1956) and in congenitally hypothymic (nude) mice (Gustowska, Ruitenberg and Elgersma, 1980). These authors noted that suppression of inflammatory

reactions in the tissues also impaired eosinophil production. The exact mechanisms of stimulation and regulation of these cells and their role in the immune response to intestinal helminth infection, is not fully understood. In vitro studies have shown that eosinophils can adhere to and damage larval helminths in cultures enriched with these cells. Attachement is mediated by anti-worm antibodies and/or complement (see Kazura and Grove, 1978; MacKenzie, Jungery, Taylor and Ogilvie, 1980) and the presence of other cell types such as macrophages may increase the damaging effect of eosinophils. Whether such mechanisms of direct damage can occur in vivo is not known. However, Grove, Mahmoud and Warren (1977) have shown that anti-eosinophil serum (AES) has no effect on the course of the intestinal stages of a T. spiralis infection in mice. Butterworth (1980) suggested that local IgE-mediated reactions at the site of the tissue stages of helminths may lead to a local accumulation of eosinophils and other components of the immune response and that the eosinophils may then contribute directly to the destruction of the invading parasites as well as eventually switching off the reaction (by the release of mediators).

In addition to polymorphonuclear cells, it has been reported that in T. spiralis infected mice the number of pyroninophilic cells in the small intestine increases significantly by day 10 post infection

(Ruitenberg, Leenstra and Elgersma, 1977). The dramatic increase in lymphocyte localization in response to parasite infection, will be discussed in chapter 5.

2. Mediators

A variety of mediators and substances are released from the cells involved in the inflammatory response and these mediators play a major role in induction of inflammatory changes. It is difficult to relate particular changes to individual factors, because these components may work in combination with each other to induce the environmental changes in the intestine. In this section some of the factors which seem to be important in the complex mechanisms of the inflammatory response are considered briefly.

The mediators released from mast cells which may have a role in the generation of the inflammatory reaction, and may also directly or indirectly, effect the parasite, are primarily the vasoactive amines (i.e. histamine and 5HT). It has been shown that these vaso-active amines are released from rat mast cells in response to helminth infection. For example, in rats infected with N. brasiliensis the level of histamine and 5HT in the small intestine increases over the period of worm expulsion (Wells, 1962; Keller, 1971; Befus, Johnston and Bienenstock, 1979b). Some of the above authors have

suggested that mast cell hyperplasia and the resulting elevation of amine levels in the gut have some major role in immunologically mediated worm expulsion. Wakelin (1978a) concluded that there are three possible mechanisms by which these mediators could influence helminth infection, a) biogenic amines could affect the worms directly, b) permeability changes could alter the environment to the detriment of the worms and c) increased permeability could allow anti-worm antibodies to reach the worms.

In support of the view that amines are involved in worm expulsion, it has been shown that amine antagonist drugs prevent worm expulsion in guinea pigs infected with Trichostrongylus colubriformis (Rothwell, Dineen and Love, 1971). In addition, Campbell, Hartman and Cuckler, 1963b) noted a prolongation of infection with T. spiralis following the administration of anti-histamine and anti-5HT drugs. It must be remembered, however, that the effects of anti-amine drugs are not specific and that they may also affect other components involved in the response.

Other factors released from inflammatory cells which are involved in the mediation of the inflammatory process include prostaglandins, slow reacting substance of anaphylaxis (S R S.A) and eosinophil chemotactic factors (E.C.F.) (see Askenase, 1980). There are, of course, other factors released which may also be involved

in the response, but these have not been as well documented.

Inflammatory exudate contains a number of cell types capable of synthesising prostaglandins which may act as soluble mediators of inflammation causing a variety of effects (Stenson and Parker, 1980). In parasite infections Dineen and Kelly (1976) showed increased levels of prostaglandins in the intestinal tissue of rats during infection with N. brasiliensis, some workers have administered prostaglandins into the intestine to assess their effects on parasite infection, but conflicting results have been reported. For example, Kassai, Redl, Jecsai, Balla and Harangozo (1980) demonstrated that prostaglandins had no effect on the course of expulsion of N. brasiliensis from rats, but Dineen, Kelly, Goodrich and Smith (1974) found that administration of prostaglandins initiated an early expulsion of the parasite. Rothwell, Love and Goodrich (1977) however, have reported that prostaglandins do not play a major role in immune expulsion of T. colubriformis from guinea pigs and it would appear that if prostaglandins have any effect, it is dependent on the experimental conditions and the administered dose. Dutoit, Tronchin, Vernes and Biguet (1979) have, for example, demonstrated that the administration of high doses of prostaglandins, but not low doses amplifies the expulsive response to T. spiralis infection.

Other components such as phospholipase and peroxidase released presumably from the infiltrating polymorphonuclear and mononuclear cells, may also participate directly or indirectly in the changes which result in the eventual expulsion of a helminth infection. Larsh, Ottolenghi and Weatherly (1974) reported that during the immune expulsion of T. spiralis from mice, the level of phospholipases increases significantly at the site of infection and Castro, Roy and Schanbacher (1975) have demonstrated that peroxidases isolated from the lamina propria can, in fact, kill T. spiralis in vitro. In T. spiralis infected rats it has been shown that there is a relationship between mucosal peroxidase activity and intestinal inflammation (Smith and Castro, 1978).

3. Patho-physiological changes

A very wide variety of pathological changes are known to occur during infections with intestinal nematodes. Some of these are of particular relevance to the fate of the worms concerned and they result in profound changes of the worms environment and will be briefly discussed. In mice infected with T. spiralis there is marked villous atrophy and crypt hyperplasia. These changes commence 4 days after infection and reach maximum levels between days 10 to 12 post infection. A population of T. lymphocytes is necessary for the onset

of these events (Manson-Smith, Bruce and Parrott, 1979a). Ferguson and Jarrett (1975) described similar changes in the N. brasiliensis/rat model. In addition, there are various other changes in the intestinal environment (such as changes in the brush border enzyme activity (including maltase and disaccharidase, local acidosis, permeability and peristalsis) which may either be a direct result of the parasite presence or which may be under more complex immunological control (Castro, 1976).

Staining properties of intestinal mast cells

It is difficult to monitor all of the complex components which contribute to the intestinal changes seen during an infection with T. spiralis, because of the lack of specific staining techniques for some of the cell types involved and of sensitive techniques for measuring physico-chemical factors in the intestinal environment. It was considered essential to use a histological technique which could give satisfactory results in quantifying and distinguishing these cellular changes and it was decided that such a specific technique would be the staining of mast cells and goblet cells.

The presence of mast cells in the small intestine of rats has been known for a long time. Enerback (1966a) and Miller (1969) studied the fixation of mast cells in different tissues of the rat and found

Carnoy's solution to be a suitable fixative for mucosal mast cells. They found also that it is possible to recognize these cells by a specific staining technique (see general materials and methods). With this in mind, the methods used by the above workers for detecting intestinal mast cells in rats were used for these cells in mice and it appears that intestinal mast cells in mice and rats share similar staining properties. In rats it has been shown that the stain employed is specifically bound to the suphated acid mucopolysaccharides at low pH (Enerback, 1966a and b; Miller, 1969) and it is probable that the binding properties are similar in mice.

CHAPTER 3

B. HISTOLOGICAL EXAMINATION OF THE CELLULAR RESPONSE IN THE INTESTINAL MUCOSA

INTRODUCTION

The kinetics of a primary infection with T. spiralis in mice and rats have been discussed in chapter 1, but briefly, the number of worms remained constant until day 8 post infection with the majority of worms being found in the anterior half of the small intestine. Worm expulsion begins after this time and is complete by day 12 in NIH mice and by day 14 in Wistar rats. Rapid expulsion of a challenge infection is seen in both mice and rats with the response being longer lived in rats.

In order to assess the relationship between these events and the cellular changes in the small intestine, two histological parameters namely, mast cells and goblet cells, were considered. Although their involvement in expulsion is uncertain, they make a suitable marker for the accompanying inflammatory changes.

One of the objectives of the studies in this chapter was to examine the cellular changes associated with complete infections, i.e. a) the changes in the levels of intestinal mast cells and goblet cells during the primary infection and worm expulsion, b) the fate of the mast cells and goblet cells after worm expulsion, c) the relationship between the presence of mast cells

and goblet cells and the rapid expulsion response and d) a comparison between mice and rats of the persistence of these cellular changes.

It has been shown that there is a minimum duration of infection necessary for priming for rapid expulsion and also that if rapid expulsion is to be induced by drug abbreviated infections (each lasting three days) at least two or more of these infections must be administered. Therefore, the second objective was to study this situation in more depth by examining, a) the relationship between the duration of infection and the mast cell response, b) the fate of the mast cell response and the relationship between the presence of these cells and rapid expulsion induced by repeated infection and c) the kinetics of the mast cell response after challenge infection.

MATERIALS AND METHODS

The strain of mice or rats and the methods used for maintenance, infection, worm recovery and histology have been described in the general materials and methods.

RESULTS

A. General observations

Since infection with T. spiralis is known to be accompanied by gross inflammation of the intestine, it was decided to describe some of these changes with a view to relating these to the histological observations. Gross changes were monitored by parameters such as gut diameter, oedema and fluid accumulation and graded according to severity. In mice infected with approximately 300 T. spiralis larvae, the degree of inflammation was rated as mild (+), moderate (++) or severe (+++), as shown in Fig. 3.1. Mild inflammation was observed on days 3 and 4 especially in the anterior half of the small intestine which was slightly oedematous. By days 5 and 6 a moderate inflammation was observed which was more intense in the anterior half of the small intestine and the gut diameter was significantly increased in comparison with control mice. Severe inflammation was observed between days 7 and 10 and gradually spread to involve the entire small intestine. After this time, the inflammation subsided considerably to what would be described as a "mild" state and subsequently disappeared by days 12 to 14 at which time the worms had been expelled completely from the intestine. During the severe phase, the small intestine showed a drastic accumulation of fluid mucus. This fluid accumulation

was also observed in the caecum and large intestine between days 8 and 10 p.i. when worms can be recovered from this part of the gut (see chapter 1). There appeared to be a positive correlation between the dose of infection and the degree of severity of the inflammation. The degree of inflammation also varied between different strains of mice.

B. Cellular changes in the small intestine of mice

When larvae of T. spiralis reach the small intestine, the majority of them establish in the anterior half and complete their development in the mucosa. Analysis by the use of the light microscope has shown that the worms embed themselves in the epithelium of the villi or crypts (plate 1, page 145). Sections of intestinal tissue taken between days 2 and 4 showed a mild inflammation in which few inflammatory cells were observed. This reaction was more severe in the tissues adjacent to the worms (plate 1). By day 6 post infection, the inflammatory cellular infiltrate was increased and the majority of the cells were polymorphonuclear (such as neutrophils and eosinophils). In some sections the cellular infiltrate appeared to be localized in the region of the villi or crypts. Since there was a large variation in the intensity of the cellular infiltrate amongst individuals in a group and even in sections from the same animal,

no attempt was made to monitor the number of the cells involved. Between days 7 and 10 the level of cellular infiltration reached a maximum with a variety of myeloid and lymphoid cell types being observed. These included eosinophils, neutrophils, macrophages, mononuclear cells, plasma cells and lymphocytes. An attempt was made to stain eosinophils using Haematoxyline and eosin or the Dominici method (see general materials and methods). The Dominici method gave satisfactory results and by using this technique, numerous eosinophils were observed on day 6 p.i. (plate 2 page 145). Because of the variation between sections and the local occurrence of the eosinophils, no attempt was made to count these cells.

The gross inflammatory changes observed during worm expulsion were associated not only with cellular infiltration, but also with changes in the intestinal structure (plate 3 page 146) such as a decrease in the height of villi (villous atrophy) and an increase in the length of the crypts (crypt hyperplasia). This alteration in villous and crypt structure was accompanied by the development of oedema in the lamina propria. A general thickening and cellular infiltration of the submucosa was also observed at this time.

i <u>Kinetics of the mast cell response in mice</u>

The number and distribution of mast cells in

four segments of the small intestine of control and infected (300 larvae) mice were examined. Three control and three infected mice were killed at various times throughout the infection. To simplify representation of the data obtained, the distribution and variation in mean number of cells counted in each group of mice, is shown in the text (mean±S.D. of 20 villus-crypt units). The pattern of the mast cell response over the duration of infection, using mean values only, is shown in Fig. 3.1.

Mast cells in control (uninfected mice)

Only a few mast cells were present in the intestinal mucosa of the control mice (61±10) and these cells contained few granules in the cytoplasm. The distribution of the cells was similar in all four segments of the intestine and in all cases, the cells were localized at the bases of the crypts (plate 3 page 146), although variations in cell numbers were observed between individual villus-crypt units.

Mast cells in infected mice

After infection with T. spiralis the number of mast cells was similar to that of the controls until day 2 post infection (62±12). By day 4 of infection there was nearly a twofold increase in the numbers of mast cells (115 ±18). At this time, the cells were still localized in the crypt region, but they contained more Alcian blue staining cytoplasmic granules. Some pale blue staining

mast cells were observed in the tissue adjacent to the worms. At this time there was mild inflammation in the anterior half of the small intestine. The number of mast cells in the intestinal mucosa increased considerably by day 6 p.i. (387±47) at which time the cells were localized not only in the crypt region, but also within the villi. Most of the cells were located in the intraepithelial position and stained dark blue indicating an increased granulation. Some areas of the mucosa had considerably more cells than others. The anterior half of the small intestine showed a moderate amount of inflammation and a greater number of mast cells than the posterior half.

Between days 6 and 8 the number of mast cells increased sharply. On day 8 the mean number of mast cells was 1010±77 and the intestinal mucosa of all the gut segments was packed with mast cells, with the majority of the cells (90%) located in the intraepithelial position. The cells were packed with large granules, although some vacuolated mast cells were also observed at this time. Severe inflammation was present along the entire length of the small intestine with extensive oedema of the lamina propria. The numbers of mast cells reached maximum levels by day 10 (1142±81). Cells were observed at the tip of the villi both in the lamina propria and in the epithelium (see plate 4, page 146). There were a few pale blue staining cells in the epithelium of the villi. In some areas the mast cells appeared fragmented with their

granules scattered locally. The number of mast cells observed declined slightly by day 12 (1092±98), but was still considerably higher than control levels even by day 14 p.i. (987±104). The distribution of the cells at this time was similar to that seen on day 10 with the intestinal mucosa packed with mast cells. By day 21 the number of mast cells had declined considerably, nearly reaching the level seen on day 6 p.i. (578±63). The majority of cells seen at this time were located in the crypt region. After day 21 the numbers of mast cells continued to decline reaching control levels by day 35 (the mean numbers of cells on days 28 and 35 were 204±75 and 70±24 respectively).

During the period of increased mastocytosis the shapes of the cells were irregular and the nuclei stained pink with safranin. Mitotic figures and morphological changes such as increased granulation, cell size and cytoplasmic vacuolation, were observed at this time.

A repeat of this experiment, using two mice per group, yielded results similar to the above.

In the control mice and some of the infected mice (those killed on days 2 and 4 p.i.) there were some cells located in the epithelium or subepithelial position which contained only a few pale staining granules. These cells were not counted as mast cells (according to Miller (personal communication), these cells presumably are granulated lymphocytes) and only the cells with strong positive Alcian blue staining granules were recorded.

ii Goblet cell response in mice

The number and distribution of goblet cells in a segment from the middle of the small intestine were examined in control and infected (300 larvae) mice. Three controls and three infected mice were killed at various times post infection. Mean numbers (±S.D.) of goblet cells in 20 V.C.U. for each of the days of examination are given in the text. The patterns of the goblet cell response over the period of infection is shown in Fig. 3.2.

By incubating the remaining portions of the intestine (i.e. those not used for histological analysis), it was ascertained that the response to the primary infection in terms of worm expulsion, was similar to that described in chapter 1, with worm loss being complete by days 12-14.

Goblet cells in control (uninfected) mice

In control mice a substantial number of goblet cells were present in the epithelium of the villi and crypts. The majority of the cells, however, were located in the epithelium of the villi (see plate 5, page 147). The mean number of goblet cells per 20 V.C.U. was (182±43).

Fig. 3.1

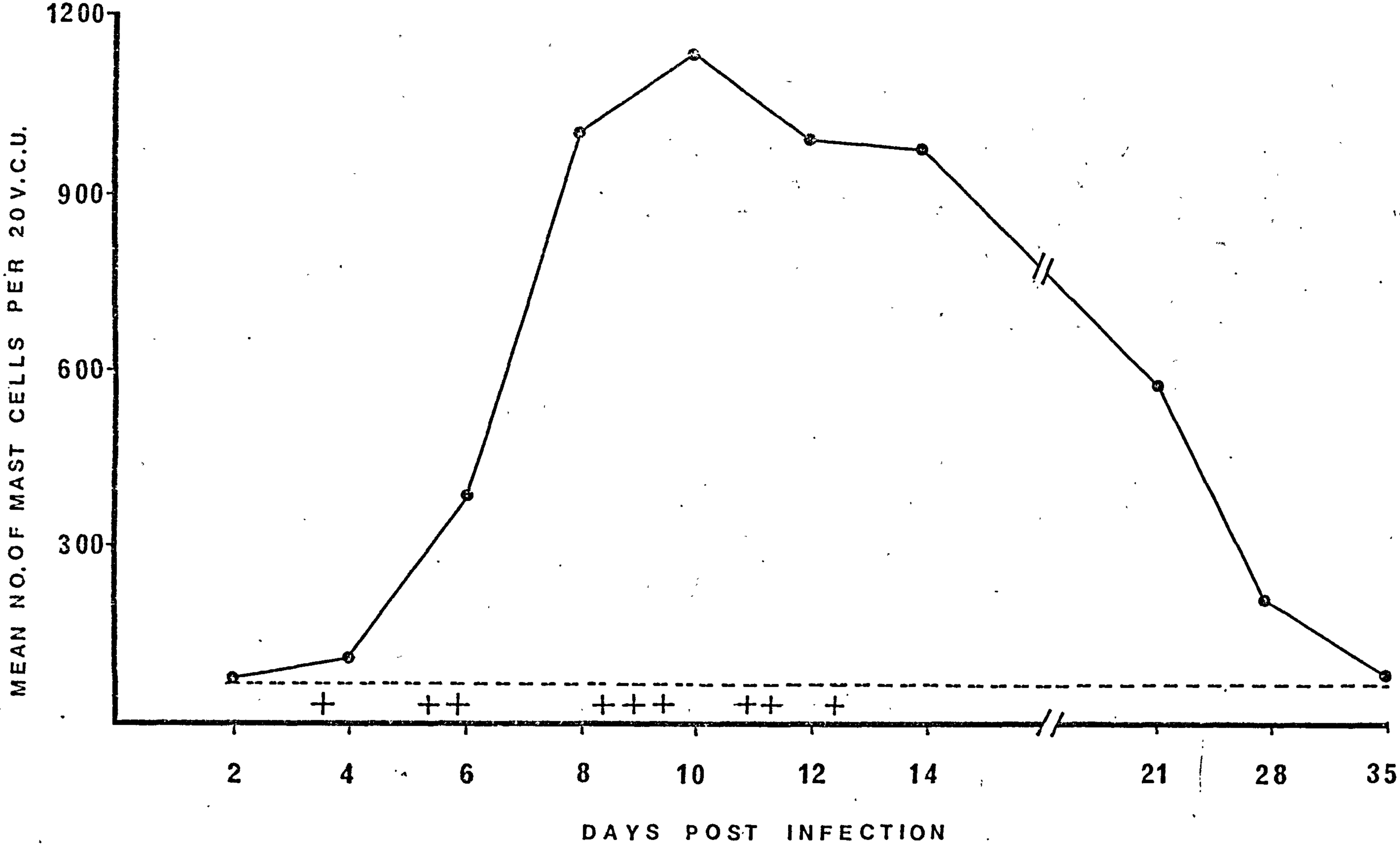
Mean numbers of mucosal mast cells per 20 V.C.U. in NIH mice infected with T. spiralis (●—●) and in control uninfected (----) mice.

The degree of inflammation is indicated as:

\+ = mild inflammation

++ = moderate inflammation

+++ = severe inflammation


1200
900
600
300
MEAN NO. OF MAST CELLS PER 20 V.C.U.
+
++
+++
++
+
2
4
6
8
10
12
14
21
28
35
DAYS POST INFECTION

Goblet cells in infected mice

After infection with T. spiralis the number of goblet cells remained similar to the controls until day 4 p.i. (202±39). By day 6 the number of goblet cells was slightly greater than in control mice (249±22). At this time however, the size of the cells was increased and the mucus contained with the cells stained more strongly, indicating an increase in mucus production. The number of goblet cells increased considerably by day 8 (402±57) and remained nearly at this level until day 10 p.i. (see plate 6, page 147). During this time the mucus content in the goblet cells was strongly stained and a layer of mucus was observed above the mucosa. This suggests that mucus production remains high from day 6 until at least day 10. Gross inflammation was observed in the gut with the accumulation of a fluid mucus. The numbers of goblet cells decreased slightly by day 12 (353±26) and by day 14 the mean number had declined to (262±23). Mucus production, however, remained high over this period. By day 21 both the goblet cell numbers and their apparent mucus production had nearly returned to control levels (234±37) with complete recovery being observed on days 28 and 35 p.i. (212±44).

No attempt was made to differentiate between goblet cells located in the epithelial lining of the

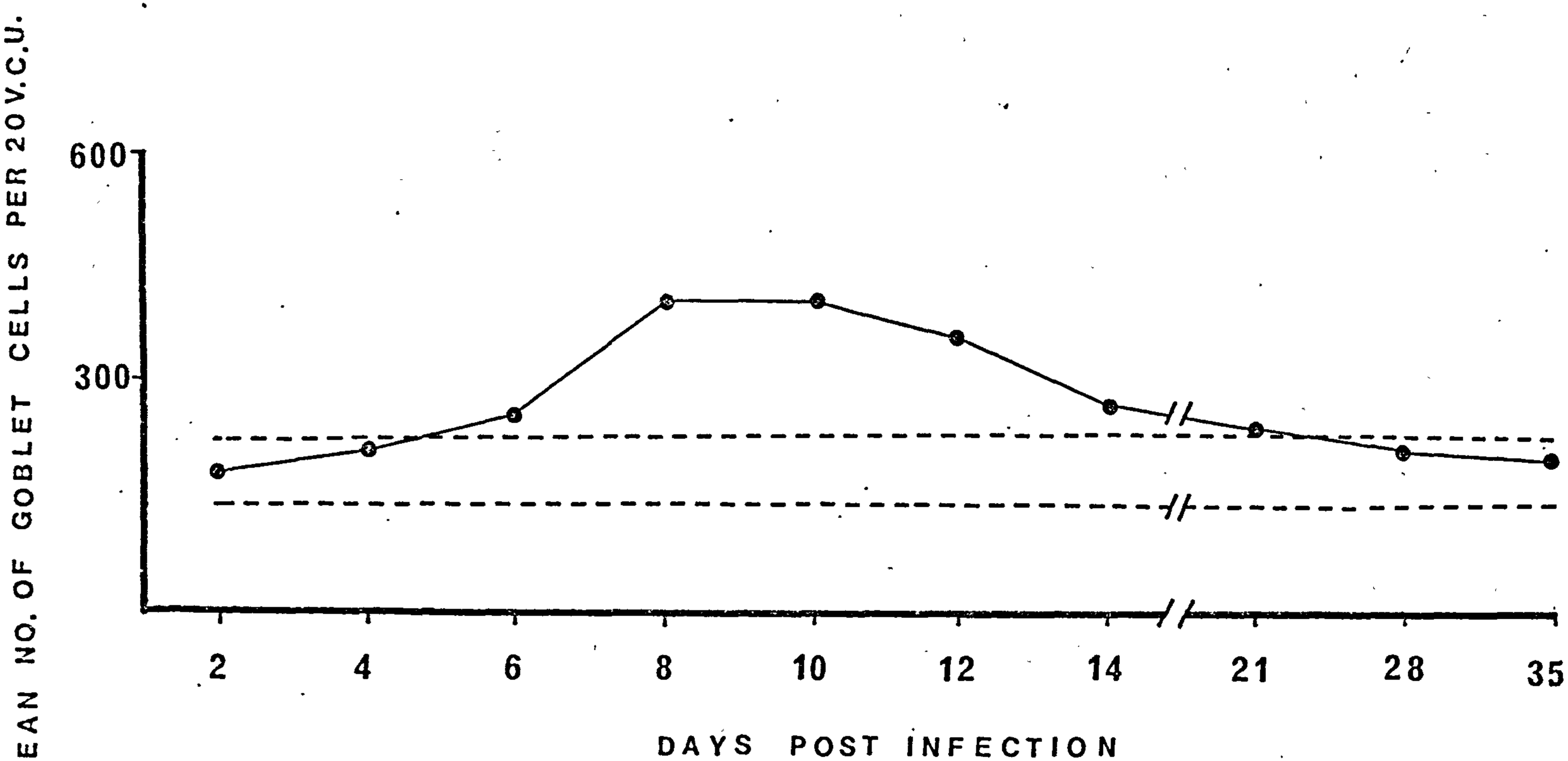
lumen and the cuboidal cells forming the acini of Brunner's glands (Wells, 1963). In these experiments all mucus-producing cells were counted as goblet cells. Cells were counted per 20 villus crypt units since the counting and expression of cells per number of epithelial cells was considered too arbitrary due to the fact that, at the light microscope level, it is difficult to distinguish and accurately count individual epithelial cells. The data obtained from counting per villus crypt unit is less arbitrary and is reproducible.

C. Kinetics of the mast cell response in rats

The numbers and distribution of mast cells in a segment from the middle of the small intestine were examined in control and in infected (2000 T. spiralis larvae) rats. Three controls and three infected rats were killed at various times p.i. Mean numbers (±S.D.) of mast cells in 20 V.C.U. for each of the days of examination are given in the text. The pattern of the mast cell response over the period of infection is shown in Fig. 3.3. By incubating the remaining portions of the intestine (i.e. those not used for histological analysis), it was ascertained that the response to the primary infection, in terms of worm expulsion, was similar to that described in chapter 1, with worm loss being complete by day 14.

Fig. 3.2

Mean numbers of goblet cells per 20 V.C.U. in NIH mice infected with T. spiralis (●——●) and in control uninfected (= = = =) mice.


MEAN NO. OF GOBLET CELLS PER 20 V.C.U.
600
300
2
4
6
8
10
12
14
21
28
35
DAYS POST INFECTION

Mast cells in control rats

In the control rats more mast cells were seen than in control mice (162±39) and they were located not only at the bases of the crypts, but also in the lamina propria of the villi (see plate 7, page 148). In addition, the mast cells in rats appeared larger and contained larger and more numerous cytoplasmic granules.

Mast cells in infected rats

Two days after infection with T. spiralis the number of mast cells did not differ from that of control rats (168±44). The number of mast cells increased slightly by day 4 p.i. (212±41). The distribution of the cells in each villus crypt unit at this time, was similar to that of control rats. Mild inflammation was observed in the intestinal mucosa with a few inflammatory cells being present in the tissues adjacent to the worms.

The number of mast cells increased nearly twofold by day 6 p.i. (325±93) and for the first time, cells were seen in the intraepithelial position as well as in the lamina propria. By this time the intestinal mucosa was moderately inflamed with an increase in inflammatory cells.

The number of mast cells increased considerably

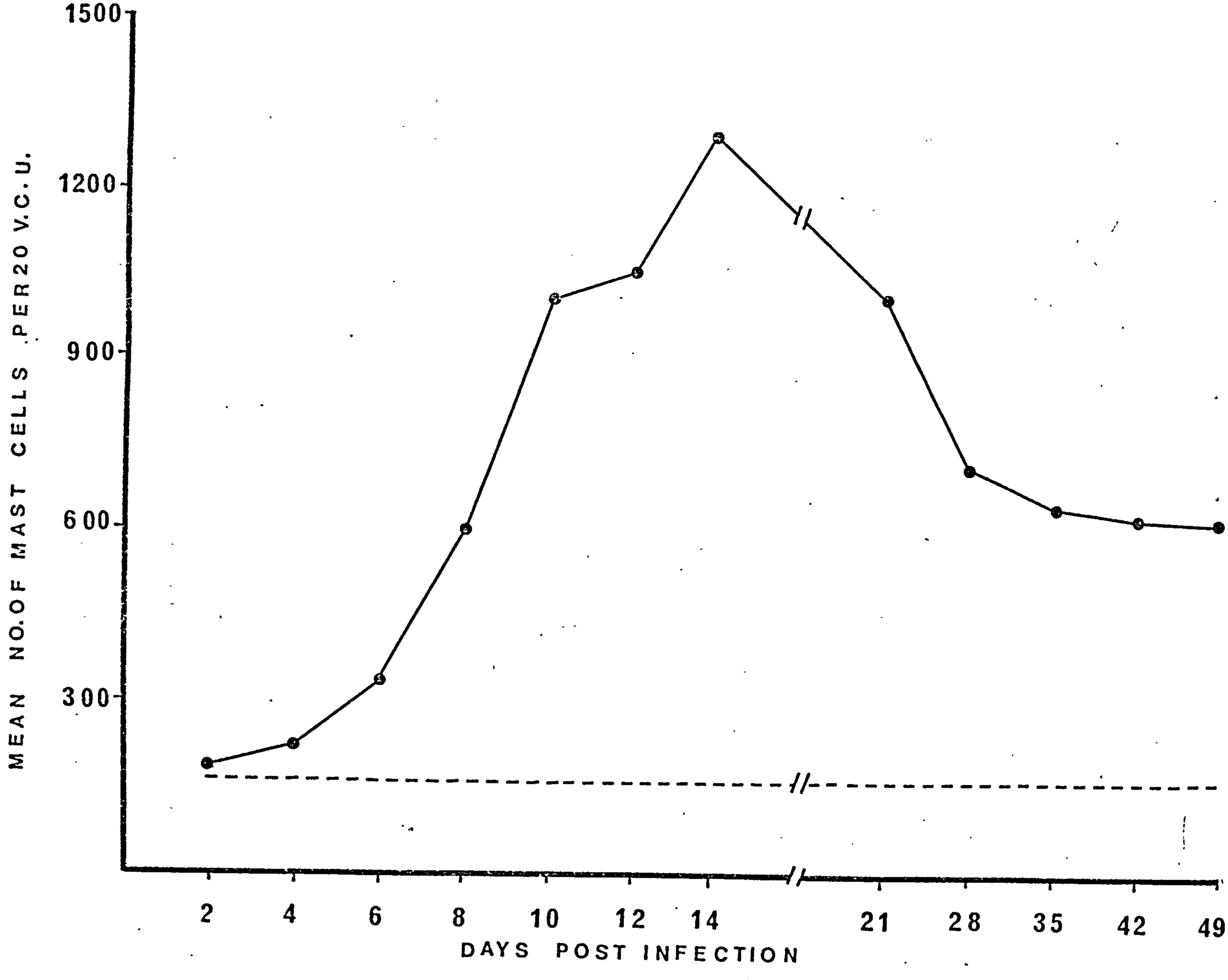
by day 8 (602±106) and had reached a level 5 times that of the controls by day 10 p.i. (1006±110). At this time, the majority of worms had been expelled and the small intestine was grossly inflamed. There was no significant increase in mast cells numbers over day 10, by day 12 (1050±56). Peak levels of mast cells were observed by day 14 (1295±126), (see plate 8, page 148). The number of mast cells decreased slightly by day 21 (1010±98), but was still above the control level by day 28 (705±53) and remained at approximately this level until the end of the experiment (day 49 - 615±60).

In contrast with mice, in rats the majority of cells were located in the lamina propria, although some pale blue staining cells were observed in the intra-epithelial position.

There was little variation between villus crypt units in terms of mast cell numbers and distribution. The cells were irregular in size and contained visible pink staining nuclei. They were packed with blue staining granules with those in the infected rats being larger than in the control animals and in some areas, the cells appeared fragmented and their granules scattered locally. It was also notable that large numbers of mast cells were found in the submucosa at the time of the peak response (see plate 10, page148).

Fig. 3.3

Mean numbers of mucosal mast cells per 20 V.C.U. in Wistar rats infected with T. spiralis (●—●) and in control uninfected (----) rats.


1500
1200
900
600
300
MEAN NO.OF MAST CELLS PER 20 V.C.U.
2
4
6
8
10
12
14
21
28
35
42
49
DAYS POST INFECTION

D. The kinetics of the goblet cell response in rats

The number and distribution of goblet cells in a segment from the middle of the small intestine, were examined in control and infected (2000 T. spiralis) rats.

The rats used in this experiment were the same as those used in the previous experiment and the sections were taken from the same piece of intestine as that which had been fixed for mast cell examination. Mean numbers (±S.D.) of goblet cells in 20 V.C.U. for each of the days of examination, are given in the text. The pattern of the goblet cell response over the period of infection, is shown in Fig. 3.4.

Goblet cells in control rats

There were obviously many more goblet cells in the control rats than in control mice (1050±81). The majority of the cells were located in the epithelium of the villi, although some cells were seen in the crypt region (see plate 9, page 149).

Goblet cells in infected rats

Two days after infection with T. spiralis the number and distribution of goblet cells were similar to that of control rats (996±106). The number of goblet cells remained at approximately this level until day 4 p.i.

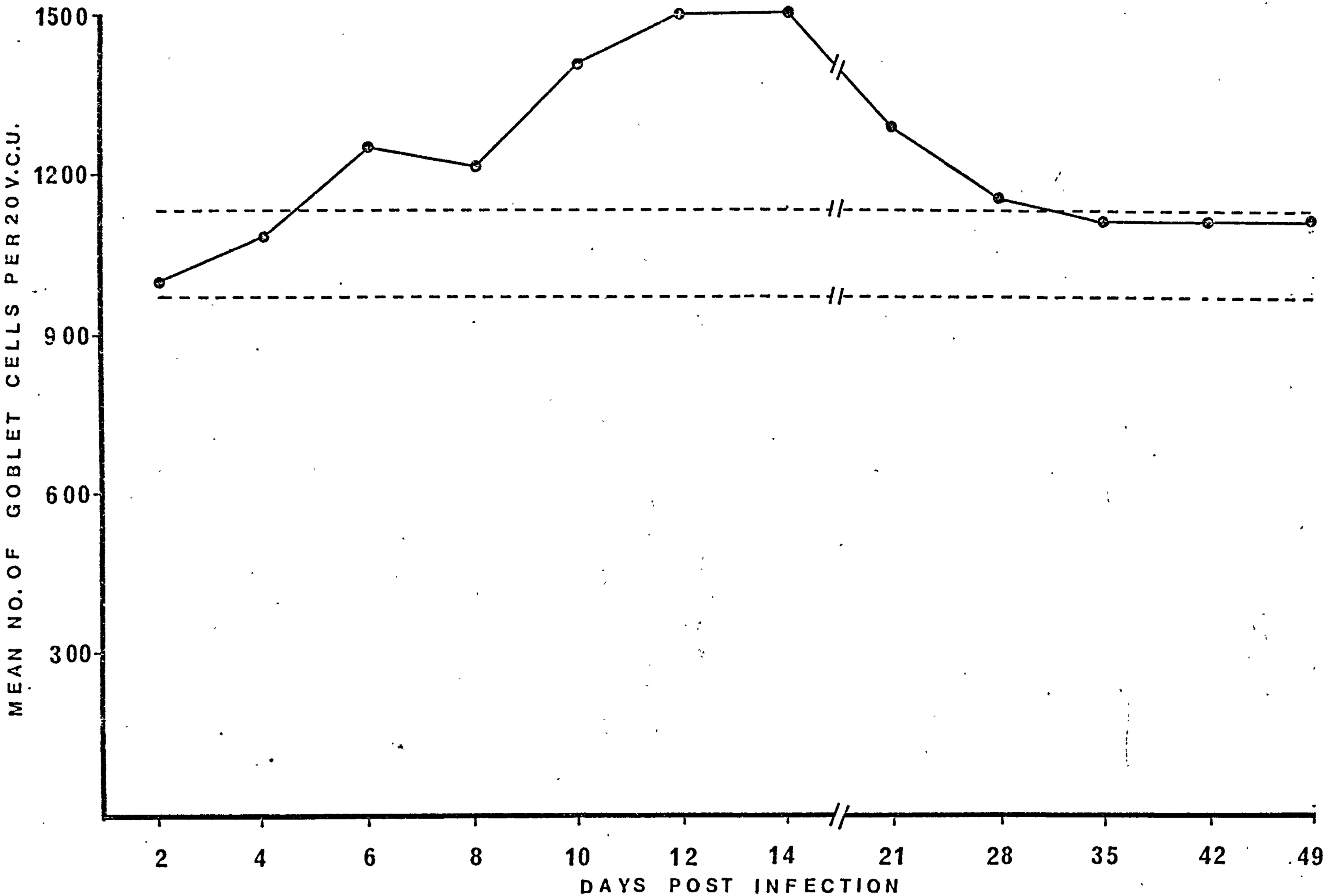
(1078±125), after which time there was a slight increase in number with the levels at day 6 (1248±98) and day 8 (1218±64) being only slightly higher than at day 4. By this time however, there was a noticeable increase in both the size and the mucus content of the goblet cells.

The number of goblet cells increased considerably by day 10 (1406±103) and reached a peak by day 12 (1492±57), remaining at this level until day 14 p.i. (see plate 10, page 149). After this time there was a slight decrease in cell numbers, but the level remained with the mean number at day 21 being 1282±49. The number of goblet cells returned to control levels by day 28 and remained at this level until the end of the experiment (i.e. day 49 - 1103±85).

During the time of increase in the number of goblet cells and for several days after, mucus production, as judged by the size and staining characteristics of the cells, was high. Even when the number of goblet cells had returned to control levels, their mucus content was greater than seen in control rats and remained so until day 49. A thick layer of mucus was evident between adjacent villi from day 12 onwards (see plate 10, page 149), and in some areas the mucus appeared to have been discharged from the cells. Since these sections of the small intestine were fixed in Carnoy's fixative, both mast cells and goblet cells stained clearly in control and infected rats as can be seen in plates 9 and 10, page 149.

Fig. 3.4

Mean numbers of goblet cells per 20 V.C.U. in Wistar rats infected with T. spiralis (●—●) and in control uninfected (= = =) rats.


MEAN NO. OF GOBLET CELLS PER 20 V.C.U.
1500
1200
900
600
300
2
4
6
8
10
12
14
21
28
35
42
49
DAYS POST INFECTION

E. Effect of duration of infection and single or multiple drug abbreviated infections upon the mast cell response in mice

It has been shown in chapter 2 that a single infection with a duration of 3 or 7 days did not induce rapid expulsion of a challenge infection given on day 14 after the primary infection. However, stimulation with a complete infection, or with two or more drug-abbreviated infections (i.e. 3 days duration) did induce rapid expulsion in mice. Further studies were then carried out to examine:

1. The relationship between the duration of infection and the mast cell response.
2. The fate of the mast cell response and the relationship between the presence of these cells and rapid expulsion induced by repeated infections.
3. The kinetics of the mast cell response after a challenge infection.

1. The effect of the duration of infection on the mast cell response

Twelve 7-week-old male NIH mice were given primary infections of 300 T. spiralis larvae. Six of these mice received the anthelmintic Mintic (see general materials and methods) on day 7 post infection, the

remaining six mice were untreated. Three mice from each group were killed on days 8 and 14. A segment (5 cm) from the middle of the small intestine was removed and examined for mast cell numbers. The rest of the gut was incubated for worm recoveries by the Baermann technique (see general materials and methods).

The mean numbers of worms recovered from mice given Mintic and from the untreated group on day 8 were 0 and 126.0 respectively. This indicates that the anthelmintic was 100% effective. No worms were recovered on day 14 from either group.

The mean number (±S.D.) of mast cells in 20 villus crypt units is shown in Fig. 3.5.

In mice given Mintic on day 7 and killed on day 8, the number of mast cells was similar to the group of mice which did not receive anthelmintic treatment (1052±104 and 1098±78 respectively). The number of mast cells was considerably lower in the group of mice given mintic on day 7 and killed on day 14 (312±70) when compared with the untreated group (854±89), indicating a rapid decline in mast cell numbers.

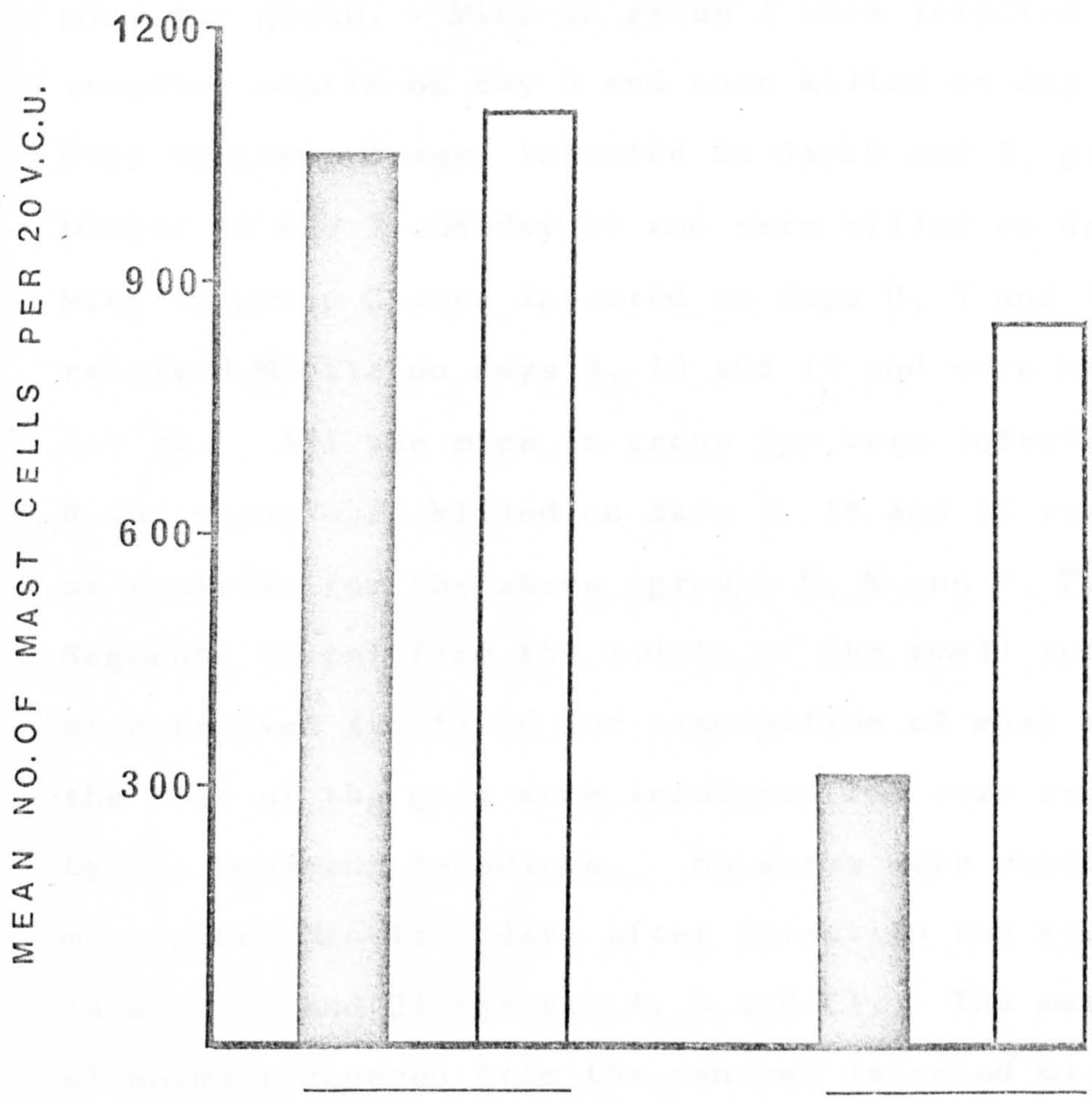
2. <u>The effect of single or repeated infections on the mast cell response</u>

For these experiments, mice were exposed to single or repeated infections (with 300 normal

Fig. 3.5

Mean numbers of mast cells per 20 V.C.U. in NIH mice infected with *T. spiralis* on day 0 and given Mintic on day 7 ▬ or untreated mice ▭

Killed on day 8 or 14 post infection.


1200
900
600
300
MEAN NO. OF MAST CELLS PER 20 V.C.U.
8
14
DAYS POST INFECTION

T. spiralis larvae) which were limited to 3 days duration.

Eighteen 7-week-old male NIH mice were divided into two groups of nine (1-2). Mice in group one were then divided into three groups (A, B and C) with three mice per group. Mice in group A were infected on day 0, received mintic on day 3 and were killed on day 7. Mice in group B were infected on days 0 and 7, given Mintic on day 3 and day 10 and were killed on day 14. Mice in group C were infected on days 0, 7 and 14, received Mintic on days 3, 10 and 17 and were killed on day 21. All the mice in group two were infected on day 0 and three were killed on days 7, 14 and 21 respectively, as controls for the above (groups D, E and F, Fig. 3.6). Segments (5 cm) from the middle of the small intestines were removed and fixed for examination of mast cells and the rest of the guts were incubated for worm recoveries by the Baermann technique. No worms were recovered from mice given Mintic 3 days after infection and killed on days 7, 14 and 21 (groups A, B and C). The mean numbers of worms recovered from the control infected mice were 138.5 and 0 on day 7 (group D) and day 14 (group E), respectively. The control animals showed normal establishment and expulsion. The numbers of mast cells in all groups are shown in Fig. 3.6. The number of mast cells remained low in mice given a 3-day infection and killed on day 7 (group A - 75±46). The number of

mast cells was considerably higher in mice given two or three drug abbreviated infections (groups B and C - 454±106 and 692±92, respectively). Mast cell numbers in the control, normally infected mice were similar to those observed previously in this chapter (group D 418±82, Group E 834±73 and group F 520±64).

3. The kinetics of the mast cell response after a challenge infection

It has been shown that the number of mast cells remains above control levels for several days after a primary infection has been expelled. To examine the effect of a challenge infection upon the mast cell response, the challenge infection should be given either after day 35 (i.e. when the number of mast cells has returned to control levels), or after the termination of the infection on day 7, as it has been shown that the number of mast cells declines sharply a few days after drug treatment. With these methods of immunization, it is possible to study the effect of a challenge infection on the mast cell response.

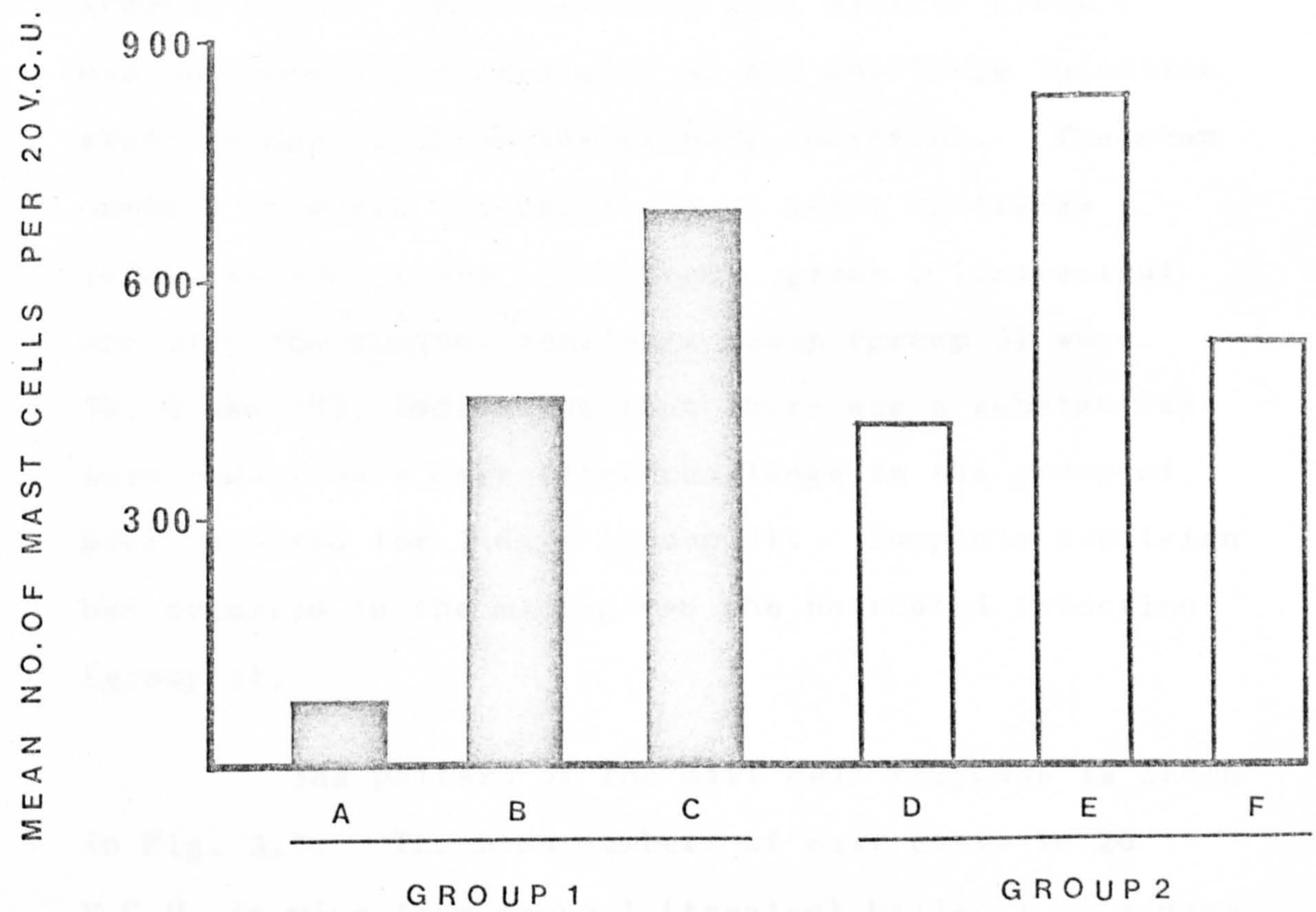
Twelve 7-week-old male NIH mice were infected with 300 T. spiralis larvae and were divided into two groups of six mice (groups 1 and 2). Mice in group 1 received Mintic on day 7 post infection and mice in group 2 were untreated (complete infection). Both groups, together with uninfected control age-matched mice (group

Fig. 3.6

Mean numbers of mast cells per 20 V.C.U. in NIH mice given one, two or three drug abbreviated infections (each of 3-day duration) ▓▓▓ or complete infection ▭.

Group 1
- A one drug abbreviated infection killed on day 7.
- B two drug abbreviated infections killed on day 14.
- C three drug abbreviated infections killed on day 21.

Group 2
- D complete infection killed on day 7.
- E complete infection killed on day 14.
- F complete infection killed on day 21.


MEAN NO.OF MAST CELLS PER 20 V.C.U.
900
600
300
A
B
C
D
E
F
GROUP 1
GROUP 2

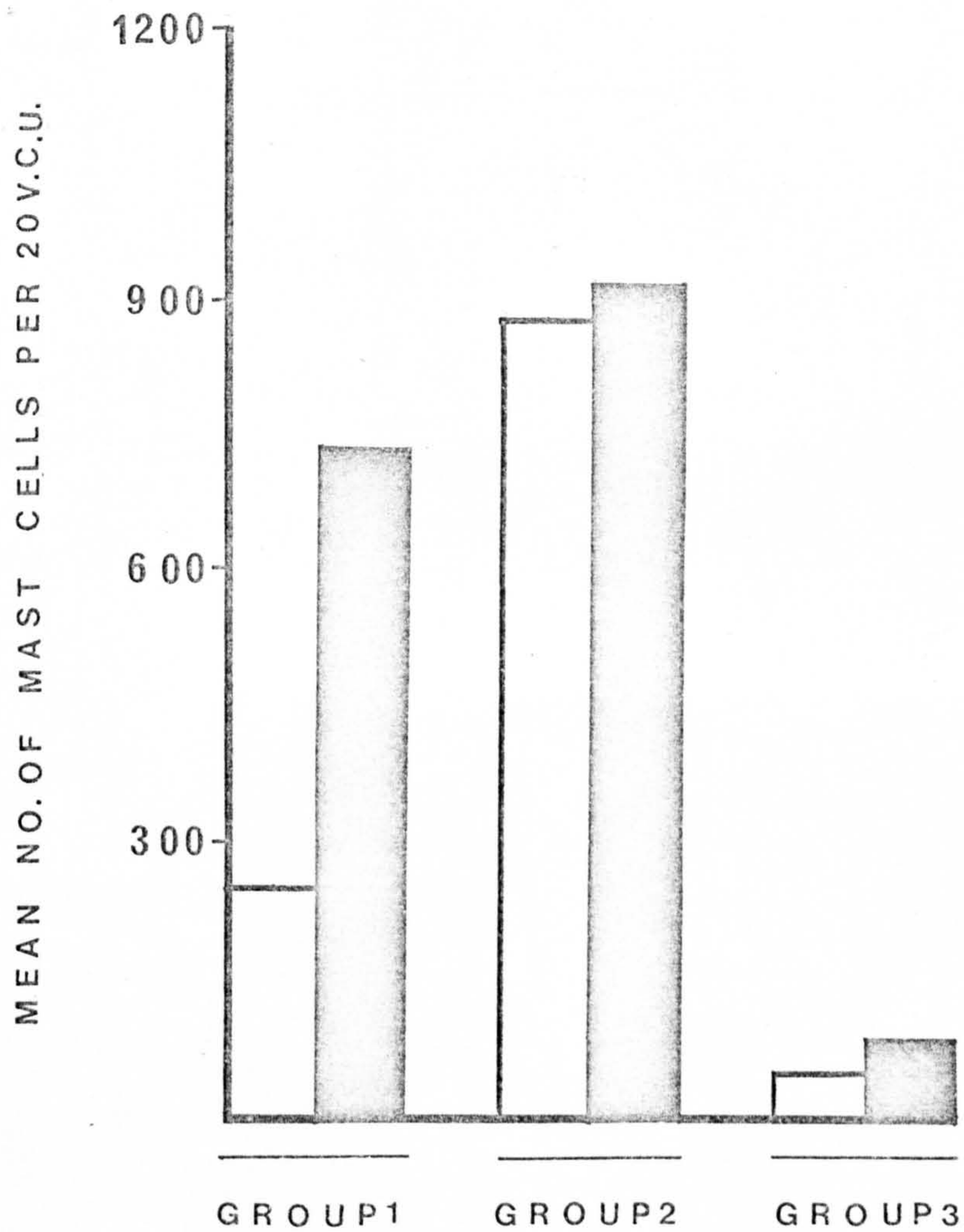
3), were then challenged on day 14 and were killed 1 or 4 days after challenge. Segments (5 cm) from the middle of the small intestines were removed for histology and the rest of the guts were incubated for worm recoveries by the Baermann technique. The mean number of worms recovered one day after challenge infection from mice in group 1 (given Mintic on day 7 p.i.), group 2 (untreated) and also the control challenge group (group 3), were 134, 2.8 and 166 respectively. This indicates that mice in group 1 did not show rapid expulsion of the challenge infection given on day 14 after the primary infection. The mean numbers of worms recovered 4 days after challenge infection from group 1 (treated), group 2 (untreated) and also the control challenge group (group 3) were 70, 0 and 153, indicating that there was a substantial worm reduction 4 days after challenge in the group of mice infected for 7 days (group 1). Complete expulsion had occurred in the mice given the untreated infection (group 2).

The pattern of the mast cell response is shown in Fig. 3.7. The mean numbers of mast cells in 20 V.C.U. in mice from group 1 (treated) killed 1 or 4 days after challenge were 252±83 and 736±92 respectively. In mice from group 2 (untreated group) killed 1 or 4 days after challenge, the numbers were882±76 and 926±69 respectively and in control challenge (group 3) killed

1 or 4 days after challenge, were 48±16 and 82±21, respectively. Thus 1 day after challenge the number of mast cells in the mice given the drug limited infection (group 1) was lower than those of mice given the complete infection, but by day 4 post challenge, the mast cell numbers were similar in both groups.

Fig. 3.7

Mean numbers of mast cells per 20 V.C.U. in NIH mice infected with T. spiralis on day 0 and given Mintic on day 7 (group 1) or untreated (complete) infection (group 2). They were then challenged on day 14 together with uninfected control (group 3) and were killed on day 1[redacted] or day 4[redacted] post challenge.


MEAN NO. OF MAST CELLS PER 20 V.C.U.
1200
900
600
300
GROUP1
GROUP2
GROUP3

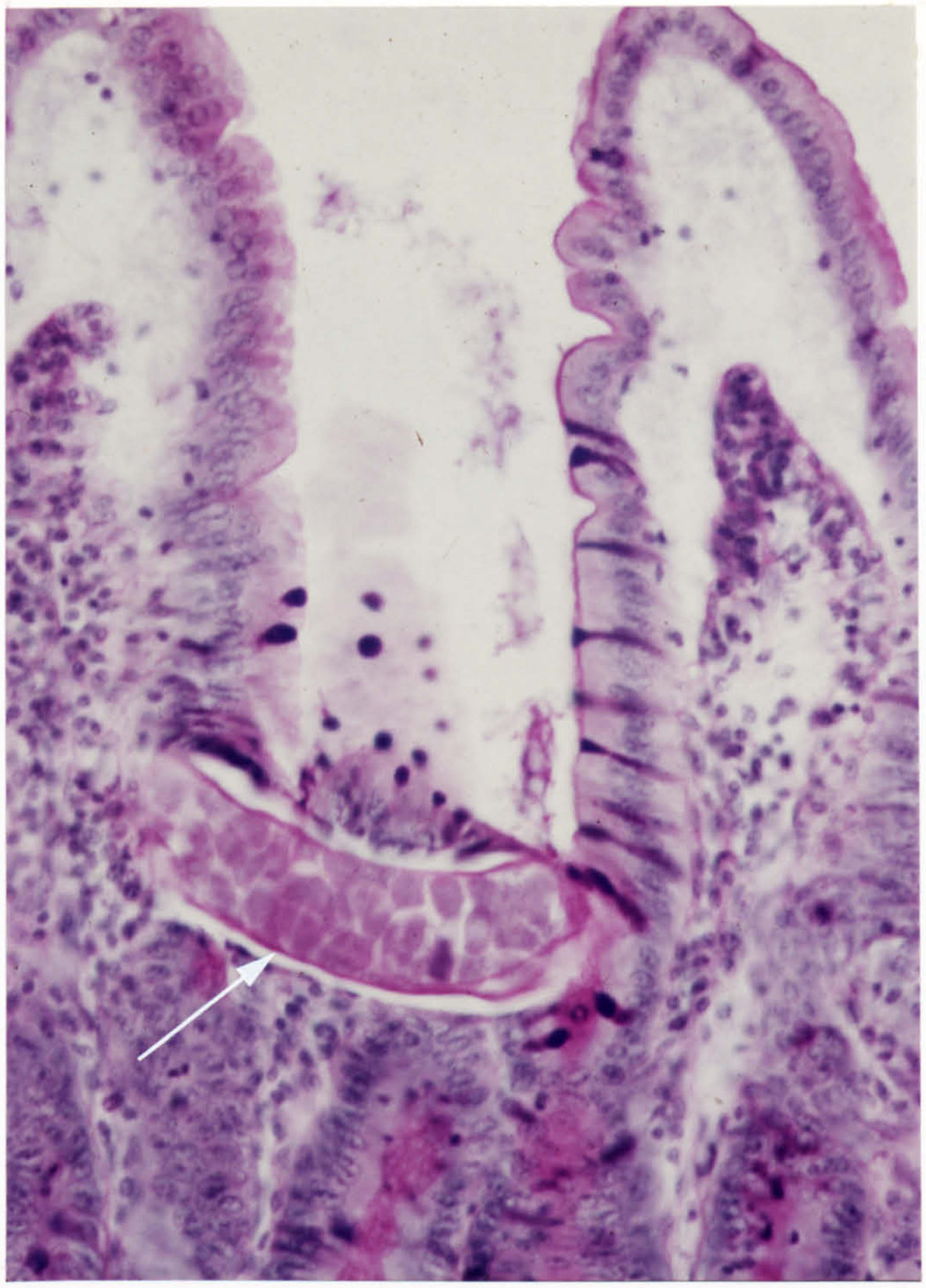
Photomicrographs of Dominici stained section of mouse small intestine.

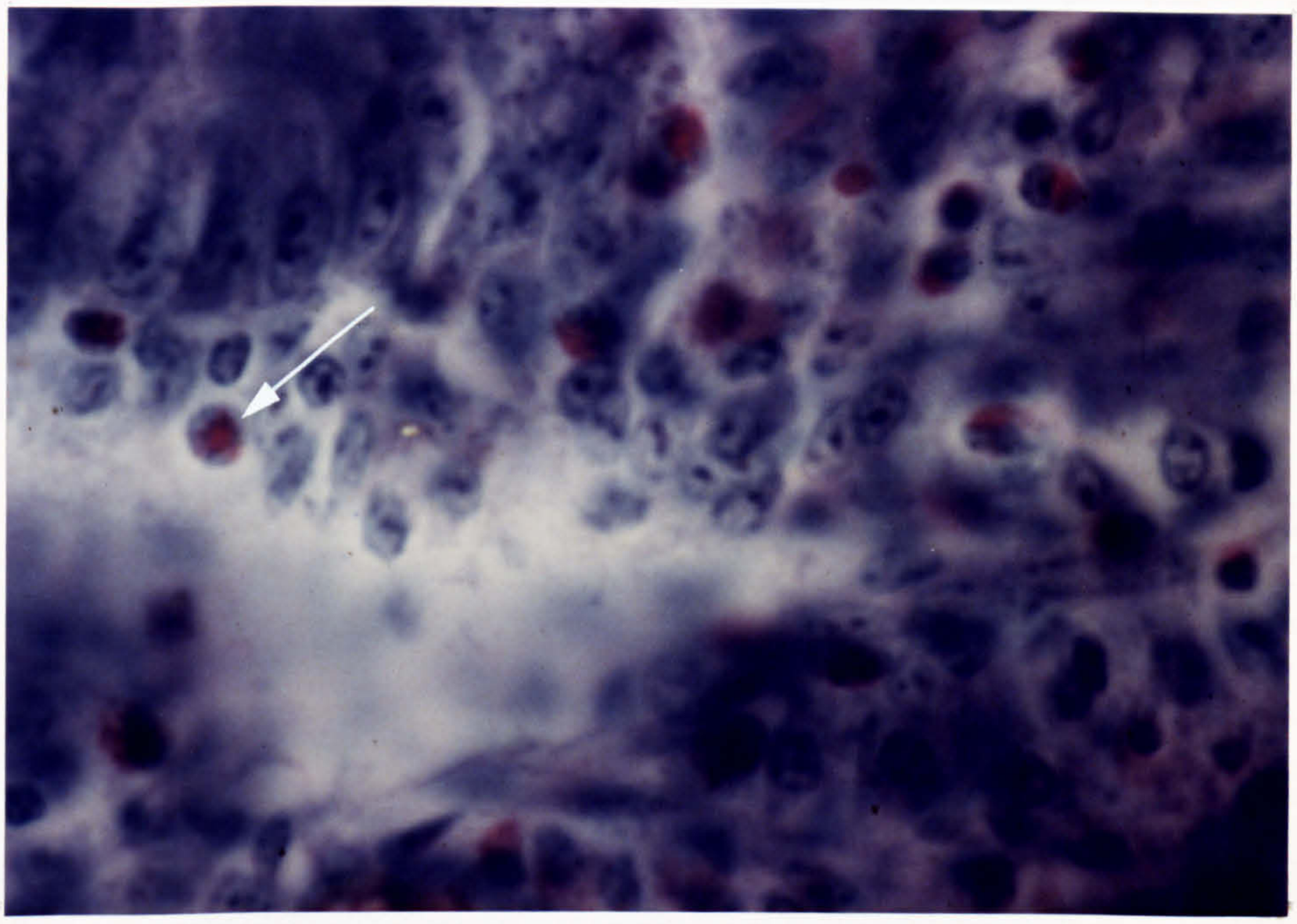
Plate 1

Section from mice infected with T. spiralis and examined on day 4 post infection. Note the section of an adult worm which has been indicated with an arrow (X350).

Plate 2

Section from mice infected with T. spiralis and examined on day 6 post infection. Note the inflammatory cell infiltrate especially the eosinophils (stained red) one of which has been indicated with an arrow (X900).





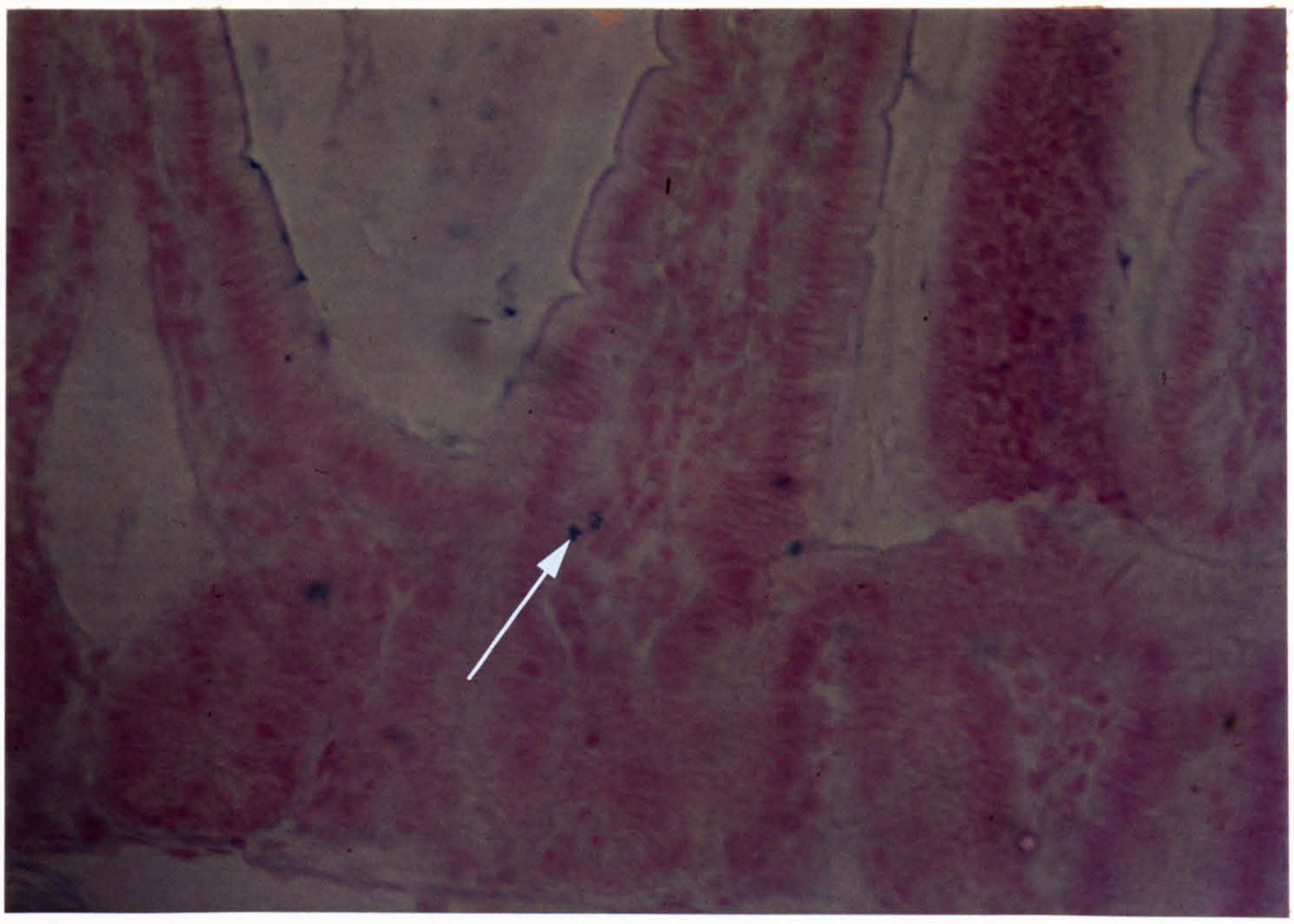
Photomicrographs of Alcian blue/Safranin stained section of mouse small intestine.

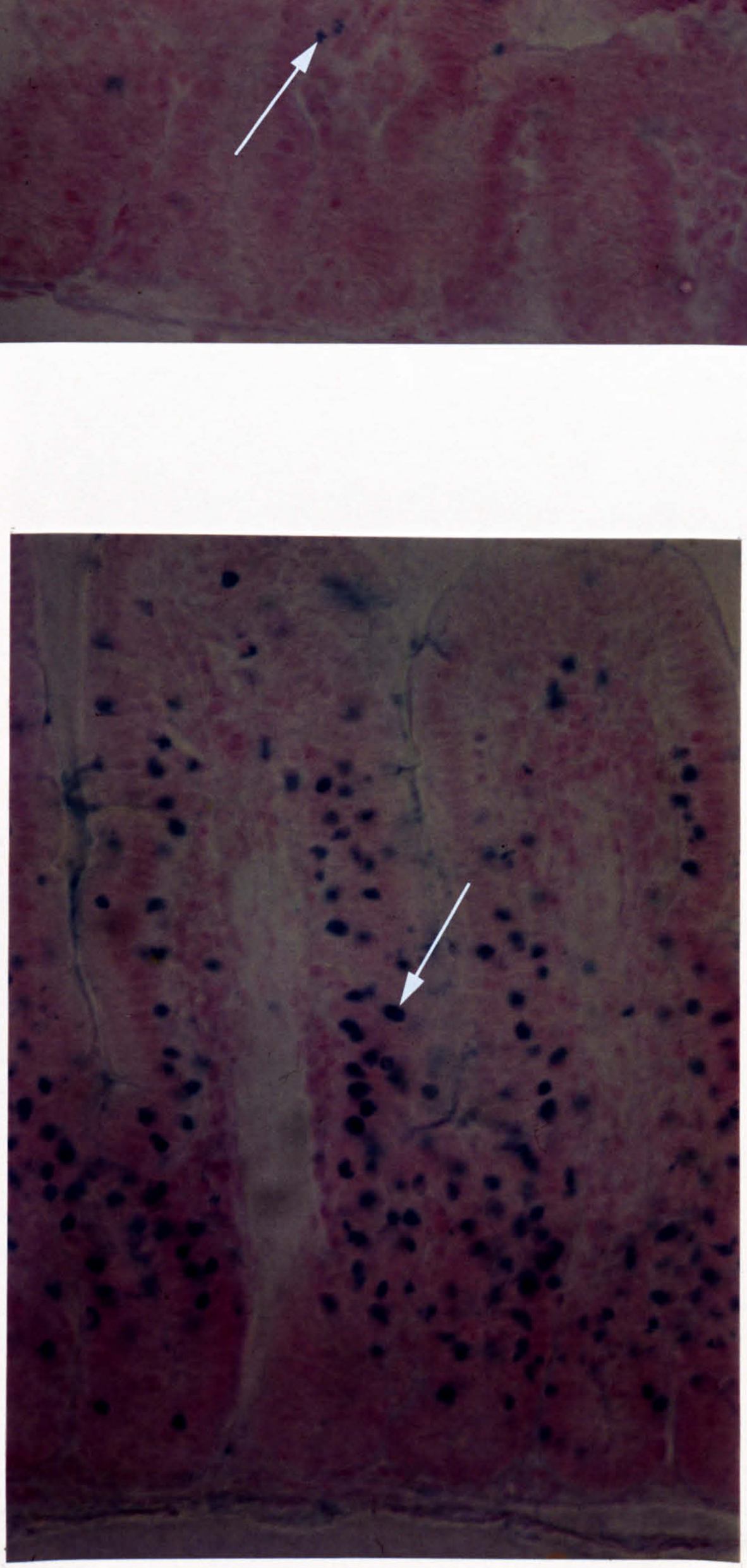
Plate 3

Section from control uninfected mice. Note the mast cells (stained blue) one of which has been indicated with an arrow (X350).

Plate 4

Section from mice infected with T. spiralis and examined on day 10 post infection. Note the mast cells (stained blue) one of which has been indicated with an arrow (X350).





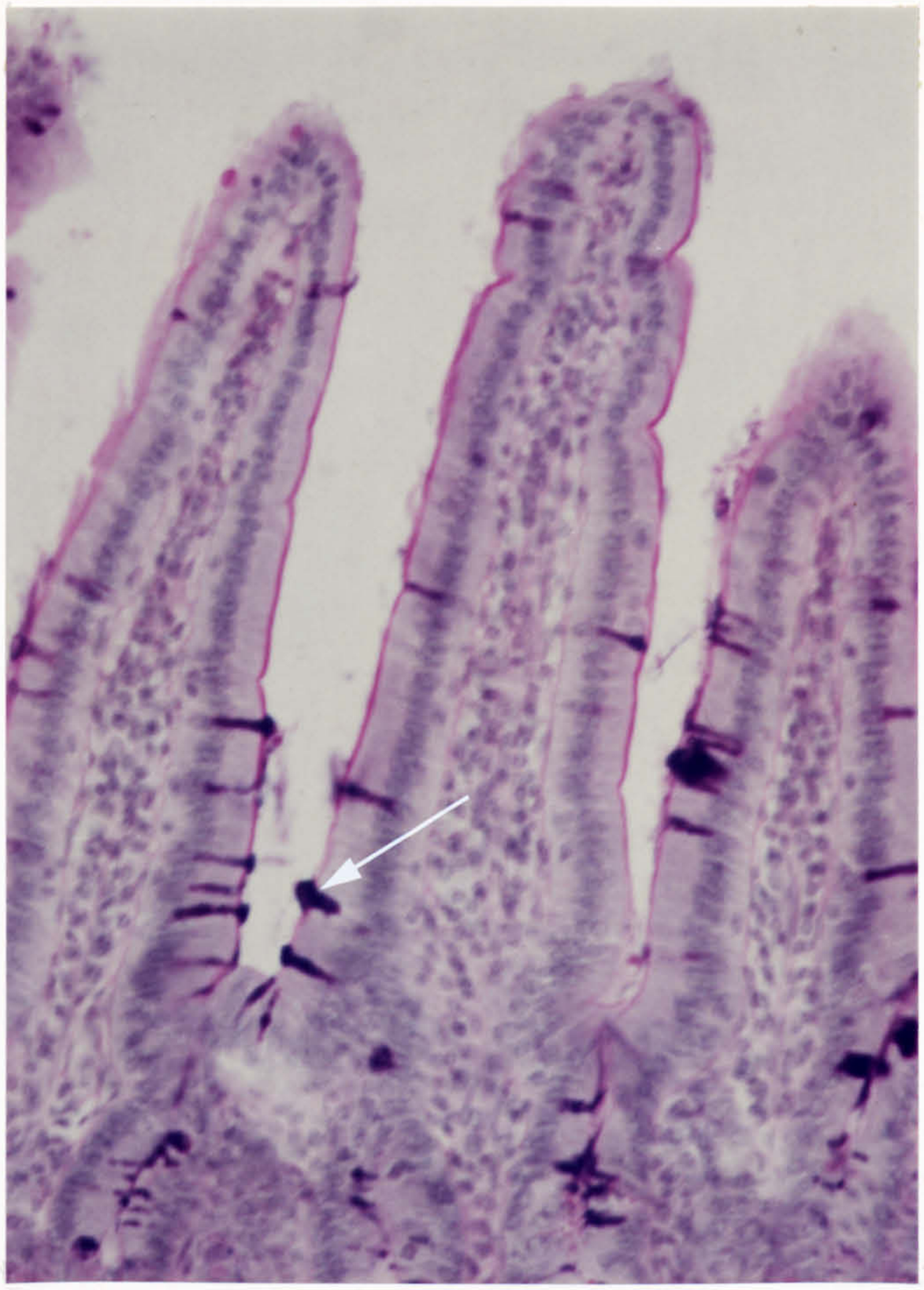
Photomicrographs of Alcian blue/PAS stained section of mouse small intestine.

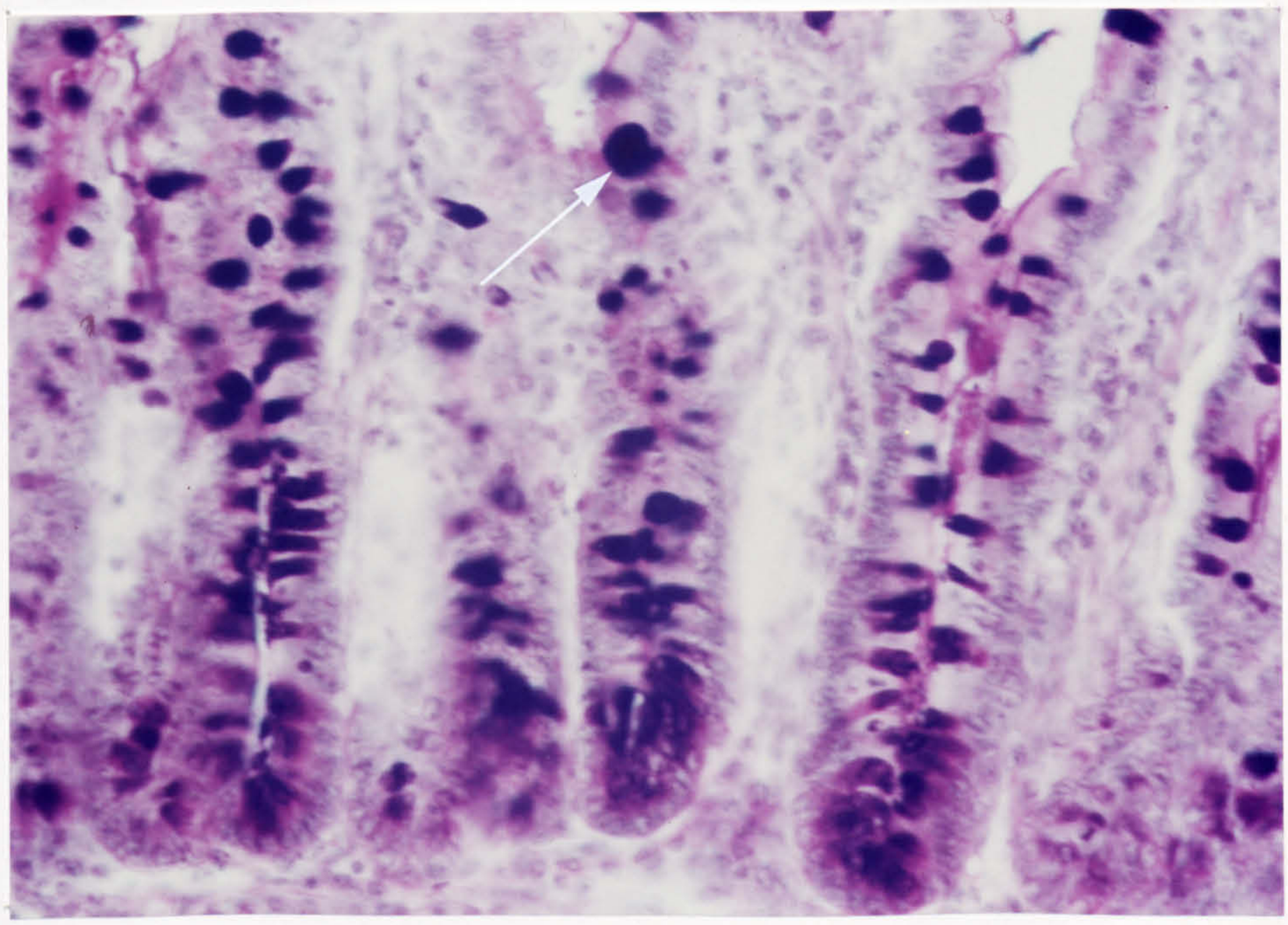
Plate 5

Section from control uninfected mice. Note the goblet cells stained purple, one of which has been indicated with an arrow (X350).

Plate 6

Section from mice infected with T. spiralis and examined on day 10 post infection. Note the goblet cells stained purple, one of which has been indicated with an arrow (X650).





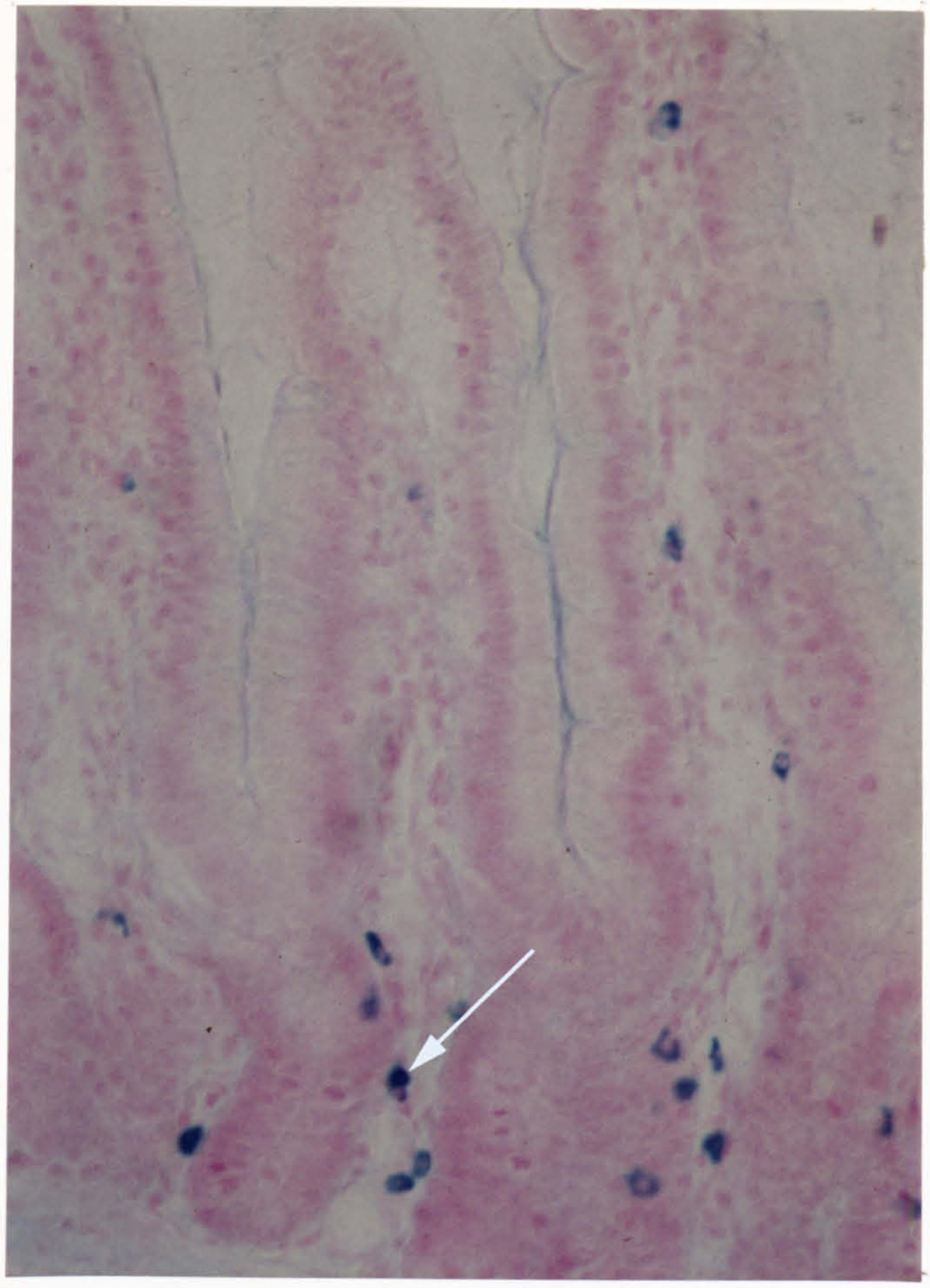
Photomicrographs of Alcian blue/Safranin stained section of rat small intestine.

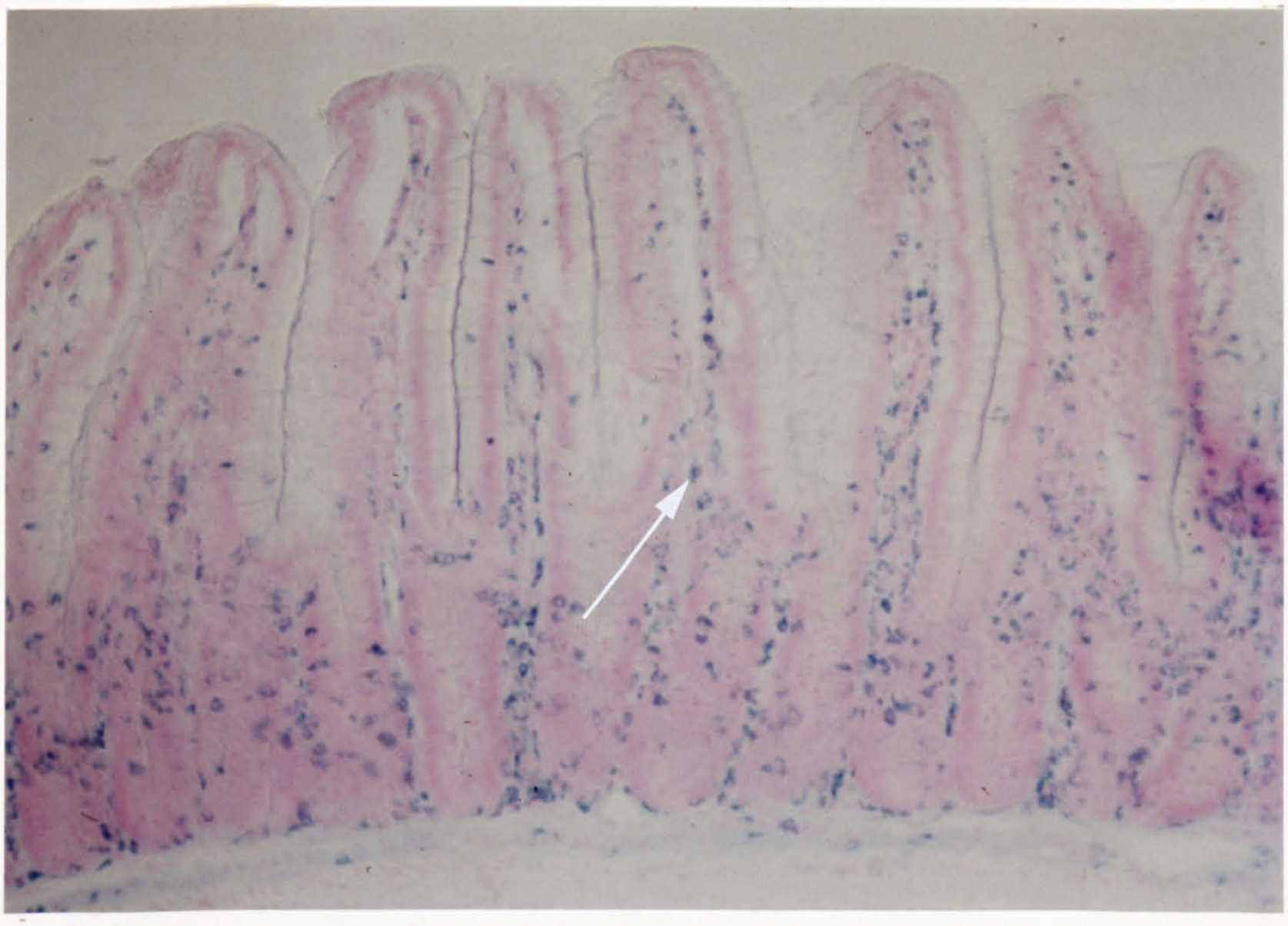
Plate 7

Section from control uninfected rats. Note the mast cells stained blue, one of which has been indicated with an arrow (X350).

Plate 8

Section from rats infected with T. spiralis and examined on day 14 post infection. Note the mast cells stained blue, one of which has been indicated with an arrow (X150).





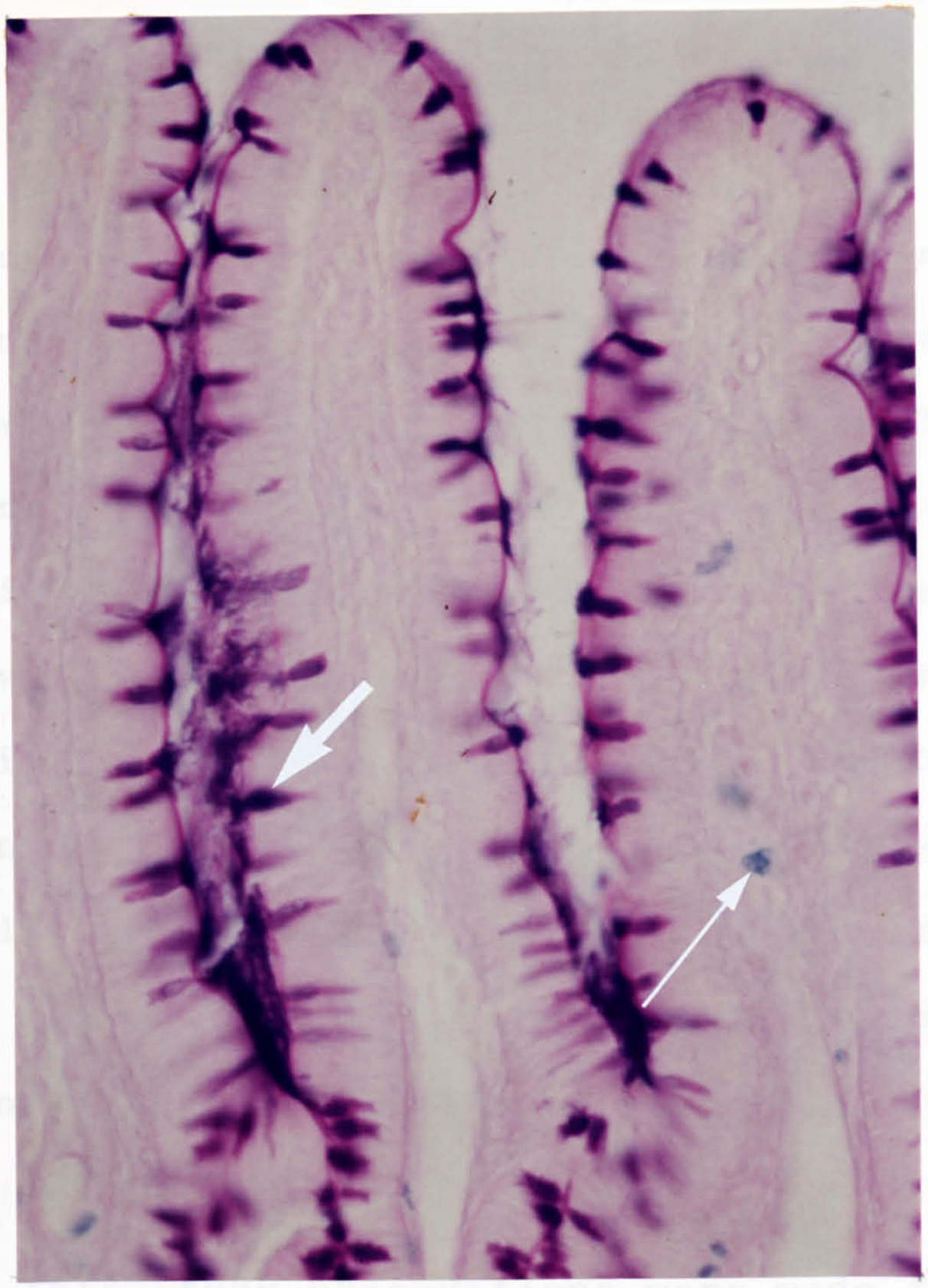
Photomicrographs of Alcian blue/PAS stained section of rat small intestine.

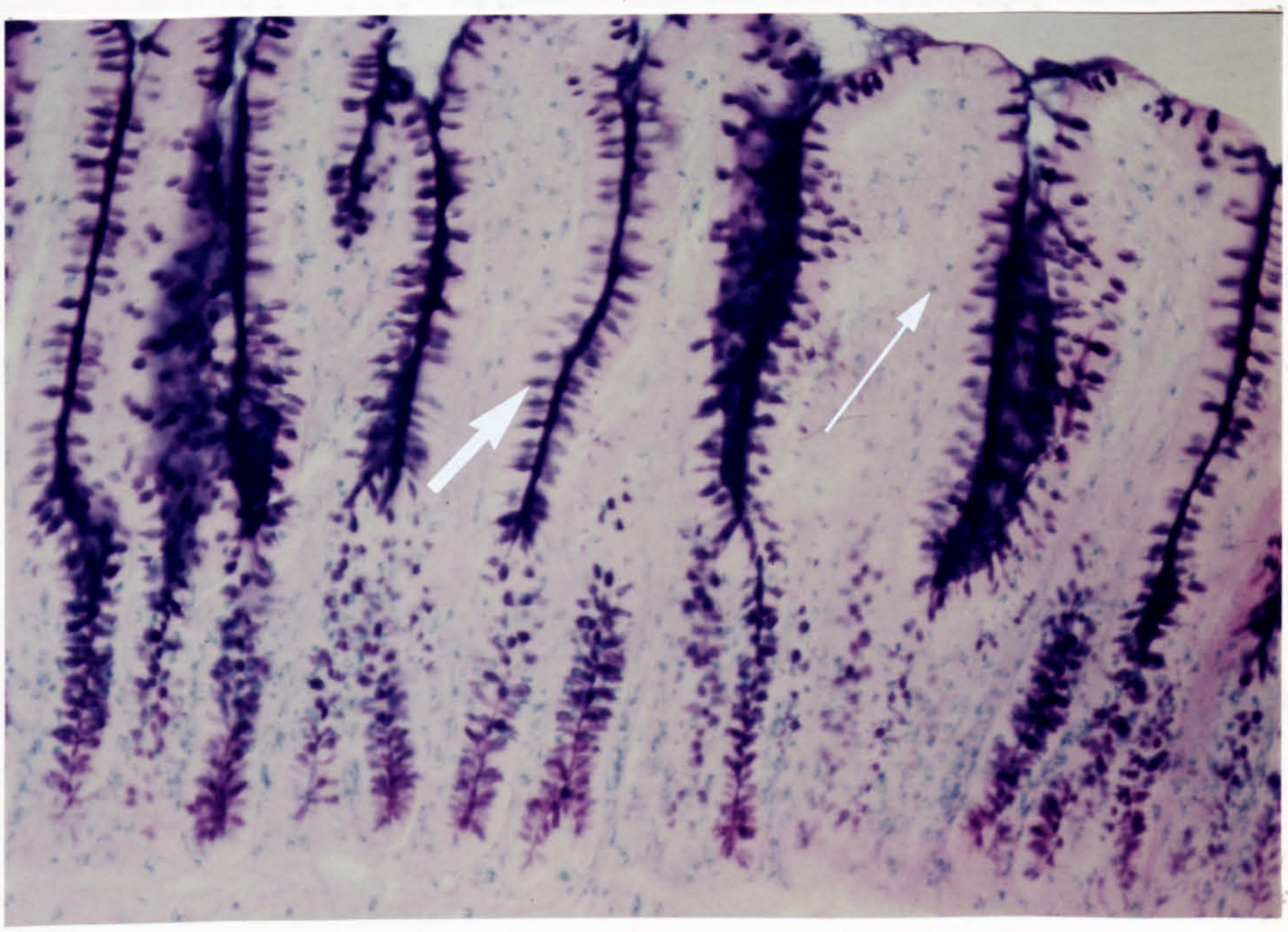
Plate 9

Section from control uninfected rats. Note the goblet cells stained pink (large arrow) and the mast cells stained blue (small arrow) (X350).

Plate 10

Section from rats infected with T. spiralis and examined on day 14 post infection. Note the goblet cells stained purple (large arrow) and the mast cells stained blue (small arrow) and the numerous mast cells present in the submucosa (X150).





DISCUSSION

The loss of T. spiralis during primary infections in both mice and rats is associated with gross inflammatory changes in the intestine. These changes occur initially in the anterior half of the small intestine, where the worms are located, but then spread to involve the whole gut. It is well established that the inflammation is mediated by thymus dependent immune responses (Wakelin, 1978a) and is characterised by marked cellular infiltration of the mucosa (Larsh and Race, 1975). In addition, there are profound physico-chemical changes in the intestinal environment (Castro, 1976).

In this study, observations were made to characterise the degree of inflammation present in the small intestine during the period of infection. The response was evident in three stages. A mild or early reaction was observed by day 4 post infection and the intensity of the response increased by day 6 when moderate inflammation was observed. Between days 8 and 10, severe inflammation was manifest with an accumulation of fluid-mucus in the gut. The inflammation subsided very quickly and disappeared between days 12 and 14, at which time the worms had been completely expelled from the intestine. Therefore, there is a direct association of inflammation and subsequent worm rejection.

Inflammatory changes were also apparent when the gut was studied histologically and during the severe inflammation, the mucosa and lamina propria contained a variety of inflammatory cells. These observations confirm those obtained by Larsh and Race (1975) who also studied the response of mice to T. spiralis by histological examination. Other workers have measured the degree of inflammation by a combination of histology and measurement of the changes in physiological factors, finding a correlation between worm expulsion and the presence of these factors. For example, Smith and Castro (1978), who measured the level of peroxidase activity (presumably contained in polymorphonuclear and mononuclear cells other than lymphocytes), in the intestinal mucosa of rats during T. spiralis infection, noted there was a relationship between increased levels of peroxidase activity and inflammation. Stewart has noted (personal communication) increased levels of peroxidase activity in the intestinal mucosa of mice infected with T. spiralis between days 6 and 7 post infection and Larsh et al. (1974) demonstrated that the phospholipase level in the intestinal tissues corresponded strikingly with the degree of inflammation. Several attempts have been made to measure the peroxidase activity in the intestinal tissues of NIH mice during T. spiralis infection, but the results were unsatisfactory because of wide variation. The reasons for this variation are not known, but may possibly be due to the lower degree of sensitivity of the test in mice.

In order to monitor the inflammatory changes generated during infection as well as their persistence and possible involvement in rapid expulsion (see chapters 1 and 2) in mice and rats, two histological parameters, namely the number of mast cells and goblet cells in the intestinal mucosa were examined. In mice the numbers of both cell types increased sharply before worm expulsion and maximum numbers were observed on day 10 post infection, whereas in rats, the rise in numbers of both cells occurred as expulsion took place and reached maximum levels on day 14 post infection. A similar increase in the number of mast cells has been described by Ruitenberg and Elgersma (1976) in B10LP mice and by Tronchin et al. (1979) in CBA mice, though the rise in the latter was post expulsion.

Levels of both cells fell quickly after worm expulsion. In mice, goblet cells reached control levels by day 14 and mast cells between days 28 and 35. In contrast, in rats, goblet cell numbers did not reach control levels until day 28 and mast cell numbers were still raised at day 49. Prolongation of the intestinal mastocytosis induced by nematode infections, has also been described by Miller (1969), Mayrhofer (1979a) and Macdonald et al. (1980) after primary infection with N. brasiliensis in rats.

The increase in mast cell numbers described here

was in both intraepithelial and lamina propria cells, but most of the cells observed at the time of the peak response in mice were intraepithelial in position, whereas in rats, the majority of the cells were located in the lamina propria. As many more goblet cells were present in control rats than in control mice, the maximum number reached during primary infection differed very considerably between the two species. It was striking in both species that the actual size of goblet cells increased during the infection and that this increase persisted longer in rats, even when cell numbers had returned to control levels. Initial experiments (chapter 1) showed that challenge infections given shortly after the expulsion of the primary infection, when the gross inflammatory changes have disappeared, were expelled rapidly (within 24 hours). In mice this response to challenge was short lived, i.e. challenge infections given more than 16 days after the primary infection were not subjected to rapid expulsion. In rats, however, the rapid expulsion response was more long-lasting and was shown to operate against challenges given up to 49 days after the primary infection. The rapidity with which challenge infections are expelled, suggests that either the intestinal environment is so altered by the primary infection that conditions are unsuitable for subsequent parasite establishment, or, that the intestine is primed in such a way that the stimulus provided by the challenge evokes an almost instantaneous response.

It is tempting to relate the ephemeral rapid expulsion response in mice to the shorter duration of cellular changes associated with intestinal inflammation. In these terms, rapid expulsion would be the direct result of the environmental changes arising during the primary infection. The persistence of the response in rats may be due to persistence of cellular changes, the most marked of which being the increased number of mast cells. It is possible that these cells may exert a continuing influence on the goblet cell population. It is known that mediators released from mast cells can increase the production and release of mucus from goblet cells. Such mediators can be elicited by the action of anaphylatoxin (Weir, 1974) or by IgE mediated anaphylaxis following the introduction of antigen into the immune intestine (Lake et al., 1979 and 1980). If it can be assumed that the challenge larvae do provoke a reaction on entry into the intestine and there is physiological data to support this (Castro, Hessel and Whalen, 1979), then the effects of mucus may be not only to act as a mechanical barrier to penetration, but also to influence gut mobility, thus facilitating expulsion of the parasite. Lee and Ogilvie (1981) have presented evidence to show that the failure of challenge larvae to become established, is due to their being trapped in the intestinal mucus layer of immune rats (possibly by antibody) and then being expelled by peristalsis. More recently

Miller et al. (1981) reported a similar result in rats infected with N. brasiliensis.

The data presented here show that there are clear species differences in the induction and persistence of the mast cell and goblet cell responses and also that these differences appear in the ability to respond rapidly to challenge infection. However, it must be emphasised that in mice, at least, there is evidence that rapid expulsion is genetically determined (Bell and McGregor, 1980c) and therefore, the species difference may not be so great when other strains are used. In addition, it is known that mice vary in their ability to mount a mast cell response to infection with T. spiralis (Alizadeh and Wakelin, 1981b). Although the mast cell response has been examined in a small number of strains of rats infected with N. brasiliensis (Nawa and Miller, 1979), there is at present, little evidence on the ability of different strains of rats to mount a mast cell response and also to express rapid expulsion. Therefore, it would be useful to examine these responses in different strains of rats.

The experiments dealing with the effect of the duration of infection on the mast cell responses have shown that a 7-day intestinal exposure to T. spiralis was sufficient to induce a mast cell response, but that the levels of these cells declined sharply within a few

days of anthelmintic treatment. This indicates that continuing antigenic stimulation is necessary for the proliferation and maintenance of the mast cell response. When the period of infection was increased (i.e. complete infection), stimulation continued and the mast cell response persisted for a longer period of time. These results confirmed those obtained by Mayrhofer (1979a) with rats infected with N. brasiliensis, who noted that after drug treatment, mast cell numbers decreased very quickly. It is not known why, after parasite expulsion, the levels of mast cells remained high for several days, perhaps there is a threshold of stimulation necessary to induce the response and when the mast cells reach maximum levels, they persist for a longer period of time. In support of this, when the infection was limited to 3 days, the numbers of mast cells did not exceed control levels when examined 4 days after drug treatment. Thus the persistence of infection is accompanied by a proliferation of the mast cell response and it is possible that, directly or indirectly, the mast cells are involved in the environmental changes which accompany infection and the persistence of these cellular changes may have some influence on a subsequent challenge infection. Indeed, when the primary infection was limited to 7-day duration, the challenge infection was not subjected to rapid expulsion (given on day 14) and at this time, it is clear that the levels of mast

cells are lower than those observed with a complete infection. Interestingly, two or three drug-abbreviated infections each lasting 3 days, were sufficient stimulation to induce a mast cell response which resembled that of a complete infection. It is unlikely that the effectiveness of this method of immunization was related to the total duration of infection (6 or 9 days) and it is possible that the effect was due to the fact that the second infection given on day 7 caused an accelerated mast cell response. More interestingly, infections given on day 14 after two drug-abbreviated infections were subjected to a rapid expulsion response (as shown in chapter 2), and it seems that this infection has some influence on the mast cell response, with the levels of mast cells quickly rising to a higher level than seen after the two drug-abbreviated infections. These findings may explain the mechanisms of rapid expulsion which have been observed in this study and also in chapter 2. They also indicate that the mast cell response to secondary infection is clearly accelerated both in onset and in magnitude.

These results have been confirmed by the use of an anthelmintic to limit infection to 7 days with challenge infection given on day 14 post infection. The results show that one day after challenge, the number of mast cells present in the intestinal mucosa was very

low (due to the early termination of the infection), but 4 days after challenge infection, the number of mast cells increased considerably in comparison with the control group given only the challenge infection. This acceleration in the onset of the mast cell increase in a secondary infection could be due to a "memory" response as has been suggested by Mayrhofer (1979a) for the comparable response in rats infected with N. brasiliensis.

CHAPTER 4

SPECIFICITY OF THE INDUCTION AND EXPRESSION OF RAPID EXPULSION IN MICE

INTRODUCTION

It has been shown that inflammatory changes in the gut induced by one species of parasite can effect the expulsion, or growth of another species. Laboratory models which have been examined include concurrent infection with Trichinella spiralis and Nippostrongylus brasiliensis (Kennedy, 1979) and T. spiralis and Hymenolepis diminuta (Christie, Wakelin and Wilson, 1979; Behnke, Bland and Wakelin, 1977).

The restriction of rapid expulsion in mice to the short period of time following expulsion of a primary infection might imply that larvae were failing to establish because of residual non-specific inflammatory changes in the intestine. To test this, experiments were carried out to discover whether T. spiralis could establish in mice previously exposed to infection with N. brasiliensis and Nematospiroides dubius, and also to see whether these latter parasites and Trichuris muris could establish in mice previously exposed to T. spiralis. Both N. dubius and N. brasiliensis generate inflammatory changes in the intestine (Liu, 1965; Ogilvie and Jones, 1971). Since the time course of infection with the latter species (N. brasiliensis) in NIH mice is very similar to that of T. spiralis (personal observations), it may therefore act as a comparison for the residual effects of intestinal inflammation. N. dubius on the other hand, is long lived and produces chronic inflammatory changes.

MATERIALS AND METHODS

The strain of mice and the methods used for the maintenance of T. spiralis, method of infection, and worm recovery have been described in the general materials and methods. Extracts of T. spiralis (larval antigen) were kindly prepared by R.K. Grencis.

Nematospiroides dubius

This parasite was maintained as a stock infection in outbred CFLP male mice at the Wellcome Laboratories (Glasgow). Third stage infective larvae of N. dubius were obtained from faecal cultures according to Jenkins and Behnke (1977). Mice were orally infected with the required number of third stage larvae in 0.2 ml of distilled water. For irradiation of larvae the required number of larvae were irradiated at 25 Krad as described in the general materials and methods.

Worms were recovered by the Baermann technique (see the general materials and methods) but the incubation was increased to 4-6 hours and the temperature was raised to 45-50^{0}C for 30 minutes after the removal of the intestine from the beaker to prevent the worms from becoming entangled with one another.

Nippostrongylus brasiliensis

The parasite was maintaned as a stock infection

in Wistar rats at the Wellcome Laboratories (Glasgow). Third stage infective larvae were obtained from faecal cultures according to Jennings, Mulligan and Urquhart (1963). Mice were infected by subcutaneous injection of a suspension of larvae in 0.1 ml of balanced salt solution; worms were recovered by the Baermann technique.

Trichuris muris

The parasite was maintained as a stock infection in mice at the Wellcome Laboratories (Glasgow). The methods used for maintenance and infection of T. muris were based on the technique of Wakelin (1967) and the eggs were kindly prepared by T.D.G. Lee.

For worm recoveries experimental mice were killed and the caecum and colon removed and frozen at -20°C for at least 12 hours. For examination the organs were allowed to thaw to room temperature, placed in a petri dish of water, slit open, agitated to remove lumen contents, then transferred to another dish and scraped with curved forceps to disrupt the mucosa and allow the worms to be seen. The dishes were allowed to settle and examined under an X12 magnification dissecting microscope. Worms were removed using a Pasteur pipette.

Anthelmintics

Methyridine was used to remove T. spiralis as

described in the general materials and methods. Pyrantel/embonate (strongid-p-paste, Pfizer) at a dose of 100 mg/kg body weight was used to remove adult N. dubius from infected mice.

RESULTS

a. Effect of primary infections of N. dubius, N. brasiliensis or T. spiralis on the rapid expulsion of challenge infections of T. spiralis in NIH mice

Four groups of 10 NIH mice were used. Group 1 was given a primary infection of T. spiralis larvae, group 2 was infected subcutaneously with 600 N. brasiliensis larvae, group 3 was given two infections of N. brasiliensis (one infection with 600 larvae on day 0 and a second with 300 larvae on day 7) to ensure the presence of worms throughout the experimental period, and group 4 was infected with 300 N. dubius. Four mice from group 1 were killed on day 7 of a primary infection to examine worm establishment, when the mean worm recovery was 180.0±27.0. Normal worm establishment of both N. brasiliensis and N. dubius infection was checked by killing mice on day 7 for N. brasiliensis and on day 14 for N. dubius, mean worm recoveries were 105.8±20.0 and 286.8±55.0 respectively. The group infected with N. dubius was treated with pyrantel on days 10, 12 and 13 to remove adult worms.

All the four groups, together with a control group, were given a challenge infection of 300 T. spiralis on day 14 of the initial infection and were killed 24 hours later (Table 4.1). Only the mice given a primary infection

Table 4.1

Effect of a primary infection with *Nippostrongylus brasiliensis* or *Nematospiroides dubius* on subsequent challenge with *Trichinella spiralis* in NIH mice

Primary infection Day 0	No. of worms recovered 24 hours after challenge with 300 *T. spiralis* on day 14	
	Mean	S.D.
T. spiralis 320 larvae	2.3*	2.4
N. brasiliensis 600 larvae	112.5	73.0
N. brasiliensis 600 larvae + 300 larvae day 7	109.8	47.8
N. dubius 300 larvae[a]	137.6	27.5
None	136.0	6.6

[a] Anthelmintic used to remove worms before challenge

* Mean significantly lower than control

$p < 0.05$

of T. spiralis (group 1) showed rapid expulsion of the challenge infection.

b. Effect of primary infections with T. spiralis upon the establishment and survival of subsequent challenges with N. dubius, N. brasiliensis, T. muris or T. spiralis in NIH mice

In the reciprocal experiments, 48 mice were divided into 8 groups. Four of these were given a primary infection of 300 T. spiralis. On day 14, group 1a, together with a control group (1b), was challenged with 300 T. spiralis, group 2a, together with a control group (2b), with 300 N. dubius, group 3a, together with a control group (3b), with 500 N. brasiliensis and group 4a, together with a control group (4b), with 300 T. muris. Group 1a and its control (1b) were killed 24 hours after challenge; groups 2a and 4a and their controls (2b and 4b) were killed 10 days after challenge, to permit recovery of adult worms, and group 3a and its control (3b) 3 days after challenge, to allow the entry of larval N. brasiliensis into the intestine (Table 4.2).

The results showed that only T. spiralis was unable to establish and survive after previous infection with this species, both N. dubius and N. brasiliensis developed as normal primary infections, similar to the

Table 4.2

Effect of a primary infection with *T. spiralis* on a subsequent challenge with *T. spiralis*, *Nippostrongylus brasiliensis*, *Nematospiroides dubius* or *Trichuris muris* in NIH mice.

	Primary infection day 0	Challenge infection day 14		No. of worms recovered after challenge	
				Mean	S.D.
Group 1 a	*T. spiralis*	*T. spiralis*	300 larvae	2.4*	4.6
Group 1 b	none	*T. spiralis*	300 larvae	95.6	31.6
Group 2 a	*T. spiralis*	*N. brasiliensis*	500 larvae	103.0	31.7
Group 2 b	none	*N. brasiliensis*	500 larvae	146.6	46.3
Group 3 a	*T. spiralis*	*N. dubius*	300 larvae	248.0	58.2
Group 3 b	none	*N. dubius*	300 larvae	252.8	41.0
Group 4 a	*T. spiralis*	*T. muris*	300 larvae	53.4*	20.5
Group 4 b	none	*T. muris*	300 larvae	139.0	15.9

* Mean significantly lower than corresponding controls $p < 0.05$

group 1 killed 1 day after challenge ; group 2 killed 3 days after challenge

groups 3 and 4 killed 10 days after challenge

controls. The recovery of T. muris, however, was significantly reduced after previous infection with T. spiralis.

c. Effect of immunization with T. spiralis antigen and intestinal priming with N. dubius infection on subsequent challenge infection with T. spiralis

Bell and McGregor (1980b) have reported that the rapid expulsion response in rats could be produced after immunization with larval antigen and intestinal priming with N. dubius. Experiments were designed to test this in NIH mice and the experimental design is shown on the next page.

Mice were injected I.P. with 300 μg of larval antigen (Ag), one injection with and one without Freund's complete adjuvant (FCA). Five days after the last immunization, they were infected orally with 300 normal (group A) or irradiated (25 Krad) N. dubius larvae (group B - irradiation at this level affects the N. dubius in a manner which induces a strong immunity in mice). Control groups received either antigen alone (group C), or irradiated or normal N. dubius (groups D and F respectively). Group G was infected with 300 T. spiralis on day 0. All the groups were given anthelmintic (pyrantel - groups A-E; Mintic-groups F and G) on day 12 and challenged, together with a group of control mice (group F), with 300 T. spiralis larvae and killed 24 hours later.

Group*	Treatment		N. dubius	Anthelmintic	T. spiralis	Killed
	Ag+FCA day-10	Ag day-5	day 0	day 12	day 14	1 day later
A	+	+	normal larvae 300	+	+	+
B	+	+	irradiated larvae 300	+	+	+
C	+	+	-	+	+	+
D	-	-	irradiated larvae 300	+	+	+
E	-	-	normal larvae 300	+	+	+
F Control	-	-	-	+	+	+
G	-	-	T. spiralis 300	+	+	+
H	-	-	Ag+FCA day 0 Ag day 7		+	killed 7 days later
I Control	-	-	-		+	killed 7 days later

Ag = larval antigen FCA = Freund's complete adjuvant * six mice per group

To test the ability of antigen to immunize mice, additional groups of mice were given antigen with FCA on day 0 and a boost of antigen alone on day 7 and challenged, together with controls, on day 14 with 300 T. spiralis larvae. All these mice were killed 7 days after challenge (groups H and I).

The mean worm recoveries 1 day after challenge were: group A - 140.0±25.0; group B - 140.8±16.4; group C - 150.5±20.6; group D - 153.8±31.2; group E - 150.6±18.5; group F - 153.8±28.0 and group G - 0.6±0.5. The results show that rapid expulsion occurred only in mice given a primary infection of T. spiralis larvae 14 days before challenge infection (group G). None of the other groups showed rapid expulsion.

The mean worm recoveries 7 days after challenge from groups H and I which were immunized with antigen 14 days before the challenge infection, were 106.0±31.6 and 245.7±12.0 respectively, indicating that the mice were substantially immune and that worm expulsion had begun in immunized mice.

DISCUSSION

The results presented here show that priming provided by N. dubius or by N. brasiliensis failed to induce a rapid expulsion response against a challenge infection of T. spiralis and that the rapid expulsion of a T. spiralis challenge occurred only when the priming infection was T. spiralis. In reciprocal experiments it was found that a primary infection with T. spiralis did not induce rapid expulsion of heterologous challenge infections of N. dubius and N. brasiliensis, although worm recoveries from mice given a heterologous challenge of T. muris were considerably lower than controls.

It has been shown with several systems that inflammatory changes in the gut induced by one species, result in an acceleration of worm expulsion or have an effect on the growth and survival of other species which are present concurrently (see introduction of this chapter).

It should be remembered that in most of these studies, either two species were present concurrently or challenge infections of the second species were given when gross inflammatory changes were present in the gut. However in the experiments designed here, the homologous or heterologous challenge infections were given when the primary infection had been expelled and the gross inflammatory changes had disappeared and it was

remarkable that the rapid expulsion response was specific to T. spiralis after priming with this species. This indicates that if rapid expulsion is dependent on residual inflammatory changes in the local intestinal environment, these changes could well be different from those produced by heterologous infections and are quite specific to T. spiralis. However, the failure to induce rapid expulsion with N. dubius may be explained by the immunosuppression of host induced by this parasite. Behnke, Wakelin, Wilson (1978) have reported that infection with N. dubius inhibits the expulsion of T. spiralis from mice and Hagan (1980) has proposed that the immunosuppression caused by infection with N. dubius may be due to an effect of the parasite on lymphocyte responsiveness/effectiveness. It is possible that infection with N. dubius interferes with the inflammatory response or the inflammatory mediators associated with T. spiralis infection.

Interestingly, a challenge infection with T. muris established less successfully, or perhaps established and was partially expelled when given on day 14 after T. spiralis primary infection. It is possible that the expulsion of T. muris resulted from a residual inflammatory change provided by T. spiralis or that there is immunologically specific cross reaction between the two species. In fact, Lee, Grencis and Wakelin (1982) have found antigenic similarities between two species and they concluded that the expulsion of a

heterologous challenge infection is the result of a specific response to shared antigens. With this in mind, if the rapid expulsion of T. muris resulted from changes induced by T. spiralis these changes must have affected T. muris in a specific manner, as they were without effect on the other species tested.

It is unlikely, for two reasons, that priming with T. muris would provide the local intestinal changes which appear to be necessary for the rapid expulsion response and which would operate against a T. spiralis challenge infection, since T. muris is a parasite of the large intestine and does not appear to elicit gross inflammatory responses.

In rats, Bell and McGregor (1980a and b) have suggested that rapid expulsion of T. spiralis is dependent on the specific and non-specific components of the immune response. For example in parabiotic rats, it has been shown that the rapid expulsion response in one partner required intestinal stimulation (e.g. by N. dubius), but also, additional immunologically specific stimulatory factors which can be transferred systemically to that partner when the other rat is infected with T. spiralis. Further experiments by these authors, in normal rats, have indicated that the stimulus for rapid expulsion can be provided by the injection of larval T. spiralis antigen coupled with the local changes induced by N. dubius

infection. In the present work, attempts were made to induce rapid expulsion by injecting larval antigen and by priming the intestine with normal or irradiated (25 Krad) N. dubius larvae. Neither normal nor irradiated larvae with or without antigen, induced rapid expulsion in mice. One reason may be incorrect timing of the injection of the antigen to stimulate immune response, or perhaps there was immunosuppression induced by N. dubius (see above). The success of Bell and McGregor (loc. cit.) in using this protocol of immunization in rats for the induction of rapid expulsion, may be due to the fact that N. dubius may not cause the same degree of immunosuppression in rats as it does in mice, but may also reflect the fact, now well established, that there are profound differences in response to T. spiralis between rats and mice.

CHAPTER 5

THE MECHANISMS OF RAPID EXPULSION AND THE EFFECTS OF ADOPTIVELY TRANSFERRED IMMUNE MESENTERIC LYMPH NODE CELLS (MNLC) ON RAPID EXPULSION AND ON THE MAST CELL RESPONSE TO INFECTION

PREFACE

In the previous chapters the basic parameters of rapid expulsion were examined. A series of experiments was then carried out to gain further information about the process itself and to investigate the ways in which it might be brought about. The results of these experiments are presented in this chapter under two main headings:

A: The mechanisms underlying rapid expulsion.

B: The effects of adoptively transferred MLNC on rapid expulsion and on the mast cell response to infection.

A. MECHANISMS OF RAPID EXPULSION

INTRODUCTION

Russell and Castro (1979) have shown that in immune rats T. spiralis is expelled within the first 15 minutes post challenge (p.c.) They concluded from this that the parasites were prevented from establishing in the intestinal mucosa. In more recent work Castro, Hessel and Whalen (1979) and Bullick, Frizzell and Castro (1981) have suggested that this results from an active response by the host upon challenge infection. Lee and Ogilvie (1981) found, however, that in immune rats, challenge infections were in fact trapped in the intestinal mucus layer. A similar picture in the N. brasiliensis/rat model has been described by Miller, Huntley and Wallace (1981). Thus there is controversy as to whether rapid expulsion is a passive process or one involving an actively induced secondary response. If the latter is true, then there should be measurable immunological and physiological changes in the intestine and these may well result from the release of mediator factors. The rapid expulsion of repeated challenge infections has been demonstrated by Bell and McGregor (1979a) and this suggests that if there are mediators of rapid expulsion, they are not readily exhausted.

In investigating the mechanisms of rapid expulsion in the mouse, it was considered necessary to see whether measurable changes occurred in the host as a result of challenge, i.e. whether there was an active secondary response.

It has been shown that there are changes both at a cellular and physico-chemical level in the intestinal mucosa during a primary infection of T. spiralis. One of the earliest measurable changes is an enhancement of the basic ability of lymphoblasts (both TDL, i.e. thoracic duct lymphocytes and MLNC) to localise preferentially in the lamina propria of the small intestine. In the T. spiralis/NIH model lymphoblast localisation is known to reach a peak at 2-4 days p.i. This localisation is thought to occur predominantly at the most heavily parasitised area of the mucosa, the major cell type being T. lymphoblasts (see Manson-Smith, Bruce, Rose and Parrott, 1979b; Rose, Parrott and Bruce, 1976). A later change, which is also a useful index of host stimulation by infection, is the appearance of dividing lymphoid cells in the draining MLN. Therefore, in this chapter experiments were designed to examine:-

1) whether a challenge infection given on day 14 can establish in the intestinal mucosa of immune mice. This was accomplished by determining the relative worm numbers in the

lumen and in the intestinal mucosa.

2) The ability of a challenge infection given on day 14 to influence MLN lymphoblast homing. As changes in homing result from the presence of worms in the mucosa, this parameter was used to measure the success of the challenge in achieving normal establishment.

3) The stimulation of MLN cells, after challenge infection, by measuring blast cell activity in vitro.

4) The fate of repeated challenge infections given at various times to determine whether exhaustible mediators were involved.

MATERIALS AND METHODS

The strain of mice and the methods used for maintenance, infection and worm recovery have been described in the general materials and methods.

1. Fecundity

Female worms were recovered by the standard Baermann technique (see general materials and methods), but incubation was limited to 45-60 minutes. Fecundity was determined *in vitro* by culturing groups of fifty worms (twenty-five worms from each beaker) in conical flasks in medium containing 8 ml of HBSS and 2 ml of heat inactivated foetal calf serum (FCS, Gibco, Europe). The flasks were incubated for 4 hours at 37°C. After incubation, the female worms were removed by filtration, the culture fluid centrifuged and the newborn larvae resuspended in a volume of 1 ml of HBSS. Four samples each of 0.05 ml were then placed on a microscopic slide, examined microscopically and the numbers of newborn larvae were counted. The results are expressed as the number of larvae released per female worm, per hour (larvae/♀/h).

2. Measurement of adult worms

Adult worms were recovered by the Baermann technique, placed in tubes containing HBSS and stored

overnight at 4°C for immobilization. The worms were then measured from Camera Lucida drawings using at least forty female worms from each experimental group. The results are expressed as the mean length of the worms in mm.

3. Irradiation

Groups of up to 4 mice confined in a flat Perspex box, were exposed to gamma irradiation from a 500 Ci ^{60}Cobalt source for the appropriate length of time (dose rate 1.05 Krad/minute). Irradiated mice were given antibiotic (Terramycin, Pfizer) in their drinking water (see the general materials and methods).

N.B. 1 Krad = 10 Gray

4. Immunosuppression

Cortisone acetate (Cortistab, The Boots Company Ltd.) given subcutaneously, was used at a dose of 100 mg/kg body weight.

Cyclophosphamide (Endoxana, W.B. Pharmaceuticals Ltd.) was given intraperitoneally at 300 mg/kg body weight and reserpine (BDH) was given by intraperitoneal injection at a dose of 1 mg/kg body weight.

5. Preparation and transfer of cell suspensions

a. MLN cells

Donor mice were killed in chloroform and the

mesenteric lymph nodes were stripped of connective tissue and fat in situ and then excised and placed into a tube containing HBSS on ice.

Cell suspensions were prepared by finely dicing the nodes with scissors and squeezing them with a rubber tipped syringe plunger through a fine mesh nylon sieve into a Petri dish containing medium 199 (M.199, Gibco, Europe). The Petri dish was raised at one side and left to stand for 4-5 minutes. This allowed cell clumps and debris to sink to the bottom of the dish. The supernatant was collected and centrifuged at 200 g for 5 minutes and the cells were resuspended in a known volume of fresh medium 199 containing inactivated foetal calf serum (FCS, Gibco, Europe) and 10 I.U./ml heparin (B.D.H.). One drop of this suspension was mixed (1 in 20) with 0.1% solution of Trypan blue in HBSS and the number of live (i.e. dye excluding) cells counted in a haemocytometer. Viability was usually greater than 90%. The concentration of cells in the suspension was then adjusted to give the required number in 0.2-0.25 ml. Cells were injected intravenously (I/V) into recipient mice via a lateral tail vein.

b. Bone-marrow cells

Mice were killed and skinned and the back legs removed. All the muscles were trimmed from the bone. The cells were prepared by flushing the long bones of

the limbs with HBSS and then proceeding as described above.

6. Cell separation

Cell suspensions and medium M.199 with FCS were prepared as decribed above.

Separation into non-adherent (T cell enriched) and adherent (B cell enriched) fractions was carried out by passing the cells through sterile nylon wool columns. Nylon wool columns were prepared as described by Julius, Simpson and Herzenberg (1973). Briefly 30 ml disposable syringes (Plasti Pak) were packed with 2.5 gram of scrubbed nylon fibres (Fenwal Laboratories, Illinois). The syringes were then filled with 25 ml HBSS and autoclaved before use. The columns were washed through with HBSS containing 5% FCS and 10 I.U. heparin/ml. The columns were then filled completely with this medium, sealed with parafilm and pre-incubated at 37°C for $1\frac{1}{2}$ hours. The cell suspension was filtered through a glass wool column to remove debris and dead cells, centrifuged for 5 minutes at 200 g and resuspended in approximately 10 ml of the above medium. A suspension of no more than $5x10^8$ cell was then carefully layered into the nylon wool columns, which were then sealed with parafilm and incubated for 45-60 minutes at 37°C. After incubation the non-adherent cells (T cell-enriched population) were washed off the columns with approximately 50 ml of warm medium.

This suspension was centrifuged for 5 minutes at 200 g and resuspended in fresh medium.

The adherent cell population (B cell enriched) was removed by the technique of Handwerger and Schwartz (1974) in which the nylon wool was removed from the syringe into a beaker and teased apart with forceps in cold medium. The nylon wool was removed and cells washed in fresh medium. The concentration of cells in the suspension was then adjusted to give the required numbers in 0.2-0.25 ml. This technique allowed the recovery of approximately 50% of the cells applied to the columns, with a ratio of nonadherent:adherent cell populations of 2:1.

7. Fluorescent labelling of Ig bearing cells

Samples of $1x10^6$ cells were washed twice in HBSS and resuspended in .9 drops of HBSS to which was added 1 drop of 0.1% sodium azide and 1 drop of a 1:5. (V/V) dilution (HBSS) of fluorescein conjugated goat anti-mouse immunoglobulin antiserum (Nordic Immunology, London). After 30 minutes incubation at 4^oC in the dark, the cells were washed twice in HBSS and resuspended in 3 drops of 50% glycerine in HBSS. One drop of the suspension was placed on a glass slide and covered with a coverslip for examination. The labelled cells were examined with a Leitz Ortholux fluorescence microscope fitted with a Ploem incident light fluorescent system.

Cells were examined under white light to determine total numbers and then under ultraviolet light to detect fluorescing cells. Cells exhibiting crescent, ringed or speckled fluorescence were considered positive. Suspensions of MLNC normally contained 50-60% Ig bearing cells.

8. Anti-Thy 1.2 treatment

Anti-Thy 1.2 monoclonol antibody (Clone F7D5) was obtained from Olac Ltd. Guinea pig complement was obtained from Grand Island Biological Company. Suspensions of washed cells (enriched B cell population) at a concentration of $1x10^7$ cell per ml were incubated for 30 minutes at room temperature with an equal volume of antiserum at a dilution of 1:1000 (V/V) in medium 199. The cells were then spun down and resuspended in a 1:10 dilution (V/V) of unabsorbed guinea pig complement in medium 199 at a concentration of $1x10^7$ cell/ml and incubated for 45 minutes at 37°C. Following incubation the cells were washed twice in medium 199 before use. Following treatment with Anti-Thy 1.2 serum the number of Ig bearing cells in the B cell enriched population was 80-90%.

9. Trypan blue (cell viability)

The stock solution consisted of 2 g Trypan blue

(BDH) made up to 100 ml with deionized water. This was diluted 1:20 (V/V) in HBSS for use in the dye exclusion test.

10. Cell labelling for in vivo injection

Lymphocytes were prepared from the MLN as described above except that the medium used was prepared by taking 100 ml RPMI 1640 (without glutamine or bicarbonate, Flow Laboratories) and adding 0.8 ml of 7.5% sodium bicarbonate (Flow Laboratories), 1.0 ml of 200 mm L-glutamine (Flow Laboratories), 5 ml of heat inactivated FCS and 16 mg Crystamycin (6 mg penicillin and 10 mg streptomycin, Glaxo Ltd., Greenford). One ml of medium containing $5x10^7$ cells was then added to 4 ml aliquots of medium containing 185 k/Bq of 5-[^{125}I] Iodo-2'-deoxyuridine in 0.9% NaCL(specific activity 185G Bq/mg, Radiochemicals Center, Amersham), giving a concentration of 37 k/Bq isotope to $1x10^7$ cells/ml. The cultures were incubated for 2 hours at $37^{o}\pm0.5$C and cell suspensions were harvested, centrifuged at 200 g for 5 minutes at room temperature and after removal of the supernatant, washed 3 times and resuspended in medium 199 without FCS and heparin. Cells were counted, assessed for viability and $2.3x10^7$ viable cells were injected (I/V) into recipient mice. An injected dose equivalent was retained for counting.

11. Counting of activity after in vivo injection

Recipient mice were killed 24 hours after injection of labelled cells and the small intestine, MLN, spleen, liver and large intestine were removed, rinsed in HBSS and placed in plastic vials for counting. The organs together with the injected dose equivalent and two empty vials for background, were counted in a Packard Tricarb Scintillation spectrometer. The results are presented as % injected dose (%ID) or % total recovered radioactivity (%TR) as shown below:

$$\%ID = \frac{\text{ORGAN COUNT-BACKGROUND COUNT}}{\text{INJECTED DOSE COUNT-BACKGROUND COUNT}} \times \frac{100}{1}$$

$$\%TR = \frac{\text{ORGAN COUNT-BACKGROUND COUNT}}{\text{TOTAL COUNT (ALL ORGANS)- 5xBACKGROUND COUNT}} \times \frac{100}{1}$$

12. Cell labelling for in vitro assays

In order to asses blast cell activity *in vitro* lymphocytes and medium were prepared as described above. Suspensions of MLNC from a group of mice were pooled and triplicated samples of $2x10^7$ cells were placed in tubes and labelled with isotope. The methods used for labelling and counting were as described above. Results are expressed as counts per minute. for each tube (C.P.M.).

RESULTS

1. The establishment of a challenge infection in immune mice

Attention was focussed on the rapidity of expulsion of a challenge infection given shortly after primary infection (see chapter 1) and it was therefore decided to re-examine the establishment of a challenge infection given 14 days after a primary infection.

In this experiment, six 7-week-old male NIH mice were infected with 300 T. spiralis larvae on day 0. Fourteen days later these mice, together with six non-infected, age-matched controls, were challenged with 300 T. spiralis larvae. Both groups were killed 2-3 hours after infection and the entire small intestines, removed and divided into anterior and posterior halves. The intestinal contents (washings) were recovered by flushing the intestine with 10 ml cold HBSS using a 10 ml syringe fitted with a blunted 21 g needle. The washings from each half were collected separately in 50 ml beakers and worm recoveries noted. Washed intestines were then processed using the Baermann technique (see general materials and methods) for estimating the number of worms remaining in the intestine.

A comparison of the total worm recoveries (shown in Table 5.1) indicated that the majority of the larvae were expelled from immune mice within 2-3 hours

post challenge. In the immune mice the number of worms recovered from the washings was more than twice that recovered by the Baermann technique. In contrast, the majority of the worms in control mice were recovered by the Baermann technique, with very few worms being recovered in the washings. It was also noted that the majority of the worms recovered in the washings from the immune mice, were from the posterior half of the small intestine. However, the majority of the worms recovered by the Baermann technique from the control mice were located in the anterior half of the small intestine. Thus the reversal of normal worm distribution found in immune mice indicated that most of the larvae had moved to the posterior half and this was emphasised by even more larvae being recovered by the Baermann technique from this segment of the small intestine.

2. <u>The effect of a challenge infection on lymphocyte migration patterns</u>

As mentioned in the introduction, the enhanced localisation of MLN cells in NIH mice infected with <u>T. spiralis</u> is related to the presence of the parasite in the intestine, the accumulation of blast cells being much greater in the region of the small intestine which the parasites are located (Manson-Smith <u>et al</u>., 1979b).

Table 5.1

Number of Trichinella spiralis recovered from anterior and posterior regions of the small intestine (by Baermann technique and by washing out the intestine 2-3 hours after challenge) from NIH mice given a challenge infection on day 14 of a primary infection.

	Number of worms												
	Washings						Baermann						
Group of mice	Anterior Mean	Anterior S.D.	Posterior Mean	Posterior S.D.	Total Mean	Total S.D.	Anterior Mean	Anterior S.D.	Posterior Mean	Posterior S.D.	Total Mean	Total S.D.	Total by both techniques Mean
Immune	6.6	3.2	32.4	28.1	39	31.2	4.4	3.3	13.0	11.2	17.4	14.5	56.4
Control	2.4	2.5	4.6	4.5	7	6.9	152.2	36.4	3.4	3.0	155.6	36.2	162.6

The MLN donors used in the following experiments were NIH mice which had been infected for 4 days with approximately 300 T. spiralis larvae. It has been shown that large numbers of blast cells can be recovered from the MLN at this time (Grencis, 1981) thus providing a cell population which will take up a sufficient amount of radiolabel to give substantial counts after injection into recipients. An initial experiment was carried out in which labelled cells were transferred to recipient mice which received different infection regimes.

Sixteen 7-week-old male NIH mice were divided into four groups of four mice each (A, B, C and D). Two groups (B and D) were infected with 300 T. spiralis on day 0. On day 14 after primary infection, the mice of group D together with control uninfected mice (group C) were challenged with 300 T. spiralis; group B mice were not given a challenge infection and group A mice were kept as control uninfected mice (naive). All the mice were given $2x10^7$ labelled immune cells 6 hours after administration of challenge infection and were killed 24 hours later. The organs (small intestine, MLN, spleen, liver and large intestine) were removed, placed in scintillation vials and the activity counted on a Packard spectrometer (see materials and methods). The results in terms of percentage (%) injected dose of isotope recovered and % of total recovery per organ,

are shown in Fig. 5.1 and 5.2, respectively. The % recoveries of injected isotope from the small intestine and the other organs in mice infected for 14 days (group B) were similar to those of control uninfected mice (group A). Furthermore, the recovery from the small intestine and other organs in the group of mice given a challenge infection on day 14 (group D) was similar to that of challenge control mice (group C). The values for the percentage of total isotope recovered from each organ, Fig. 5.2, shows that there was a similar distribution of labelled cells in each of five organs between all groups.

In the previous experiment, it was shown that there was no enhancement of lymphocyte migration to the intestine and other organs of infected mice given labelled MLN cells 6 hours after the challenge infection (day 14) and assessed 24 hours later. However, it has been reported that peak lymphocyte localisation in the small intestine occurs between days 2 and 4 after primary infection, when assessed 24 hours later, see Manson-Smith et al. (1979b). Therefore, in accordance with this finding, it was decided to study lymphocyte localisation 3 days after challenge as assessed 24 hours later.

Sixteen 7-week-old male NIH mice were divided into four groups of four mice each (A, B, C and D). Two

Fig. 5.1

Lymphocyte migration patterns in T. spiralis infected mice. Data expressed as percentage recovery of the injected dose of ^{125}IUdR.

All mice received labelled cells. Groups B and D were infected 14 days previously and groups C and D were given challenge infections (day 14) 6 hours before the cell transfer (Note: this is a secondary infection for group D). Mice were killed 24 hours after cell transfer.

Recipients	Group
Uninfected control	A
Primary infection day 14	B
Primary infection day 1	C
Challenged day 14	D

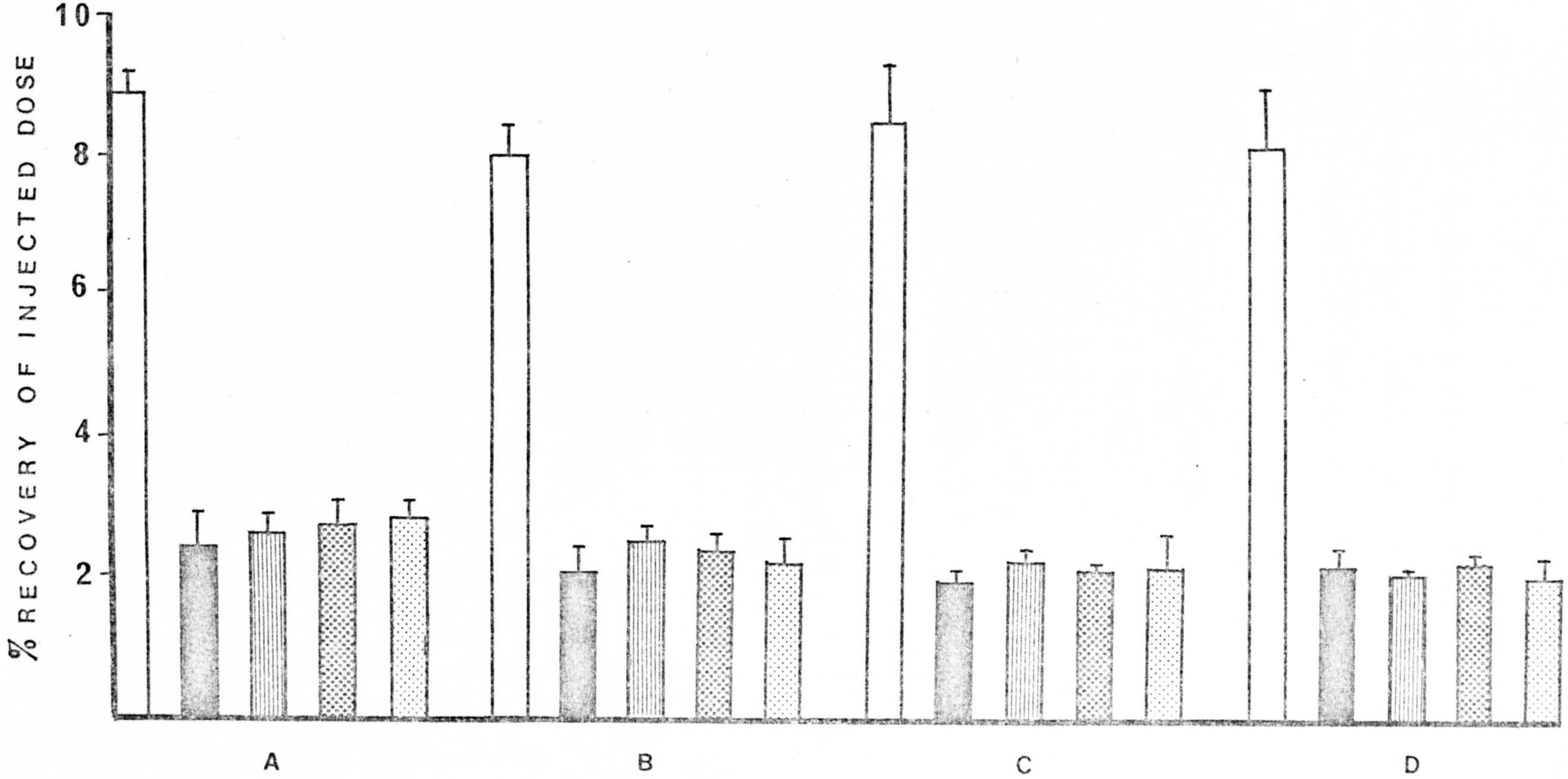
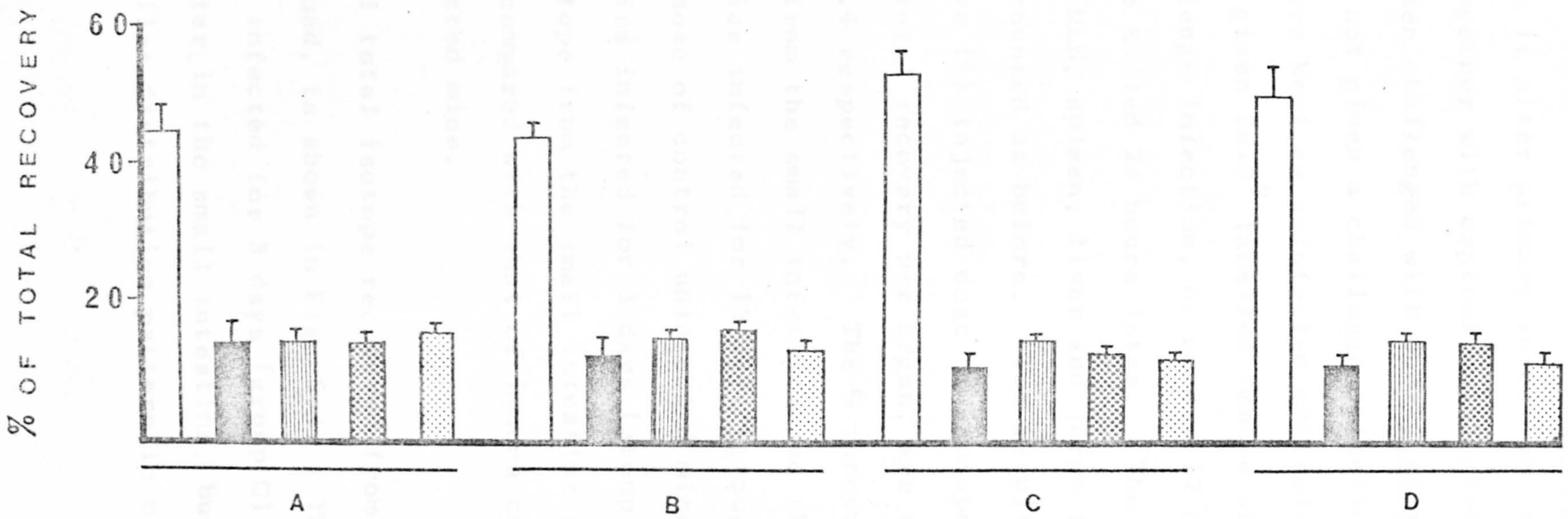

% RECOVERY OF INJECTED DOSE
10
8
6
4
2
A
B
C
D

Fig. 5.2

Lymphocyte migration patterns in T. spiralis infected mice. Data expressed as percentage of total recovery of ^{125}IUdR.

All mice received labelled cells. Groups B and D were infected 14 days previously and Groups C and D were given challenge infections (day 14) 6 hours before cell transfer (Note: this is a secondary infection for group D). Mice were killed 24 hours after cell transfer.

Recipients	Group	small intestine
Uninfected control	A	MLN
Primary infection day 14	B	spleen
Primary infection day 1	C	liver
Challenged day 14	D	large intestine


% OF TOTAL RECOVERY
60
40
20
A
B
C
D

groups (B and D) were infected with 300 T. spiralis on day 0. On day 14 after primary infection, the mice of group D together with control uninfected mice (group C), were then challenged with 300 T. spiralis. Group B mice were not given a challenge infection and mice in group A were kept as uninfected controls.

All the mice were given $2x10^7$ labelled immune cells 3 days after challenge infection, or on day 17 of primary infection, and were killed 24 hours later. The organs (small intestine, MLN, spleen, liver and large intestine) were removed and counted as before. The results in terms of percentage (%) injected dose of isotope recovered and % of total recovery per organ, are shown in Fig. 5.3 and 5.4 respectively. The % recoveries of injected isotope from the small intestine and the other organs in mice infected for 17 days (group B), were similar to those of control uninfected mice (group A). In mice infected for 3 days (group C), the % recovery of isotope from the small intestine showed an increase when compared with that of immune challenged, or control uninfected mice.

The % of total isotope recovered from each of five organs examined, is shown in Fig. 5.4. The results show that in mice infected for 3 days (group C), the % recovery was greater in the small intestine, but all groups show a similar distribution pattern in other organs.

Fig. 5.3

Lymphocyte migration patterns in T. spiralis infected mice. Data expressed as percentage recovery of the injected dose of ^{125}IUdR.

All mice received labelled cells. Groups B and D were infected 14 days previously and groups C and D were given challenge infections (day 14). Cells were injected 3 days later (Note: this is a secondary infection for group D). Mice were killed 24 hours after cell transfer.

Recipients	Group
Uninfected control	A
Primary infection day 17	B
Primary infection day 3	C
Challenged day 14	D

small intestine
MLN
spleen
liver
large intestine

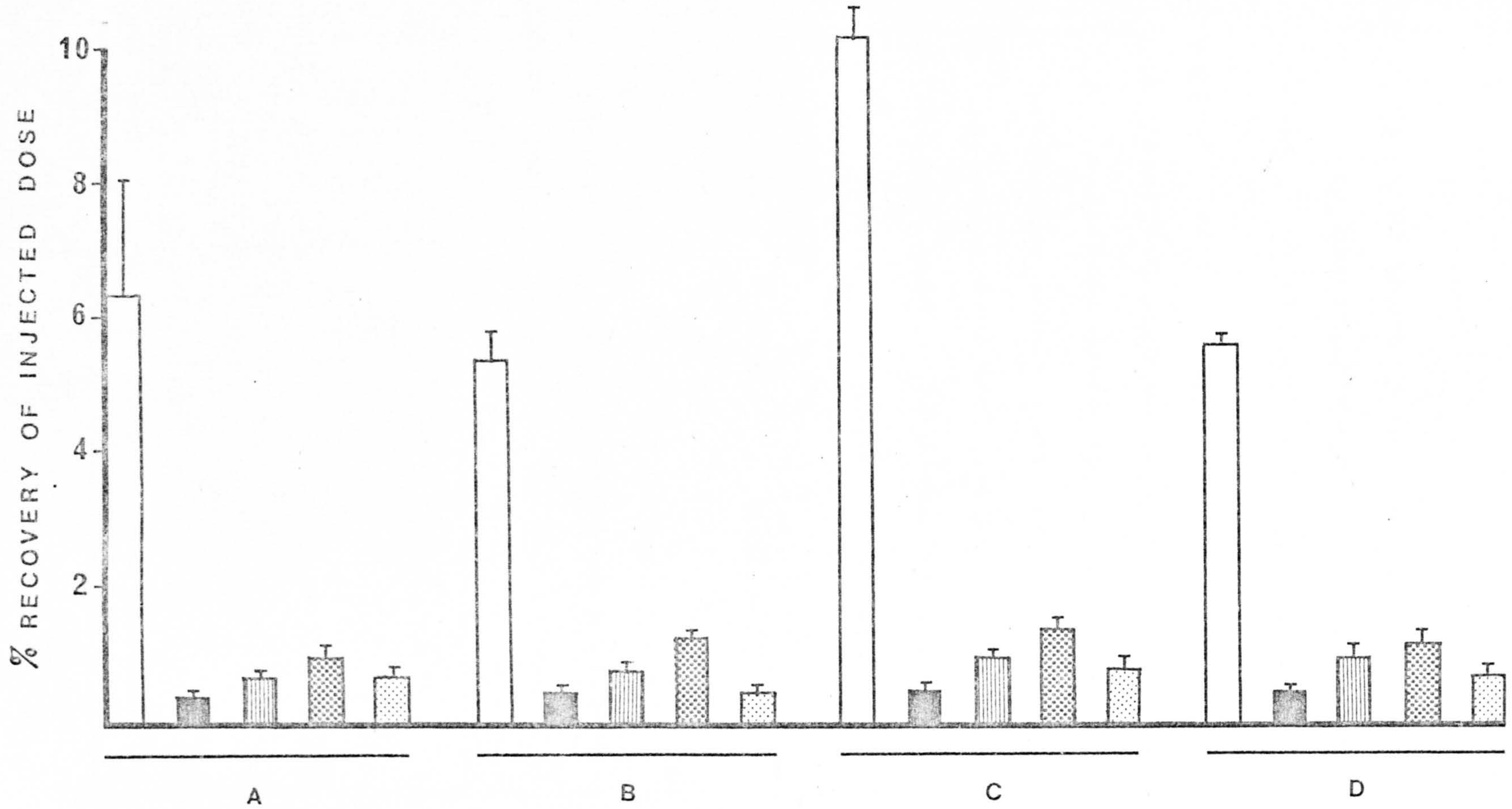
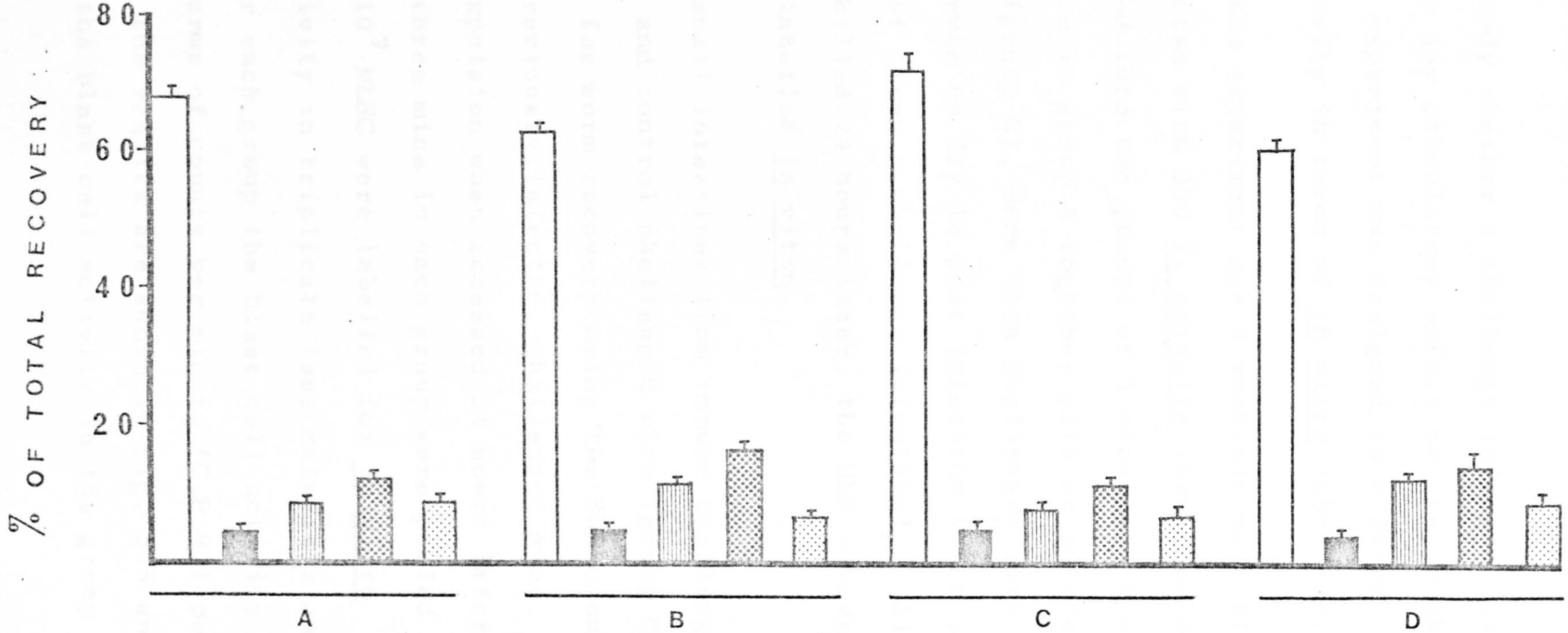

% RECOVERY OF INJECTED DOSE
10
8
6
4
2
A
B
C
D

Fig. 5.4

Lymphocyte migration patterns in T. spiralis infected mice. Data expressed as percentage of total recovery of ^{125}IUdR.

All mice received labelled cells. Groups B and D were infected 14 days previously and groups C and D were given challenge infections (day 14). Cells were injected 3 days later (Note: this is a secondary infection for group D). Mice were killed 24 hours after cell transfer.

Recipients	Group		
Uninfected control	A	MLN	
Primary infection day 17	B	spleen	
Primary infection day 3	C	liver	
Challenged day 14	D	large intestine	
		small intestine	


% OF TOTAL RECOVERY
80
60
40
20
A
B
C
D

3. Stimulation of MLN cells after challenge infection

To study whether a challenge infection given on day 14 p.i. has any stimulatory effect on the lymphocytes in the MLN, an experiment was designed to examine lymphocyte activity in terms of *in vitro* labelling.

In this experiment six 7-week-old male NIH mice were infected with 300 *T. spiralis* larvae on day 0 and were divided into two groups of 3 mice each (groups A and B). Mice in group A together with an uninfected control group (group C), were then challenged with 300 *T. spiralis* larvae on day 14 post infection (mice in group B were not given a challenge infection). All the mice were killed 24 hours later, the MLN removed and MLN cells labelled *in vitro*.

The small intestines from immune challenged mice (group A) and control challenged mice (group C) were incubated for worm recovery using the Baermann technique. Previously infected, challenged mice showed rapid expulsion when assessed 24 hours later. The MLNC from three mice in each group were pooled and cultures of $2x10^7$ MLNC were labelled for *in vitro* blast cell activity in triplicate (see materials and methods). For each group the blast cell activity was expressed in terms of counts per minute (C.P.M.) per $2x10^7$ cells. The results are shown in Fig. 5.5 and indicate that the blast cell activity in the group of

Fig. 5.5

In vitro blast cell activity of MLN from T. spiralis infected mice. Data expressed as ^{125}IUdR counts per minute for each tube. Cells were taken (i) 24 hours after a challenge infection given on day 14 of a primary infection (group A), (ii) 15 days after a primary infection (group B) and (iii) 24 hours after challenge of a control group given no primary infection (group C).

Group

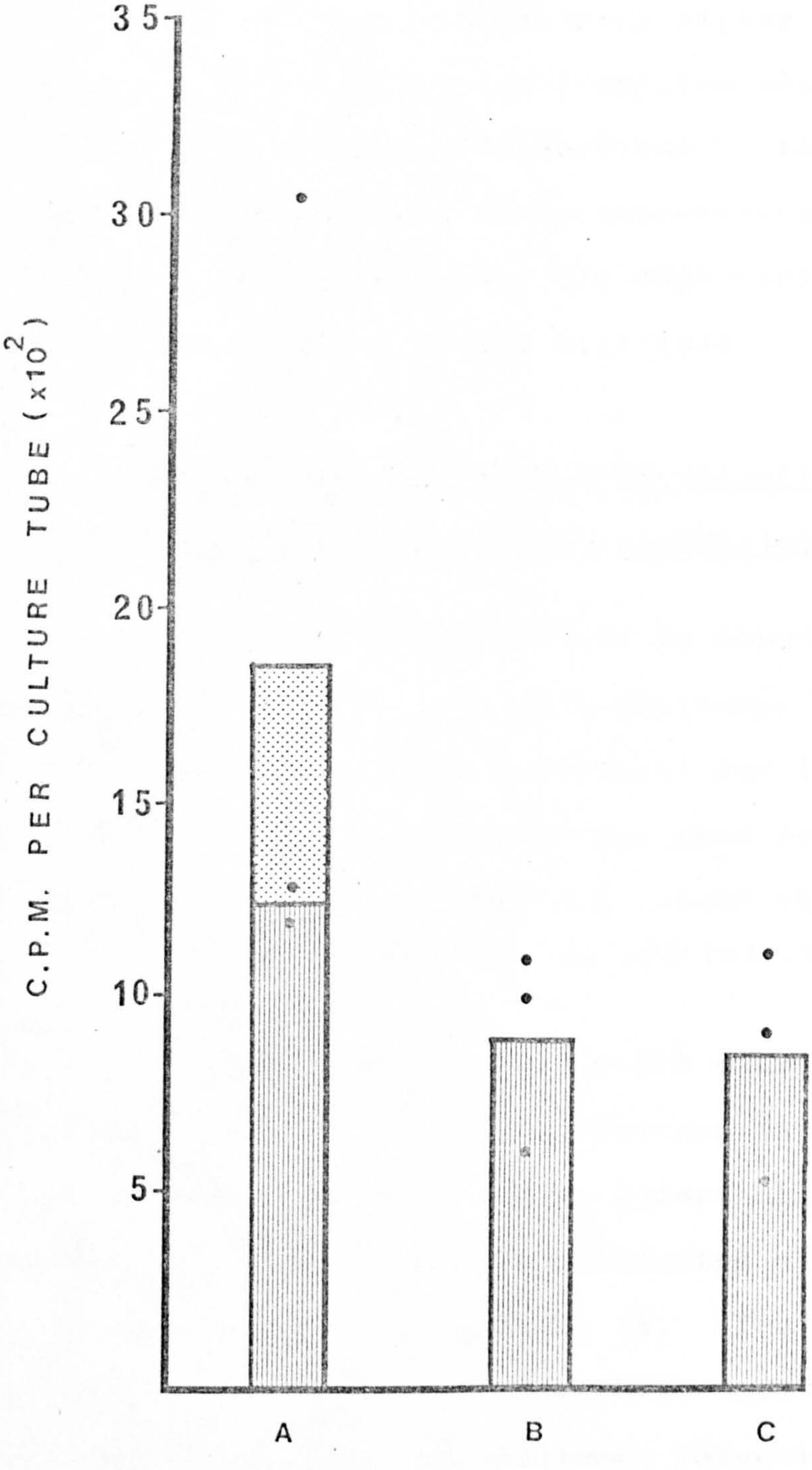
A infected day 0, challenged day 14

B infected day 0, no challenge

C uninfected, challenged day 14

Hatched bars = mean values of 3 cultures for groups B and C, or 2 cultures for group A.

Stippled bar = mean value of 3 cultures for group A.


C.P.M. PER CULTURE TUBE ($\times 10^2$)
35
30
25
20
15
10
5
A
B
C

mice infected for 15 days (group B) was similar to that of control challenged mice (group C). In the immune challenged mice (group A) one of the three cultures gave a count considerably higher than the other two. The mean of the lower two was still higher than the mean of groups B and C, this probably reflects a true difference between group A and groups B and C, although the high variance in group A makes statistical analysis difficult.

4. <u>The fate of repeated challenge infections given at various times after initial challenge</u>

In NIH mice the ability to mount the response leading to rapid expulsion of a challenge infection was short lived and persisted only until day 16 p.i. (see chapter 1). Experiments were designed to examine the capacity of mice to respond to a second challenge infection given at various times after an initial challenge infection given on day 14.

Forty eight 7-week-old NIH mice were given a primary infection on day 0 and divided into eight groups of six mice each. Four groups of these mice, together with four groups of uninfected mice were challenged on days 14, 15, 16 or 18. The four remaining infected groups were challenged on day 14 and were also given a second challenge infection on days 15, 16, 18 or 21 (1, 2, 4 and 7 days after the initial

challenge). All the primary and challenge infections were with approximately 300 T. spiralis larvae and all the mice were killed 24 hours later. The results are shown in Table 5.2. Rapid expulsion was expressed until day 16 after primary infection. A second challenge infection given 1 or 2 days after the initial challenge on day 14 was also expelled rapidly, but a challenge infection given 4 days after the initial challenge was only partially expelled. Mice given a second challenge infection on day 21 (7 days after initial challenge) did not show rapid expulsion.

Table 5.2

Rapid expulsion of repeated challenge infections given at various times after the initial challenge

Group	Initial challenge day 14	Day of second challenge after the initial challenge				Worm recoveries 24 hours later	
		1	2	4	7	Mean	S.D.
1 Immune	+	-	-	-	-	17.7*	12.6
2 Control	+	-	-	-	-	157.2	7.7
3 Immune	+	+	-	-	-	3.8*	2.5
4 Control	none	+	-	-	-	132.8	32.6
5 Immune	none	+	-	-	-	14.5*	10.5
6 Immune	+	-	+	-	-	2.2*	2.6
7 Control	none	-	+	-	-	140.8	22.7
8 Immune	none	-	+	-	-	31.2*	25.4
9 Immune	+	-	-	+	-	60.5*	34.3
10 Control	none	-	-	+	-	148.0	25.2
11 Immune	none	-	-	+	-	141.1	27.3
12 Immune	+	-	-	-	+	137.1	24.5
13 Control	none	-	-	-	+	139.4	19.9

*Means significantly lower than the corresponding controls $p < 0.05$

B. THE EFFECTS OF ADOPTIVELY TRANSFERRED MLNC ON RAPID EXPULSION AND ON THE MAST CELL RESPONSE TO INFECTION

The immune response elicited by infection with intestinal nematodes may confer protection upon the host by interfering with the growth or reproduction of the worms and most importantly, may cause the expulsion of worms from the intestine (Wakelin, 1978a). The immunological mechanisms which are involved in these events and the components of these mechanisms have been studied for many years.

It has been shown in a variety of host-parasite systems, that both antiworm antibodies and sensitised lymphocytes can be involved in worm expulsion, although the relative importance of each varies between systems. Evidence that other components may also be involved, has come from experiments in which the ability of the host to respond has been destroyed by irradiation and then restored by transferring, singly or in combination, serum, sensitised lymphocytes and bone marrow cells. For example, antiworm antibodies are known to be involved in the expulsion of N. brasiliensis from rats. Sensitised lymphocytes are also necessary and their action may require co-operation with non-lymphoid, bone marrow-derived cells, although this is controversial (Dineen and Kelly, 1973; Ogilvie, Love, Jarra and Brown, 1977).

In the case of T. spiralis, antiworm antibodies appear to be of less importance and sensitised lymphocytes are thought to be the major immunological component in worm expulsion. Wakelin and Wilson (1977b) suggested that the lymphocytes did not act directly on the worm, but co-operated with some other component of the host response in order to bring about expulsion. Evidence was presented to show that this other component was non-lymphoid and bone marrow derived (see Wakelin and Wilson, 1977a and b).

The lymphocyte component of worm expulsion can be transferred by taking cells from either the MLN or TDL and injecting these into recipient animals. The success of transfer is then measured by an accelerated expulsion of worms. Experiments of this kind have been carried out in several host parasite systems, for example, in the N. brasiliensis/rat model (Dineen, Ogilvie and Kelly, 1973; Ogilvie et al., 1977; Nawa and Miller, 1978), in the T. spiralis/mouse or rat model (Wakelin and Lloyd, 1976b; Love, Ogilvie and Maclaren, 1976; Crum, Despommier and McGregor, 1977), in the Trichuris muris/mouse model (Wakelin, 1975) and in the Trichostrongylus colubriformis/guinea pig model (Dineen and Wagland, 1966).

Cell fractionation studies have shown that, on the whole, T-enriched lymphocytes from MLN or TDL

are more successful in the transfer of accelerated worm expulsion than B-enriched lymphocytes (see Wakelin and Wilson, 1979a; Nawa, Parish and Miller, 1978; Wakelin, 1978a). However, in rats infected with T. spiralis, Despommier, McGregor, Crum and Carter (1977) have proposed that B cells are more effective in transferring immunity than T cells and they therefore postulated that expulsion is mediated by IgA antibody.

The role of mast cells in parasitic infection and their possible involvement in the rapid expulsion response, have been discussed previously (see chapter 3). It is known that the intestinal mast cell response to parasite infection is thymus dependent (Mayrhofer, 1979a and b; Ruitenberg and Elgersma, 1976). The origin of the mucosal mast cell is not known, but Guy-grand, Griscelli and Vassalli (1978) proposed that there is a relationship between intestinal T-lymphocytes and mucosal mast cells and that mast cells may be the final products of T cells.

More recently, in rats infected with N. brasiliensis, Nawa and Miller (1979) showed that an accelerated intestinal mast cell response could be transferred with immune TDL and that the T cell fraction from TDL (sIg^- cells) was the effective cell population, not the B cell fraction (sIg^+ cells). They concluded that either intestinal mast cells are derived from T-cells or that transferred immune TDL regulate the differen-

tiation of mast cells. In addition, in the N. brasiliensis/rat system and in the T. spiralis/mouse system, Befus and Bienenstock (1979) and Alizadeh and Wakelin (1981a) respectively, have found that immune MLNC can transfer intestinal mastocytosis.

The responses mediated by T-lymphocytes that lead to worm expulsion are still controversial. In the case of T. spiralis is is likely that T cells mediate inflammatory changes in the intestine and that these changes render the intestinal environment unsuitable for worm survival (Larsh and Race, 1975; Wakelin and Wilson, 1979a and b).

The cellular basis for the generation of these changes is unknown, but a potentially important component may be the intestinal mastocytosis which T cells mediate.

The experiments discussed in this section were designed to examine the involvement of immune lymphocytes in rapid expulsion and in the accompanying mast cell response. The experiments fall into three categories:

1) The effect on the rapid expulsion response of suppressing the host with immune suppressive drugs or irradiation.

2) The effect upon the mast cell response of transferring immune MLNC or separated T and B cell populations into irradiated and unirradiated mice.

3) The relationship between rapid expulsion and the mucosal mast cell response in recipients of immune MLNC.

1. Effect of immunosuppressive drug treatment and irradiation on rapid expulsion

Attempts were made to block the rapid expulsion response in immune mice by using a variety of immunosuppressive drugs (i.e. cortisone, cyclophosphamide and reserpine) or by irradiation (850 rad) administered before challenge infections.

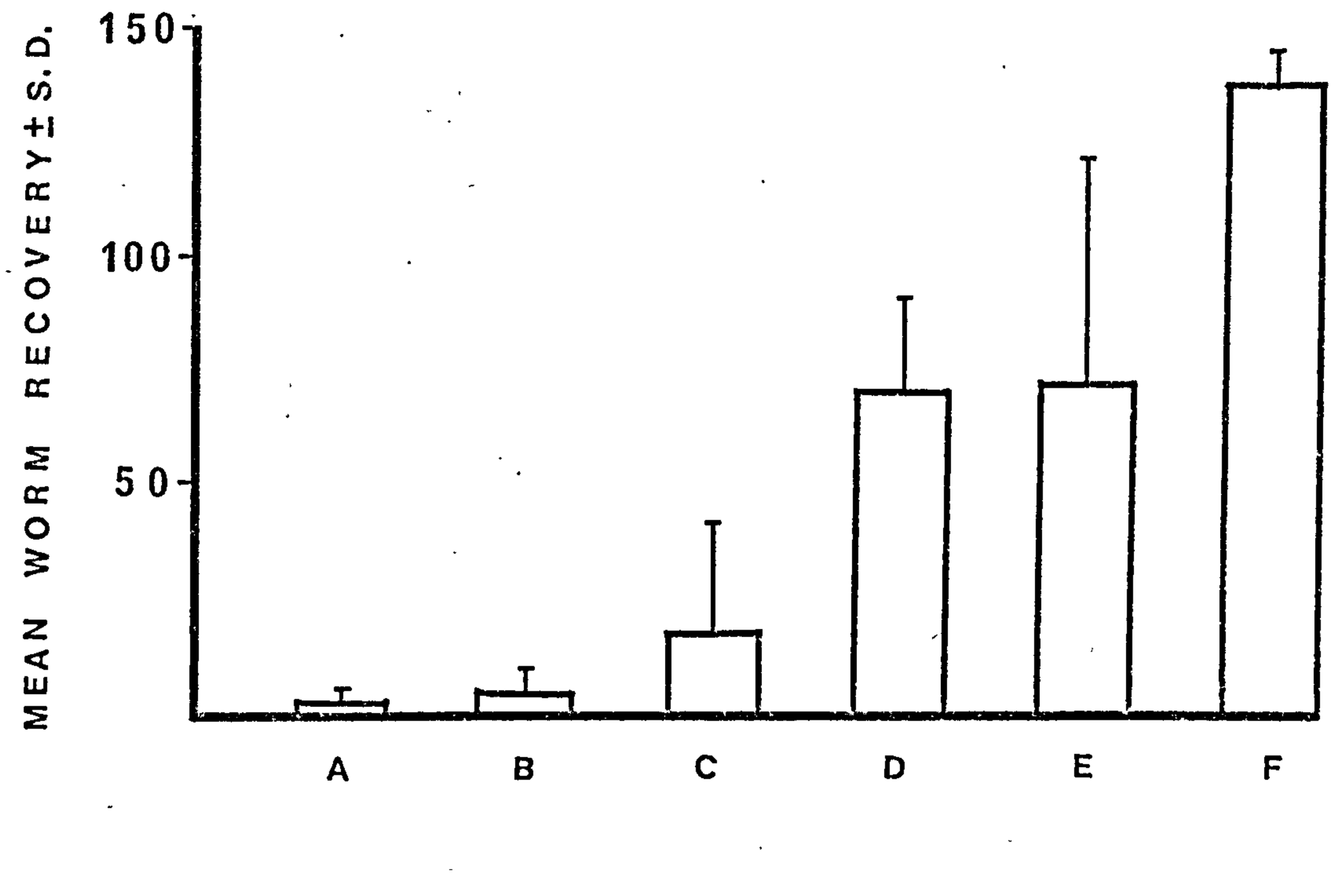
Thirty 7-week-old male NIH mice were infected with 300 T. spiralis larvae on day 0 and were divided into five groups of six mice each (A, B, C, D and E). Group A mice were not given any treatment, groups B, C and D were given two single doses of reserpine, cyclophosphamide or cortisone, respectively, on days 12 and 13 p.i. Mice from group E were irradiated (850 rad) on day 12 (see materials and methods). All five groups together with uninfected age-matched controls (group F) were challenged with 300 T. spiralis larvae on day 14 p.i. and were killed 24 hours later. The results are

Fig. 5.6

Effect of immunosuppression, by drug treatment or irradiation given 2 days before a challenge (secondary) infection, on rapid expulsion in NIH mice.

Group

A	no treatment
B	reserpine
C	cyclophosphamide
D	cortisone
E	irradiation
F	control (previously uninfected)


MEAN WORM RECOVERY ± S.D.
150
100
50
A
B
C
D
E
F

shown in Fig. 5.6. Rapid expulsion was partially prevented in mice treated with cortisone or given irradiation. Reserpine and cyclophosphamide were found to be ineffective.

2. Adoptive transfer of immune MLNC in irradiated and unirradiated mice

When mice are exposed to 400 rad irradiation, expulsion of T. spiralis is prevented and the worms survive for more than 3 weeks (Wakelin and Wilson, 1980 and personal observations). It is known that irradiation interferes with the development and expression of immunity. In a series of experiments, attempts were made to examine a) the effect of transferring immune MLNC upon primary worm expulsion in irradiated and unirradiated mice, b) the effect of transferring immune cells upon the intestinal mast cell response in irradiated and unirradiated mice, and c) the rapid expulsion of a challenge infection and the effect of transferring immune cells on this response in irradiated mice.

a) The effect of transferring immune cells upon worm expulsion in irradiated and unirradiated mice

Thirty-six 7-week-old male NIH mice were divided into six groups of six mice each. Two groups of these mice were irradiated with 400 rad. On the following

day one irradiated and two unirradiated groups were given $3.2x10^7$ immune MLNC and all the mice were then infected with approximately 300 T. spiralis larvae. Unirradiated recipients and control mice were killed on days 1 and 8 p.i. Irradiated recipients and control mice were killed on day 12 p.i. The results are shown in Table 5.3. In unirradiated mice given immune cells, loss of the worms had begun by day 8 and in irradiated recipients loss of the worms had begun by day 12 p.i.

b) The effect of transferring MLNC upon the intestinal mast cell response in irradiated and unirradiated mice

i Unirradiated mice

Forty-five 7-week-old male NIH mice were infected with 300 T. spiralis larvae and were divided into three groups of fifteen mice each. The mice in groups 1 and 2 received immune or control MLNC on the day of infection, group 3 mice did not receive cells. An additional forty-five age-matched uninfected NIH mice were divided into three groups of fifteen mice each. Two groups received immune or control cells, the third group acted as normal controls. Three mice in each group were killed on days 2, 4, 6, 8 and 10 p.i. A segment (5 cm) from the middle of the small intestine was removed and examined for the mast cell response.

Table 5.3

Transfer of immunity with immune MLNC in irradiated (400 rad) and unirradiated mice.

	Worm recoveries after infection					
	Day 1		Day 8		Day 12	
Group	Mean	S.D.	Mean	S.D.	Mean	S.D.
Unirradiated mice						
no cells	185.2	24.5	165.3	40.6	n.d.	
immune cells	160.7	18.2	62.5*	38.0	n.d.	
Irradiated mice						
no cells	n.d.		n.d.		171.6	39.5
immune cells	n.d.		n.d.		80.0*	27.4

* Mean significantly lower than control

$p < 0.05$

n.d. = not done

The mean numbers of mast cells per 20 V.C.U. for each group is shown below and the overall pattern of the response is shown in Fig. 5.7.

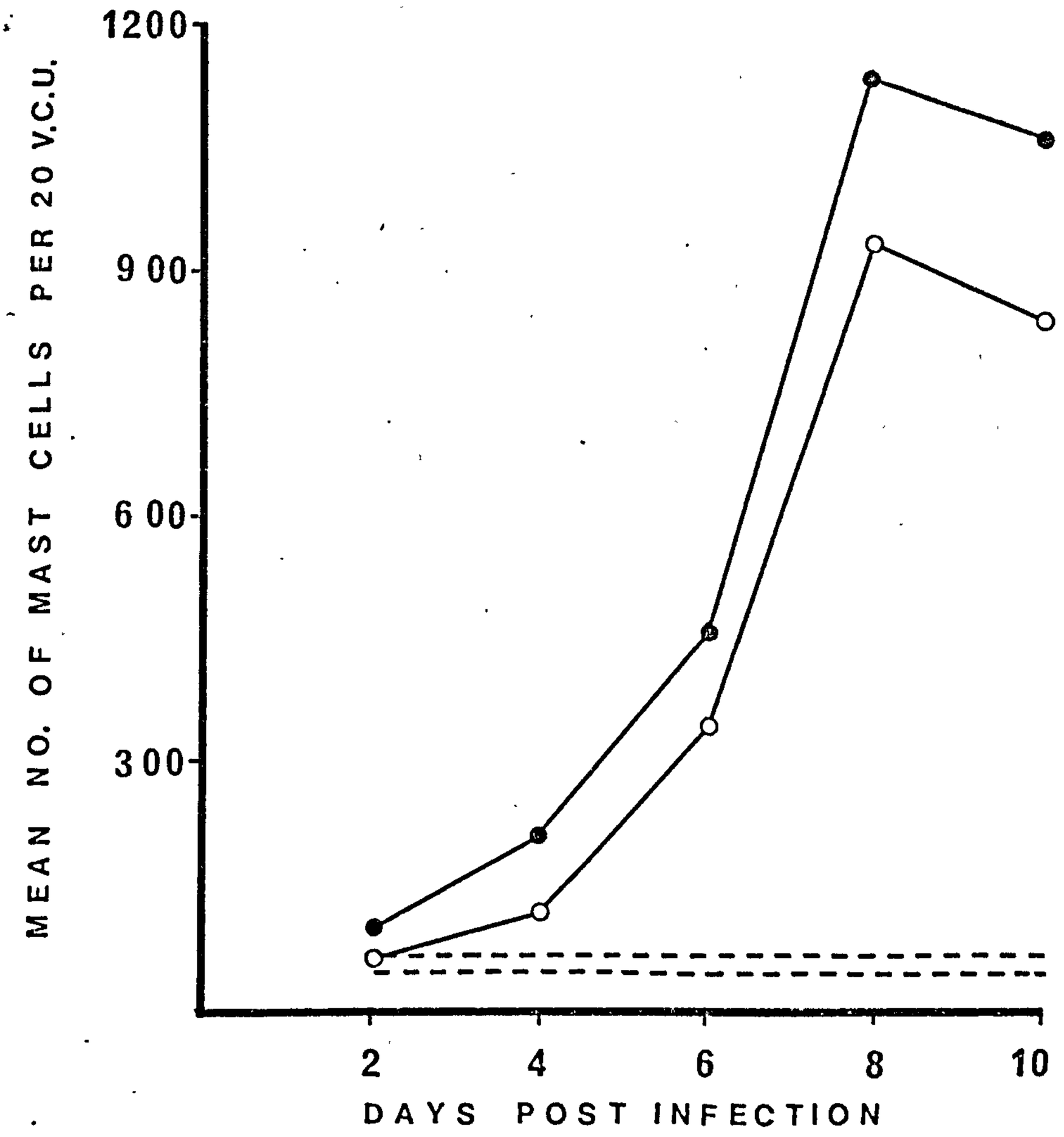
Mean number (±S.D.) of mucosal mast cells per 20 V.C.U. in unirradiated mice.

Days of post infection	Groups of mice: Infected (no cells)		Infected recipients of immune MLNC	
	Mean	S.D.	Mean	S.D.
2	56	8	98	10
4	102	17	215	12
6	342	38	450	45
8	936	64	1132	57
10	838	46	1063	61
Uninfected control	52	10	45	12

Mast cell numbers increased considerably by day 6 p.i. and had reached a peak by day 8 in the infected group (no cells). The mast cell response was accelerated in mice infected and given immune MLNC being approximately double the level in control infected mice on days 2 and 4 p.i. The number of mast cells remained at higher levels in infected recipients of immune cells on days 6, 8 and 10 post infection. Control MLNC did not accelerate mastocytosis in infected mice, cell numbers were virtually identical with those in mice infected only and the counts

Fig. 5.7

Mean mast cell numbers per 20 V.C.U. in unirradiated NIH mice. Infected mice given no cells (O——O) or given immune MLNC (●——●). ═══ Mast cell numbers in uninfected mice given no cells, or given immune or control MLNC.


1200
900
600
300
MEAN NO. OF MAST CELLS PER 20 V.C.U.
2
4
6
8
10
DAYS POST INFECTION

have been omitted from the data. In the absence of infection transfer of cells, whether control or immune, had no effect on mast cell numbers.

ii Irradiated mice

Forty-five 7-week-old male NIH mice were irradiated with 400 rad and infected with 300 T. spiralis larvae one day later. They were divided into three groups of fifteen mice each. Two groups received immune or control MLNC on the day of infection, the third group did not receive cells. An additional forty-five age-matched uninfected controls were similarly divided into three groups of fifteen mice each. Two groups received immune or control cells, the third groups did not receive cells. Three mice in each group were killed on days 2, 4, 6, 8 and 10 p.i. and a segment (5 cm) from the middle of the small intestine was removed and examined for the mast cell response. The mean number of mast cells per 20 V.C.U. for each group is shown below. The overall patterns of the mast cell response is shown in Fig. 5.8.

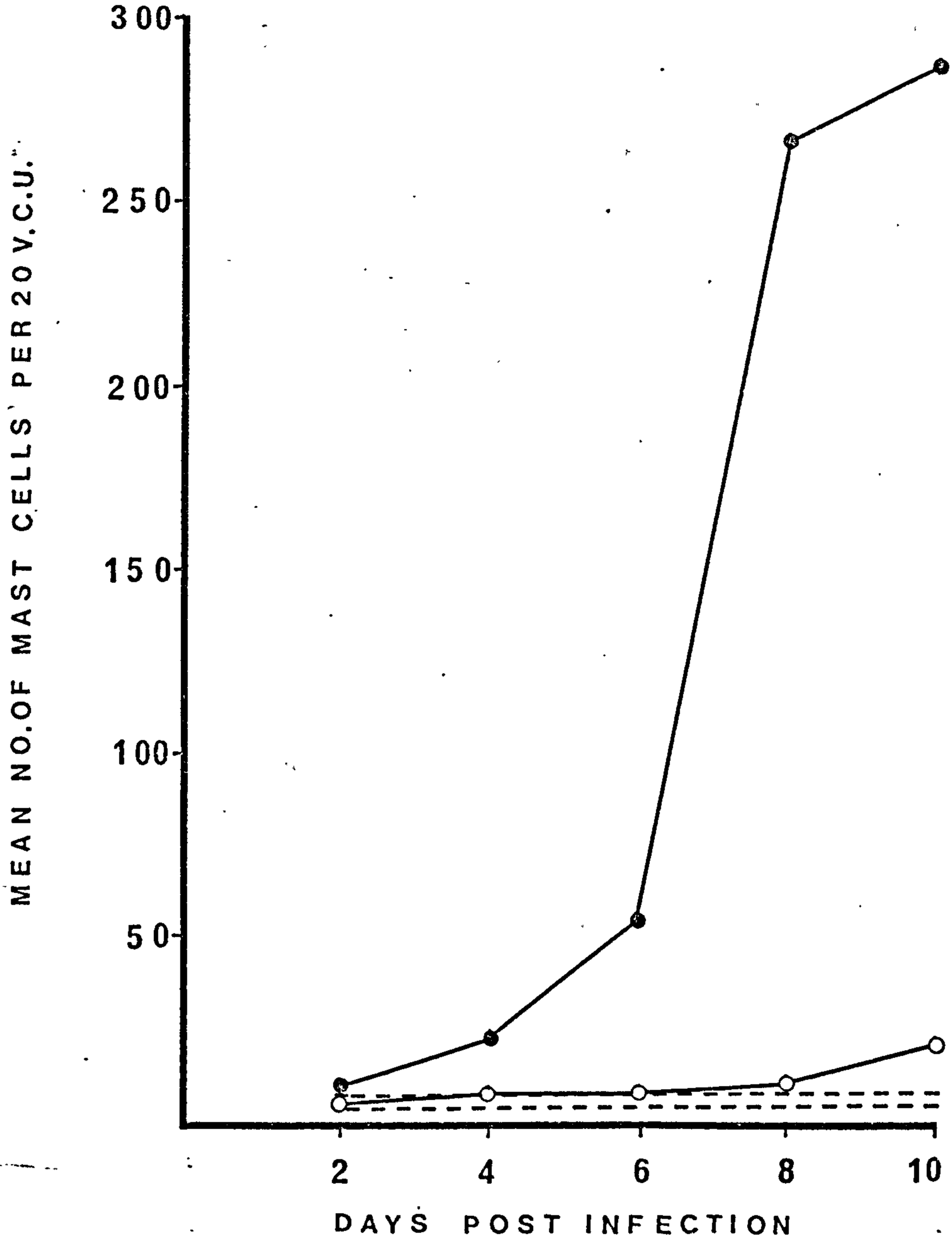
Mean number (±S.D.) of mast cells per 20 V.C.U. in irradiated mice

Days of post infection	Infected (no cells)		Infected recipients of immune MLNC	
	Mean	S.D.	Mean	S.D.
2	7	2	9	3
4	8	4	22	8
6	8	3	54	15
8	10	4	265	18
10	21	10	286	22
uninfected control	3	1	4	2

In irradiated mice, the numbers of mast cells were considerably lower than in unirradiated mice (see the above results). Irradiation delayed intestinal mastocytosis in infected mice and no rise was observed until day 10 p.i. In irradiated infected recipients of immune MLNC, mastocystosis began to develop by day 4 and was still rising by day 10 p.i. At this time, mast cells were observed in discrete patches in the mucosa. The number of mast cells in irradiated recipients of control MLNC was similar to that of irradiated infected mice, cell numbers were virtually identical with those in mice infected only and the counts have been omitted from the data. In the absence of infection transfer of cells, whether control or immune, had no effect on mast cell numbers.

Fig. 5.8

Mean mast cell number per 20 V.C.U. in irradiated NIH mice. Infected mice no cells (O—O) or given immune MLNC (●—●). ≡≡≡ Mast cell numbers in uninfected mice given no cells or given immune or control MLNC.


300
250
200
150
100
50
MEAN NO.OF MAST CELLS' PER 20 V.C.U.
2
4
6
8
10
DAYS POST INFECTION

c) Rapid expulsion in irradiated mice and the effect of transferring MLNC on this response

The results of the above experiments showed that irradiated (400 rad) mice did not reject a primary infection in the "normal" time. The transfer of immune cells into such mice does result in an accelerated worm expulsion, but this is delayed by 2-4 days compared with normal unirradiated recipients.

Experiments were therefore designed to examine the fate of rapid expulsion in irradiated mice and also to determine which components associated with rapid expulsion are affected by irradiation.

Forty-eight 7-week-old male NIH mice were divided into groups of six mice each. Four groups (1-4) were irradiated with 400 rad and one day later two groups of these mice (groups 2 and 3) received 3.2×10^7 immune or control MLNC, respectively. Two unirradiated groups (groups 6 and 7) also received immune MLNC. Three irradiated groups (i.e. cell recipient groups 2 and 3 and control group 1) together with two unirradiated groups (i.e. cell recipient group 6 and control group 5) were infected with 300 T. spiralis on the day of cell transfer. The remaining mice (groups 4, 7 and 8) were not infected. On day 12 p.i. all the infected and uninfected groups were given a single treatment of

methyridine (see general materials and methods) to remove any residual worms. On day 14 all the mice were challenged with 300 T. spiralis larvae and were killed 24 hours later. The results are shown in Table 5.4. Rapid expulsion did not occur in either irradiated mice or irradiated recipients of control MLNC. Immune MLNC restored the rapid expulsion response in irradiated mice. Non infected recipients of immune cells did not show rapid expulsion of a challenge infection.

d) The effect of T and B cells upon the mast cell response in irradiated mice

The results from previous experiments showed that immune MLNC were capable of transferring a mast cell response in irradiated infected mice and an accelerated mast cell response occurred in unirradiated infected mice. Further transfer experiments using T and B enriched populationsof MLNC were used to identify the lymphocyte population responsible for the transfer of the mast cell response in irradiated mice.

Twenty-four 7-week-old male NIH mice were irradiated with 400 rad and infected with 300 T. spiralis one day later. They were divided into four groups of six mice each (A, B, C and D). Group A mice did not receive cells, group B mice were given $2x10^7$ unseparated immune cells, group C mice received $2x10^7$ T cells and group D mice received $2x10^7$ B cells. All the mice were

Table 5.4

Effect of MLNC upon rapid expulsion in irradiated mice

Group		Cells transferred		Worm recoveries 24 hours after challenge	
		IMLNC	CMLNC	Mean	S.D.
Irradiated mice					
1 - infected	challenged	-	-	127.5	12.3
2 - infected	challenged	3.2×10^7	-	30.0*	22.6
3 - infected	challenged	-	3.2×10^7	129.8	28.4
4 - uninfected	challenged	-	-	157.7	36.0
Unirradiated mice					
5 - infected	challenged	-	-	12.5*	22.0
6 - infected	challenged	3.2×10^7	-	10.8*	7.4
7 - uninfected	challenged	3.2×10^7	-	120.9	22.4
8 - uninfected	challenged	-	-	148.5	41.2

*Mean significantly lower than uninfected challenged control

$p < 0.05$

Immune cells (IMLNC)

Control cells (CMLNC)

killed on day 12 p.i. A segment (5 cm) from the middle of the small intestine was removed, from three to four mice in each group, and examined for the mast cell response (see below). The rest of the guts were incubated for worm recoveries by the Baermann technique and worm fecundity was measured (see materials and methods).

In addition, 24 unirradiated mice were infected with approximately 300 T. spiralis on day 0 and were divided into four groups of six mice each. Two groups of these mice received $2x10^7$ unseparated immune cells on the day of infection, the other groups received no cells. One recipient group and a control was killed on day 8 p.i. to determine the effectiveness of cell transfer. The mean number of worms recovered was 109±46 and 240±30 respectively and worm fecundity was 1.6 and 3.2 larvae/female/hour, indicating that immunity had been transferred successfully. Both irradiated mice and irradiated recipients of unseparated immune MLNC and T or B cells failed to reject their worms by day 12 p.i., but worm fecundity was reduced in unseparated cell recipients(i.e. the fecundity in irradiated controls and irradiated recipients was 7.2 and 1.2 larvae/female/hour).

The other two remaining groups of unirradiated mice (groups E and F) were killed on day 12 p.i. to

determine the number of mast cells present and to compare the result with those obtained from irradiated mice killed at this time.

The mean numbers (±S.D.) of mast cells per 20 V.C.U. for each group is shown below and the overall pattern of the mast cell response is shown in Fig. 5.9.

Group	Irradiated mice	Mean number (±S.D.) of mast cells per 20 V.C.U. on day 12 p.i.	
		Mean	S.D.
A	no cells	40	21
B	unseparated immune MLNC	381	34
C	T-cell	224	25
D	B-cell	45	17
	Unirradiated mice		
E	no cells	1020	84
F	unseparated immune MLNC	1124	52

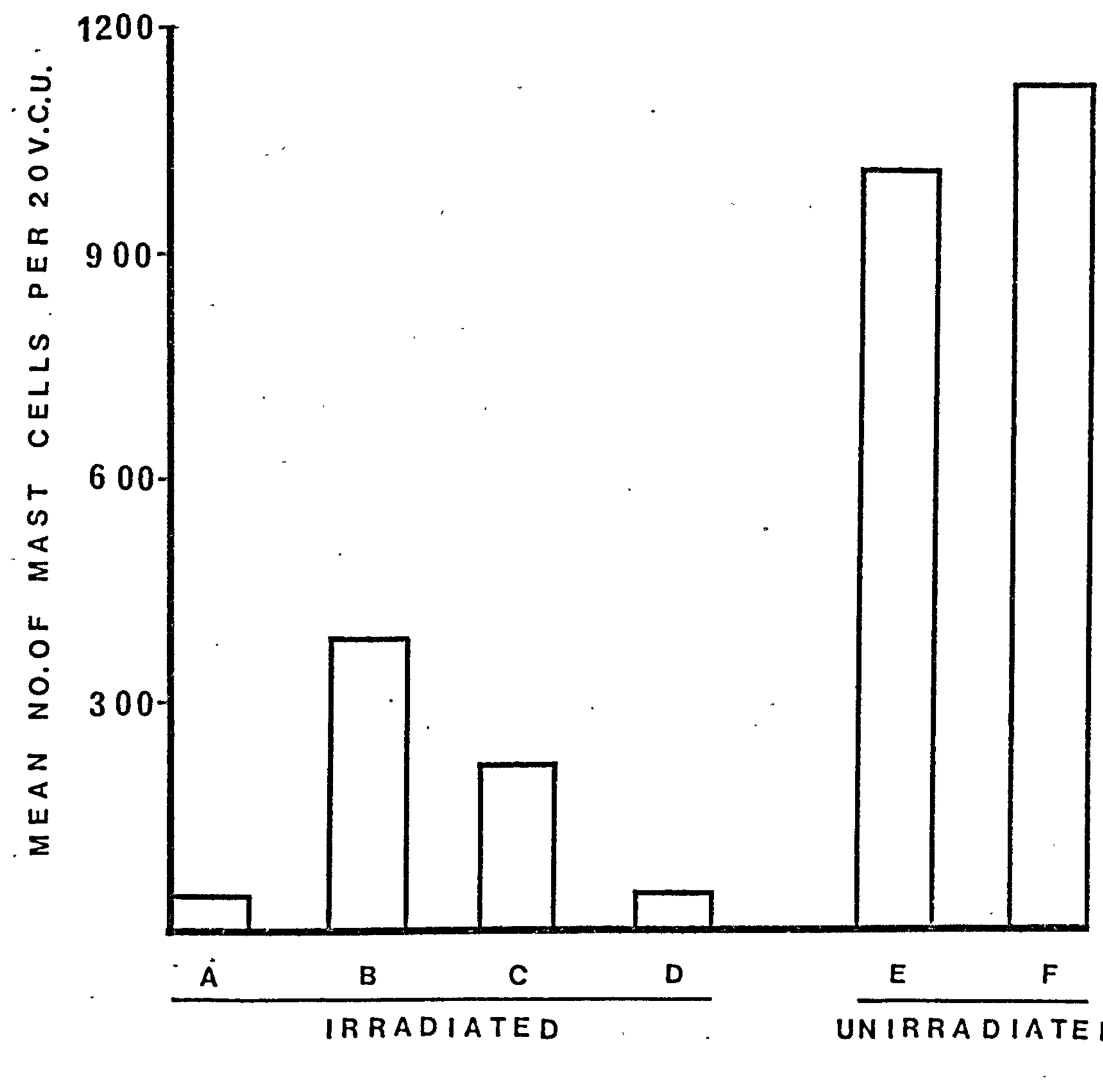
Irradiated recipients of unseparated MLNC and T cell enriched MLNC (groups B and C) developed mastocytosis by day 12 p.i., but the number of mast cells in irradiated recipients given the B cell enriched fraction (group D) was similar to the control group (group A), i.e. irradiated no cells. In unirradiated mice given either no cells (group E) or immune cells (group F) the

number of mast cells was considerably higher than in the irradiated mice, even when these had been given unseparated MLNC or T cells.

Fig. 5.9

The effect of adoptive transfer of separated populations of immune MLNC upon the mast cell response in irradiated (400 rad) infected mice. All the mice were killed on day 12 post infection. Immune MLNC given to mice irradiated 1 day previously and infected on day of cell transfer.

Group	Recipients
A	irradiated, no cells (control)
B	irradiated, given unseparated immune MLNC
C	irradiated, given T cells
D	irradiated, given B cells
E	unirradiated, no cells
F	unirradiated, given unseparated immune MLNC


MEAN NO.OF MAST CELLS PER 20 V.C.U.
1200
900
600
300
A
B
C
D
E
F
IRRADIATED
UNIRRADIATED

e) The effect of adoptive transfer of immune MLNC upon the mast cell response and on rapid expulsion in irradiated, bone marrow reconstituted mice

A. Mast cell response

The results from the above experiments using mice irradiated at 400 rad showed that immune MLNC transferred an enhanced mast cell response, but only to a limited degree. It is possible that immune MLNC may give rise directly to mast cells, act as helper cells or co-operate with other components of the host response (presumably bone marrow-derived components). To examine these possibilities, it was decided to increase the level of irradiation to further deplete the mice of immuno-competent cells and of bone marrow stem cells.

Thirty-six 7-week-old male NIH mice were divided into six groups of six mice each. Four groups (A, B, C and D) were irradiated with 650 rad and 1 day later, together with one group of unirradiated mice (group E), they were infected with 300 T. spiralis larvae; mice in group F were kept as unirradiated uninfected controls. Mice in group A received no cells, mice in group B received 3.5×10^7 immune MLNC, mice in group C received 3.5×10^7 immune MLNC and 1×10^7 syngeneic bone marrow cells from uninfected donors and mice in group D

received only $1x10^7$ bone marrow cells. All the mice were killed on day 9 p.i. A segment (5 cm) from the middle of the small intestine was removed from 3 or 4 mice in each group and examined for the mast cell response. The mean numbers (±S.D.) of mast cells per 20 V.C.U. is shown below and the overall pattern of the mast cell response is shown in Fig. 5.10. The rest of the guts were incubated for worm recoveries using the Baermann technique, the fecundity and the length of the worms were also measured.

Group	Irradiated mice	Mean numbers (±S.D.) of mast cells per 20 V.C.U. on day 9 post infection	
		Mean	S.D.
A	no cells	5	2
B	immune MLNC	282	19
C	bone marrow cells	7	3
D	immune MLNC and bone marrow cells	305	23
	Unirradiated mice		
E	no cells	985	92
F	Control uninfected mice	49	10

Irradiated recipients of immune MLNC alone (group B) or immune MLNC and bone marrow cells (group D) developed mastocytosis by day 9 p.i. The number of

mast cells in irradiated recipients of bone marrow cells (group C) was similar to that of controls given no cells (group A). The number of mast cells in unirradiated infected mice (group E) was considerably higher than in irradiated mice or in control unirradiated mice (group F).

None of the mice in the irradiated groups had rejected their worms by day 9 p.i., but the majority of the worms had been rejected from unirradiated groups at this time. However, the growth and fecundity of the worms in irradiated recipients of immune cells had been markedly reduced (Table 5.5) indicating that immunity had been successfully transferred.

The ability of immune MLNC to transfer immunity was determined in additional groups of unirradiated mice given 3.5×10^7 immune cells, infected with 300 T. spiralis and killed 8 days later. The numbers of worms recovered in recipients and controls were 100 ± 20 and 156 ± 31, respectively; fecundities were 1.8 and 4.5 larvae/female/hour respectively; indicating that immune cells had considerably affected worm survival and fecundity.

B. Rapid expulsion

To examine the rapid expulsion of a challenge infection in mice given 650 rad irradiation before

infection, an additional eighteen NIH mice were divided into three groups of six mice each. One group was irradiated and given $3.5x10^7$ immune MLNC and $1x10^7$ bone marrow cells on the following day. This group and one unirradiated group were infected with 300 T. spiralis larvae on the day of cell transfer and then, together with control uninfected mice, given a single treatment of methyridine (see general materials and methods) on day 12 p.i. They were challenged with 300 T. spiralis on day 14 p.i. and were killed one day later. The numbers of worms recovered were 12 ± 14, 91 ± 50 and 130 ± 15 in the unirradiated challenged, irradiated challenged recipients, and unirradiated challenged controls, respectively. These results indicate that rapid expulsion occurred only in immune unirradiated mice. However, worm numbers were reduced in irradiated recipients when compared with the control challenge group.

Fig. 5.10

Effect of adoptive transfer of immune MLNC upon the mast cell response in irradiated (650 rad) bone-marrow (BM) reconstituted infected mice. All mice were killed on day 9 post infection.

Group	Irradiated mice
A	No cells
B	immune MLNC only
C	BM reconstitution only
D	immune MLNC plus BM
	Unirradiated mice
E	no cells
F	control uninfected mice

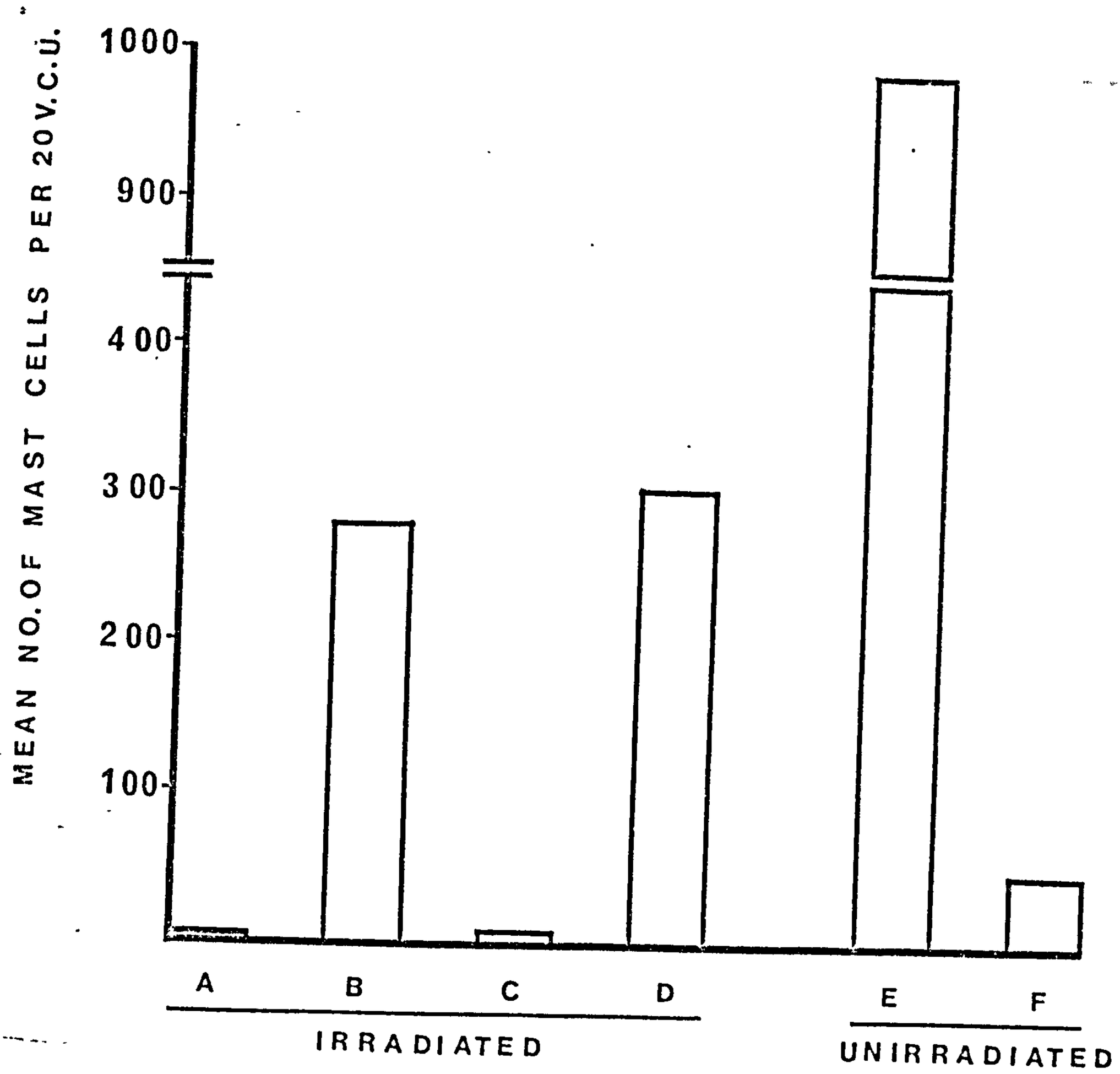

MEAN NO.OF MAST CELLS PER 20 V.C.U.
1000
900
400
300
200
100
A
B
C
D
E
F
IRRADIATED
UNIRRADIATED

Table 5.5

Effect of transferring immune MLNC and bone marrow (BM) on fecundity and length of worms in mice irradiated with 650 rad before infection with T. spiralis

	Day 9 post infection	
Group	Fecundity larvae/female/hour	Female length mm
Unirradiated mice		
No cells	3.9	1.8
Irradiated mice		
No cells	5.7	2.8
B.M.	5.4	2.9
Immune MLNC	1.8	2.0
Immune MLNC+BM	1.8	2.0

DISCUSSION

Previous results (chapter 1) showed that rapid expulsion of T. spiralis only occurred within a short period of time after challenge in immune mice. In the experiments described in this chapter, attempts were made to see whether a challenge infection given on day 14 p.i. could actually establish in the intestinal mucosa. For this reason, both washing out and the Baermann technique were used for worm recovery enabling the percentage of the worms that remained in the intestinal mucosa to be determined. It was found that in immune mice the majority of challenge larvae were expelled within 2-3 hours after challenge and only 34% of the worm population remained in the intestine (expressed as a percentage of the total worm recovery in control challenge mice). Of these, 24% were recovered by flushing out (presumably those worms that remained in the lumen) and 10% were recovered by the Baermann technique, which implies that this proportion of the worms was in the intestinal mucosa.

The mechanism underlying the rapid expulsion response is controversial and currently two views have been proposed for the mechanisms and components which are involved in this response. A passive mechanism for rapid expulsion was suggested by Lee and Ogilvie (1981). They reported that a challenge infection given to immune

rats was prevented from establishing in the intestinal mucosa, and concluded that the mucus layer played an important role in the response. However, in the T. spiralis/ rat model, substantial numbers of worms were recovered from the deep intestinal mucosa, which may suggest that a proportion of the challenge infection did establish. Although it is not clear whether or not there is increased mucus production upon a challenge infection, in vivo studies of mucus trapping by the same authors showed that a few larvae were also trapped in the mucus of uninfected rats. The factors which contribute to mucus trapping are not known, but in vitro studies showed that a similar number of larvae were trapped in the mucus of immune and control rats when incubated with immune or control serum. Attempts were made in the present work to develop techniques for the study of mucus trapping in immune mice, however, sufficient quantities of intestinal mucus could not be obtained from these animals. Notwithstanding, considering the large increases in mast cells and goblet cells seen in mice, it is probable that a passive mechanism is operating in the mouse system, (see chapter 3).

Active mechanisms which may be induced by a challenge infection of T. spiralis have been suggested by Castro et al. (1979). They have proposed that physico-chemical changes and alterations in epithelial function (Bullick et al., 1981) are associated with the rapid

expulsion response. The phenomenon of mucus trapping of challenge infections in rats infected with N. brasiliensis has been studied by Miller et al. (1981). They concluded that, following challenge, a sequence of events leading to a rapid expulsion may result as a consequence of a local intestinal anaphylaxis, which may have an additive effect on the environmental changes in the intestine. At present there is not sufficient data to support an active mechanism for rapid expulsion in mice, therefore, both mechanisms described above should be considered in the analysis of this response. The cellular and physico-chemical changes which result from a primary infection, can be assumed to be prerequisite for both of these mechanisms involved in rapid expulsion.

In respect to an active mechanism, experiments were carried out in an attempt to determine the influence which an infection had upon the localisation of I/V injected immune MLNC. The pattern of homing of lymphoblasts was similar in uninfected mice and mice infected with T. spiralis for 14 days. When immune MLNC were injected 6 hours after challenge, the extent of lymphoblast localisation had not changed in either the small intestine or other organs that were measured in immune and control challenged mice, and the distribution of labelled cells was also shown to be similar. It is possible that the time of injection of labelled cells in

relation to the presence of the parasite is important, because increased localisation of lymphocytes in the small intestine occurred in control mice which had been infected with T. spiralis for 3 days, thus confirming the work of Manson-Smith et al. (1979b), but not in immune mice. It is clear that a challenge infection given on day 14 is subjected to rapid expulsion and these results may imply that the factors associated with increased lymphocyte localisation were not stimulated by the challenge larvae. These factors are not known, but it has been suggested that they are not parasite specific (Rose et al., 1976; Love and Ogilvie, 1977) and are not related to the increased blood flow which accompanies infection (Ottaway, Manson-Smith, Bruce and Parrott, 1980). They may be dependent on environmental, chemotactic factors, hormonal influence and possibly other factors (Bienenstock and Befus, 1980). It is possible that lymphocytes are trapped in these organs rather than becoming localised or homing. However, the fate of these cells after localisation is not known.

The experiments did not investigate whether a challenge infection given after the period of rapid expulsion (after day 16) can, in fact, cause an increase in lymphoblast localisation or whether enhanced localisation of lymphocytes develops more quickly in a challenge infection than in a primary infection.

In vitro blast cell activity was measured on day 15 after primary infection and one day after a challenge infection given on day 14 p.i. The results showed that activity in immune challenge mice was raised but not markedly higher than that of challenge control mice. This may suggest that, because the majority of the challenge infection was expelled rapidly, there were insufficient worms to stimulate a normal increase in blast cell activity in the MLN. However, the variation in the readings of radio labelled cells which appeared in the results of pooled lymphocytes from immune, challenged mice, make it difficult to draw any firm conclusions. It is possible that an inappropriate time was chosen for these measurements. There is evidence to suggest that blast cell activity increases within 2 days of a challenge infection given on day 21 p.i., whereas in primary infection this increase is not observed until between days 4 and 8 p.i. (Grencis, 1981). In addition, Grencis (manuscript in preparation) has data showing that an increased secondary blast cell response can be demonstrated when a challenge infection is given on day 13 post infection. Thus it is possible that there is a correlation between the presence of parasites and the development of blast cell response in MLN upon challenge infection.

A second challenge infection given 1 or 2 days after an initial challenge on day 14 p.i., was also

expelled rapidly indicating that whatever factors are involved in the response, they had not been exhausted by one challenge and were capable of affecting a second challenge infection. Interestingly, when a second challenge infection was given 4 days after an initial challenge, it was partially subjected to rapid expulsion, whereas an initial challenge at this time was not. It is possible that the first challenge infection amplified the "mediators" of rapid expulsion and caused the expulsion of the second challenge infection. A similar result has been obtained in rats by Bell and McGregor (1979b) who have shown that immunized rats were capable of resisting two challenge infections given from 12-72 hours after an initial challenge and this has been interpreted as showing that the response does not depend upon exhaustible mediators.

To examine the nature of the rapid expulsion response and the immunological components which may be associated with this response, mice were given immunosuppressive drugs or irradiation (850 rad) before challenge infections. Rapid expulsion was partially prevented by cortisone and by lethal levels of irradiation given 2 days prior to challenge infection, but reserpine and cyclophosphamide were ineffective. Irradiation and cortisone treatment given at this time should not markedly alter the titre of pre-existing antibodies present (Taliaferro, Taliaferro and Jaroslow, 1964) and

therefore it is reasonable to suggest that these treatments might have exerted their effect by altering cellular components involved in the rapid expulsion response. It is known that cortisone and irradiation suppress the inflammatory response and in particular effectively prevent mastocytosis in parasitic infection (this thesis, Befus and Bienenstock, 1979; Larsh and Race, 1975; Coker, 1956 and Topham, personal communication).

The timing and level of treatment with immunosuppressive drugs are known to be important factors governing effective suppression of the immune response. Thus it is possible that cyclophosphamide and reserpine failed to prevent rapid expulsion because of an inadequate regimen. Certainly it is known that reserpine can act on mast cells and their products (Lee and Ogilvie, 1981). Both of these drugs may suppress the inflammatory response, but the complexity of their modes of action make it difficult to relate effects to particular components of the immune response.

To analyse some of the cellular components which may be involved in the rapid expulsion response, initial experiments were carried out to examine the effect on a primary infection of transferring immune MLNC. The results described in this chapter confirm those obtained by Wakelin and Lloyd (1976b) and Wakelin and

Wilson (1977a), i.e. they indicate that immune cells are capable of transferring an effective immunity to recipient mice.

Analysis of the action of immune cells and of their possible interaction with other components of the immune response in worm expulsion, requires the use of recipients which are relatively immunologically incompetent. To this end, irradiation was used to inactivate the response in intact mice. However, the action of irradiation cannot be explained in simple terms. There is evidence to suggest that irradiation affects primarily cellular components, and lymphocytes are highly sensitive (see Taliaferro et al., 1964; Anderson and Warner, 1976), but treatment influences many other physiological and regulatory mechanisms. This method of rendering an animal immunologically incompetent, has been used successfully in analysing the components of the immune response involved in the N. brasiliensis/rat model (Dineen and Kelly, 1973; Ogilvie et al., 1977), in the T. muris/mouse model (Wakelin, 1975 ; Wakelin and Selby, 1976) and in the T. spiralis/mouse model (Wakelin and Wilson, 1977b, 1979a and 1980).

In the present work, attention was focussed on the effect of transferring immune cells on the mucosal mast cell response and on the rapid expulsion of challenge infection. The results are discussed in the

following order:

a. The role of the cells on worm expulsion from a primary infection and on the rapid expulsion response.

The experiments described here showed that in normal recipients, immune MLNC confer an immunity which leads to accelerated worm expulsion by day 8 post infection. In irradiated recipients of immune MLNC, worm expulsion occured between days 8 and 12. Challenge infections given on day 14 after infection were expelled rapidly from both unirradiated mice and recipients of immune cells, but rapid expulsion did not occur in either irradiated mice or normal recipients of control MLNC. Irradiated mice showed rapid expulsion only when they received immune cells 1 day after irradiation, but control cells were ineffective.

In NIH mice, the mesenteric lymph nodes are grossly enlarged on day 8 post infection and it appears that this is a consequence of a lymphocyte proliferation which reaches peak levels at this time, (i.e. blast cell numbers are increased between days 4 and 8 p.i. as measured by <u>in vitro</u> labelling - Grencis, 1981). It is known that when these cells are injected, they localise preferentially in the small intestine between days 2 and 4 and that this localisation is characteristic predominantly of T. lymphoblasts (Rose et al., 1976).

What factors do these immune cells supply which both accelerates worm expulsion in normal mice and restores a primary worm rejection or rapid expulsion of challenge infection in irradiated (400 rad) mice? In addition, how do these cells express the response in recipient mice?

It is known that activated lymphocytes and anti-worm antibodies in conjunction with the inflammatory response are involved in the primary-type expulsion of T. spiralis from mice. The factors which are involved in rapid expulsion are possibly also connected with the above mechanisms and it is clear that these components can be supplied by immune MLNC. Transfer of these cells may supply the components necessary to generate the response directly, or the cells may co-operate with other components. Mesenteric lymph node cells taken from mice after infection are known to contain both T and B lymphoblasts. In both unirradiated and irradiated (400 rad) mice, it has been shown that expulsion of T. spiralis can be generated by a T cell-enriched fraction of MLNC (Wakelin and Wilson, 1979a). Of course in this system a role for anti-worm antibodies in worm expulsion cannot be ignored because some B cells are known to be radioresistant (Pilarski and Cunningham, 1975), but Wakelin and Wilson (1979a) concluded that the contribution of B cells to the expulsion of T. spiralis

from mice is a minor one. From this evidence, it is likely that the factors which restored the rapid expulsion response in irradiated mice are also primarily dependent upon the T cell population of transferred MLNC.

In the primary-type expulsion of T. spiralis, it has been suggested that T cells may act as helper cells or contribute to generation of cellular changes in the intestinal mucosa (Wakelin and Wilson, 1979a). In addition, there is evidence to suggest that generation of some cellular changes (mast cells and goblet cells) in N. brasiliensis infected rats is also dependent on the T cell population of TDL (Miller and Nawa, 1979a; Nawa and Miller, 1979).

In the T. spiralis/mouse system, it has been demonstrated that the other components involved in worm expulsion, are bone-marrow derived. For example, restoration of expulsion from sublethally irradiated mice (600) is achieved when the mice are given both MLNC and bone-marrow cells, (Wakelin and Wilson, 1977a). Bone-marrow cells are also involved in the expulsion of N. brasiliensis (Dineen and Kelly, 1973).

The results presented here showed that neither worm expulsion nor rapid expulsion of a challenge infection occurred in mice irradiated (650 rad) and given

only immune MLNC, but fecundity and growth in a primary infection were affected. This evidence confirms that obtained by Wakelin and Wilson (1980) who showed that an anti-worm immunity, but not worm expulsion; was evident in irradiated (600 rad) recipients. The role played by bone-marrow derived cells is not known, but it has been suggested that if worm expulsion occurs as a result of inflammatory changes in the intestinal mucosa, bone-marrow cells may contribute to the renewal of those non-lymphoid components which are affected by irradiation and which co-operate with lymphocytes to bring about primary worm expulsion and perhaps also the rapid expulsion response. The reason for the failure of mice irradiated and reconstituted with MLNC and bone-marrow to expel a challenge is not known, but it is possible that the cellular components involved in this response were not completely restored.

b. The role played by immune cells in the mast cell response to infection

The evidence presented here showed that in infected unirradiated mice, the number of mast cells increased considerably by day 6 and reached a peak level between days 8 and 10 post infection. In recipients of immune MLNC an accelerated mast cell response occurred by day 4 and the numbers of mast cells remained higher than

in control infected mice (no cells) up to day 10 p.i. In irradiated infected mice the mast cell response was delayed until day 8, and even then, by day 10 p.i. the numbers of mast cells had reached only control (uninfected) levels.

In infected irradiated recipients of immune MLNC the mast cell response was partially restored and was comparatively high by day 10, but the number of mast cells was still lower than in unirradiated mice. In addition, the number of mast cells in recipients of control MLNC was similar to that of control (infected, no cells) throughout the experiments. The level of mast cells in control (uninfected) recipients of either immune or control MLNC, was similar to that of uninfected mice.

These results can be summarised as follows: 1) immune MLNC transferred immunity (in terms of worm expulsion) to unirradiated and irradiated (400 rad) recipients, 2) the mast cell response could be transferred with immune MLNC in irradiated and unirradiated mice, 3) the mast cells or precursors of mast cells, are radio-sensitive, 4) antigenic stimulation is necessary for differentiation of mast cells, 5) rapid expulsion was restored in irradiated (400 rad) recipients of immune MLNC and 6) there is a relationship between transfer of immune MLNC, the mast cell response and subsequent rapid

expulsion of challenge infection. Therefore, whatever mechanisms are involved in the rapid expulsion, i.e. passive or active mechanisms (see earlier), the results imply that mast cells or their products, may play an important role. Nawa and Miller (1979) and Miller and Nawa (1979a) have reported similar results from their work in the N. brasiliensis/rat model and have suggested that immune TDL not only regulate the mast cell response but also, directly or indirectly, regulate the differentiation of intestinal goblet cells.

Further experiments with separated immune MLNC were carried out to identify the lymphocyte populations responsible for transferring the mast cell response. In these experiments, irradiated (400 rad) mice were used because mast cells are depleted in mice given this dose of irradiation and exogenous bone-marrow cells are not needed for the restoration of the immune system. Interestingly, the mast cell response was transferred successfully with enriched T. cells, but not with B. cell. Thus it is possible that either T. cells act independently or act as helper cells. However, the number of mast cells in mice given unseparated immune MLNC, was higher than in thosegiven the T. cell-enriched population and it is possible that co-operation of both B and T cells is necessary for the full response. The experiments by Nawa and Miller (1979) also demonstrated that immunoglobulin negative cells (sIg^-) from immune TDL were able

to transfer the mast cell response in N. brasiliensis infected rats. However, both Miller (1979) and Befus and Bienenstock (1979) have reported that immune serum can also transfer the mast cell response in rats. The underlying mechanism for this is not known. Askenase (1980) has suggested that T. dependent antibodies (presumablye IgE) may be involved in this response, but it is possible that antigen-antibody complexes may have some influence.

An attempt was made to determine whether cells other than MLNC (lymphoid cells) are involved in transferring the mast cell response, or whether MLNC co-operate with bone marrow-derived components in transferring this response. Immune MLNC or bone marrow cells were transferred to mice irradiated with 650 rads and it was found that transfer of immune MLNC alone, or together with bone marrow cells, was effective, but that bone marrow cells alone had no effect. Clearly MLNC alone had the capacity to restore a limited response. This may not mean that mast cells originate directly from lymphocytes, but may imply that donor MLNC contain mast cell precursors. Production of such precursors, presumably from bone marrow, may therefore require a longer period of time than was provided in this experiment.

In T. spiralis infected mice and in N. brasiliensis infected rats, substantial numbers of mast cells can be found in the mesenteric lymph nodes (personal observation; Keller, Cottier and Hess, 1974;

Befus, Johnston and Bienenstock, 1979). Befus and Bienenstock (1979) have suggested that it is unlikely that the mast cells which populate the mucosa after transfer of MLN cells, are derived by direct homing of mature cells, instead the origin of these cells is likely to be mastopoetic precursors present in the MLN. Miller (1971) has also noted that intestinal mast cells differentiate and proliferate from mastoblast precursors. It is possible that immune MLNC are able to act as helper, activator or precursor cells in regulating the intestinal mast cell response. In vitro studies by Denburg, Befus and Bienenstock (1980) showed that mast cells differentiated in vitro from MLN suspensions of N. brasiliensis infected rats exposed to antigenic or mitogenic (PHA) stimuli and they suggested that the MLN of infected rats contain precursor cells which may be involved in the intestinal mastocytosis.

The origin of mast cells is not known, but it is well documented that they are T cell dependent (see chapter 3) and that T cells or their products, may positively influence the tissue accumulation or differentiation of mast cells.

The relationship between T. cells and the intestinal mast cell response in nematode infection has been studied in several laboratories. Recently it has been reported in mice, that 24 hours after injection of

radio-labelled T. lymphoblasts, the mucosa contains cells which carry the label and that some labelled cells also stain with Alcian blue (Guy-Grand, et al., 1978). However, Askenase (1980) suggested that because T. cell markers were absent from these cells, it is not possible to prove directly that they were derived from T. blasts. To investigate the origin of mucosal mast cells and the involvement of T. cells in this response, it should be possible to devise experiments in which cells from mice that have a chromosome marker (e.g. T6 T6 CBA mice) are transferred into compatible irradiated mice. In this way, progeny of the cells could be recognised and their fate determined.

A possible relationship between epithelial T. cells and mast cells has recently been suggested by Askenase (1980). He proposed that there are two populations of recirculating, gut-homing cells involved in this response, a radiosensitive mast cell precursor and a radio-resistant T. cell that induces differentiation of the precursor into mucosal mast cell when stimulated by nematode antigen in the intestinal environment.

Whatever mechanisms are proposed for the differentiation of mast cells, if the immune expulsion of T. spiralis is caused by T cell dependent inflammatory changes (Larsh and Race, 1975; Wakelin and Wilson, 1979a), it is possible that T cells may contribute to worm

expulsion by generating inflammatory changes in the intestinal mucosa and express this response through the mast cells or give a signal to bone-marrow components which in turn, participate in these environmental changes.

CHAPTER 6

MOUSE STRAIN VARIATION IN INTESTINAL MAST CELL RESPONSE TO PRIMARY INFECTION WITH T. SPIRALIS

The results described in this chapter have been presented at the British Society for Parasitology and will be published in abstracted form in Parasitology, 1981.

INTRODUCTION

In a variety of systems it has been shown that genetically controlled factors operate to influence the host response to intestinal nematode parasites, (Wakelin, 1978b). The mechanisms through which genetic control is exposed have rarely been determined, but it has been reported that when mice are genetically selected for high and low antibody production and infected with T. spiralis there are differences in antibody levels, macrophage activity and intestinal mast cell responses (Ruitenberg, Perrudet-Badoux, Boussac-Aron and Elgersma, 1980). Strain variation in anaphylactic antibody production (IgG_1 and IgE) during infection with T. spiralis has been reported by Rivera-Ortiz and Nussenzweig (1976) and strain differences have also been observed in lymphoblast localisation during a T. spiralis infection (Manson-Smith, Bruce, Rose and Parrott, 1979b).

The role played by the major histocompatibility complex (MHC)-linked genes in controlling the response to parasite infection is not known, but Wassom, David and Gleich (1979 and 1980) have shown (in mice) that the level of resistance to infection with T. spiralis (as measured by the number of encysted larvae) is influenced by several genes mapping within the mouse MHC (H-2) complex. However, it is clear that genes mapping outside the H-2 complex (non H-2 genes) also contribute to this

resistance and in fact, Wakelin (1980a) has suggested that the major genetic control of time of worm expulsion is determined by such genes. It is known that the expulsion of T. spiralis is associated with T cell-mediated inflammatory responses and it has been proposed that non H-2 genes control the generation of inflammatory changes whereas H-2 genes may control overall lymphocyte responsiveness to infection (Wakelin and Donachie, 1980). Thus the major difference between rapid responder (worm expulsion occurring within 2 weeks) and slow responder (expulsion within 3 weeks) strains is a non H-2 linked, genetically determined ability to generate intestinal inflammatory responses to infection.

A variety of components are involved in the inflammatory response to T. spiralis (see chapter 3), but attention has been focussed here on the role played by mast cells since they provide an easily quantifiable parameter and it has been shown that in T. spiralis infected NIH mice the rise of mast cells coincides with observable inflammation in the intestine (see chapter 3). It has also been demonstrated that with T. spiralis and N. brasiliensis, an acceleration of the mast cell response can be achieved by the transfer of immune MLNC or TDL (this thesis; Befus and Bienenstock, 1979; Nawa and Miller, 1979; Brown, Bruce, Manson-Smith and Parrott, 1981).

The experiments described in this chapter were

designed to examine 1) the kinetics of the mucosal mast cell response in rapid responder (NIH, DBA_1 and CBA) mice and slow responder (B10G, B10.BR, B10D2 and C57BL/10) mice; 2) the influence of H-2 or non H-2 genes on the generation and control of the intestinal mast cell response to T. spiralis infection; 3) the effect upon the mast cell response of transferring immune MLNC between and within rapid responder and slow responder mice and 4) the component (lymphoid or myeloid) through which genetic control of mastocytosis operates.

MATERIALS AND METHODS

Inbred strains of mice were obtained from Hacking and Churchill Ltd. or Olac 1976 Ltd. F_1 hybrids (obtained by mating female B10G with male NIH mice) and DBA_1 mice as well as additional B10 and NTH mice were bred in the Wellcome Laboratories (Glasgow).

The haplotype of the strains of mice used are given in the figures for each experiment. In the majority of experiments mice were used at approximately 7-10 weeks.

Methods used for the maintenance of T. spiralis, infection, worm recovery and histology have been described in the general materials and methods.

Methods used for preparation and transfer of immune mesenteric lymph node cells (MLNC) and for irradiation of mice, have been described in chapter 5.

RESULTS

1. The kinetics of the mucosal mast cell response in different strains of mice

In the experiments described below, mice were infected with approximately 300 T. spiralis larvae and were killed on days 8, 12 and 16 after primary infection. Each group consisted of between 5-7 mice. A 5 cm section of the small intestine was removed from three mice per group and examined for the mast cell response. The entire small intestine of the remaining mice in each group and the rest of the small intestine of the former mice were incubated for worm recoveries as described previously.

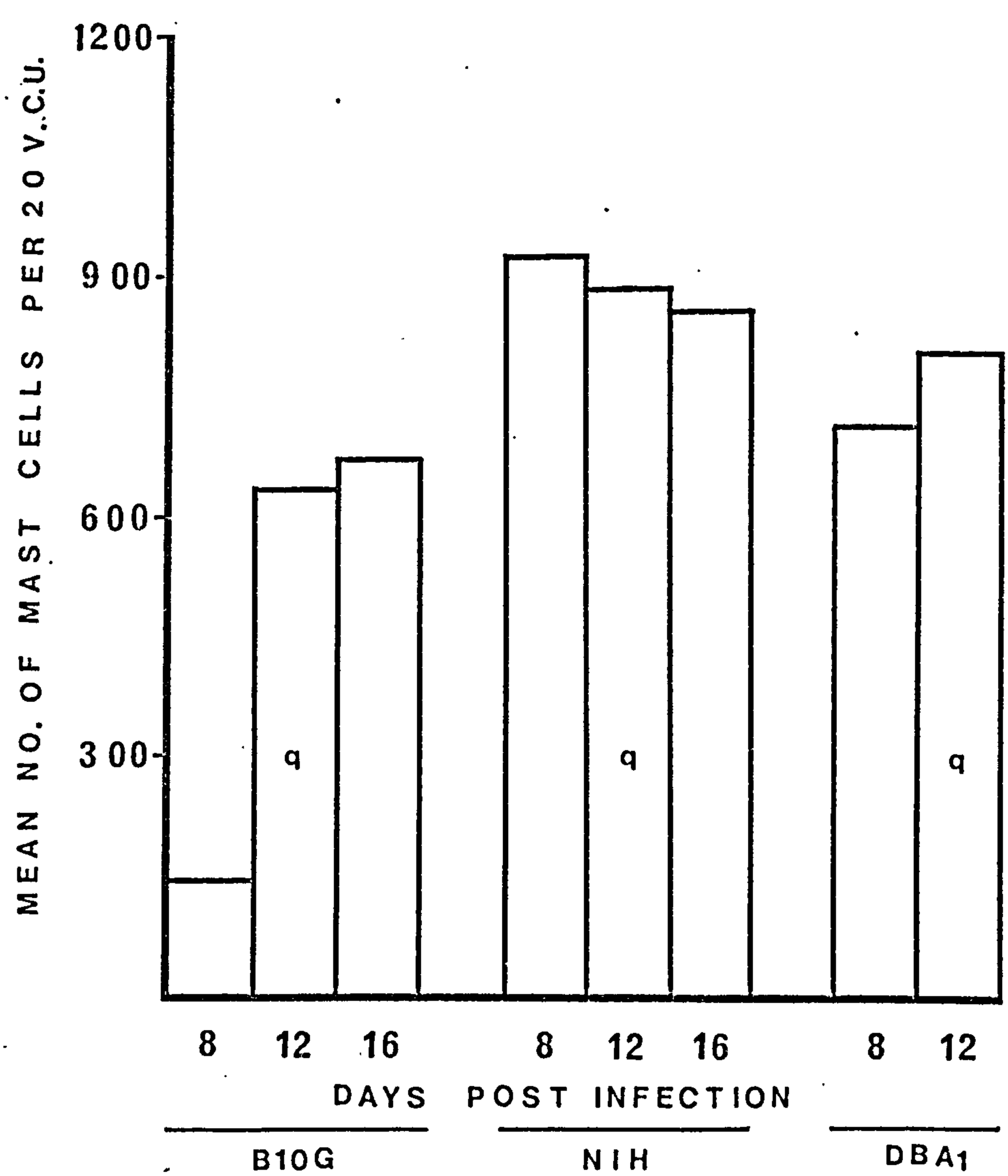
A. Kinetics of mast cells in H-2 identical strains of mice with different genetic backgrounds

The strain of mice used were NIH, DBA_1 and B10G, all carrying the $H\text{-}2^q$ haplotype. In terms of worm expulsion NIH and DBA_1 mice are rapid responders and B10G mice are slow responders (Wakelin, 1980a).

The mean number (±S.D.) of mast cells per 20 V.C.U. for each strain over the period of infection, is shown below and the overall pattern of the mast cell response is shown in Fig. 6.1.

Fig. 6.1

Mean numbers of the mast cells per 20 V.C.U. in rapid responder (NIH and DBA_1) and slow responder (B10G) mice after infection with T. spiralis.


MEAN NO. OF MAST CELLS PER 20 V.C.U.
1200
900
600
300
q
q
q
8
12
16
8
12
16
8
12
DAYS POST INFECTION
B10G
NIH
DBA1

Mean number (±S.D.) of mast cells per 20 V.C.U.

		Days post infection					
		8		12		16	
Strain	Haplotype	Mean	S.D.	Mean	S.D.	Mean	S.D.
B10G	q	140	50	625	86	662	98
NIH	q	905	102	867	70	845	80
DBA_1	q	704	76	792	104	not done	

The results show that B10G mice had a lower mast cell response than NIH or DBA_1 mice on day 8 post infection, but the level of mast cells was similar to that of NIH and DBA_1 on days 12 and 16 post infection. Few mast cells were present in the intestinal mucosa of control uninfected mice of each strain and these values have been omitted from the figure.

Worm recoveries indicated that worm loss in NIH and DBA_1 mice occurred between days 8 and 12 post infection. However, in B10G mice the majority of the worms were still present on day 16 post infection.

B. <u>Kinetics of the mast cell response in strains of mice of identical genetic background (B10) which carry different H-2 haplotypes</u>

In this experiment similar numbers of mice

were used and group treatments were as above. The strains of mice used were C57BL/10, B10.D2, B10.BR and CBA. With the exception of the CBA mice, all the mice are of the B10 background, but carry different H-2 haplotypes (see below). In terms of worm expulsion, CBA mice are rapid responders and all of the B10 background mice are slow responders (see Wakelin, 1980a).

The mean number (±S.D.) of mast cells per 20 V.C.U. for each strain of mice over the period of infection is shown below and the overall pattern of the mast cell response is shown in Fig. 6.2.

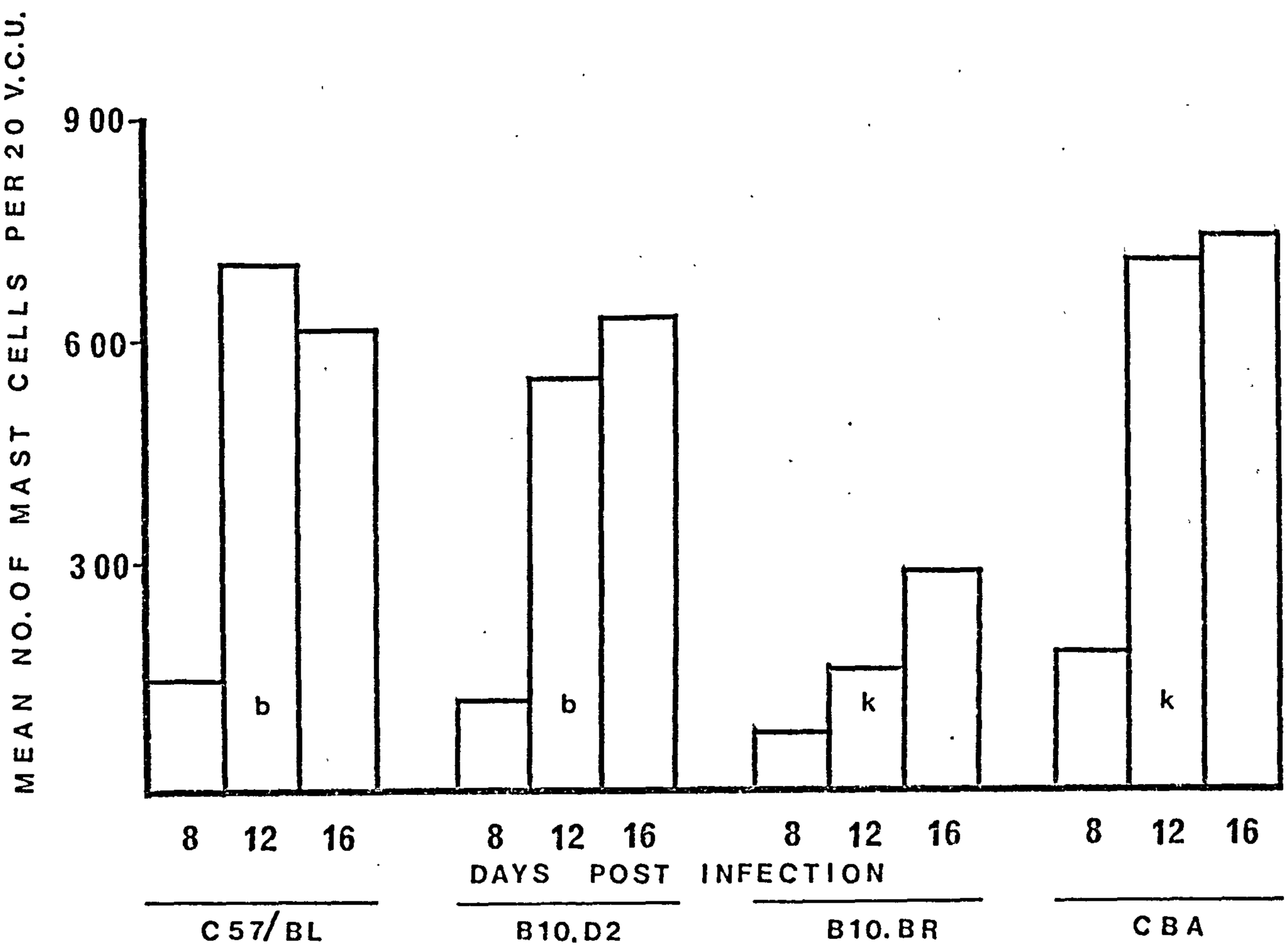
Mean number (±S.D.) of mast cells per 20 V.C.U.

		Days post infection					
		8		12		16	
Strain	Haplotype	Mean	S.D.	Mean	S.D.	Mean	S.D.
C57BL/10	b	148	92	690	80	602	75
B10.D2	d	118	85	542	130	623	82
B10.BR	k	75	50	155	43	285	47
CBA	k	176	67	694	78	728	74

All mice with the B10 background showed a low level mast cell response when examined on day 8 post infection, but the level had risen to that of rapid

Fig. 6.2

Mean numbers of mast cells per 20 V.C.U. in slow responder mice (C57/BL, B10.D2, B10.BR) of identical genetic background (B10) but carrying different H-2 haplotypes and also in CBA mice (rapid responder, H-2^k) after infection with T. spiralis.


MEAN NO. OF MAST CELLS PER 20 V.C.U.
900
600
300
b
b
k
k
8 12 16
8 12 16
8 12 16
8 12 16
DAYS POST INFECTION
C57/BL
B10.D2
B10.BR
CBA

responders mice (NIH and DBA_1) by day 16 post infection. The levels of mast cells in B10.BR mice remained consistently lower than the other strains. The number of mast cells in CBA mice was considerably lower than comparable values in NIH or DBA_1 on day 8 post infection, but was similar to that of C57BL/B10 and B10.D_2 mice on days 12 and 16 post infection (compare Fig. 6.1 and Fig. 6.2). Few mast cells were present in the intestinal mucosa of control uninfected mice of each strain and the data have been omitted from the figure.

Worm recoveries indicated that on day 16 post infection the majority of the worms were still present in the intestines of the B10 background mice, but in the CBA mice most of the worms were rejected between days 12 and 16 post infection.

C. Kinetics of the mast cell response to infection with T. spiralis in B10G x NIH F_1 hybrids

To examine whether the mast cell response during a T. spiralis infection is genetically determined, F_1 hybrids between B10G and NIH mice were used (see materials and methods). Mice of each strain together with F_1 hybrids were killed and examined on days 8 and 12 post infection.

The mean number (±S.D.) of mast cells per

20 V.C.U. for each group of mice is shown below; the overall pattern of the mast cell response is given in Fig. 6.3.

Mean number (±S.D.) of mast cell per 20 V.C.U.

	Days post infection			
	8		12	
Strain	Mean	S.D.	Mean	S.D.
B10G	115	42	652	67
NIH	976	130	895	76
F_1 (B10GxNIH)	585	115	948	83

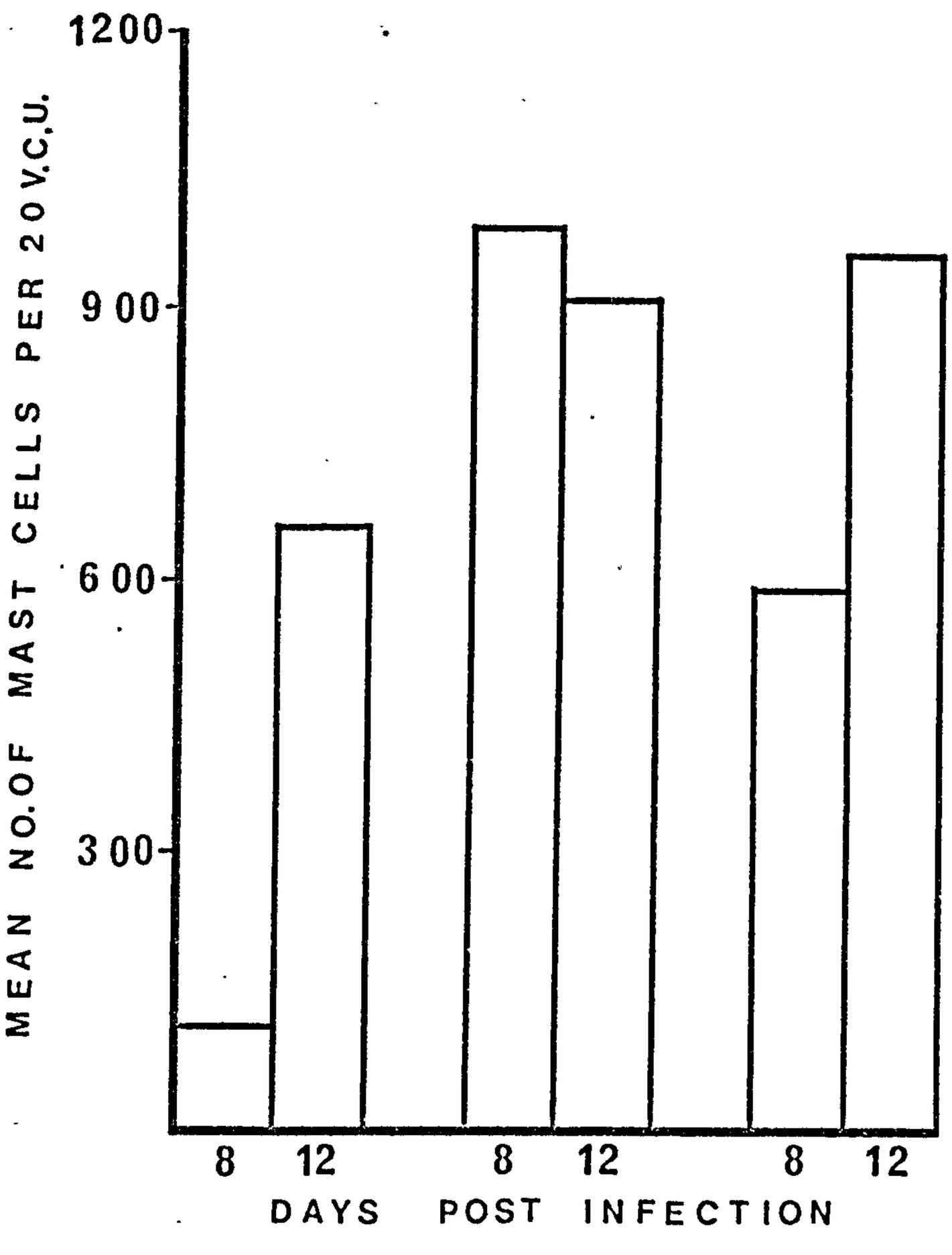
F_1 hybrids showed considerably higher mast cell numbers than the B10G mice, but the numbers were lower than in NIH mice on day 8 post infection. By day 12 post infection, the level of mast cells in the F_1 hybrid mice was similar to that of the NIH mice and still higher than the B10G mice.

2. <u>The effect of transferring immune B10G or NIH MLNC upon the mast cell response in normal mice</u>

It has been shown that immune MLNC from the rapid responding NIH mice are capable of accelerating worm expulsion from the slow responding B10G mice (both carry H-2^q haplotype). Expulsion, however, was slower

Fig. 6.3

Mean numbers of mast cells per 20 V.C.U. in B10G mice (slow responders), NIH mice (rapid responders) and F_1 hybrids between B10G and NIH after infection with T. spiralis.


1200
900
600
300
MEAN NO.OF MAST CELLS PER 20 V.C.U.
8
12
8
12
8
12
DAYS POST INFECTION
B10G
NIH
F1 (B10GXNIH)

in B10G recipients of NIH cells than in homologous recipients (see Wakelin and Donachie, 1980). The following experiments were carried out to examine the effect upon the mast cell response of transferring immune MLNC between and within rapid responder and slow responder mice.

The cells responsible for accelerated worm expulsion in both B10G and NIH mice are present in the MLN between days 4 and 8 post infection (Wakelin, 1977a; Wakelin and Donachie, 1980) and it is known that these cells are also capable of transferring an accelerated mast cell response in NIH mice at least. MLNC from uninfected donors have no effect upon either worm expulsion or the mast cell response (see chapter 5). In these experiments, cells were taken from donors which had been infected with approximately 300 T. spiralis larvae for 6 days.

a. B10G recipient mice

Nine B10G mice were divided into three groups, each containing three mice (groups 1-3). Mice from group 1 received no cells; group 2 received $2x10^7$ immune cells from B10G donors, while group 3 received $2x10^7$ immune cells from NIH donors.

b. NIH recipients

Nine NIH mice were divided as above into three groups of three mice (groups 4-6). Mice from group 4

received no cells; group 5 received $2x10^7$ immune cells from NIH donors and group 6 received $2x10^7$ immune cells from B10G donors. All the mice in groups 1-6 were infected on day of cell transfer (day 0) and were killed 6 days after infection.

The mean number (±S.D.) of mast cells for each group is shown below and the overall pattern of the mast cell response, is shown in Fig. 6.4.

Mean number (±S.D.) of mast cells per 20 V.C.U. 6 days after infection

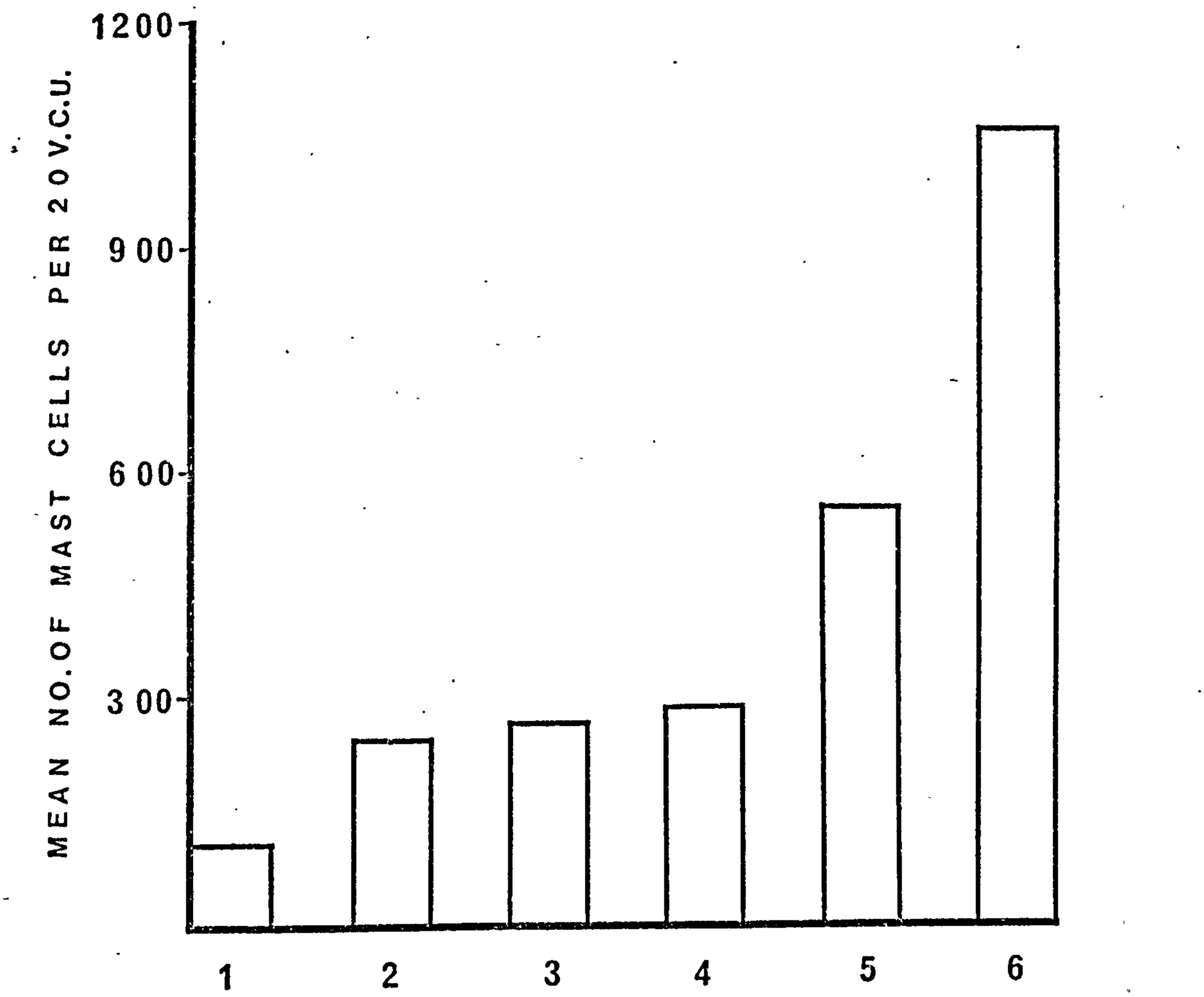
Group	Recipients	Donors	Mean	S.D.
1	B10G	No cells	105	48
2	B10G	B10G cells	242	57
3	B10G	NIH cells	260	40
4	NIH	no cells	286	32
5	NIH	NIH cells	544	61
6	NIH	B10G cells	1042	96

In control B10G mice (group 1 - no cells) the number of mast cells was lower than in comparable NIH mice (group 4 - no cells). Immune cells from either B10G or NIH donors resulted in an increase in the numbers of mast cells in B10G recipient mice (groups 2 and 3 respectively), but in comparison with NIH mice given immune cells from NIH or B10G mice the numbers of mast cells remained

Fig. 6.4

Mean numbers of mast cellsper 20 V.C.U. in normal recipients, B10G or NIH mice received no cells, or immune MLNC from B10G or NIH donor mice. All mice were killed on day 8 post infection. Immune MLNC given to mice on day 0 and mice were infected.

Group	Recipients	Donors
1	B10G	no cells
2	B10G	B10G cells
3	B10G	NIH cells
4	NIH	no cells
5	NIH	NIH cells
6	NIH	B10G cells


MEAN NO.OF MAST CELLS PER 20 V.C.U.
1200
900
600
300
1
2
3
4
5
6

relatively low. In NIH mice the numbers of mast cells rose to high levels (groups 5 and 6 respectively), with the B10G cells, in fact, being more effective than the NIH cells (compare groups 5 and 6).

3. The effect of transferring immune B10G and NIH MLNC on the mast cell response in irradiated mice

To distinguish between the possibilities that the mast cell response in recipients was induced by the cells transferred or arose by direct transformation of these cells, cell transfers were carried out using irradiated recipients. Irradiation (400 rad) is known to ablate the intestinal mast cell response to infection and the transfer of homologous immune cells can partially restore the mast cell response in irradiated NIH mice (see chapter 5).

Here again adoptive transfer of immune cells was reciprocal between rapid and slow responding strains of mice. The experimental design was similar to that of the above experiment, but recipients (NIH or B10G mice) were irradiated (400 rad) on day -1 and received immune cells and infection on day 0. Mice were killed and the mast cell response examined on day 8 post infection.

The mean number (±S.D.) of mast cells per 20 V.C.U. for each group is shown below and the overall

pattern of the mast cell response is shown in Fig. 6.5.

Mean number (±S.D.) of mast cells per 20 V.C.U. in irradiated recipient mice 8 days after infection

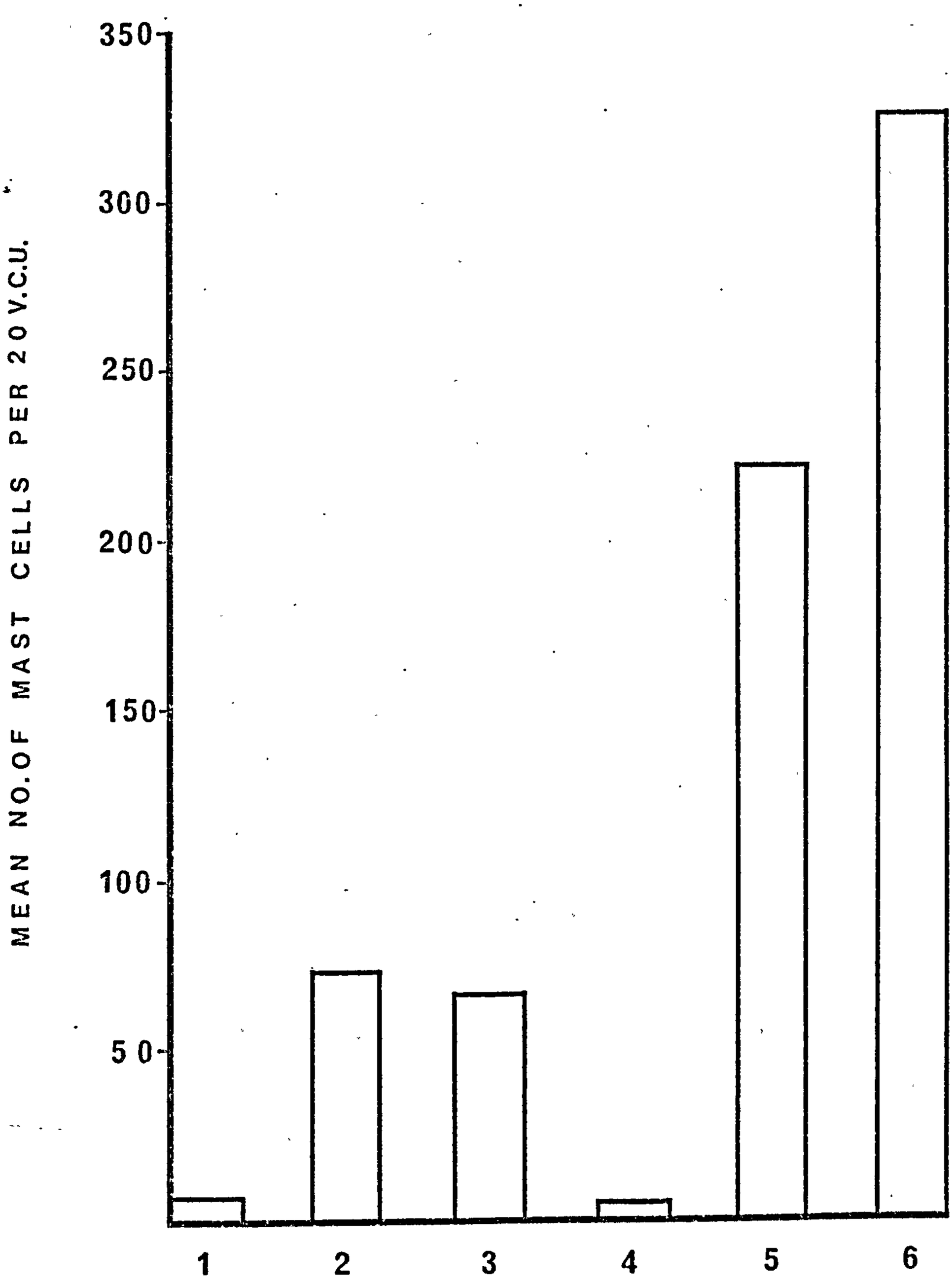
Group	Recipients	Donors	Mean	S.D.
1	B10G	No cells	6	3
2	B10G	B10G cells	72	25
3	B10G	NIH cells	66	18
4	NIH	No cells	5	2
5	NIH	NIH cells	218	30
6	NIH	B10G cells	321	27

Mast cells were almost completely absent in the irradiated control B10G and NIH mice. There was, however, an increase in the mast cell response in B10G mice given B10G or NIH immune cells (groups 2 and 3 respectively), although, as expected in irradiated mice, the number of cells was much lower than would be expected in normal mice. Immune cells from NIH and B10G mice also gave rise to an enhanced mast cell response in NIH recipient mice (groups 5 and 6) and the number of mast cells was higher in these mice than in B10G recipients (groups 5 and 6 as compared with groups 2 and 3). As before the mast cell response was greatest in NIH mice which received B10G cells (group 6).

Fig. 6.5

Mean numbers of mast cells per 20 V.C.U. in irradiated (400 rad) B10G or NIH mice which received no cells or immune MLNC from B10G or NIH donor mice. All the mice were killed on day 8 post infection. Immune MLNC were given to mice irradiated on day -1 and infected on the day of cell transfer (day 0).

Group	Recipients	Donors
1	B10G	No cells
2	B10G	B10G cells
3	B10G	NIH cells
4	NIH	no cells
5	NIH	NIH cells
6	NIH	B10G cells


MEAN NO.OF MAST CELLS PER 20 V.C.U.
350
300
250
200
150
100
50
1
2
3
4
5
6

4. <u>The effect on worm expulsion of transferring immune MLNC from NIH and B10G mice into NIH or B10G recipients</u>

The ability of immune MLNC to transfer immunity was determined using additional groups of mice given either NIH or B10G immune cells.

Fifteen NIH mice were divided into three groups of five mice each (groups 1-3). Mice from group 1 received no cells; group 2 received $2x10^7$ NIH cells; and group 3 received $2x10^7$ B10G cells. All the mice were infected on the day of cell transfer (day 0) and were killed on day 8 post infection.

The mean number of worms recovered is shown in Table 6.1. The number of worms in the cell recipient groups (i.e. 2 and 3) was significantly lower than in control mice (group 1 no cells), indicating that immune cells from either NIH or B10G were able to transfer immunity in NIH recipient mice. It can be also noted that B10G cells appear to be more effective than NIH cells.

A similar experimental design was used with B10G recipient mice which were given either NIH or B10G immune cells. Here,however some of the mice died during the process of transferring cells leaving insufficient numbers for the data to be included. However, three mice which were killed on day 12 post infection, showed that in B10G recipient mice given NIH cells worm loss had occurred by day 12 post infection.

Table 6.1

The effect on worm expulsion of transferring immune MLNC from NIH or B10G mice into NIH recipients

			Worm recoveries on day 8 p.i.	
Group	Recipients	Donors	Mean	S.D.
1	NIH	no cells	174.8	24.3
2	NIH	NIH	73.6*	15.4
3	NIH	B10G	37.5*	21.7

* Mean significantly lower than control

$p < 0.05$

DISCUSSION

It has been shown that the difference between rapidly and slowly responding strains of mice in their ability of expel a T. spiralis infection is under genetic control, with the rapid response being inherited as a dominant characteristic (Wakelin, 1980b). It is known that both immunological and inflammatory components are involved in the expulsion of T. spiralis from mice. Differences in time of worm expulsion between rapid responder and slow responder mice could therefore arise as a result of genetic influences in either of these components of expulsion. Further investigation by Wakelin and Donachie (1980) has shown that both the time course of expulsion and the generation of the inflammatory response are linked to the genetic background rather than to the H-2 type. i.e. are non H-2 controlled. The inflammatory components involved are not known, but it has been suggested that mast cells participate in this response (Askenase, 1977). Attention, therefore, was focussed on the role of H-2 and non H-2 genes in the generation of the mast cell response in rapid and slow responder mice.

When a number of strains of mice carrying H-2^q haplotype but different genetic backgrounds were examined B10G mice (slow responders) showed a delay in the generation of the mast cell response as assessed at day 8

post infection, when compared with NIH and DBA, mice (rapid responder mice). However, the numbers of mast cells were similar in all three strains of mice by days 12 and 16 post infection. The results obtained from the congenic strains of mice, which share the same slow-responder background genes (B10) but differ in H-2 haplotypes, showed that the mast cell response was low on day 8 post infection, but similar to rapid responders when assessed on days 12 or 16 post infection. One congenic strain, B10.BR mice ($H\text{-}2^k$), showed even lower mast cell numbers during the period of examination than the other strains of B10 mice. This greater delay of the mast cell response is not linked with the $H\text{-}2^k$ haplotype because the mast cell response in CBA mice, which also carries the $H\text{-}2^k$ haplotype, was similar to that of the other B10 background mice, i.e. a delay in the generation of mast cell response as assessed at day 8 post infection, but a subsequent rise after this time. The results presented here show that the strain characteristics of the mast cell response are genetically determined, and that non H-2 genes may control the generation of the mast cell response in the early phase of infection.

In NIH and DBA_1 mice (rapid responders) it was noted that worm rejection occurred between days 8 and 12 post infection, but in all B10 background mice (slow responders) the majority of the worms were still present

on day 16 post infection, confirming the result of Wakelin (1980a).

F_1 hybrids between B10G and NIH mice showed an intermediate pattern of the mast cell response, which resembled that of NIH mice (rapid responders) suggesting the response is inherited as a dominant characteristic (although it appears with less than complete penetrance). Wakelin (1980a) also noted that the pattern of worm rejection in F_1 hybrids between B10G and NIH mice was similar to that of NIH mice.

The effect upon the mast cell response of adoptive transfer of immune MLNC between and within rapid and slow responder strains of mice showed some interesting results which can be summarised as follows:

1. Immune lymphocytes from rapid responder (NIH mice) enhanced the mast cell response in normal or irradiated NIH mice, but had only a small effect on the number of mast cells in B10G mice.

2. Immune lymphocytes from B10G mice gave rise to an enhanced mast cell response in normal and irradiated NIH mice, but had less effect in B10G mice.

It is clear that enhanced acceleration of the mast cell response is independent of the strain of donor from which the immunocompetent cells were obtained, as

both NIH and B10G cells were able to cause mastocytosis in recipient mice of the homologous and heterologous strains. The generation of the mast cell response appear to be influenced more by the identity of the recipient than by the origin of the cells, thus when B10G recipient mice were given NIH or B10G cells the level of mast cells was always lower than in NIH mice given either B10G or NIH cells.

It is possible that the delayed mast cell response in slow responder mice may be due to some positive suppression, but at present this cannot be differentiated from a slow generation of the non-lymphoid components of the response. Certainly slow responders appear to develop the lymphoid component as rapidly as do rapid responders.

The results showed that accelerated worm expulsion occurred in NIH recipients of either NIH or B10G cells. Wakelin and Donachie (1980) have also demonstrated that immune MLNC from slow responder mice (B10G) transferred immunity to rapid responders as effectively as did homologous cells. Therefore, if expulsion of T. spiralis is associated with a T-dependent inflammatory response which is controlled by non H-2 genes, the results suggest strongly that there is a close correlation between this response and intestinal mastocytosis, although, at present it is not possible to identify the nature of this correlation.

In general it appears that B10G non-H-2 genes regulate intestinal mastocytosis at a slower rate and at a lower level; NIH non H-2 genes regulate a faster response. The interaction between nonlymphoid and lymphoid components which causesthe expression of such diversity can, at present, be only a matter for speculation.

It could be that there are some inherent properties of the myeloid precursors which regulate their response to lymphocyte signals. Alternatively, the recipients could well be capable of suppressing or depressing lymphocyte function or affecting mast cell replication by a negative feedback mechanism.

GENERAL DISCUSSION

In preliminary studies the response characteristics of NIH mice and Wistar rats to infection with Trichinella spiralis have been defined. Primary infections in both hosts were expelled towards the end of the second week of infection.

The mechanisms which underlie the immune expulsion of T. spiralis from mice and rats are controversial. The available evidence suggests that the immediate cause of worm expulsion from mice is associated with inflammatory changes in the intestine (Larsh and Race, 1975; Wakelin, 1978a) although there is some evidence that in rats a direct effect of IgA antibody may play an important role (Despommier et al., 1977).

The immunity engendered by a primary infection is biphasic in expression against a challenge infection, consisting of a rapid expulsion response which eliminates the majority of the parasites and a later slow response which completes expulsion. Early or rapid expulsion appears to operate as a major protective response against T. spiralis in rats in that more than 90% of a challenge infection is rejected within a short period of time. Rapid expulsion also occurs in mice, but it does not seem to form a major lasting component of immunity to challenge infection.

The induction and expression of rapid expulsion in mice has been examined and compared with the response operative in rats.

In chapter 1 the basic parameters of the induction and expression of rapid expulsion were defined. The stage specificity of the induction and expression of rapid expulsion was examined in chapter 2. As has been discussed in chapters 1 and 2, the rapidity of the response was postulated to result from the inflammatory changes induced by the primary infection. The third chapter of the thesis therefore deals mainly with attempts to monitor the inflammatory changes in the intestine by histological examination of mast cell and goblet cell numbers during infection, as well as the onset and persistence and possible involvement of those cells in the rapid expulsion response.

Due to the complexity of the experimental results involved, a summary of the major findings of these three chapters is presented as a comparison of the rapid expulsion response between mice and rats (see table below).

Comparison of rapid expulsion of T. spiralis in NIH mice and Wistar rats

Parameters	Mice	Rats
1. Effectiveness	More than 90%	More than 90%
2. Persistence	After a single complete infection expressed until day 16 p.i.	After a single complete infection expressed at least for 7 weeks
3. Size of primary infection	Required large numbers of larvae	Required small numbers of larvae
4. Priming with irradiated larvae	Did not induce rapid expulsion	Induced rapid expulsion
5. Minimum duration of primary infection for priming	9 days	Less than a week
6. Size of challenge infection	Effective against both large and small challenge infections	(Effective against both large and snall challenge infections; Bell and McGregor, 1979a)
7. Number of drug abbreviated infections necessary to induce rapid expulsion	More than two	One
8. Persistance of rapid expulsion induced by repeated drug abbreviated infections	Expressed at least for 5 weeks	Expressed at least for 9 weeks
9. Stage specificity	Expressed against both larvae and adult	(Not expressed against adult, Bell and McGregor, 1979b)
10. Cellular response	Increased levels of both mast cells and goblet cells after primary infection	Increased levels both mast cells and goblet cells after primary infection
11. Persistence of cellular response	Declined quickly	Remained above control levels for a prolonged period

The most prominent observable cellular response was the increase in numbers of mast cells in the intestinal mucosa. In a primary infection the increase in the mast cell numbers coincided with inflammatory changes in the intestine and subsequent worm expulsion.

The use of drug abbreviated infections showed that:

1) The onset and persistence of the mast cell response is dependent on the duration of infection.

2) Repeated infections (3 days duration) result in an increase in the number of mast cells.

3) The mast cell response was amplified in terms of rapidity of onset and magnitude after a challenge infection in immune mice (which suggests a "memory" for the mast cell response).

If the rapid expulsion of a challenge infection results from environmental changes in the intestine induced by inflammation, then mast cells or their products may be involved in the inflammatory response itself (Askenase, 1977) or may amplify the effect of this response by influencing the release of mucus from goblet cells. It would be useful to devise a technique to study the phenomenon of mucus trapping of T. spiralis in mice, as it has been shown that this mechanism is involved in the rapid expulsion response in rats.

The presence of mast cells and perhaps reaginic antibodies raises the possibility that challenge larvae

may provoke a reaction on entry into the intestine and this could be an important element in the mechanism of rapid expulsion. Such a reaction might well lead to increases in local concentrations of factors such as peroxidases and amines (which have been suggested to have a role in the inflammatory response to, and primary expulsion of, some parasites) and these may be involved in the rapid expulsion response.

The results from chapter 4 imply that the rapid expulsion response in mice was specific to a T. spiralis challenge infection after a T. spiralis primary infection. Priming provided by N. dubius or by N. brasiliensis failed to induce rapid expulsion. Thus, if rapid expulsion is dependent on inflammatory or other changes in the local intestinal environment, these changes are quite different from those produced by unrelated parasites and are specific to T. spiralis infection. The differences between mice and rats in terms of the specificity of the response and the involvement of specific and non-specific factors (Bell and McGregor, 1980a, b) in rapid expulsion may be due to the involvement of different mechanisms. Attempts were made to induce rapid expulsion by providing non-specific intestinal stimulation with N. dubius and parenteral immunization with larval antigen, but these failed to induce rapid expulsion in mice. As it has been suggested in chapter 4, this failure may be due to immunosuppression induced by N. dubius in the mice (which

would not be observed in rats) or may possibly arise from the experimental protocol and method of immunization with larval antigen employed. Further studies along these lines should include this method of immunization, but with another nematode parasite for intestinal stimulation.

The use of the "washing out" technique had the advantage of differentiating between worms loose in the lumen (and capable of being washed out) and worms which were somehow more closely associated with the mucosal tissue (and therefore not susceptible to washing out). The results indicated that the majority of the parasites failed to establish in the intestine of immune mice at the time when the mice are capable of a rapid expulsion response. This may suggest that even a minimum of inflammation is unsuitable for parasite establishment.

Challenge infections had no influence on injected MLN lymphoblast homing in immune mice, but blast cell activity was quite high in immune mice 1 day after challenge. Although there is variation in the data from this experiment, the results warrant closer examination and confirmation since, if there is an increase in blast cell activity during rapid expulsion, this may indicate that an active immune mechanism is involved in the response.

The rapid expulsion of repeated challenge infections indicates that under these conditions the

factors necessary for rapid expulsion can be persistent. The effects of immunosuppressive drugs on the rapid expulsion response highlight the importance of cellular components.

The most important findings in the second part of chapter 5 are summarised as follows:

1) Immune MLNC transferred immunity (in terms of worm expulsion) to unirradiated and irradiated (400 rad) recipient mice.

2) The mast cell response could be transferred with immune MLNC to irradiated and unirradiated mice and an enriched T cell but not B cell fraction of the immune MLN cells also transferred this response. It is possible that immune MLNC, particularly T cells, are able to act as helpers, activators or precursor cells in regulating the intestinal mast cell response.

3) Control MLNC were not effective in transferring a mast cell response to either irradiated or unirradiated mice.

4) The mast cells or precursors of mast cells are radio-sensitive and antigenic stimulation is necessary for differentiation of mast cells.

5) Bone marrow cells alone were not effective in transferring the mast cell response to irradiated (650 rad) mice.

6) Rapid expulsion was ablated by irradiation (400 rad) but could be restored by the transfer of immune, but not control, MLNC.

It appears that there is relationship between the transfer of immune MLNC, the mast cell response and subsequent rapid expulsion of challenge infection. It was concluded at the end of chapter 5 that if "primary type" expulsion and "rapid expulsion" are associated with T cell dependent inflammatory changes in the intestine, the major expression of this response could be through the mast cells. If this is so, it would be interesting to investigate the nature of these cells especially their morphology, degranulation, IgE binding capacity, origin, histochemical characteristics and the mechanisms which control differentiation and proliferation.

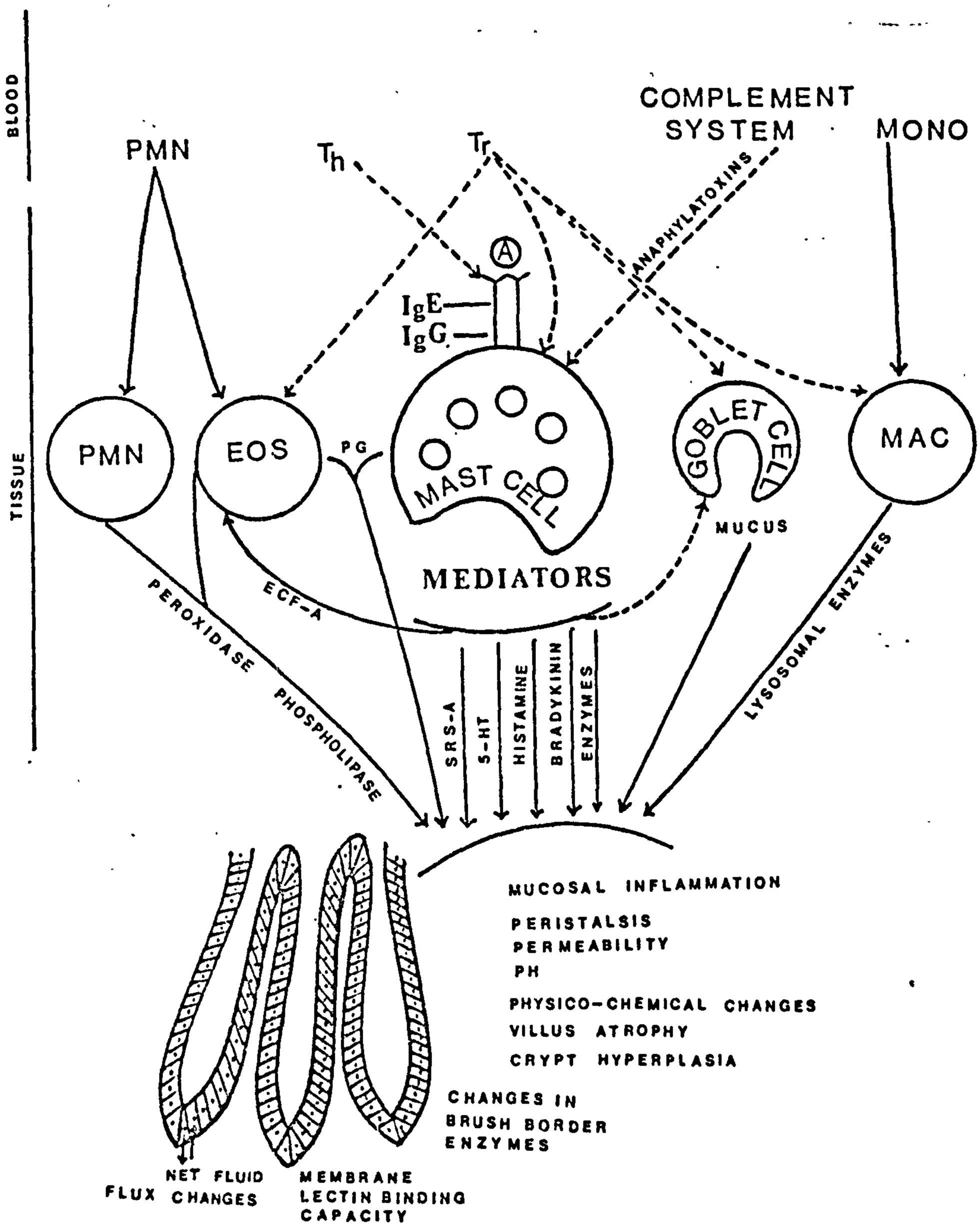
The studies of the mast cell response to infection with T. spiralis in slow and rapid responder mice showed interesting results. The strain characteristics of mast cell responses appear to be genetically determined and non-H-2 genes may control the generation of mast cell responses in the early phase of infection. It is possible that the delayed mast cell response in slow responder mice may be due to a positive suppression or to slow generation of either the lymphocyte or bone marrow components.

It is known that a variety of specific and non-specific factors are involved in resistance to

T. spiralis. In an attempt to clarify a response of such enormous complexity a diagrammatic summary is provided to relate the numerous components which combine to expel T. spiralis from its host (p. 283).

Fig. D.1

A	Antigen
B	B cell
EFC-A	Eosinophil chemotactic factor-A
EOS	Eosinophils
MAC	Macrophages
MONO	Monocytes
PG	Prostaglandins
PMN	Polymorphonuclear leukocytes
SRS-A	Slow-reacting substance-A
T_h	T helper cell
T_r	T regulatory cell


BLOOD
TISSUE
PMN
Th
Tr
COMPLEMENT SYSTEM
MONO
ANAPHYLATOXINS
A
IgE
IgG
PMN
EOS
PG
MAST CELL
GOBLET CELL
MAC
MUCUS
MEDIATORS
ECF-A
PEROXIDASE
PHOSPHOLIPASE
LYSOSOMAL ENZYMES
SRS-A
5-HT
HISTAMINE
BRADYKININ
ENZYMES
MUCOSAL INFLAMMATION
PERISTALSIS
PERMEABILITY
PH
PHYSICO-CHEMICAL CHANGES
VILLUS ATROPHY
CRYPT HYPERPLASIA
CHANGES IN BRUSH BORDER ENZYMES
NET FLUID FLUX
MEMBRANE CHANGES
LECTIN BINDING CAPACITY

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MECHANISM OF RAPID EXPULSION OF TRICHINELLA SPIRALIS FROM MICE

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INTRODUCTION

When challenged with T. spiralis immediately after a primary infection, NIH mice show a strong and rapid expulsion response (RE). RE is coincident with high mast cell numbers in the intestinal mucosa (1). The mechanisms underlying RE were examined, using a) repeated challenge of previously infected mice, b) immune suppressive treatments and c) adoptive transfer with immune mesenteric lymph node cells (IMLNC) in normal and irradiated recipients.

MATERIALS AND METHODS

Male NIH inbred mice, 7 weeks old, were used in groups of six. The methods used have been described previously (2); the infective dose was 300 larvae/mouse. IMLNC taken from donors 8 days after infection were prepared and transferred as described (3). Mice were irradiated at 1050 rad/min using a 60 Co source. Drugs were given as follows: cortisone acetate (Boots) subcutaneously at 2.5 mg/kg body weight, cyclophosphamide (WB Pharmaceuticals) intraperitoneally (IP) at 300 mg/kg and reserpine (BDH) IP at 1 mg/kg.

Ten cm portions of the middle small intestine were processed for histology by the Swiss roll technique, fixed in Carnoy's, embedded in paraffin wax, sectioned at 5 µm and stained for mast cells with alcian blue. Numbers of alcian blue-staining cells were counted in 20 villus-crypt units (VCU). Mean values were compared by the Student's t test; $P > 0.05$ was considered non-significant.

RESULTS

Rapid expulsion in repeated challenge infections

Challenge infections given on Days 14 or 16 of a primary infection were expelled rapidly within 24 hours. Mice given an initial challenge infection on Day 14 and a second challenge on Days 15, 16 or 18 post infection (PI) showed RE of the repeat challenge on Days 15 and 16, but on Day 18 the parasites were only partially expelled (Table 1).

Effect of immunosuppressants and irradiation on rapid expulsion

Groups of mice were given cortisone, cyclophosphamide or reserpine on

Days 12 and 13 PI, or irradiated (850 rad) on Day 12 PI. These mice, together with control infected and uninfected mice, were challenged on Day 14 PI and killed 24 hours later. RE was partially prevented only in cortisone-treated or irradiated mice.

Adoptive transfer experiments

The results can be summarized as follows:

1. In unirradiated mice given IMLNC (3.2×10^7) loss of the worms had begun by Day 8. In irradiated (400 rad) recipients loss had begun by Day 12 PI (Figs. 1 and 2).
2. Mast cell numbers were significantly increased by Day 6 and reached a peak by Day 8 in control infected mice. The response was accelerated in mice given IMLNC; mastocytosis developed by Day 4 and numbers of mast cells remained high. Control cells (CMLNC) dit not accelerate mastocytosis in infected mice. The numbers of mast cells remained at normal levels in all uninfected groups (Fig. 1).

TABLE 1: Rapid expulsion of challenge infections in infected mice

Group	Day of 1st challenge	Day of 2nd challenge			Worm recovery (% control)
	14	15	16	18	24 h later
1	none	+	-	-	10
2	+	+	-	-	2
3	none	-	+	-	20
4	+	-	+	-	1
5	none	-	-	+	94
6	+	-	-	+	48

TABLE 2: Effect of MLNC upon rapid expulsion in irradiated mice

Group	Cells transferred		Worm recoveries 24 h after challenge	
Irradiated mice	IMLN	CMLN	Mean	S.D.
1. infected challenge	-	-	127	12
2. infected challenge	3.2×10^7	-	30*	22
3. infected challenge	-	3.2×10^7	129	28
4. noninfected challenge	-	-	157	36
Unirradiated mice				
1. infected challenge	-	-	12*	22
2. infected challenge	3.2×10^7	-	10*	7
3. noninfected challenge	3.2×10^7	-	120	22
4. noninfected challenge	-	-	148	41

* Mean significantly lower than control
Immune cells (IMLNC)
Control cells (CMLNC)

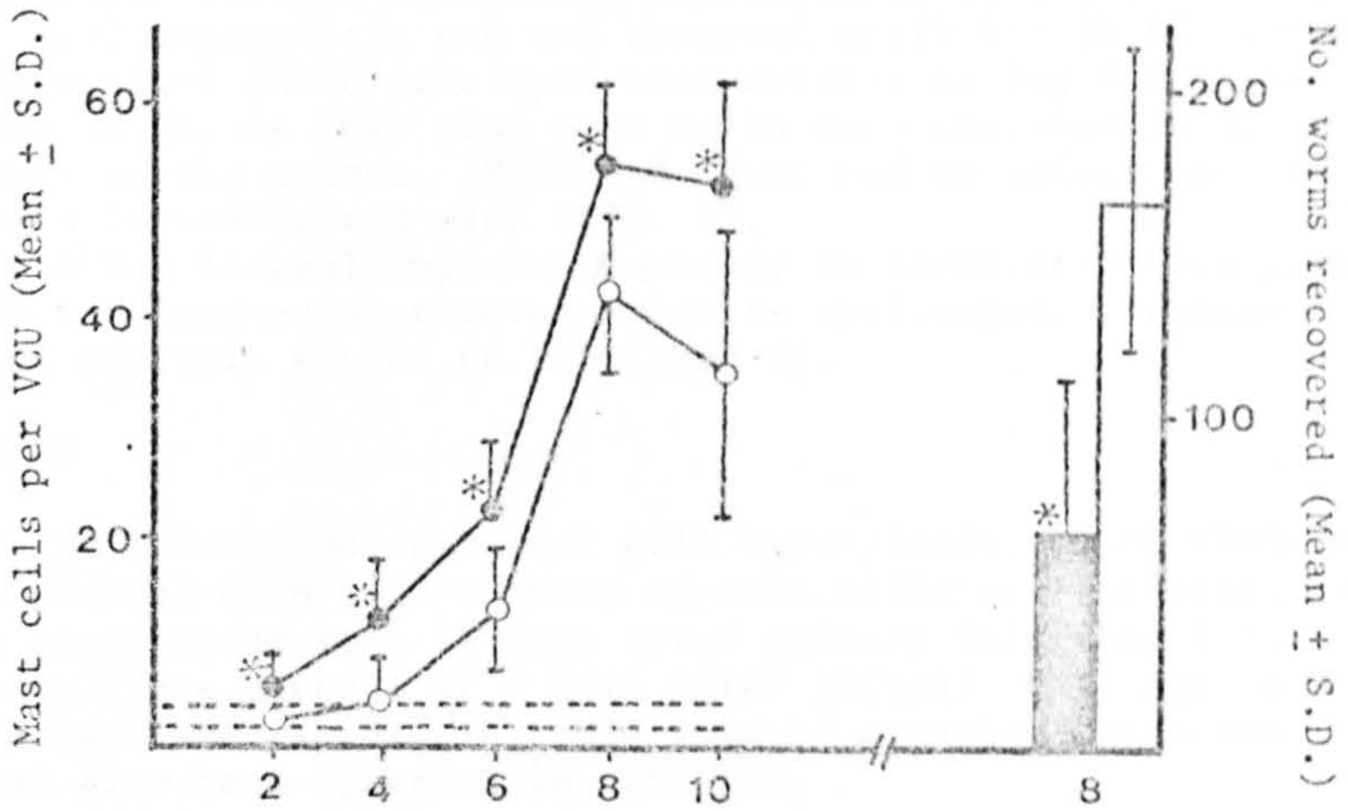

Figure 1

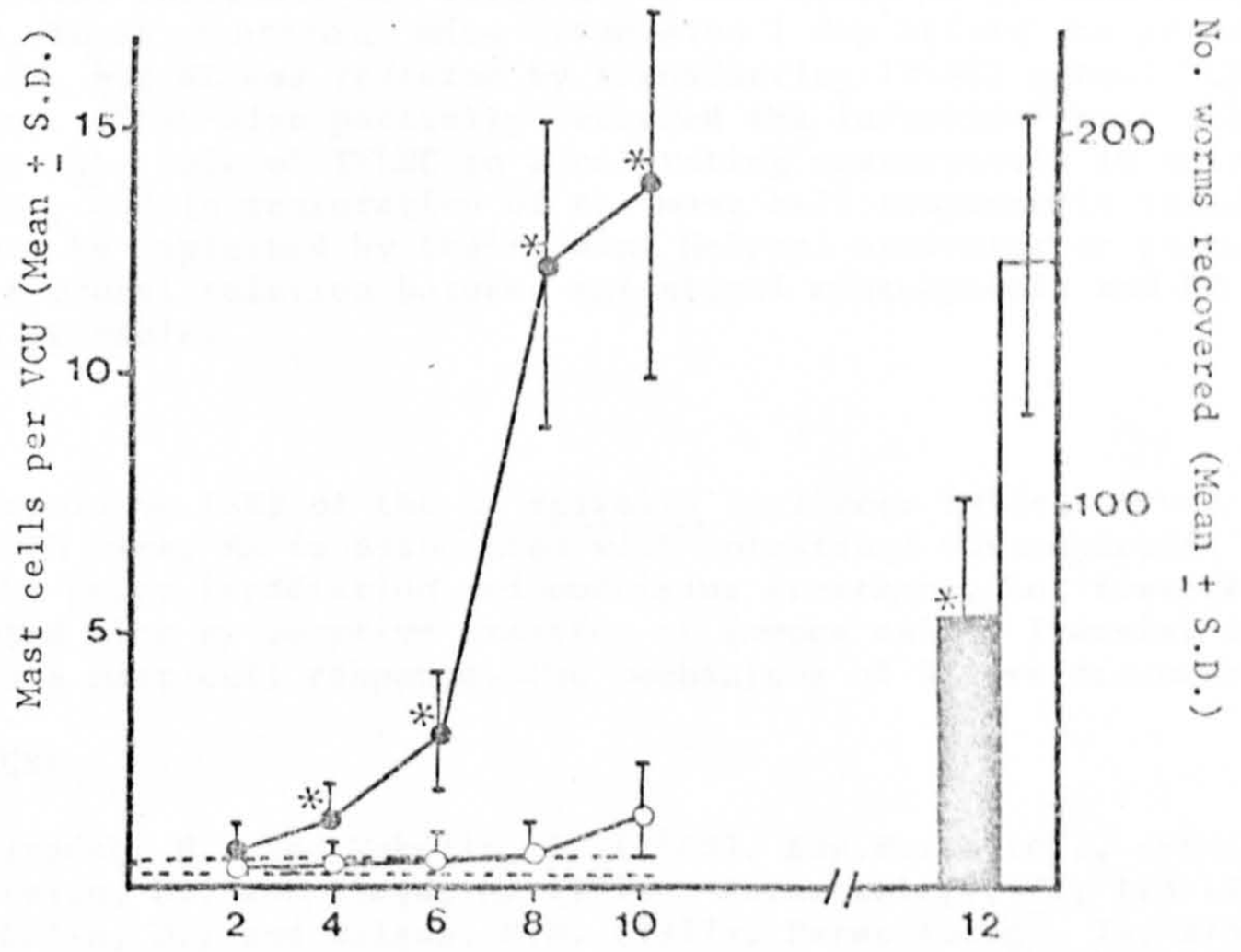

Figure 2

Mast cell numbers in non-irradiated (Figure 1) and irradiated mice (Figure 2). Infected mice given no cells (o——o) or given IMLNC (●——●). ==== Uninfected mice given no cells, IMLNC, or CMLNC. Worm recovery from control (open bars) and IMLNC recipients (closed bars).

*Mean significantly different from corresponding control

3. Irradiation delayed intestinal mastocytosis in infected mice and mast cell hyperplasia was not observed until Day 10 PI. Irradiated recipients of IMLNC developed mastocytosis by Day 4 and this peaked by Day 10 PI. At this time mast cells were observed in discrete patches in the mucosa. IMLNC and CMLNC had no effect upon mast cell numbers in uninfected mice (Fig. 2).
4. RE of a Day 14 challenge occurred only in irradiated mice given IMLNC (primary worms removed prior to challenge); irradiated infected controls showed no RE (Table 2).

DISCUSSION

T. spiralis induced mucosal mast cell hyperplasia. RE of challenge larvae occurred when high numbers of mast cells were present, but did not occur later than 16 days after primary infection (1). A second challenge given 1 or 2 days after initial challenge on Day 14 was also expelled rapidly. A rapid loss of repeated challenge infections has also been observed in rats (4).

RE was partially prevented by cortisone and irradiation (850 rad) given 2 days prior to challenge; reserpine and cyclophosphamide were ineffective. Cortisone and irradiation are known to prevent mastocytosis. No RE occurred in mice irradiated 1 day before the primary infection, but RE was restored by transferring IMLNC; normal MLNC had no effect. IMLNC also partially restored the intestinal mast cell response. The role of IMLNC in accelerating mastocytosis in unirradiated mice, and in restoration of the mast cell response in irradiated mice, may be explained by their being helper, activator or precursor cells. A causal relation between intestinal mastocytosis and RE appears probable.

SUMMARY

Rapid expulsion (RE) of the T. spiralis challenge infection was studied in NIH mice. RE is associated with intestinal mastocytosis, prevented by prior irradiation and cortisone treatment, but restored in irradiated mice by adoptive transfer of immune cells. Transfer also restores a mast cell response. The mechanisms of RE are discussed.

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COMPARISON OF RAPID EXPULSION OF *TRICHINELLA SPIRALIS* IN MICE AND RATS

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Abstract—ALIZADEH H. and WAKELIN D. 1982. Comparison of rapid expulsion of *Trichinella spiralis* in mice and rats. *International Journal for Parasitology* 12: 000–000. Primary infections of *Trichinella spiralis* in both NIH mice and Wistar rats resulted in increased levels of mucosal mast cells and goblet cells. In mice the numbers of both cell types rose sharply before worm expulsion (days 8–10), remained at an increased level for a short time and declined quickly, reaching control levels on day 14 for goblet cells and between days 28 and 35 for mast cells. In contrast, in rats, the numbers of goblet cells and mast cells increased during worm expulsion and remained above control levels for a prolonged period. Challenge infections given shortly after expulsion of a primary infection (day 14) were expelled rapidly, worm loss being virtually complete with 24 h. In mice this response to challenge was short-lived and persisted only until day 16 after primary infection. After this time, challenge worms were expelled more slowly after infection. In rats the rapid expulsion response was expressed for at least 7 weeks after primary infection. Mice and rats showed differences in the conditions of infection necessary to prime for rapid expulsion, mice requiring larger and longer duration primary infections, but the expression of the response appeared to be similar in both species. In mice it was shown that rapid expulsion of *T. spiralis* was a response evoked specifically by prior infection with this species: infections with other intestinal nematodes had no effect. Similarly, the effect upon challenge infection was also specific to *T. spiralis*. The rapidity with which challenge infections are expelled suggests that either the specific inflammatory changes generated during primary infection result in an environment that is unsuitable for establishment of subsequent infections or that challenge infections provide a stimulus that can provoke an almost instantaneous response in the primed intestine. The relationship of the observed cellular changes to such mechanisms is discussed.

INDEX KEY WORDS: *Trichinella spiralis*; nematode parasite; mouse; rat; immunity; rapid expulsion; mast cell; goblet cell; inflammation; specificity.

INTRODUCTION

THE ENTERAL and parenteral stages of *Trichinella spiralis* induce strong protective responses in rodent hosts and there is increasing evidence that these responses are stimulated and expressed in a stage specific manner (Bell, McGregor & Despommier, 1979). During primary infections immunity is associated with a reduction in reproductive capacity of the adult worms and leads ultimately to their expulsion from the intestine (Wakelin, 1978). In secondary infections the expression of immunity is dependent upon the experimental conditions employed. In mice, challenge immunity is most often reflected in reduced worm growth and reproduction and an accelerated expulsion from the intestine (Wakelin & Lloyd, 1976), but when challenge is given late after a primary infection there may be a marked suppression of the expulsive component (Grove, Hamburger & Warren, 1977). In rats, challenge immunity appears to act primarily against the initial stages of infection and the majority of worms may be lost within a very short period, the phenomenon of rapid expulsion (McCoy, 1940; Love, Ogilvie & McLaren, 1976). This aspect of immunity has been studied in an extensive series of papers by Bell & McGregor (1979a, b, 1980a, b) and by Castro and co-workers (Russell & Castro, 1979; Castro, Hessel & Whalen, 1979).

Earlier work indicated that rapid expulsion could occur in mice (Wakelin *et al.*, 1976; Alizadeh & Wakelin, 1981) but that it did not seem to form a major component of challenge immunity and this conclusion is supported by the recent work of Kennedy (1980). In this paper some of the conditions necessary for the induction and expression of rapid expulsion in mice have been examined and compared

with those operative in rats. In order to assess the relevance of inflammatory events to this form of immunity, the onset and persistence of changes induced in the intestine by primary infections have been monitored by two histological parameters, namely the numbers of mast cells and of goblet cells in the mucosa.

MATERIALS AND METHODS

Animals and infections. Male inbred NIH mice (Hacking and Churchill Ltd.) aged approximately 7 weeks were used throughout. Male Wistar rats (bred at the Wellcome Laboratories, Glasgow) were used at 8-9 weeks old. Animals were killed by an overdose of chloroform vapour. The strain of *T. spiralis* and the methods used for maintenance, infection and worm recovery have been described by Wakelin & Lloyd (1976). Unless otherwise stated, primary and challenge infections were with approx. 300 larvae. The maintenance, methods of infection and recovery of *Nematospiroides dubius* have already been described by Jenkins & Behnke (1977). Third stage infective larvae of *Nippostrongylus brasiliensis* were obtained from faecal cultures according to Jennings, Mulligan & Urquhart (1963). Mice were infected by subcutaneous injection of a suspension of larvae in 0·1 ml of balanced salt solution.

Anthelmintics. Methyridine (mintic and promintic, I.C.I.) was used to remove *T. spiralis*. Mintic was given orally to mice at a dose level of 1000 mg/kg body weight; promintic was injected subcutaneously into rats at a dose of 300 mg/kg. Pyrantel embonate (strongid-p-paste, Pfizer) at a dose of 100 mg/kg was used to remove adult *N. dubius* from infected mice. The efficacy of the drugs was determined in infected mice given anthelmintic and killed 1 day later; no worms were observed in the gut at this time. Neither of these drug treatments has any effect upon subsequent infection with *T. spiralis* (Alizadeh, unpublished results).

Statistics. The statistical significance of difference between mean worm recoveries was determined using Students' *t* test. A value of $P > 0{\cdot}05$ was considered to be non-significant.

Histology. Mice or rats were autopsied, the small intestine removed entirely and divided into four segments. Each segment was opened longitudinally and rolled on a syringe plunger with the mucosal surface out (Swiss roll technique of Reilly & Kirsner, 1965). For examination of mast cells, tissues were fixed in Carnoy's fixative for 3–6 h, embedded in paraffin wax and sectioned at 5 μm. Sections were stained with Alcian blue (0·1% w/v in 0·7 N-HCl, Alcian blue 8 GX, Gurr, London) and safranin O (0·5% w/v in 0·125 N-HCl, michrome No. 405, Gurr, London) by modification of the method of Enerback (1966) for staining mast cells. For quantitative purposes all Alcian blue positive cells were recorded, whether in the lamina propria or intraepithelial in position and, as the precise identity of cells in these locations is presently disputed (Askenase, 1980), they are referred to collectively as mucosal mast cells. In each intestinal segment 20 villus crypt units were counted. For staining of goblet cells the segments of small intestine were prepared as for the Swiss roll technique. Tissues were fixed in Bouin Hollande for 24 h, embedded in paraffin wax and sectioned at 5 μm, sections were stained with Periodic acid/Schiff (PAS) and Alcian blue by the methods described by Mowry (1963) and were counter stained with haematoxylin. For quantitative purposes the numbers of mucus producing cells located in the epithelium of the villi and crypts were examined. In each intestinal segment 20 villus crypt units were counted.

RESULTS

The course of a primary infection of *T. spiralis* is similar in both NIH mice and Wistar rats. The pattern in mice has been described by several workers (Wakelin & Lloyd, 1976; Manson-Smith, Bruce, Rose & Parrott, 1979; Kennedy, 1980) and that in rats by Russell & Castro (1979) and Love *et al.* (1976). Preliminary experiments were carried out to confirm these results and the details of both are summarized in Figs. 1 and 2. Briefly, approximately 50% of larvae administered become established in the anterior half of the small intestine and remain there for about 8 days. After this time, when the gut is grossly inflamed, worms are lost rapidly. Worm loss is normally complete by days 12–14 after infection by which time gross inflammatory changes in the intestine have disappeared.

Fate of challenge infections given at intervals after primary infection in mice and rats

Rapid expulsion in rats has been defined as loss of the majority of a challenge infection within 24 h. To identify the times at which rapid expulsion occurred in mice, a total of 30 animals was divided into six groups and the groups were given primary infections at intervals of 35, 28, 21, 18, 16 or 14 days before challenge. All the mice, together with a control group, were challenged on the same day and killed 24 h later (Table 1). Rapid expulsion occurred only in those groups which had been challenged 14 and 16 days after the primary infection. In contrast, in a similar experiment using rats given a primary infection of 2000 larvae and challenged with 500 larvae, rapid expulsion occurred at all time points tested between days 14 and 49 (Table 1).

An attempt was made to induce rapid expulsion on day 21 of a primary infection in mice by increasing the initial infection dose to 500 larvae. The mice were challenged with 300 larvae, but again there was no rapid expulsion, mean worm recoveries being $106 \pm 7{\cdot}6$ and $116{\cdot}0 \pm 11{\cdot}0$ for the challenged and control mice respectively. The immune status of mice at this time was confirmed in a further experiment in which mice were challenged on day 20 of a primary infection but killed 4 days later, i.e. at the time at which the accelerated loss characteristic of conventional challenge immunity should have commenced. The mean worm recoveries at this time were $199{\cdot}0 \pm 44{\cdot}9$ and $70{\cdot}0 \pm 35{\cdot}8$ for control and challenge mice respectively, indicating that the failure

to show rapid expulsion at this time was not associated with any loss of immune capacity.

Time at which worms are lost during the process of rapid expulsion in mice

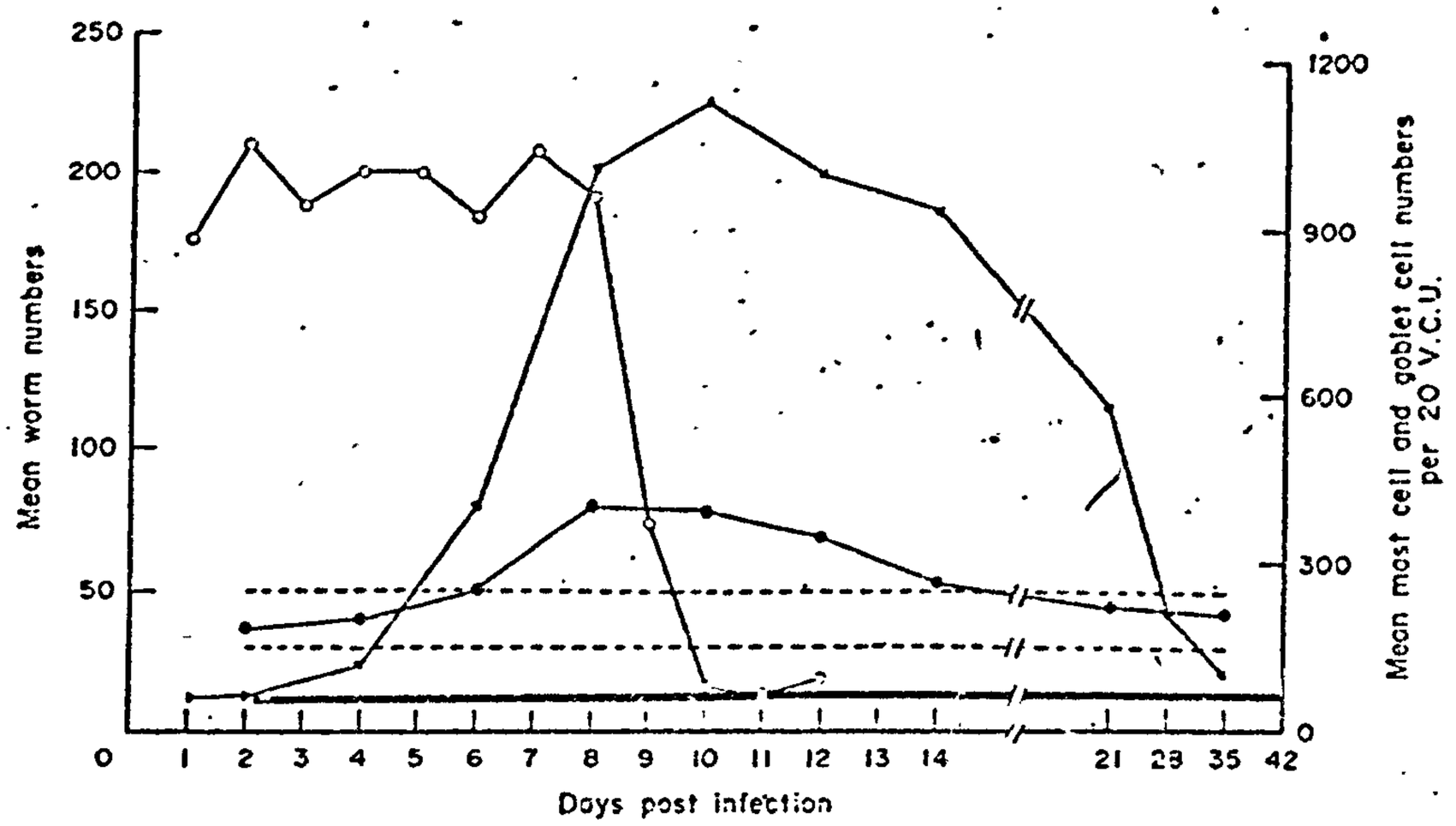

FIG. 1. The course of primary infection with *T. spiralis* in NIH mice. Number of intestinal worms (O——O), number of mucosal mast cells (▷——▷) and goblet cells (●——●) /20 villus crypt units (V.C.U.). Number of mast cells (——) and goblet cells (- - - -) in control uninfected mice.

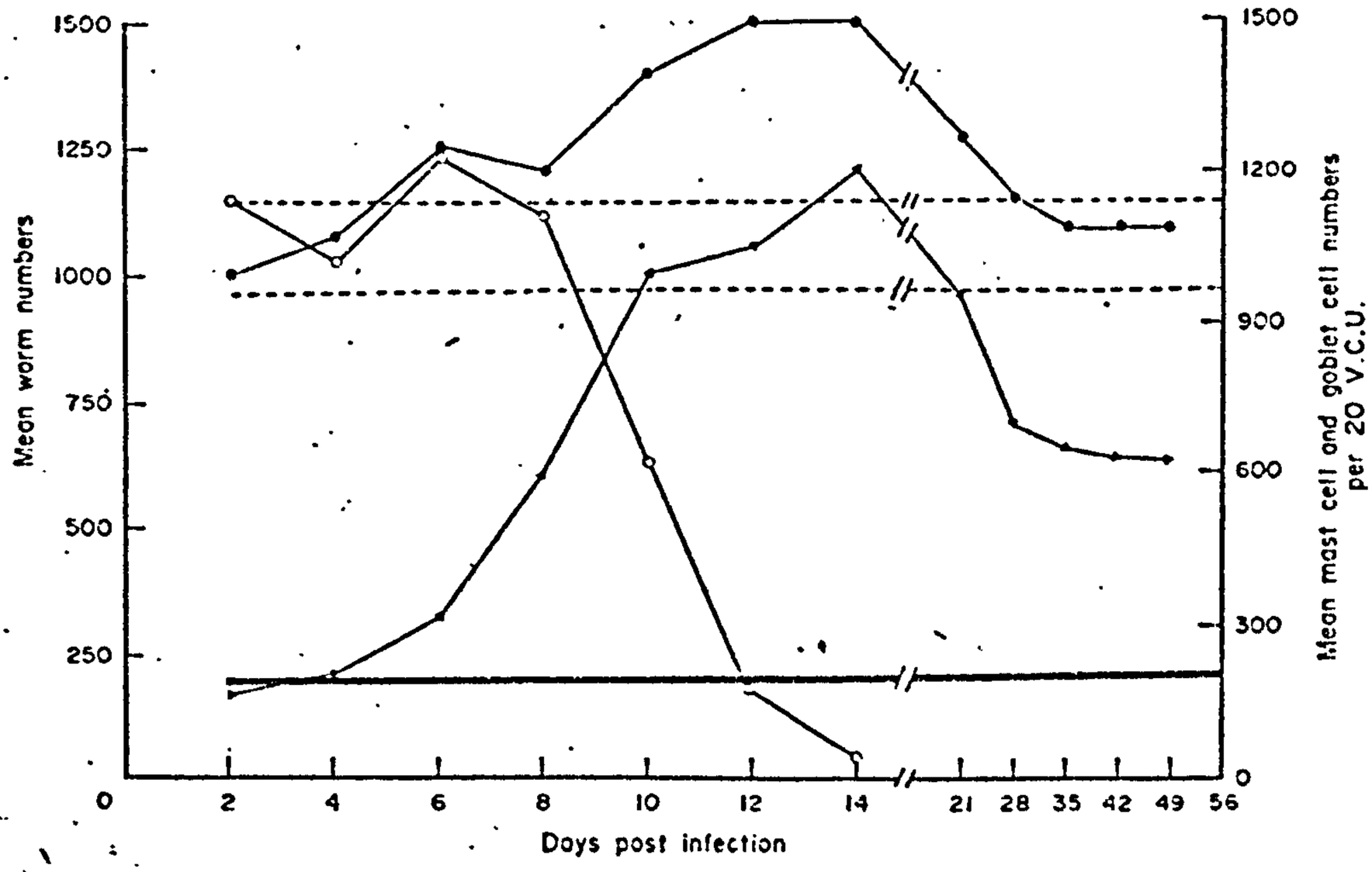

FIG. 2. The course of a primary infection with *T. spiralis* in Wistar rats. Number of intestinal worms (O——O), number of mucosal mast cells (▷——▷) and goblet cells (●——●)/20 villus crypt units (V.C.U.). Number of mast cells (——) and goblet cells (- - - -) in control uninfected rats.

TABLE 1—RAPID EXPULSION OF CHALLENGE INFECTIONS OF *T. spiralis* GIVEN AT INTERVALS AFTER A PRIMARY INFECTION IN NIH MICE AND WISTAR RATS

Groups	Challenge infection	No. of worms recovered 24 h after challenge			
		Mice		Rats	
	Day	Mean	S.D.	Mean	S.D.
1	14	3·2	6·6	3·8	5·7
2	16	8·0	5·2	—	—
3	18	153·0*	37·4	—	—
4	21	176·4*	22·3	4·8	5·2
5	28	135·5*	25·9	26·3	2·0
6	35	131·6*	14·3	2·4	2·5
7	42	—	—	3·4	3·2
8	49	—	—	5·8	5·1
9	Challenge infection only	159·6	25·9	188·0	24·3

*Means not significantly different from control $P>0·05$.

The loss of worms during rapid expulsion in rats takes place in less than 24 h when the challenge infection is given orally (Bell & McGregor, 1979a) and may be shown to occur within 1 h when larvae are injected directly into the intestine (Russell & Castro, 1979). In an initial experiment, infected mice were challenged, together with controls, 14 days after a primary infection and killed in groups of six at 1·5, 6 and 24 h later. (By 1·5 h almost all orally administered larvae have passed into the small intestine.) The results are shown in Table 2. Equivalent numbers of larvae were recovered from both groups 1·5 h after challenge, but by 6 h the worm recovery was significantly lower in previously infected mice. By 24 h very few worms were recovered. A similar result was obtained in a replicate experiment.

TABLE 2—TIME COURSE OF LOSS OF *T. spiralis* FROM NIH MICE GIVEN A CHALLENGE INFECTION ON DAY 14 AFTER PRIMARY INFECTION

Hours after challenge infection	No. of worms			
	Control mice		Challenged mice	
	Mean	S.D.	Mean	S.D.
1·5	137·0	31·8	105·0	29·7
6	139·0	40·6	46·0*	52·8
24	157·2	7·7	17·7*	18·9

*Mean significantly lower than control $P<0·05$.

To confirm that the absence of worms at 24 h post challenge in previously infected mice reflected expulsion from the intestine and not a failure to recover larvae from the mucosa, a further experiment was carried out. Mice given a primary infection were challenged on day 14 and larvae recovered by the Baermann technique after 6 and 24 h. After each recovery period the intestines were then digested in pepsin-HCl. Very few worms remained in the mucosa at 6 h and none were present at 24 h.

Effect of size and duration of primary infection upon the induction of rapid expulsion

In the first experiment of this series, 56 mice were divided into four groups and given primary infections of 50, 150, 300 or 500 larvae respectively. Four mice from each group were killed on days 7 and 14 to determine the establishment and survival of each infection. The remaining mice and a control group were challenged on day 14 and killed 24 h later (Table 3). The results show that the lowest level of primary infection (50 larvae) did not prime for rapid expulsion, but when a larger infection was used the challenge was expelled rapidly.

TABLE 3—RAPID EXPULSION IN NIH MICE GIVEN DIFFERENT LEVELS OF PRIMARY INFECTIONS AND CHALLENGED 14 DAYS AFTER PRIMARY INFECTION

Size of primary infection	Primary infection				Challenge infection 24 h after challenge	
	Day 7		Day 14			
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Experimental groups						
50	31·2	8·8	0	0	165·0	39·9
150	95·7	12·6	0	0	8·8*	20·2
300	191·2	24·2	0	0	0·6*	1·3
500	363·2	11·7	0	0	0*	0
Control group						
None	—	—	—	—	149·8	33·1

*Mean significantly lower than control $P<0·05$.

A similar experiment was then carried out using rats. Groups of six rats were infected with 200, 500 or 2000 larvae (infections similar in terms of larvae/g body weight to those used in mice) challenged, together with control rats, with 500 larvae on day 14 and killed 24 h later (Table 4). Rapid expulsion occurred in all the previously infected rats, regardless of the level of the primary infection.

TABLE 4—RAPID EXPULSION IN WISTAR RATS GIVEN DIFFERENT LEVELS OF PRIMARY INFECTION AND CHALLENGED 14 DAYS AFTER PRIMARY INFECTION

Size of primary infection	No. of worms recovered 24 h after challenge	
	Mean	S.D.
Experimental groups		
2000	3*	3·5
500	2*	1·7
200	16·8*	15·0
Control group		
None	141·2	11·0

*Mean significantly lower than control $P<0·05$.

The effect of the duration of the primary infection in priming for rapid expulsion was then examined. Twenty-four mice were infected and divided into four groups. Groups 1, 2 and 3 were given methyridine to remove their infections on days 7, 9 and 13 respectively, group 4 received no treatment. All the groups, and a group of controls were challenged on day 14 and killed after 24 h (Table 5). Removal of worms on day 7 of the primary infection did not allow the mice to expel the challenge infection, but rapid expulsion was evident in mice treated on days 9 and 13, as well as in the untreated group.

TABLE 5—THE EFFECT OF DURATION OF PRIMARY INFECTION ON THE RAPID EXPULSION OF A CHALLENGE GIVEN ON DAY 14 IN NIH MICE

Group	Anthelmintic during primary infection	No. of worms recovered 24 h after challenge Mean	S.D.
1	day 7	128·0	20·5
2	day 9	40·0*	16·4
3	day 13	2·6*	2·0
4	None	4·2*	3·4
5	None—challenge control	138·0	8·4

*Mean significantly different from control $P < 0.05$.

A similar, but more limited experiment was carried out in rats given a primary infection of 2000 larvae and challenged with 500 larvae. In rats treated with methyridine on day 7 and challenged on day 14, rapid expulsion still took place, the mean worm recoveries being 27·3 ± 39·0 in the experimental rats and 188·0 ± 24·0 in controls.

Effect of size of challenge infection upon expression of rapid expulsion in mice

In rats, rapid expulsion is known to be effective against both small and large challenge infections (Bell & McGregor, 1979a). To find whether this held true for mice, a group of 18 animals was given a primary infection and, on day 14, divided into three groups which were then challenged with 30, 300 or 600 larvae respectively. Three challenge control groups were similarly infected and all the mice were killed after 24 h (Table 6). Expulsion of the smallest challenge was as complete as that of the largest.

TABLE 6—RAPID EXPULSION OF LOW- AND HIGH-LEVEL CHALLENGE INFECTIONS OF *T. spiralis* FROM NIH MICE

Size of challenge infection	No. of worms recovered 24 h after challenge on day 14 Control mice Mean	S.D.	Challenged mice Mean	S.D.
30	13·8	3·3	0·1*	0·4
300	139·5	45·2	1·0*	2·3
600	371·0	36·7	12·0*	15·1

*Mean significantly different from corresponding controls $P < 0.05$.

Specificity of the induction and expression of rapid expulsion in mice

The restriction of rapid expulsion in mice to a short time period following expulsion of a primary infection might imply that larvae were failing to establish because of residual non-specific inflammatory changes in the intestine. To testt his, experiments were carried out to discover whether *T. spiralis* could establish in mice previously exposed to infection with other intestinal nematodes (*Nematospiroides dubius* and *Nippostrongylus brasiliensis*) and whether these latter species could establish in mice previously infected with *T. spiralis*. Both *N. dubius* and *N. brasiliensis* generate inflammatory changes in the intestine (Liu, 1965; Ogilvie & Jones, 1971). The time course of infections with the latter species in NIH is very similar to that of *T. spiralis* (unpublished observations) and therefore makes a good comparison for the residual effects of intestinal inflammation. *N. dubius* on the other hand is long-lived and produces chronic inflammatory changes.

In the first experiment four groups of mice were used. Group 1 was given a primary infection of *T. spiralis*, group 2 was infected with 600 *N. brasiliensis*, group 3 with 600 *N. brasiliensis* on day 0 and a further 300 on day 7, and group 4 was infected with 300 *N. dubius*. The establishment of these infections was determined in mice killed on day 7, for *T. spiralis* and *N. brasiliensis*, and on day 14 for *N. dubius*. The group infected with *N. dubius* was treated with pyrantel on days 10, 12 and 13 to remove the adult worms. All of the groups, together with a control group, were challenged with *T. spiralis* on day 14 of the initial infection and killed 24 h later (Table 7). Only the mice given a primary infection of *T. spiralis* showed rapid expulsion of the challenge infection.

In the reciprocal experiment, 36 mice were divided into six groups and three of these (1a, 2a and 3a) were given a primary infection of *T. spiralis*. Group 1a was challenged with *T. spiralis* on day 14, group 2a with 300 *N. dubius* on day 14 and group 3a with 500 *N. brasiliensis* on day 14. Group 1a and its control (1b) were killed 24 h after challenge, group 2a and its control (2b) 10 days after challenge, to permit recovery of adult worms, and group 3a and its control (3b) 3 days after challenge (Table 8) to allow the entry of larval *N. brasiliensis* into the intestine.

The results show that only *T. spiralis* was unable to establish and survive after previous infection with this species. Both *N. dubius* and *N. brasiliensis* developed as well in previously infected as in control mice.

Inflammatory changes in the intestine during and following primary infection with T. spiralis

The experiments described above show that the phenomenon of rapid expulsion in mice exhibits pecificity. This element of specificity may indicates

that the intestinal changes induced by infection with *T. spiralis* are deleterious to challenge infections only with that species because they interfere with specific physico-chemical or morphological requirements necessary for worm establishment. Many of the inflammatory changes that occur in the intestine during infection with *T. spiralis* are complex and difficult to measure (Castro, 1976). Two relatively simple parameters are the numbers of mast cells and of goblet cells in the mucosa, and these parameters have been monitored over an extended period during and following primary infections in mice and rats.

TABLE 7—EFFECT OF PRIMARY INFECTIONS WITH *Nippostrongylus brasiliensis*, *Nematospiroides dubius* OR *T. spiralis* ON SUBSEQUENT CHALLENGE WITH *T. spiralis* IN NIH MICE

Primary infection day 0	No. of worms recovered 24 h after challenge with 300 *T. spiralis* on day 14 Mean	S.D.
N. brasiliensis 600 larvae	112·5	73·0
N. brasiliensis 600 larvae + 300 larvae day 7	109·8	47·8
N. dubius 300 larvae*	137·6	27·5
T. spiralis 320 larvae	2·3†	2·4
None	136·0	6·6

*Anthelmintic used to remove worms before challenge.
†Mean significantly lower than control $P < 0·05$.

TABLE 8—EFFECT OF A PRIMARY INFECTION WITH *T. spiralis* ON A SUBSEQUENT CHALLENGE WITH *T. spiralis*, *Nippostrongylus brasiliensis* OR *Nematospiroides dubius* IN NIH MICE

Primary infection day 0		Challenge infection day 14		No. of worms recovered after challenge Mean	S.D.
Group 1	(a) *T. spiralis*	*T. spiralis*	300 larvae	2·4*	4·6
	(b) None	*T. spiralis*		95·6	31·6
Group 2	(a) *T. spiralis*	*N. brasiliensis*	500 larvae	103·0	31·7
	(b) None	*N. brasiliensis*		140.0	46·3
Group 3	(a) *T. spiralis*	*N. dubius*	300 larvae	248·0	58·2
	(b) None	*N. dubius*		252·0	41·0

*Mean significantly lower than control $P < 0·05$.
Group 1: Killed 1 day after challenge.
Group 2: Killed 3 days after challenge.
Group 3: Killed 10 days after challenge.

Mast cells (*Figs.* 1 *and* 2). In control NIH mice very few mast cells were present. Those cells that were present were located at the bases of crypts and were evenly distributed along the length of the intestine. After infection the numbers of cells increased significantly by day 6 and reached a peak by day 10. The numbers remained high for several days but had declined by 50% at day 21 and reached control levels by day 35. More mast cells were observed in the anterior half of the small intestine than in the posterior half on day 6, but by day 10 all parts of the intestine were infiltrated with mast cells and at this time the majority of cells (>90%) were located in the intra-epithelial position. Control rats showed a higher base-line count of mast cells than did NIH mice, but the general pattern of the rise in cells followed that described above. Peak numbers were apparently reached by day 14 after infection, but the subsequent decline was much slower than in mice and even by day 49 the mast cell count was nearly four times higher than controls. Whereas in mice the majority (90%+) of cells were intra-epithelial, in rats the majority of the intestinal mast cells were located in the lamina propria and few (≃10%) were present in the intra-epithelial position.

Goblet cells (*Figs.* 1 *and* 2). In control mice a substantial number of goblet cells were present in the epithelium of villi and crypts. However, there were many more goblet cells in the former than in the latter. After infection the number of cells remained at control level until day 8, but increased significantly between day 8 and day 10. The number of cells declined to control level by day 14 and remained at this level until the end of the experiment (day 35).

During the rise in the number of goblet cells, and for several days after that, the size of the cells was increased, their mucus content stained strongly and a layer of mucus was present over the mucosa. These facts suggest that, at this time, mucus production was high. Even on day 14, when the number of cells had returned to control levels, mucus production still appeared to be increased. Normal conditions were not apparent until after day 21. In control rats the number of goblet cells/villus crypt unit was higher than in control NIH mice. After infection the number of cells had increased significantly by day 12 and reached a peak by day 14. The number of cells remained high until day 21 but returned to control levels by day 28 and remained at this level until the end of the experiment (day 49). During the time of increase in the number of goblet cells and for several days after that mucus production was high as judged by the size and staining characteristics of the cells. Even when the numbers of goblet cells had returned to control levels, their mucus content was greater than in control rats and remained so until day 49. A thick layer of mucus was evident between adjacent villi from day 12 onwards.

DISCUSSION

The loss of *T. spiralis* from primary infections in both mice and rats is associated with gross inflammatory changes in the intestine. These changes occur initially in the anterior half of the small intestine, where the worms are located, but then spread to involve the whole gut. It is well established that the inflammation is mediated by thymus dependent immune responses (Wakelin, 1978) and is characterized by marked cellular infiltration of the mucosa, (Larsh & Race, 1975). In addition there are profound physico-chemical changes in the intestinal environment (Castro, 1976).

Initial experiments showed that challenge infections, given shortly after expulsion of a primary infection when the gross inflammatory changes had disappeared, were expelled rapidly, within 24 h. In mice this response to challenge was short-lived; challenges given more than 16 days after primary infection were not subject to rapid expulsion, instead worms were expelled more slowly. In rats, however, the rapid expulsion response was persistent and was shown to operate against challenges given up to 49 days after primary infection.

This distinct difference between the species was evident also in the conditions necessary to elicit the rapid expulsion response. A proportionately larger primary infection was necessary to evoke rapid expulsion in mice (Tables 3 and 4) and this infection had to persist for at least 9 days in order to be effective (Table 5). Indeed, as Bell & McGregor (1979b) have shown, exposure to preadult infections lasting only 48 h will elicit rapid expulsion in rats whereas this duration of infection is quite ineffective in NIH mice (Alizadeh, unpublished results). Thus the difference between mice and rats in priming for rapid expulsion cannot be accounted for by the minor variation which exists in the courses of complete primary infections in the two hosts.

In contrast to the species, differences in priming the expression of rapid expulsion seems to be similar in both rats and mice. The data reported here shows that loss of worms from mice occurred between 1·5 and 6 h after infection, a time course that is similar to that in rats (Russell & Castro, 1979). Rapid expulsion was effective against both small and large challenge infections (Table 6), as previously established for rats (Bell & McGregor, 1979a).

The rapidity with which challenge infections are expelled suggests that either the intestinal environment is so altered by the primary infection that conditions are unsuitable for subsequent parasite establishment, or that the intestine is primed in such a way that the stimulus provided by the challenge evokes an almost instantaneous response. The recovery at 1·5 h after challenge of an equivalent number of larvae from both immune and control mice (Table 2) might imply normal establishment, as it is known (Despommier, Sukhdeo & Meerovitch, 1978) that larval penetration into the mucosa is extremely rapid, but there are insufficient experimental data to justify this conclusion at present, and it is equally possible that the larvae recovered were in the lumen rather than in the mucosa. Thus both mechanisms of rapid expulsion must be considered. For this reason an attempt was made to monitor parameters of the inflammatory changes generated during primary infection in order to measure their persistence and their possible involvement in rapid expulsion.

In both mice and rats infection resulted in raised levels of mast cells and goblet cells, but there were significant differences in the time and persistence of these increases. In mice both cell types increased sharply before expulsion, whereas in rats the rise occurred as expulsion took place. Levels of both cells fell quickly after worm expulsion in mice, goblet cells reaching control levels by day 14 and mast cells between days 28 and 35. In contrast in rats, goblet cell numbers did not reach control levels until day 28 and mast cell numbers were still raised at day 49. As many more goblet cells were present in control rats than in control mice, the maximum numbers reached during primary infection differed very considerably. It was striking in both species that the actual size of goblet cells increased during the infection and this increase persisted longer in rats, even when cell numbers had returned to control levels.

It is tempting to relate the ephemeral rapid expulsion response in mice to the shorter duration of the cellular changes associated with intestinal inflammation. In these terms, rapid expulsion would be the

direct result of environmental changes arising during the primary infection. However, it was remarkable that the rapid expulsion response in mice was specific to *T. spiralis* challenge infections after *T. spiralis* primary infections (Tables 7 and 8), priming provided by *N. dubius* or by *N. brasiliensis* failed to induce rapid expulsion. Likewise priming with *T. spiralis* did not induce rapid expulsion of these species. Thus, if rapid expulsion is dependent on inflammation or changes in the local intestinal environment, these changes are quite specific. The persistence of the response in rats may be due to persistence of cellular changes, the most marked of which is the increased number of mast cells. It is possible that these cells may exert a continuing influence on the goblet cell population. It is known that mediators released from mast cells can increase the production and release of mucus from goblet cells. Such mediators can be elicited by the action of anaphylatoxins (Weir, 1974) or by IgE-mediated anaphylaxis following the introduction of antigen into the immune intestine (Lake, Bloch, Neutra & Walker, 1979; Lake, Bloch, Sinclair & Walker, 1980). If it can be assumed that challenge larvae do provoke a reaction on entry into the intestine, and there is physiological data to support this (Castro *et al.*, 1979), then the effects of mucus may be not only to act as a mechanical barrier to penetration but also to influence gut mobility, thus facilitating expulsion of the parasite. Lee & Ogilvie (1981) have presented evidence to show that the failure of challenge larvae to become established is due to their being trapped in the intestinal mucus of immune rats (possibly by antibody) and then being expelled by peristalsis.

The data presented here show that there are clear species differences in ability to respond rapidly to challenge. However, it must be emphasized that in mice, at least, there is evidence that rapid expulsion is genetically determined (Bell & McGregor, 1980c). In addition, it is also known that mice vary in their ability to mount mast cell response to infection with *T. spiralis* (Alizadeh & Wakelin, in press). There is at present little evidence for strain differences in rapid expulsion in rats, but it will be necessary to examine a wider spectrum of strains before making general statements on the ability of the two species to make this response to infection.

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