# THE IMMUNE RESPONSE OF THE MOUSE TO THE INTESTINAL TREMATODE DIPLOSTOMUM PHOXINI (FAUST,1918)

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## Summary

It was found that starvation of CFLP and NIH mice for six hours prior to infection was sufficient to produce a marked improvement in the level and consistency of establishment of oral infections of D.phoxini metacercariae, if the mice were starved from 6 AM, allowing stomach emptying to occur before infection.

In contrast to spermatogenesis, the detection of vitellogenesis and oogenesis in D.phoxini in the NIH mouse was markedly delayed compared with that reported in the duckling.

There was no difference in establishment or loss of a 200 metacercarial oral infection in male and female NIH mice. Loss began on day 6 and was complete by day 11 pi. Growth of flukes was complete by day 3. Flukes were largely confined to the anterior 10 cm of small intestine until the loss phase, when some attached to the region 10-20 cm post pylorus before being lost.

The method used detected a very low rate of egg production which declined rapidly after the onset of the loss phase of infection, although at this time there was not a corresponding decline in the percentage of flukes bearing eggs.

Implantation of metacercariae into different regions of the small intestine led to the following conclusions.

1. Establishment was best 30-60% post pylorus, and very poor in the posterior 40% of the small intestine. Inconsistent establishment in the anterior duodenum could be due to lack of preincubation, combined with other factors.

2. Recovery of flukes five days after transplantation of metacercariae was best in those implanted in the anterior 30% of the small intestine and the percentage of egg bearing flukes was highest in the anterior 10 cm of small intestine.

3. Flukes that survived until day 5 were the same size if found near to the site of implantation. Those which had moved in a posterior direction were smaller.

Reduction of infection size from 400 to eight metacercariae resulted in a four day delay in expulsion of primary infection, which otherwise occurred normally. Some delay in growth of flukes in a 400 metacercarial infection may have been due to changes in the gut preceding expulsion.

Cortisone acetate treatment delayed the onset of main fluke loss, which occurred after day 13 pi, by which time a normal primary infection was completely removed. 11% of flukes still remained on day 25 pi in treated mice. It is suggested that this represents loss due to senescence, or to a delayed, reduced immunological response. Flukes in cortisone acetate-treated mice were large (comparable to those from low level infections) and remained in the anterior 10-15 cm of the small intestine. Serial transplantation also resulted in increased longevity of flukes but losses were greater, probably due to the trauma of recovery and transplantation.

Transplantation of flukes undergoing expulsion resulted in their re-establishment in naive donors, though once again losses occurred. Transplanted flukes then had a longevity similar to that of flukes in an oral primary infection.

The results indicate

1. Expulsion of a primary infection is host-mediated.
2. <u>D.phoxini</u> is highly immunogenic as the expulsion of light infections (eight metacercariae) is similar to that of infections initiated by 400 metacercariae.
3. Damage to flukes is reversible, as shown by the establishment and survival of flukes in the process of expulsion, upon transfer to naive hosts.

The characteristics of a 200 metacercarial secondary infection (administered three weeks after a 200 metacercarial primary infection) are described. After normal establishment, rejection occurred between two and four days post infection. Fluke development was impaired, functional vitellaria did not form and eggs were not produced. Growth stopped before day 2 pi. No waning of immunological memory occurred when the interval

between primary and secondary infections was increased to seven months.

Reduction of the size of the immunizing infection to as few as five metacercariae resulted in no reduction in the effect of the immune gut on the rate of expulsion of secondary infection, although the inhibitory effect on fluke growth was less marked. Abbreviation of a 200 metacercarial to 15h duration apparently did not diminish resistance of mice to subsequent reinfection.

The effects of the immune gut on the growth, development and longevity of D.phoxini were found to be reversible when flukes from a secondary infection were transplanted into a naive host.

The effect of the immunized gut on transplanted, almost mature (three day old) flukes from primary infection was less marked than the effect on metacercariae surgically implanted into the duodenum, survival of the three day old flukes in immune mice was almost as long as would be expected in primary infection, and development proceeded to completion.

Serum from mice infected with D.phoxini eight days previously failed to transfer immunity. Immunity transferred adoptively by IMLNC was manifested as an acceleration of expulsion, and a retardation of vitelline development and reduced growth of flukes in recipient mice compared with controls. As few as $1x10^7$ IMLNC affected expulsion. $2x10^7$ IMLNC affected body length and vitelline development also.

IMLNC taken from donor mice between days 2 and 6 of a primary infection were most effective. After day 6, efficacy declined, however IMLNC taken from donor mice on day 21 after primary infection unexpectedly had some effect on recipient challenge infection. IMLNC taken 12 hours after secondary infection were effective but those from days 6 and 12 of secondary infection were not. IMLNC transferred less than two days before challenge of recipient mice did not transfer immunity.

T-lymphoblast activity was high and cellularity of the MLN increased following primary and secondary infections, but these changes were not consistently correlated temporally with the efficacy of IMLNC. T-lymphoblasts were

ineffective but a population of mainly non-dividing B cells was effective in transferring immunity adoptively.

Histopathological changes in the mouse intestine associated with D.phoxini infection were characterized, and the effect of adoptively transferred immunity (via IMLNC) on these parameters was studied.

Infection was characterized by marked globule leukocyte proliferation and eosinophilia which preceded and accompanied the expulsion phase of infection. Both responses occurred more rapidly in secondary than in primary infection. By comparison, the response of lamina propria mast cells was delayed and very limited, and was not marked in secondary infection.

The response of goblet cells to infection was minor, and irregular during normal infection, however it is possible that mucus production by individual cells may be increased during infection.

Adoptive transfer of immunity led to an acceleration of all cellular responses. sIg+ve MLNC transferred immunity most effectively and generated a level of inflammation which was severe compared with normal infection, and was uncharacteristic as it involved increased goblet cell differentiation. The poor ability of T cells to transfer immunity might have been attributable to low viability and/or selective depletion during cell separation.

High variability was observed in the number of plasma cells in the intestine during infection. The most marked increases occurred in lgG, and lgM secreting plasma cells during primary infection and lgG, during secondary infection.

# ABBREVIATIONS

| | |
|---|---|
| BM | bone marrow |
| BSA | bovine serum albumin |
| CTMC | connective tissue mast cells |
| DH | delayed hypersensitivity |
| GC | goblet cells |
| GL | globule leucocytes |
| h. | hours |
| HBSS | modified Hanks balanced salt solution |
| 5-HT | 5-hydroxytryptamine |
| IEL | intra-epithelial lymphocytes |
| IS | serum from previously infected hosts (e.g.mice) |
| LPMC | lamina propria mast cells |
| met. | metacercaria(e) |
| MLDC | mesenteric lymph duct cells |
| MLNC | mesenteric lymph node cells |
| IMLNC | " " " " from previously infected hosts |
| PBS | phosphate buffered saline |
| PG | prostaglandin |
| PLB | phospholipase B |
| post. | posterior |
| p.p. | post-pylorus |
| SC | spleen cells |
| S.D. | standard deviation |
| S.I. | small intestine |
| SRS-A | slow releasing substance of anaphylaxis |
| TDL | thoracic duct lymphocytes |

| | |
|---|---|
| vitell. | vitellaria |
| $1^{o}$ | primary infection |
| $2^{o}$ | secondary infection |

## General Introduction

Most of the present knowledge of the immune responses of vertebrates to intestinal helminths has been derived from the intensive study of several experimental host-parasite systems, consisting of nematode and cestode species maintained usually in rodents, and occasionally in birds: however convenient laboratory models facilitating the study of the immune response to intestinal trematodes have not been well established, in spite of the existence of several such parasite species which are severely debilitating to man and domestic animals. It was for this reason that a study of the immune response of the mouse to Diplostomum phoxini was undertaken. Similar studies have been initiated in some other host-intestinal trematode systems: study of the immune response of the mouse to Echinostoma revolutum, a parasite of broad host-range, has shown that 66-100% establishment of the parasite occurs, and primary infections persist for at least 20 days. Spontaneous cure, and an anamnestic response to homologous reinfection have been demonstrated (Sirag, Christensen, Frandsen, Monrad and Nansen 1980), however there is some evidence of parasite-induced immunotolerance manifested as a delay in rejection of large primary infections, and an immunosuppressive effect on a concurrent infection of Schistosoma mansoni (Christensen, Nydal, Frandsen and Nansen 1981).

The immune response of the duck to Apatemon gracilis minor was investigated by Raisyte (1968) and Blake (1973, 1974). Unfortunately, less than 50% establishment of parasites was attainable in this system, but egg production and parasite longevity have both been useful in the characterisation of this system. Spontaneous cure of a 200 metacercarial infection began on day 12 pi, preceded over several days by a decline in egg production. Corticosteroid treatment apparently inhibited these effects of the immune response. Severe reduction of egg production occurred in secondary infection. Thymectomy depressed the immune response, but bursectomy of three day old birds had no detectable effect on infection. Inflammatory lesions (mastocytosis, eosinophilia and increased gut permeability and mucus production) and an increase in numbers of mucosal plasma cells, were associated with infection.

Plagiorchis elegans will establish in the mouse. This host-parasite relationship was studied by Gorman (1981). 90% establishment of administered parasites was achieved. Transplantation studies showed that this parasite was rejected immunologically.

Diplostomum phoxini is a trematode occurring naturally in the small intestine of fish-eating birds, but Berrie (1960) demonstrated that D.phoxini could also establish in the mouse. The metacercaria of D.phoxini was described by Matare (1910), André (1918) and Ashworth and Bannerman (1927). The life cycle was demonstrated by Arvy and Buttner (1954) and Rees (1955, 1957). The eggs are operculate and hatch in presence of light after embryonating for seven days at 20°c (Donges 1969). Miracidia penetrate the molluscan host (lymnaea peregra in Britain, L.auricularia in mainland Europe). Furcocercariae (described morphologically by Arvy and Buttner (1954) Rees (1957) and Donges (1969) are later released from sporocysts (Rees 1957). With the aid of penetration gland secretions, the skin of the second intermediate host (Phoxinus phoxinus) is penetrated, and the cercarial tail lost. The fact that cercariae are observed in the brain within 12 hours (Arvy and Buttner 1954) and the occurrence of haemorrhaging (Rees 1957) suggests that the brain is reached via the bloodstream. Development of the metacercaria in the brain takes 28 days at 10°c (Arvy+Buttner 1954, Rees 1957) In the infective metacercaria, all organs of attachment, and gut caeca are fully formed. Metacercariae are not enclosed in a cyst of parasite origin. Their distribution is described by Arvy (1954) and Rees (1957). After ingestion by the definitive host (natural hosts include Mergus merganser merganser and Podiceps ruficollis; experimental avian hosts include Larus argentatus, Anas boschas and Cairina moschata) metacercariae attach to the mucosa of the duodenum, and mature within three or four days. Similar rapid maturation is observed in other strigeoid species (A.gracilis minor, Holostephanus luhei, Cyathocotyle-bushiensis, D.spathaceum).

The process of maturation of D.phoxini in Anas boschas was described by Bell and Smyth (1958). The following phases were identified :-

1. Cell multiplication phase: rapid mitosis occurring in the 24 hours infection.

2. Body shaping: formation of the characteristic adult "bilobed" structure, 24-48h. pi.

3. Organogeny: genital primordia become visible 12-24h. pi.

4. Early gametogeny: the stages of spermatogenesis are visible (in aceto-orcein squashes) from 28-40h. pi.

5. Late gametogeny: mature spermatozoa appear in the testes 40-48h. pi.

6. Eggshell formation and vitellogenesis: vitellaria give positive histochemical (diazo) reaction for phenolic eggshell precursors at 55-60 h. pi.

7. Oviposition: the appearance of fully formed eggs occurs at 60-72 h. pi.

D.phoxini has been used extensively for in-vitro studies of helminth development, being particularly suitable because of the ready availability of metacercariae, the advanced development of the infective metacercaria, the rapid maturation of the adult and the small size of the organism (Bell and Hopkins 1956). Development to the oviposition stage was achieved in media containing egg yolk and albumen by Bell and Smyth (1958), (but this is not the case with other strigeoids (Basch, Dicorn za and Johnson 1973)). Simplification of this complex and variable medium was attempted by Hopkins and Wyllie, Wilson 1960, Wyllie, Williams and Hopkins 1960: but was largely unsuccessful; however substitution of other nutrients such as yeast extract in glucosaline and albumen medium, promoted the maturation of male genitalia and spermatogenesis (Wyllie, Williams and Hopkins 1960). It was suggested that this effect may have been due to the presence of B vitamins in yeast extract. The best growth of D.phoxini in-vitro is obtained in semi-solid media, and particle size is critical. Development was poor in liquid and diphasic media, and on the chorioallantoic membrane of chick embryo (Kannangara and Smyth 1974). Pretreatment with pepsin and trypsin was found to be deleterious, and duck bile, duck liver macerate and duck intestinal extract had no beneficial effect.

The morphology of adult D.phoxini has been described in detail by Rees

(1955). The presence of the "adhesive organ" is characteristic of the suborder Strigeida Railliet 1919 (classification of Dubois (1953, 1964, 1970) summarized by Blair (1974)). The body consists of well defined anterior and posterior lobes, a characteristic of the superfamily Strigeoidea (in contrast with the Cyathocotyoidea, in which there is no such division of the body).

1. The posterior lobe accommodates paired male and single female genitalia with vitellaria forming a mid ventral band, and a terminal genital atrium. Large tanned, operculate eggs are produced. Up to five eggs may be observed in utero in the avian host (Berrie 1960).

2. The anterior lobe is flattened and contains two lateral branches of vitellaria but is otherwise highly adapted for attachment and feeding. In addition to characteristic digenean oral and ventral suckers and the adhesive organ (also referred to as "holdfast" or"tribocytic organ") there are lateral organs which may also aid attachment, the lappets ("pseudosuckers"), on the anterior margin of the forebody. The relative contribution of the adhesive organ to attachment and nutrition is thought to vary between different genera, depending on the presence or absence of lappets. In simpler strigeoids such as C.bushiensis and H.luhei (Cyathocotyloidea), parasites of the avian rectum and caecum, the adhesive organ is the sole organ of attachment (Erasmus and Ohman 1963, Ohman 1966b), however Diplostomum and A.gracilis minor, parasites of the small intestine, possess lappets which in these organisms are thought to be the primary organs of attachment (Ohman 1965, Erasmus 1968, Blake 1974). In Diplostomum and Apatemon the whole anterior lobe is involved in attachment. The ventral sucker is thought to be important in the initial attachment of A.gracilis minor to the duck intestine (Blake 1974).

The adhesive organ of D.phoxini is acetabular in form. It has been described by Erasmus (1970). The lateral walls bear stout spines which might function in abrading the mucosal epithelium. The ventral surface is highly convoluted to form a chambered structure, lined with microvilli. This represents an enormous increase in surface area which would be compatible with secretory or absorptive functions. The poorly developed fibrous layer associated

with the basal plasma membrane allows intimate contact between the adhesive organ surface and the excretory lacuna system, for which a solute transport as well as a hydraulic function has been postulated. Gland cells within the body of the adhesive organ contain proteinaceous secretion bodies which are passed via cytoplasmic processes into the tegument, and are thought to be discharged into the chambered exterior of the parasite. Acid phosphatase activity is associated with the gland cell endoplasmic reticulum and the chamber walls, but not with the secretion granules.

The structure of the lappets was described by Erasmus (1969). These structures are very mobile and the associated musculature is highly developed (Rees 1955). The outer surface of the tegument is extended to form finger-like processes. Forebody gland cells of two types are associated with the lappets. The basal nucleated portions lie in the forebody and discharge their secretion bodies at the surface of the tegument, via long, neck-like processes, probably aided by muscular contraction. "Type A" cells are thought to secrete esterase, and "Type B" a carbohydrate complex.

In four species of strigeoid trematode examined, extracorporeal digestion is thought to contribute to nutrition (Ohman 1966), disintegrated host tissue being taken in via the oral sucker, and further digestion occurring in the intestinal caeca. Involvement of the lappets of Diplostomum sp in extra-corporeal digestion has been suggested by Lee (1962) and Ohman (1965), however Blake (1974) suggested that in A.gracilis minor these organs primarily aid attachment rather than serving a digestive function. The possibility of direct absorption by the adhesive organ has not been discounted.

Limited histochemical tests have demonstrated enzyme activity associated with the lappets and adhesive organ of D.phoxini, however the relationship between identified enzymes, parasite nutrition and pathology of infection is not clear. Acid phosphatase (Erasmus 1970) and non-specific esterase (Lee 1962) are associated with the adhesive organ in adult D.phoxini. Phosphatase and esterase activity is characteristic of the adhesive organ and lappets of other strigeoid species studied. The presence of esterase and phosphatase

in the caecal cells of some species is thought to be associated with digestion.

In all apecies studied (C.bushiensis, H. luhei, A.gracilis minor, D.spathaceum) attachment of parasites resulted in the erosion or dissolution of the mucosal epithelium at the site of attachment. The enlargement of local blood vessels was also a commonly noted feature of infection. The avian intestine infected with A. gracilis minor was studied in detail (Blake 1974) and inflammatory changes in gut permeability and cellular infiltrations were noted.

The work in this thesis forms a preliminary study of the host-parasite relationship between D.phoxini and the mouse. In order to study the immune response involved, it was necessary first to attain a consistent and preferably high level of establishment of metacercariae, and secondly to define the basic parameters of development, fecundity, distribution and longevity of the parasite in the mouse. The roles of parasite senescence and of host immunity in the loss of D.phoxini from the mouse were then evaluated, using the immunosuppressive and anti-inflammatory drug cortisone acetate and the technique of serial transplantation. The effects of acquired immunity on flukes in secondary infection, and the duration of immunological memory were investigated. An attempt was made in this system, to establish a means of transferring immunity adoptively or passively in an attempt to characterise further the components involved in protective immunity. Histopathological changes in the gut associated with infection were investigated in an attempt to identify components of the host immune/inflammatory system which are directly or indirectly involved in the response to infection (and eliminate those which are not). Finally the results obtained with this intestinal fluke/rodent system were compared with those of similar investigations carried out in other systems, principally involving nematodes and cestodes.

# CHAPTER ONE

## Introduction

Prior to any investigation of the immune response of the mouse to D.phoxini being undertaken, it is necessary to ensure consistent and efficient establishment of metacercariae administered orally, and to characterise the basic parameters of fluke development, fecundity and longevity, and the location of D.phoxini in the mouse intestine.

Preliminary work in this laboratory (Hopkins, unpublished) has indicated that poor establishment of D.phoxini metacercariae following oral infection is the major drawback in the establishment of the D.phoxini/mouse system as a convenient laboratory model. Similar losses of 50% metacercariae were observed by Blake (1974) infecting ducklings with A.gracilis minor. In-vitro studies (Bell and Hopkins 1956) have indicated that under the right conditions an appropriate increase in temperature is sufficient to initiate the development of adult D.phoxini from the metacercaria. The subsequent degree of development is then determined by the fulfillment of complex physico-chemical and nutritional requirements.

It has been suggested (Hopkins) that the three most plausible reasons for variable establishment of D.phoxini in mice are:

1. Weakness in the mouse gut of an undefined "attachment stimulus" which may in addition to temperature, be responsible for the attachment of metacercariae to the intestine of the definitive host.
2. Presence in infected minnow brains of a variable proportion of immature, non-infective metacercariae, a factor influenced greatly by the time of year, and which could be offset by an appropriate delay, allowing maturation of metacercariae before the fish concerned were used.
3. Damaging effects of the mammalian stomach on metacercariae during oral infection.

An attachment stimulus effective for D.phoxini has not been characterized. Its existance would be difficult to prove, and would be more easily inferred by a process of elimination of other possibilities.

Metacercariae for infection were selected on a morphological basis. "Mature" metacercariae were comparatively dark in colour and granular in appearance, and the gut caeca and organs of attachment were fully formed and the body shape more clearly defined and less distensible than that of "immature" metacercariae. It became unnecessary for controlled experiments to be done to eliminate "immaturity" of metacercariae as a factor responsible for variability in establishment of oral infections.

Conditions prevailing in the mammalian stomach and the avian proventriculus and gizzard render these areas of the gut generally unfavourable as a habitat for gastrointestinal helminths. Nematodes (Ascaris sp, H.contortus, Trichostrongylus sp, Hyostrongylus rubidus and several spiruroidea) have been more successful than digenea in colonising this region of the gut, but a few digenetic flukes do occur there (e.g. Riberoia ondatrae in the proventriculus of fish eating birds, Euparyphium melis in the stomach of mink). Intestinal helminths passing through the stomach are usually protected by a cyst or cuticle. Although it would normally be surrounded by host (brain) tissue, the absence of a protective cyst wall might predispose D.phoxini metacercariae to the effects of mechanical abrasion, low pH and enzymic activity in the stomach. Stomach pH varies between species, and with time after feeding, however stomach pH is generally low, and must remain $< 5$ for pepsin to be active. In contrast, the pH of the duodenum is higher than that of the stomach, in-vitro studies have shown that D.phoxini metacercariae will develop between pH 6 and 8, the optimum pH varying with the medium (Bell 1958, Wilson 1960), however tolerance of low pH has not been investigated in detail.

In other systems, the maintenance of a transient increase in stomach pH at the time of infection has facilitated the establishment of parasites which would otherwise barely establish, e.g. Bacha (1964) found that administration of sodium bicarbonate solution increased the establishment of Zygocotyle lunata in the rat.

It was decided that starvation of mice prior to infection might hasten

the transit of metacercariae through the stomach. The aim was to achieve good establishment as a preliminary to further work, rather than to document the factors affecting establishment.

The parameter of longevity of D.phoxini in the final host has been largely ignored by previous workers. Infections frequently consisted of a given number of infected minnow brains rather than a known number of metacercariae. Specimens were recovered for study between four and six days after infection of young ducklings (Arvy and Buttner 1954, Rees 1955). Berrie (1960) recovered established D.phoxini from mice and gulls in addition to ducklings, but none of these authors investigated the longevity of the parasite in these hosts. Early unpublished work in this laboratory showed that an oral infection of 200 metacercariae administered to CFLP mice was completely lost by day 11-12 pi. Poor establishment obscured the kinetics of parasite loss which was thought to commence on day 7 pi.

Sex of host and administered sex hormones, are known to exert an effect upon the growth of parasites or course of infection in some host-parasite systems (reviewed by Reddington, Stewart, Kramer and Kramer 1981, also see Sadun (1948, 1951), Behnke (1975), Dobson and Owen (1978), Novak, Collins Sitepu and Dobson 1982 and Evans 1980), however the effect of sexual behavioural characteristics such as aggression, which may induce stress, may also be important in this context. In general, parasitic nematodes have been found to establish and reproduce better in male hosts (Solomon 1969, Mankau and Hamilton 1973, Reddington et al 1981). The course of D.phoxini infection in male and female mice is compared.

Little is known of the distribution of D.phoxini in the intestine. D.phoxini was recovered from the small intestine of Anas boschas and Cairina moschata by Arvy and Buttner (1954). Rees (1955) stated that distribution was restricted to the duodenum, however her recovery technique consisting of visual inspection of the gut with no prior incubation would not be reliable in the case of so small a trematode. Highet and Hopkins (unpublished) found that after oral infection of CFLP mice, D.phoxini was

found almost exclusively in the anterior 10-20 cm of the small intestine.

Whether this represents attachment to the first habitable region of the gut, and the extent to which flukes are able to seek out specific sites is unknown. In the present work, the distribution of flukes in the anterior small intestine is observed during infection and in an attempt to determine the extent to which preferential sites exist or are sought by the flukes, metacercariae are inserted surgically into various regions of the small intestine, and their subsequent development and movement checked.

D.phoxini adults in mice carry few eggs in utero (Berrie 1960). The eggs are large and operculate and therefore cannot be recovered by flotation methods. Although occasional eggs may be observed in faecal smears, egg production from 200 fluke infections has proved difficult to measure. The method described herein is the only technique used with any success, during the course of this study.

## Materials and Methods.

Animals used were CFLP 3 star males (Bantin and Kingman, or Hacking and Churchill) or NIH (Hacking and Churchill). Mice were not infected until at least six weeks of age, at which time they were considered to be immunologically mature.

Minnows were collected from Milngavie reservoir. All were found to be infected with *D. phoxini* metacercariae though the size of infection varied greatly. The fish were maintained in running water in an outside tank, and fed on either dried daphnia or powdered rodent diet.

### Oral infection procedure

Except where stated otherwise, food was withheld from mice immediately prior to infection (commencing sometime between 5-0 pm on the day prior to infection, and 6.30 am on the day of infection). During this time the animals were housed on metal grids to prevent refection of faeces and bedding shavings. Mice were infected after midday, and food and normal housing restored not less than half an hour after infection.

To obtain metacercariae, a minnow was decapitated and the brain exposed by a mid dorsal incision. The brain was removed and placed in modified Hanks Balanced Salt Solution (HBSS) at room temperature. Metacercariae were released by gentle teasing of the tissue. Infective metacercariae were selected on a morphological basis as described in the introduction. Infections of 200 metacercariae were routinely used. These were drawn into portex tubing (bore 0·5mm) attached to the needle of a 1 ml syringe. The stomach tube was inserted into a mouse under light ether anaesthesia, and the metacercariae administered in 0.15 ml of modified HBSS. Once the fish brain had been removed, metacercariae were administered as quickly as possible, although in exceptionally heavily infected brains, metacercariae may have been immersed in HBSS for up to half an hour before they were administered.

Kannangara and Smyth (1974) reported that *D. phoxini* metacercariae could be stored at 4°c for at least two months and still retain viability, but no attempt was made in this study to store metacercariae prior to use.

Infection by laparotomy:

Metacercariae for transplantation were obtained as above. Flukes for transplantation were recovered from donors by slitting the proximal 10-15 cm of the small intestine longitudinally and incubating in modified HBSS at 37-39°C for ten minutes or until flukes became dislodged from the villi. The period of incubation was kept to a minimum. Flukes or metacercariae were drawn into a portex tube (bore 0.5mm) attached to the needle of a 1 ml syringe, and implanted immediately.

Recipient mice were anaesthetised by intraperitoneal injection of 0.1 ml/10g body weight of a 10% solution of "Sagatal" (May and Baker Ltd., containing 60 mg/ml pentobarbitone) in modified HBSS containing 17% ethanol. The skin of the upper abdomen was shaved and swabbed with 70% ethanol. An incision was made through the skin and body wall near the midline, and a loop of the small intestine was exposed by means of a hook. The gut was pierced using a 16 guage hypodermic needle. The tube was then inserted into the intestinal lumen and the flukes or metacercariae injected in a direction away from the stomach in 0.1 ml modified HBSS. The tube was withdrawn and the lesion repaired using a 6/0 mersilk (Ethicon Ltd) suture. The surgical area was sprayed with antibiotic (Rikospray, Riker Labs). The body wall and skin were each closed with 6/0 sutures, the surfaces treated with "Rikospray" and the skin sealed with "Nobecutane" (BDH). The infection tube was then inspected for parasites, thus the exact number administered to each mouse could be determined.

Recovery of parasites:

Mice were killed by ether anaesthesia followed by cervical dislocation. The anterior small intestine was cut into four or five sections, each five cm in length. These were immersed in modified HBSS at 37-39°C, then slit longitudinally. The gut segments were allowed to incubate for at least 15 minutes before examination under dissecting microscope (×25)
Flukes which had detached from the gut were removed by pipette and immediately immersed in 70% ethanol in which they could be stored indefinitely.

The gut segments were shaken vigorously to detach any remaining flukes, and in the case of mice killed 24h or less pi, the mucosa was removed by scraping between two glass slides, and was then compressed between the slides and checked under the microscope for any remaining flukes.

Examination of flukes:

1. Measurement: Flukes were fixed in 70% ethanol for at least 24h before measurement. Those which were grossly contracted or distended were not measured, though there were no fixed criteria to standardise the rejection of these flukes, which were very few in number. Flukes were transferred to a slide, in 70% ethanol, and mounted with a coverslip. It was decided not to compress the flukes in any way so sufficient alcohol was maintained under the slide to prevent compression by the coverslip. The total length of the organism and the length and width of the posterior lobe were measured using an ocular micrometer.

2. Vitelline development: The stable diazotate "Fast Red Salt B" (G.T.Gurr, London) was used to couple with phenolic eggshell precursors (Johri and Smith 1956). A freshly prepared 1% aqueous solution of Fast Red salt B was allowed to flood a slide prepared as above, while absorbent tissue was used to draw 70% alcohol from under the slide. Vitellaria were stained red, and eggs orange, within one or two minutes. Absence or presence of anterior and posterior vitellaria was noted. Severe depletion was also noted, however lack of standardised criteria made this rather subjective.

3. Spermatogenesis: The presence of mature spermatozoa may be confirmed by observation of compressed living organisms. For the observation of spermatogenesis, slightly squashed aceto-orcein mounts were prepared (Bell and Hopkins 1956). Fresh living specimens were placed in a few drops of 1% aceto-orcein on a microscope slide and incubated for about 15 minutes at 39°C. The time required for staining varied with each batch of stain and with the age of the stain. Firm pressure was then applied to the coverslip. Spermatids and sperm morulae were then visible at x1000 magnification under an oil-immersion lens.

4. Egg production: When recording eggs in-utero it was found that

Statistical treatment of results :

When necessary, the parameters of fluke length, vitelline development and proportions of flukes bearing eggs are analysed with the use of Students t test. Fluke recoveries are compared using the non-parametric Wilcoxon test. In both cases a value of $p < 0.05$ is considered significant.

unless the recovery of flukes was rapid, flukes tended to expel their eggs during incubation in BSS. A modification of the "Formol-Ether technique" (described by Muller,1975) was used to estimate or detect egg production. Pairs of mice were caged on grids and 24h samples of faeces were collected and suspended in 10-15 ml of 10% formalin, for at least 24h. The faeces were broken down using a spatula, and filtered through a 250 mesh sieve. The filtrate and washings were aliquoted in 7 ml volumes. To each was added 3 ml of diethyl ether. Each tube was shaken vigorously for 40 secs. prior to spinning at 300 rpm for 60 secs. The ether layer containing fatty faecal deposits was removed by suction after ringing with a fine pipette. The remaining supernatant was also removed by suction, leaving <5mm depth at the bottom of each tube. After agitation to resuspend eggs, the sample was examined by means of a Mc Master chamber. The total number of eggs recovered was recorded. After collection of faeces, mice were killed and the flukes recovered from their small intestine and counted. A value for the output per fluke per 24h was then estimated.

Modified Hanks Balanced Salt Solution (HBSS)

This was modified by excluding glucose and sodium bicarbonate, and increasing the remaining salts pro rata to an osmotic pressure of 300 m-osmole (Hopkins and Stallard 1974).

| | | |
|---|---|---|
| Solution I | NaCl | ·168g |
| | KCl | 8g |
| | $KH_2PO_4$ | 2g |
| | $Na_2HPO_4$ | 4g |
| | 0.2% phenol red | 200ml |

Made up to 2 litres with deionised water

| | | |
|---|---|---|
| Solution II | $CaCl_2.2H_2O$ | 3.92g |
| | $MgCl_2.6H_2O$ | 2.00g |

Made up to 2 litres with deionised water

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

## Results

The effect of food withdrawal (starvation) prior to infection, on the establishment of an oral infection of D. phoxini metacercariae:

Groups of six to eight NIH or CFLP mice were starved for a six hour period prior to infection (200 met in 0.15 ml HBSS unless otherwise stated). Establishment was compared with that of metacercariae given to control (non-starved) mice.

Groups of mice were :

A Eight NIH, starved from 6AM, infected at noon.

B Eight NIH, starved and infected as above (metacercariae administered in 0.05ml HBSS).

C Eight NIH, not starved, infected at noon.

D Eight NIH, starved from 9AM, infected at 3PM.

In addition to the stipulated period of starvation, no mice (including controls) were given access to food for 30-40 mins. following infection. At the time of infection, three mice from each group were killed and their stomach contents assessed visually. The remaining mice were killed 21 hours pi and fluke burdens counted.

The experiment was repeated using groups of six or seven CFLP mice. Group B was omitted, and only one mouse in each group was killed for observation of stomach contents.

The fluke recoveries were compared using student's t test. Results are shown in Table 1-1.

The usefulness of the results is reduced by several faults in experimental design.

1. The method of assessment of stomach contents was inadequate. Dry or even wet weight would have been of more use.

2. Controls (non-starved) should have been killed at 3PM as well as at noon.

Absence of food for a six hour period prior to infection did not lead to a statistically significant change in establishment if food was withdrawn

at 9AM, however withdrawal of food at 6AM resulted in a marked increase in the number of metacercariae which established.

Only the stomachs of mice starved from 6AM were empty. The stomach contents in the other groups were variable and although starvation from 9AM appeared to have resulted in a decrease in stomach contents, no data are available to substantiate this suggestion.

It was observed that mice of both strains were active at 6AM whereas at 9AM activity had ceased. All groups of mice were inactive when collected for infection.

In the mice tested, variation of the volume of HBSS in which D. phoxini was administered between 0.05ml and 0.15ml had no effect on establishment.

TABLE I-I

The effect of starvation prior to infection on the establishment of an oral infection of D.phoxini metacercariae:

| Mice/ group(X) | Food withdrawal: Started: | Duration: | Establishment / mouse mean ± SD | P | Volume HBSS(ml) | Stomach contents at time of infection |
|---|---|---|---|---|---|---|
| 6 +I CFLP | 9AM | 6 h | 82 ± 33 | < 0.I | 0•I5 | substantial |
| 6 +I " | 6AM | " | I4I ± 20 | < 0•00I | " | empty |
| 5 +I " | - | - | 6I ± 2I | | " | substantial |
| 5 +3 NIH | - | - | 7I ± 50 | | 0•I5 | small variable amounts(none empty) |
| 5 +3 " | 6AM | 6 h | I73 ± 9 | < 0•002 | " | empty |
| 5 +3 " | " | " | I57 ± I6 | < 0•0I | 0•05 | empty |
| 5 +3 " | 9AM | " | 67 ± 23 | < 0•I | 0•I5 | variable amounts (none empty) |

X = number of mice infected + number of mice killed to assess visually the quantity of stomach contents at the time of infection.

## The development of D. phoxini in NIH mice.

Female NIH mice aged nine or ten weeks were infected with 200 metacercariae. Groups of five mice were autopsied 38, 42, 55, 60, 76, and 86h pi and flukes recovered from the anterior 10cm of the small intestine. The presence of spermatids, spermatozoa, vitellaria and eggs was determined by the procedures described (see materials and methods). Ten flukes from each mouse were examined for each characteristic.

Aceto-orcein preparations were made and spermatids at various stages of development observed at 42h and 55h pi in all flukes examined. Spermatozoa were first observed at 42h pi, at which time all flukes recovered from three mice, and 70% and 40% of flukes from the remaining two mice. Spermatids only were observed in the remaining flukes.

Data relating to observations of vitellaria and eggs are given in Table 1-2. The technique used showed no vitelline development in any specimens until 68h pi when 38% of flukes showed only small traces of stained vitellaria. At this time the diazo-positive reaction was confined to the anterior lobe and the region between the anterior and genital lobe. By 76h pi, 80% of flukes contained well developed vitellaria, but no eggs were present. At 86h pi over 90% of flukes contained complete vitellaria and 51% contained one or more eggs in utero.

TABLE I-2

Development of D.phoxini in the NIH mouse: Appearance of (fast red salt B - incorporating) vitellaria and eggs

| Time(h)pi | Mean ± SD % of recovered flukes containing : (a)"complete" vitellaria | (b) traces of vitellaria | (c) eggs |
|---|---|---|---|
| 55 | 0 | 0 | 0 |
| 60 | 0 | 0 | 0 |
| 68 | 2 ± 6 | 38 ± I2 | 0 |
| 76 | 80 ± I0 | I8 ± I2 | 0 |
| 86 | 9I ± 9 | 8 ± 6 | 5I + I5 |

Establishment and course of a primary infection of D. phoxini in male and female NIH mice.

Male and female laboratory bred, seven to eight week old NIH mice were each infected with 200 metacercariae. Groups of five or six mice of each sex were killed on days 2, 4, 6, 8, 11 and 13 pi. The number, distribution and size of the flukes in the anterior 25 cm of small intestine was determined.

Approximately 88% of administered parasites were recovered from both male and female mice, two and four days pi, after which fluke loss occurred at the same rate in both sexes. By day 11 pi, the number of flukes recovered was insignificant. (Fig. 1-3).

On days 2 and 4 pi, virtually all flukes were recovered from the proximal 10 cm of the small intestine. On day 6 pi, associated with the onset of expulsion, a shift in the position of the flukes was apparent. 37% of those remaining now occurred 10-20 cm post pylorus. As expulsion continued, this figure rose to 72% by day 8. (Fig. 1-4).

During the first two days of infection a 45% increase in body length occurred. Between days 2 and 3 the increase was 11%.

Between days 2 and 8 pi the size of the posterior lobe, measured as a proportion of total body length, remained remarkably constant. (Between 32.4 and 36.8% of total body length in all groups).

No increase in lengths of flukes recovered in the anterior 10 cms of the small intestine was apparent after day 3 pi (small pooled samples were not analysed (Fig. 1-5)). A small (9%) increase in body length of flukes recovered 10-15 cm post pylorus occurred between days 6 and 8 pi (a time when posterior movement of flukes from more anterior locations would be expected to be occurring).

There was no difference in body length between flukes occurring in the anterior 5 cm of small intestine, and 5 to 10 cm post pylorus.
On day 6 pi (when there were sufficient flukes to allow comparison), flukes

recovered 10 to 15 cm post pylorus were shorter than those recovered in the anterior 5 cm of small intestine.

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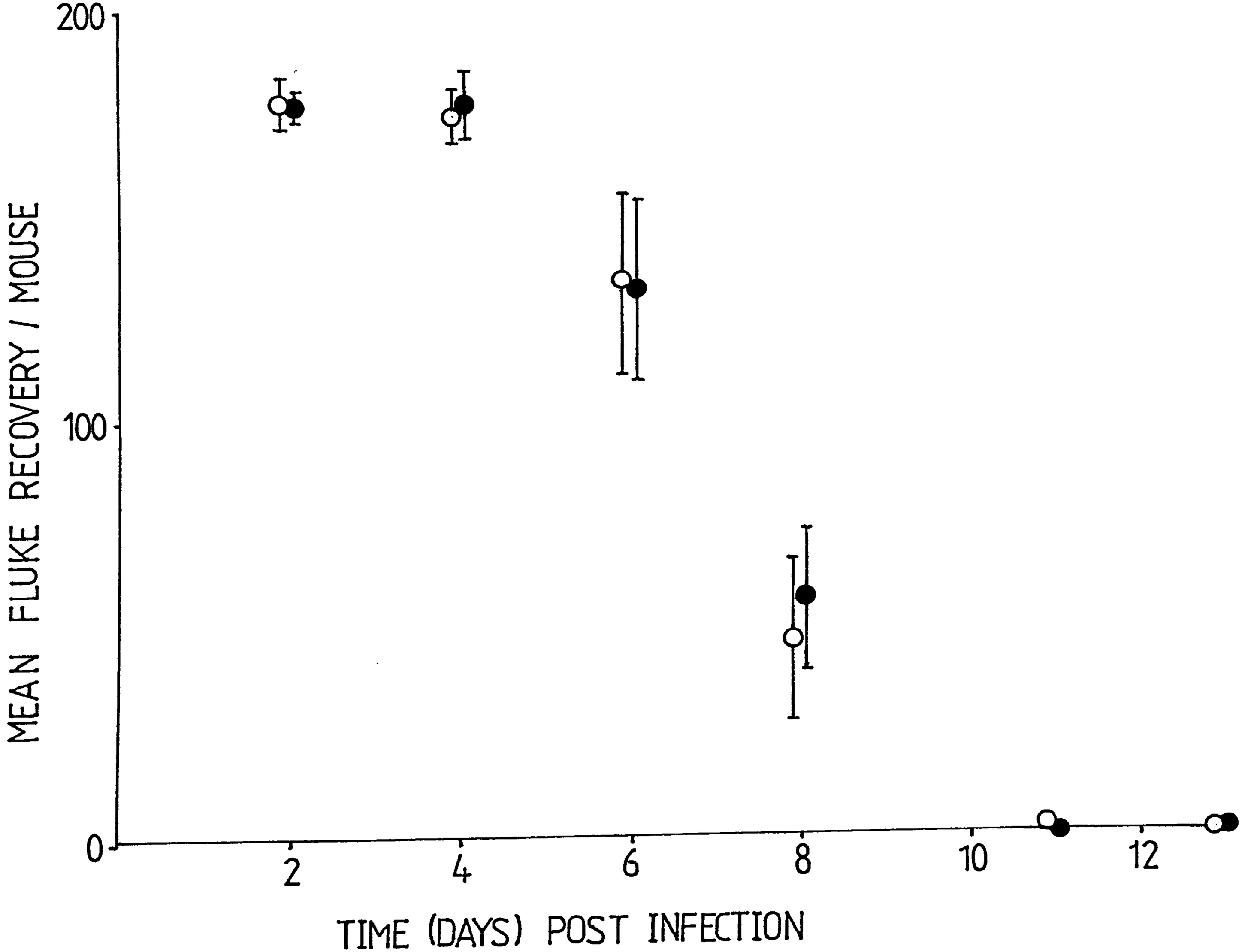
Figure 1-3

Course of a 200 metacercarial primary infection of D.phoxini in male and female NIH mice. Mean fluke recovery per mouse ± S.D.

○ = male mice.

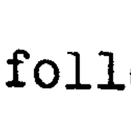
● = female mice.


200
100
0
MEAN FLUKE RECOVERY / MOUSE
2
4
6
8
10
12
TIME (DAYS) POST INFECTION

Facing page 23

Figure 1-4

Distribution of flukes in the anterior small intestine following primary infection of 200 D.phoxini metacercariae in male NIH mice.



= 0-10 cm post pylorus

=10-20 " "

Vertical bars denote standard deviation.

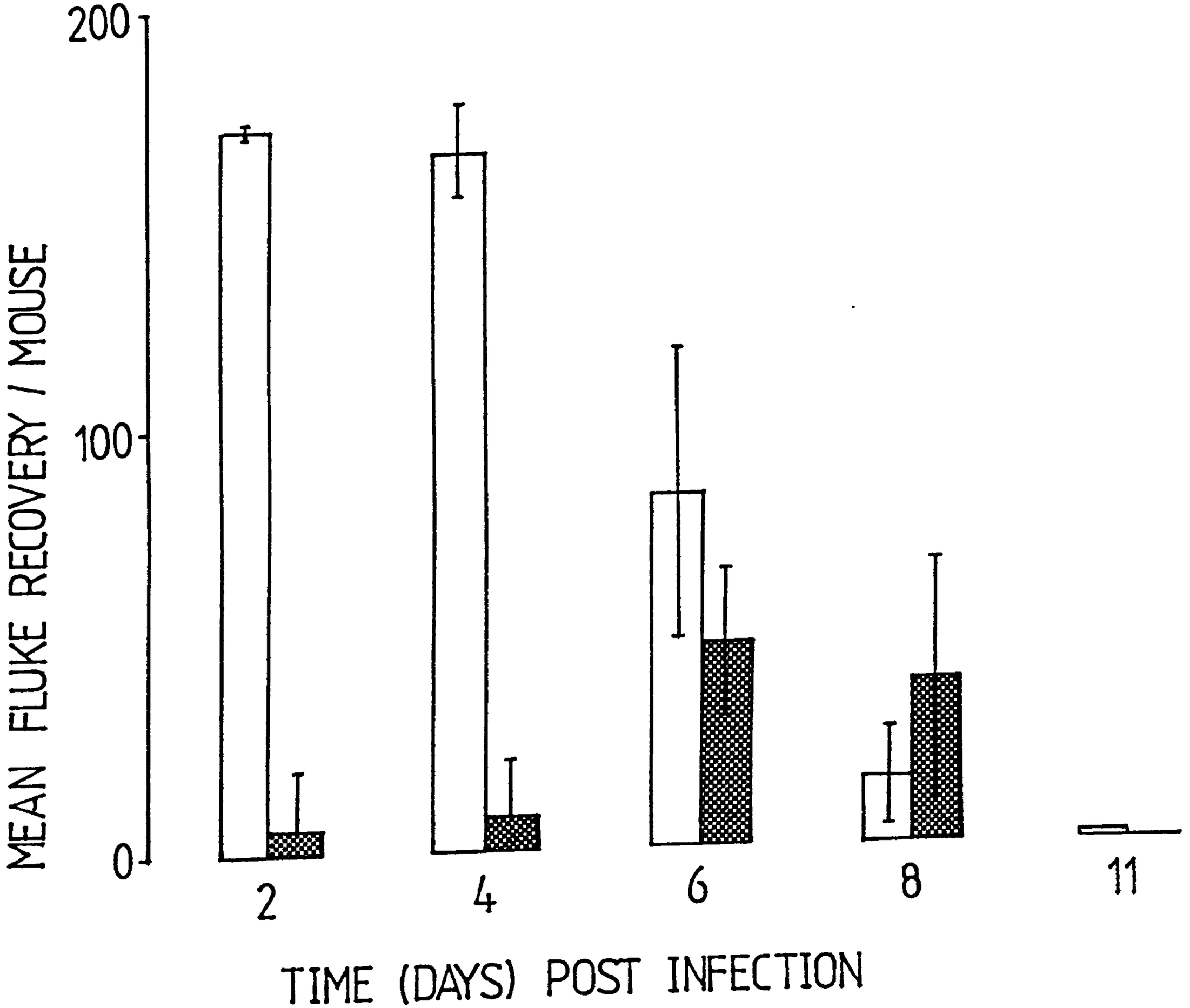

200
100
0
MEAN FLUKE RECOVERY / MOUSE
2
4
6
8
11
TIME (DAYS) POST INFECTION

TABLE I-5

The length of the body and posterior lobe of D.phoxini during a 200 metacercarial primary infection in NIH mice:

| Day pi | No. flukes measured | | | | Mean ± SD fluke length (µm) in different regions of the small intestine | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | | A | B | C | D |
| 2 | 50 | 50 | 50 | T. | 420 ± 27 | 428 ± I5 | | |
| | | | | P. | I40 ± 8 | I4I ± II | | |
| 3 | 50 | 50 | I4 | T. | 46I ± I2 | 473 ± I5 | 430 (±29) | |
| | | | | P. | I6I ± 6 | I65 ± 9 | I50 (±I8) | |
| 4 | 50 | 50 | I4 | T. | 468 ± 23 | 485 ± I9 | 455 (±44) | |
| | | | | P. | I65 ± II | I65 ± 8 | I56 (±26) | |
| 6 | 50 | 40 | 38 | T. | 470 ± I7 | 446 ± 26 | 4I0 ± 23 | |
| | | | | P. | I76 ± 8 | I67 ± I7 | I47 ± 6 | |
| 8 | 8 (sect.D | 2I I0) | 50 | T. | 5I0 (±7I) | 467 ± I7 | 447 ± 23 | 5I8 (± 6) |
| | | | | P. | I97 (±35) | I7I ± I2 | I55 ± II | I86 (±29) |

| | No.meas. | | Mean ± SD length |
|---|---|---|---|
| 0 | 30 | T. | 290 (±20) |
| (metacercariae) | | P. | 20 |

T. = total body length

P. = length of posterior lobe

A = region of small intestine 0-5 cm pp.

B = " 5-I0 "

C = " I0-I5 "

D = " I5-20 "

Egg production during the course of a 200 metacercarial primary infection in NIH mice :

The procedure for estimation of egg production was described on page 13. 36 male NIH mice were infected with 200 metacercariae each. Beginning at 72h pi, 24hour-faecal samples were taken from three pairs of mice, which were killed at the end of the sampling period, and their fluke burdens recorded. The sampling and fluke counting procedure was repeated using a further five groups of six mice, and ending on day 9 pi.

Results are summarized in Table 1-6. Expulsion of flukes began on days 5-6, and only 4% remained by day 9 pi when the last group of mice was autopsied. The rate of egg output detected was extremely low reaching a maximum of 0.65 eggs per fluke per 24h on day 5-6, at the onset of expulsion.

Egg outputs/fluke/24h calculated during the expulsion phase of infection (days 6-9) will represent maximum values in the sense that the fluke burdens are measured at the end of the faecal collection period, and therefore represent minimum values.

Because the presence of eggs in-utero had been used in past experiments as an indication of normal fecundity, it was of interest to compare fluke egg burdens with egg output. It was found that although egg output was insignificant by day 7-8, the proportion of egg bearing flukes had not declined significantly, and remained at 52% Though the number of flukes available for sampling on day 8 pi was very small, the samples were combined and it was found that the proportion of egg bearing flukes was 43% though no eggs were detected in the faeces.

TABLE I-6

Egg production during the course of a 200 metacercarial primary infection in mice

| Time pi | Eggs recovered/ 2 mice /24 h mean* ± SD | Flukes/ 2 mice mean ± SD | % of flukes bearing eggs mean ± SD | Eggs produced/ fluke / 24 h mean ± SD |
|---|---|---|---|---|
| Day 3-4 (72-96h) | 32 ± 2I | 355 ± I2 | nd | 0·09 ± 0·06 |
| 4-5 | I49 ± 45 | 347 ± I0 | nd | 0·43 ± 0·I4 |
| 5-6 | I98 ± 33 | 306 ± I6 | 64 ± 8 | 0·65 ± 0·08 |
| 6-7 | 62 ± 22 | I59 ± I2 | 58 ± 7 | 0·4 ± 0·I7 |
| 7-8 | 0,67 | 73 ± 35 | 52 ± II | 0·07 |
| 8-9 | 0 | I8 ± 4 | 43 (pooled) | 0 |

* n=3

Establishment and growth of D.phoxini metacercariae implanted into different regions of the small intestine.

Infections of 200 metacercariae were implanted into different regions of the small intestine of 45 female NIH mice aged 8-9 weeks. The point of implantation was calculated by the position of the intestinal suture, at autopsy. 22 mice were killed 24h pi, and the remaining 23 mice were killed five days pi. Fluke recoveries, and distribution were noted. Flukes were measured and their egg burdens noted. During fluke recovery procedure, the whole of the small intestine was split into five cm sections and examined. Where implantation occurred within 10 cm of the caecum, it too was examined, however although the length of the mouse small intestine was usually approximately 39 cm , no flukes were recovered distal to 35 cm post pylorus.

Results are shown in Tables 1-7 a,b,c, and Figures 1-8, 1-9, and 1-10.

Establishment of metacercariae: Table 1-7a, (Fig. 1-9).

The establishment rate of metacercariae within the anterior 5% of the small intestine was extremely variable (18.5-64.5%) whereas greater and more consistent (44-73%) establishment occurred when metacercariae were implanted 20-60% posterior to the pylorus.

Establishment of metacercariae implanted into the posterior 40% of the small intestine was very poor. 9.5% of flukes from a single infection were recovered after implantation of metacercariae 80% post-pylorus. Two other attempts to establish metacercariae at 77% and 79.5% post pylorus were unsuccessful.

Five days after implantation (Table 1-7b, Fig 1-10) the mean recovery from the metacercariae implanted in the anterior 30% of the small intestine had dropped to 34.3% and great variability in recovery was still observed. A much greater decrease in recovery (compared with 24h pi) occurred in infections established 30-60% post pylorus. Six out of seven recoveries in this group were below 31% whereas one mouse was found to be harbouring 67% of the administered infection. The removal of this one recovery from

the group would result in a drop in mean recovery from 22% to 14.4% and a decrease in S.D. from 44.7 to 11.2.

Of the metacercariae implanted in the posterior 40% of the small intestine, only one mouse was found to be harbouring flukes on day 5 pi. These had been implanted 80% post pylorus.

Position of flukes: (Tables 1-7a, 1-7b)

No anterior movement of flukes from the site of implantation occurred. A comparison of positions of flukes on days 1 and 5 pi suggests that a slight posterior shift in position occurred during the course of infection, in the case of metacercariae implanted in the anterior 70% of the small intestine. Low variable recoveries contributed to the absence of similar trends in other infections.

Body length:

In most cases fluke recovery was too low to allow statistical analysis of results (Fig. 1-8). Flukes recovered 10-20 cm pp, derived from metacercariae implanted in the anterior 30% of small intestine, were significantly shorter in body and posterior lobe length, compared with those recovered 0-10 cm post pylorus. No similar reduction in length was observed in flukes recovered 10-20 cm post pylorus derived from metacercariae, implanted 30-60% post pylorus, which had presumably not moved far from the point of implantation. The flukes recovered from a single successful implantation 80% post pylorus were unexpectedly large, and similar in size to those which had remained in the anterior 10cm of the small intestine, whereas the few flukes surviving in the same area (20-30 cm post pylorus) which were displaced from their site of implantation (anterior 30% of small intestine) were much smaller.

Egg production:

Presence of eggs in utero was used as an indicator of maturity having been attained. Results are shown on Table 1-7c. The highest percentage of egg bearing flukes was observed in the anterior 10cm of small intestine. Fewer bore eggs 15-20 cm post pylorus. All these flukes developed from

metacercariae implanted in the anterior 60% of the small intestine.

A 21% proportion of egg-bearing flukes in the single infection which established in the posterior 40% of the small intestine is very unexpected, but data from a single infection in the presence of many unsuccessful infections must be regarded with caution.

TABLE 1 - 7

(a) MEAN ± S.D. FLUKE RECOVERY / MOUSE, 24 HOURS AFTER IMPLANTATION OF 200 METACERCARIAE

| POSN OF IMPLANT. IN S.I. | No. MICE | MEAN ± S.D FLUKE RECOVERY/MOUSE 0-5cm p.p. | 5-10cm p.p. | 10-15cmp.p. | 15-20cmp.p. | 20-25cmp.p. | 25-30cmp.p. | Total |
|---|---|---|---|---|---|---|---|---|
| Anterior | 10 | 28.4± 22.6 | 37.9± 21.8 | 16.1± 19.7 | 4.7± 8 | 1.5 | 0 | 87.8± 28.3 |
| Middle | 4 | 0 | 0 | 44 ± 66.2 | 69.3± 44.7 | 13.3 ± 18 | 0.5± 1 | 127.3± 17.8 |
| Posterior | 8 | 0 | 0 | 0 | 0.4 | 2.6 ± 5.7 | 3.6± 9.1 | 6.3± 10.4 |

(b) MEAN ± S.D. FLUKE RECOVERY / MOUSE, FIVE DAYS AFTER IMPLANTATION OF 200 METACERCARIAE

| POSN OF IMPLANT. IN S.I. | No. MICE | MEAN ± S.D. FLUKE RECOVERY/MOUSE 0-5cm p.p. | 5-10cm p.p. | 10-15cmp.p. | 15-20cmp.p. | 20-25cmp.p. | 25-30cmp.p. | Total |
|---|---|---|---|---|---|---|---|---|
| Anterior | 8 | 15.5± 26.8 | 16.4± 11.5 | 11.1± 6.7 | 20.5± 20 | 5.1± 11.1 | 0 | 68.5± 37.9 |
| Middle | 8 | 0 | 2.3± 6.1 | 20.6± 37.1 | 15.4± 13.6 | 4.7± 6.7 | 0.9 | 43.9± 44.7 |
| Posterior | 7 | 0 | 0 | 0 | 0 | 0 | 4.7± 12.5 | 4.7± 12.5 |

TABLE 1 - 7 contnd.

(c) MEAN ± S.D. % OF FLUKES BEARING EGGS, FIVE DAYS AFTER TRANSPLANTATION

| POSN OF IMPLANT. IN S.I. | No. MICE | MEAN ± S.D. % OF FLUKES BEARING EGGS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0-5cm p.p. | 5-10cm p.p. | 10-15cm p.p. | 15-20 cm p.p. | 20-25 cm.p.p. | 25-30 cm p.p. |
| Anterior | 8 | 70.9± 11.5 | 52.2± 53.6 | 28.2± 25.8 | 19.2± 28.9 | 0 | |
| Middle | 8 | | (68) | 23.6± 23.2 | 23.2± 24.7 | 2.7± 7.1 | |
| Posterior | 7 | | | | | | (21) |

Anterior S.I. = region 0-30% post-pylorus

Middle " = " 30-60% " "

Posterior " = " 60-100% " "

Figures in brackets denote samples from one mouse only

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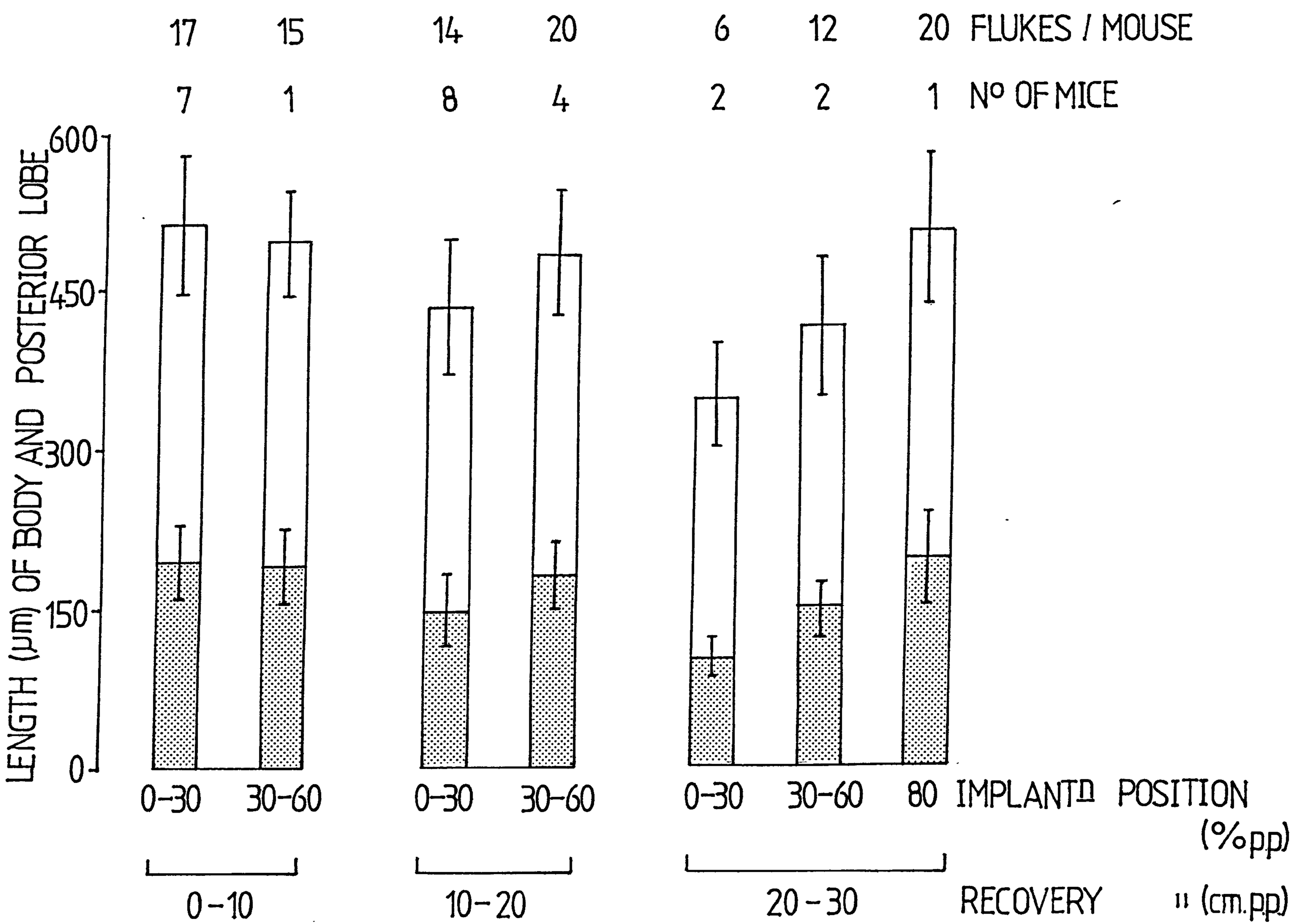
Figure 1-8

Mean $\pm$ S.D. Length of body and posterior lobe of flukes recovered five days after implantation of 200 metacercariae of D.phoxini into naive CFLP mice.

= Mean body length

= Mean posterior lobe length

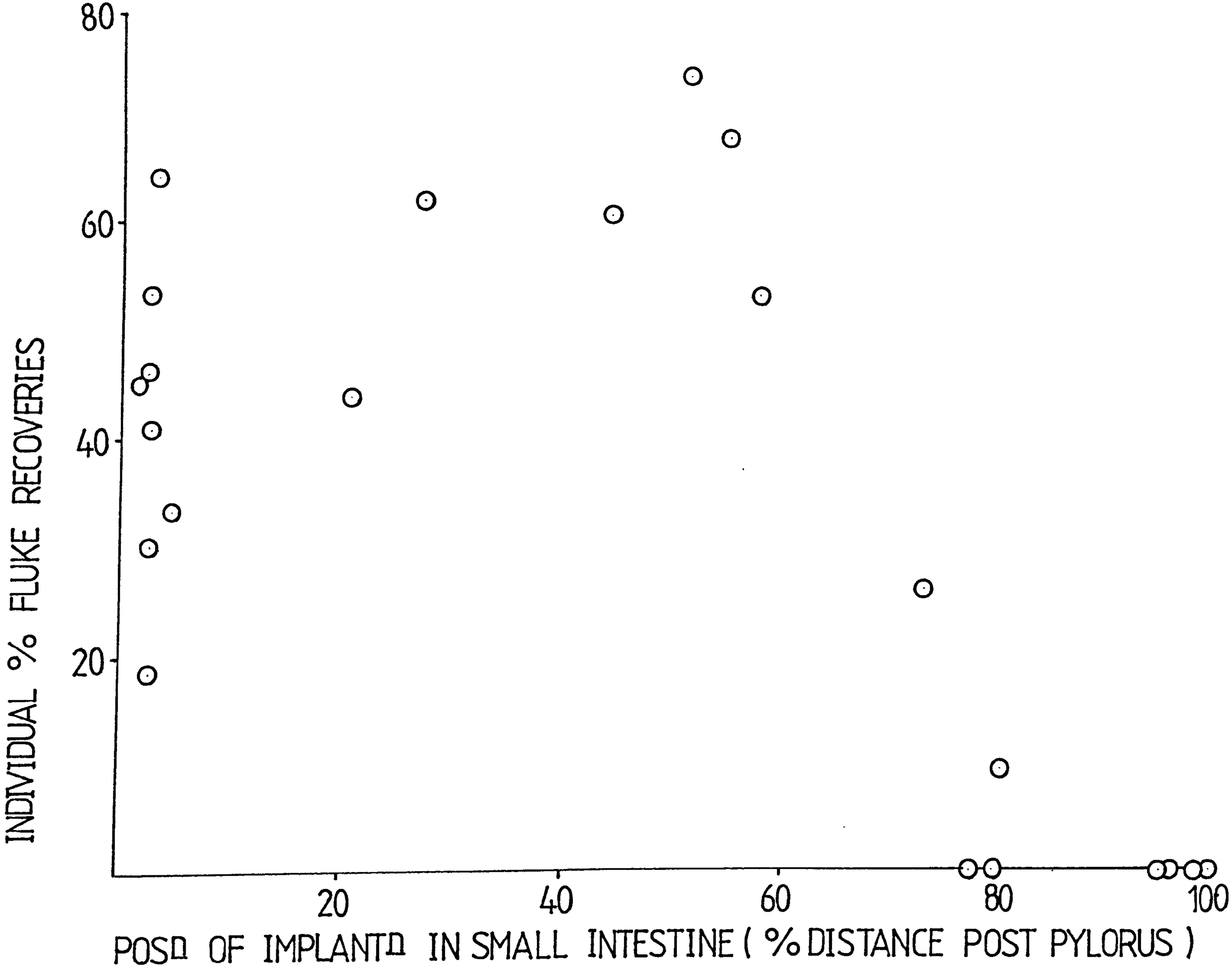

17 15 14 20 6 12 20 FLUKES / MOUSE
7 1 8 4 2 2 1 Nº OF MICE
LENGTH (µm) OF BODY AND POSTERIOR LOBE
600
450
300
150
0
0-30 30-60
0-30 30-60
0-30 30-60 80 IMPLANTED POSITION (%pp)
0-10
10-20
20-30 RECOVERY " (cm.pp)

Facing page 33

Figure 1-9

Fluke recoveries, 24h after implantation of 200 metacercariae of D.phoxini into varying regions of the small intestine of CFLP mice.

Recoveries are shown at the point of implantation.

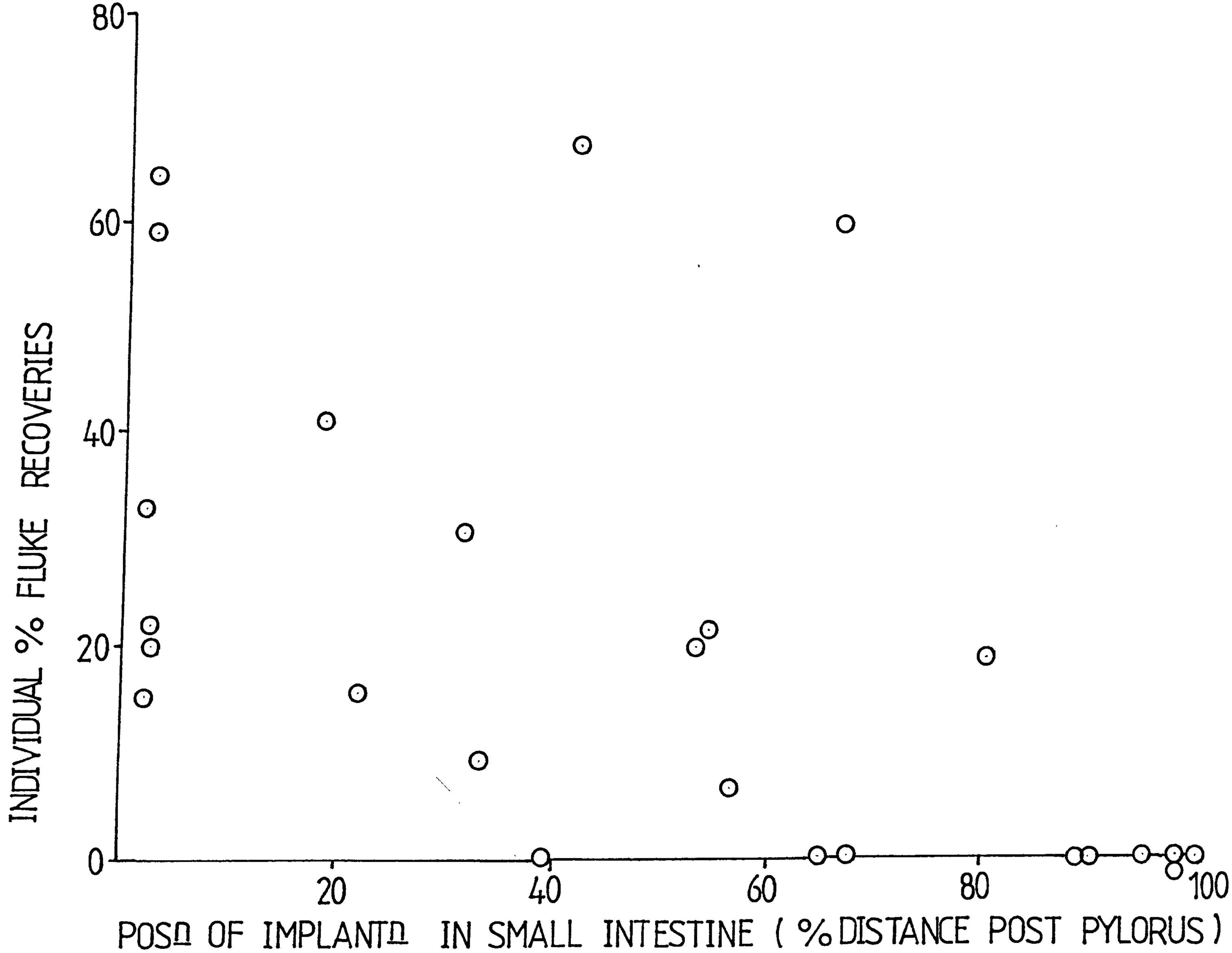

80
60
40
20
INDIVIDUAL % FLUKE RECOVERIES
20
40
60
80
100
POSN OF IMPLANTN IN SMALL INTESTINE ( % DISTANCE POST PYLORUS )

Facing page 34

Figure 1-10

Fluke recoveries, five days after implantation of 200 metacercariae of D. phoxini into varying regions of the small intestine of CFLP mice.

Recoveries are shown at the point of implantation.


80
60
40
20
0
INDIVIDUAL % FLUKE RECOVERIES
20
40
60
80
100
POSN OF IMPLANTN IN SMALL INTESTINE ( % DISTANCE POST PYLORUS )

## Discussion

It has been shown that efficient and consistent establishment of D.phoxini metacercariae can be achieved if mice are starved prior to infection, suggesting that the problems associated with establishment are caused by conditions in the mouse stomach; however the experimental design and results obtained give little indication of the precise mechanism by which starvation caused the observed improvement in establishment. The observation that a six hour period of starvation was effective when begun at 6-0 am but not if begun at 9-0 am is interesting. Infection timing in subsequent experiments (in which establishment was satisfactory) conducted during this project, varied from noon to late evening, suggesting that the time of infection is in itself not of critical importance, but this has not been proved in a controlled experiment. It is possible that a six hour period of "starvation" which totally coincides with a natural period of non-feeding and inactivity of the mice concerned may affect neither stomach contents, nor digestive physiology, whereas the incorporation of a phase normally associated with feeding and activity into the starvation period might have several consequences.

1. Stomach emptying would be completed only in mice from which food was with held during a sufficiently long period of activity prior to infection, in mice which became inactive before stomach emptying was complete, some digesta would be expected to remain.

2. The gastric emptying rate depends on the functional state of the stomach, and is faster in starved rats than in fed rats (Poulakos and Kent 1973), and once again the physiological manifestations of fasting would be expected to be apparent only when the period of fasting coincided with a period normally associated with feeding.

The results obtained show that no digesta was present in the stomachs of mice starved from 6-0 am, however the amounts of food occurring in some mice starved from 9-0 am were very small, though all contained some food. Stomach contents may present a physical barrier to the passage of metacercariae

to the pylorus. In addition, the presence of food in the stomach would be expected to prevent the acceleration of emptying associated with starvation, and indeed the presence of large fibrous particles would be expected to delay stomach emptying (Davenport 1977).

The survival of parasites in starved mice demonstrates that D.phoxini can tolerate short exposure to the conditions in the stomach, even though in the empty stomach, the gastric mucosa is acidified. (Johnson 1977). The role of enhanced gastric emptying in the establishment of D.phoxini in mice could be further evaluated by killing starved and unstarved mice within minutes of infection, and observing the time taken by metacercariae to reach the duodenum.

Although no direct comparison was made, results obtained suggested that spermatogenesis in D.phoxini in the mouse commences and is completed at a time similar to that which was reported by Bell (1956) in the duckling, but that vitellogenesis may be slightly delayed in the mouse. This is not surprising as the nutritional demands of oogenesis and vitellogenesis are probably far greater and more complex than those of spermatogenesis, and therefore these processes might be more greatly affected by adverse conditions or perhaps nutritional deficiencies in the mouse, or by the mammalian body temperature which is several degrees lower than that of a bird. Subophimum conditions in the mouse are further suggested by the small size attained by D.phoxini (Berrie 1960).

The sex of host had no detectable effect on the course of primary infection of D.phoxini. The presence of fully formed attachment organs and gut caeca in the infective metacercaria and the resultant rapid establishment of the host-parasite interface following infection, allows rapid growth, and maturation occurs within four days, after which no further growth occurs. It is possible that the host-parasite interface remains intact until the time of fluke loss, especially as extracorporeal digestion, dependent upon its integrity, is thought to be an important component of parasite nutrition. Similar inactivity was postulated by Blake (1974), studying A.gracilis minor

in the duckling. The comparatively small size of flukes which were recovered posterior to their site of establishment following both oral and surgical infections may reflect the disruption of the host-parasite interface and therefore of feeding. The nature of the factors causing such disruption is of course, unknown.

The establishment of metacercariae from an oral infection occurred almost exclusively in the anterior 10 cm of the small intestine, however following surgical implantation, establishment was most efficient and consistent in the mid-region rather than the anterior of the small intestine. Quite poor establishment of surgically implanted metacercariae might be partially due to the absence of a pre-incubation period similar to that which would follow oral infection. This might cause a delay in the attachment response of implanted metacercariae, and this effect might be enhanced by the cooling of the exposed loop of intestine during the implantation procedure.

Although conditions favouring attachment may prevail in the mid-region of the small intestine, and parasites which attached and remained stationary after implantation into any region of the small intestine were of similar size, it appears that actual losses of parasites were greatest in those infections implanted in the posterior 70% of the small intestine. Dramatic losses within 24 h. of implantation of metacercariae into the posterior 40% of the small intestine do not, however, preclude the possibility that transient attachment occurred. Insufficient flukes were recovered to allow adequate analysis of results, particularly in the posterior regions of the small intestine. It is not possible at this stage, to speculate on the factors which might stimulate early loss of flukes implanted into the mid and posterior regions of the small intestine, as so many factors change progressively along the length of the gut, for instance, villar length, nature of mucus, muscular activity, bacterial flora, physicochemical conditions and nutrient availability.

Coupled with the very limited ability of D.phoxini to survive posterior

"drift" before parasite loss occurred, is the absence of any evidence of anterior migration of D.phoxini from any point in the gut.

Egg production by D.phoxini in the mouse was suspected to be low but the egg counts obtained are inordinately low. For instance Blake (1974) estimated that A.gracilis minor in the duckling can produce over 80 eggs per 24 h. If in fact the egg production rate by D.phoxini in the mouse is as low as results suggest, this may be due to the combined effects of a suboptimal host environment (Berrie 1960) and a rapid immune response. In other systems examined, a reduction in fecundity normally precedes intestinal helminth rejection. This factor may be of importance in this system at the level of infection used, especially when it is considered that at the 200 metacercarial level of infection, rejection of primary infection begins only 2-3 days after patency is achieved. The low egg recovery on day 3-4 pi, will be partially due to immaturity of a proportion of the flukes during the initial part of this recovery period. The fact that egg production declined from a maximum at the onset of expulsion to a negligible rate within 24 h. after which time many flukes still contained eggs, further supports the view that although the appearance of eggs in utero is useful as a criterion in establishing the maturity of parasites, it is not an accurate reflection of the dynamic process of egg production, because of the possibility that in deteriorating environmental conditions, eggs may be retained in utero.

## Summary

It was found that starvation of CFLP and NIH mice for six hours prior to infection was sufficient to produce a marked improvement in the level and consistency of establishment of oral infections of D.phoxini metacercariae, if the mice were starved from 6AM, allowing stomach emptying to occur before infection.

In contrast to spermatogenesis, the detection of vitellogenesis and oogenesis in D.phoxini in the NIH mouse was markedly delayed compared with that reported in the duckling.

There was no difference in establishment or loss of a 200 metacercarial oral infection in male and female NIH mice. Loss began on day 6 and was complete by day 11 pi. Growth of flukes was complete by day 3. Flukes were largely confined to the anterior 10 cm of small intestine until the loss phase, when some attached to the region 10-20 cm post pylorus before being lost.

The method used detected a very low rate of egg production which declined rapidly after the onset of the loss phase of infection, although at this time there was not a corresponding decline in the percentage of flukes bearing eggs.

Implantation of metacercariae into different regions of the small intestine led to the following conclusions.

1. Establishment was best 30-60% post pylorus, and very poor in the posterior 40% of the small intestine. Inconsistent establishment in the anterior duodenum could be due to lack of preincubation, combined with other factors.

2. Recovery of flukes five days after transplantation of metacercariae was best in those implanted in the anterior 30% of the small intestine and the percentage of egg bearing flukes was highest in the anterior 10 cm of small intestine.

3. Flukes that survived until day 5 were the same size if found near to the site of implantation. Those which had moved in a posterior direction were smaller.

# CHAPTER TWO

Factors influencing loss of a primary infection of D.phoxini from the mouse.

The object of experiments in this chapter was to evaluate the roles of parasite senescence and the host response in the loss of a primary infection of D.phoxini from the mouse.

Earlier work (Hopkins, unpublished) has shown that an immune response is stimulated in the mouse by a primary infection of 200 metacercariae, as demonstrated by the accelerated rejection of a challenge infection given 21 days later. The effect of the immunological response on a primary infection is however not known. The possible role of senescence in fluke loss cannot be discounted, particularly in view of the short lifespan of this parasite in its avian hosts, referred to by Wylie Williams and Hopkins (1960).

Three experiments were performed in an attempt to determine the maximum longevity of D.phoxini in the mouse:

1. The influence of the number of flukes present on the longevity of a primary infection:

It has been shown in some host-parasite systems that there may exist a low "threshold" level in the number of flukes in primary infection, below which "spontaneous cure" is not stimulated. Low level (10 egg) infections of T.muris were found to persist beyond the usual two to three week period in NIH mice (Wakelin 1973). Conversely, an infection of only 10 T.spiralis larvae elicits a spontaneous cure response with loss of parasites by day 14 pi, almost as rapidly as in a heavy infection (Wakelin and Lloyd 1976). Not only is the expulsion of low level infections (five worm) of Echinostoma revolutum from the mouse thought to be host mediated (Christensen et al 1981) but in contrast to other host parasite models it was found that an increase in worm burden delayed the rejection of the primary infection. The authors suggested that this was due to parasite-induced immunotolerance. In the present work, the longevity of D.phoxini in the mouse, in primary infections varying from 200 to eight metacercariae is investigated.

2. The effect of corticosteroids on the longevity, fecundity and growth

of a primary infection of D.phoxini is investigated. These effects of corticosteroids have been studied in many mouse-intestinal parasite systems (Aspicularis tetraptera (Behnke 1975), Hymenolepis spp (Moss 1971, Hopkins, Subramanian and Stallard 1972, Hopkins and Stallard 1976), Trichuris muris (Campbell 1963, Wakelin 1970), T.spiralis (Stoner and Godwin 1953, 1954) N.brasiliensis (Kennedy 1980) and the trematode A.gracilis minor in the duckling (Blake 1974). The effects of corticosteroids upon immune and inflammatory responses have been the subject of an enormous amount of research. These effects have been found to be extremely complex and far-reaching (a reflection of the nature of the responses themselves) and are incompletely understood. The lymphoid systems of different species show great differences in sensitivity to corticosteroids: the rat and mouse are very susceptible, whereas man and guinea pig are comparatively resistant (Claman 1972, 1975). Dracott and Smith (1979) stated that :- "Corticosteroids have at least three effects on the cells of the immune system: destruction, inhibition of function and redistribution.... Susceptibility to these effects depends upon the animal species, the class or even subclass of cell, its function, location, physiological maturity and state of immunological activation".

In the mouse, corticosteroids cause reduction of recirculating cells and lymphoid tissues. This effect, mainly due to cell destruction, is greatest in lymph nodes > spleen > thymus. The localisation in the bone marrow maybe little affected (Zatz 1975, Dracott and Smith 1979). Plasma cells may be cortisone resistant (Segal et al 1972) but other B cells may be severely affected (Dracott and Smith 1979). The humoral response to T-dependent antigen was found to be highly susceptible to cortisone (Mantzouranis and Borel 1979). Accessory macrophages may be a prime target for immunosuppression. Effects on T cells are highly complex. Immunologically immature cortical thymocytes and T cell activation and proliferation seem to be sensitive. In addition almost every aspect of inflammation is affected by corticosteroid treatment. There are several recent reviews on effects of corticosteroids (Claman 1975, Fauci 1975, Zatz 1975, Mantzouranis

and Borel 1979, Dracott and Smith 1979).

3. Because of the far reaching extent of the effects of cortisone acetate on the mouse, and the possibility of direct (non immunological) effects on parasite growth and longevity it was decided to attempt to extend the longevity of D.phoxini in the immunocompetent host using the technique of serial transplantation. This has been performed in most established laboratory intestinal host-parasite models, in which the longevity of parasites was extended by their repeated removal from the donor host gut before the latter responded to their presence (Ogilvie and Hockley 1968, Ogilvie and Love 1974, Rothwell, et al 1980 , Hopkins and Zajac 1976, Howard 1977, Kennedy, Wakelin and Wilson 1978, Kennedy 1980, Kennedy and Bruce 1980). This technique has also been used to investigate the nature of the effects of the host response upon the parasite, and in particular, their reversibility. Advanced cytopathological changes in N.brasiliensis in the rat are irreversible (Ogilvie and Hockley 1968, Jones and Ogilvie 1971) whereas those occurring in T.spiralis in the mouse (Kennedy, Wakelin and Wilson 1979), T.colubriformis (Rothwell et al 1980 ), S.ratti in the rat (Moqbel et al 1980) and H.diminuta in mice (Hopkins and Zajac 1976) are reversible, with recovery occurring after transplantation to a naive host. In the present work, the ability of D.phoxini adults undergoing expulsion to re-establish in naive recipients is investigated.

## Materials and Methods

Procedures for oral infection, recovery and measurement of flukes and infection by laparotomy are described in Chapter 1.

Cortisone acetate (Cortistab, Boots Ltd) suspension (containing 25 mg/ml) was injected subcutaneously in the neck region at a dosage of 0.04 ml ( 1mg) per mouse. Administration began on day 2 of infection and continued thereafter, on alternate days, until one or two days before the day of kill.

During cortisone acetate treatment, and following laparotomy, mice were given oxytetracycline HCl ("Terramycin, Pfizer Ltd) in their drinking water at a concentration of 3g Terramycin/litre.

## Results

The effect of varying the size of primary infection, on the establishment, growth and expulsion of D.phoxini from the mouse intestine.

Female NIH mice aged seven to eight weeks were infected with 8, 20, 50 or 400 D.phoxini metacercariae. Groups of four or five mice were killed at 48h intervals after infection. Flukes were recovered from the intestine, fixed in 70% alcohol and measured.

When comparing the longevity of flukes in 8, 20, 50 and 400 metacercarial infections it was found (Fig 2-1) that whereas loss of a 400 fluke infection was underway by day 6 pi, and almost complete by day 8 pi, loss of flukes from a low level (8 met.) infection was not evident until day 10 pi. Although a four day delay occurred in the onset of expulsion of an 8 met. infection, the kinetics of the parasite loss phase were very similar to those of the 400 met. infection.

The loss of flukes from eight and 20 metacercarial infections was almost identical.

There was no increase in longevity of flukes in low level infections, apart from that which resulted from the four day delay in expulsion described above.

Growth of flukes (Table 2-2) from 20 metacercarial infections was complete by day 4 pi, whereas, although the flukes from 400 metacercarial infections were mature by this time, they were smaller, and growth continued (flukes sampled 0-10 cm pp).

The maximum size attained (day 4 pi) by flukes from 20 and 50 metacercarial infections was greater than that attained by those from 400 metacercarial infections (day 6 pi), however by day 8 pi flukes from heavy infections (400) were at least as long as those from 20 metacercarial infections.

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Figure 2-1

The effect of varying size of primary infection on the course of infection.

○ = mice given 8 metacercariae

◔ = " " 20 "

● = " " 50 "

△ = " " 400 "

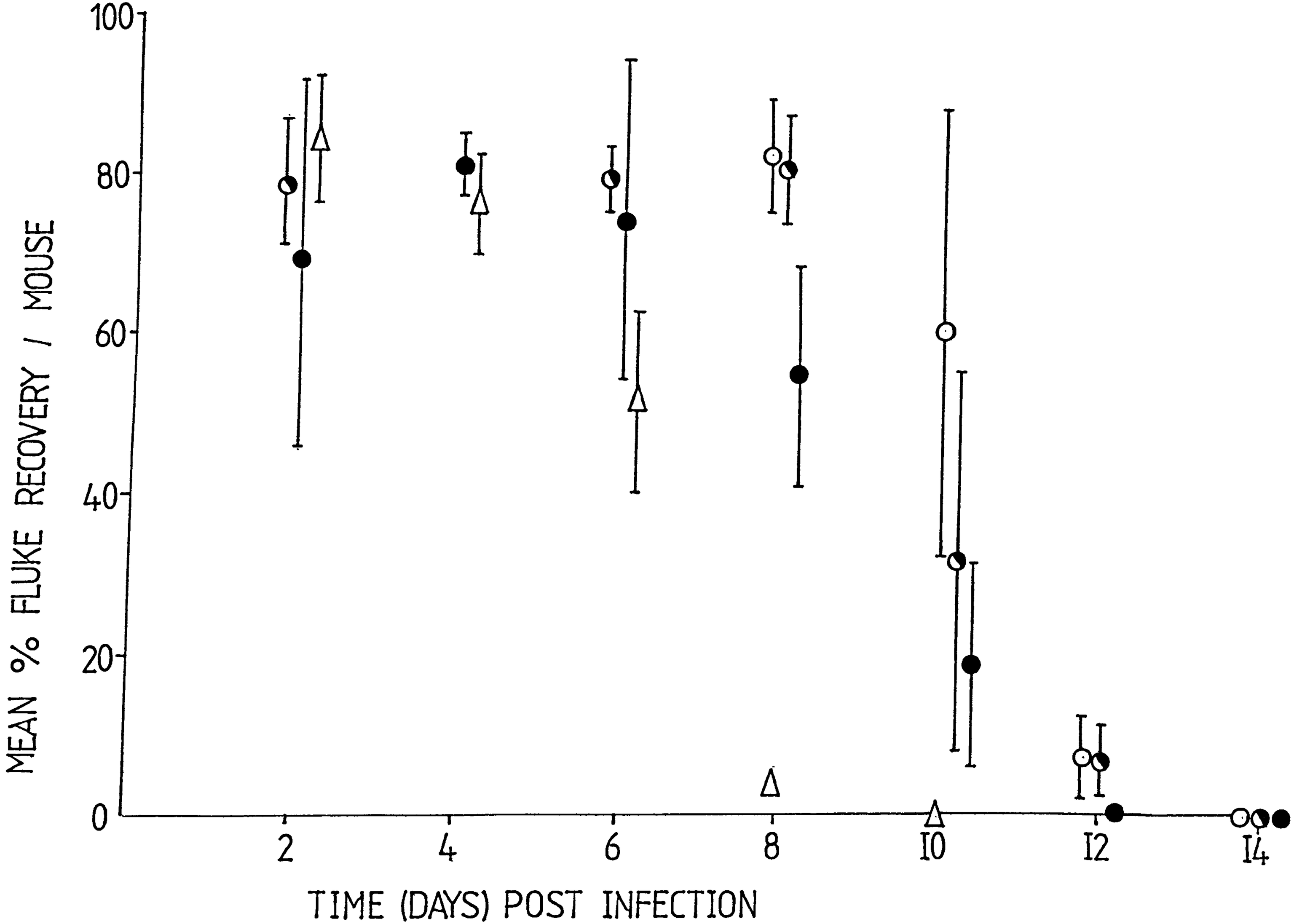
100
80
60
40
20
0
MEAN % FLUKE RECOVERY / MOUSE
2
4
6
8
10
12
14
TIME (DAYS) POST INFECTION

TABLE 2-2

The effect of varying size of primary infection on size (length) of recovered flukes

| Day p.i. | Metacerc. given | No. mice | Flukes/ mouse meas. | Recovery position (cm p.p.) | Mean ± SD fluke length (µm) Body(total) length | Posterior lobe length |
|---|---|---|---|---|---|---|
| 2 | 20 | 5 | 10 | 0 - 10 | $^{a}428 \pm 14$ | $135 \pm 12$ |
| " | 50 | 5 | 10 | " | $411 \pm 20$ | $131 \pm 8$ |
| " | 400 | 5 | 10 | " | $^{a}402 \pm 17$ | $128 \pm 8$ |
| 4 | 50 | 4 | 10 | " | $^{b}639 \pm 39$ | $^{c}258 \pm 26$ |
| " | 400 | 5 | 10 | " | $^{b}474 \pm 38$ | $^{c}168 \pm 20$ |
| 6 | 20 | 4 | 9 | " | $^{d}600 \pm 24$ | $^{f}239 \pm 15$ |
| " | 50 | 5 | 10 | " | $^{e}585 \pm 18$ | $^{g}240 \pm 9$ |
| " | 400 | 4 | 10 | " | $^{de}546 \pm 20$ | $^{fg}209 \pm 8$ |
| " | 400 | 4 | 10 | 10 - 20 | $^{de}488 \pm 8$ | $^{fg}179 \pm 11$ |
| 8 | 20 | 4 | 10 | 0 - 10 | $540 \pm 29$ | $215 \pm 9$ |
| " | 50 | 5 | 10 | 0 - 20 | $519 \pm 23$ | $192 \pm 15$ |
| " | 400 | 4 | 7 | 0 - 10 | $546 \pm 80$ | $215 \pm 50$ |
| 10 | 20 | 5 | 6 | 0 - 10 | $531 \pm 15$ | $221 \pm 15$ |

(p.p. = post pylorus)

a-g denotes means statistically different ($P \leq 0{\cdot}05$)

(comparison of flukes recovered at same time p.i.)

The effect of cortisone acetate on the course of a 200 metacercarial primary infection.

Female NIH mice (65) aged between six and seven weeks were infected with 200 D.phoxini metacercariae. Starting on day 2 pi, cortisone acetate was administered to 47 mice on alternate days, until one or two days before the day of kill.

Groups of between four and six cortisone acetate treated animals were killed on days 6, 8, 11, 13, 15, 17, 19, 22 and 25 pi. Untreated controls were killed on days 6, 8, and 11 pi. The flukes were recovered, and their number, distribution and length recorded.

Figure 2-3 shows that slight loss of flukes was noted between days 6-8 in cortisone acetate-treated animals with no further loss occurring before day 13 pi after which fluke loss increased to a maximum rate between days 17-19 before declining. Expulsion of flukes from control mice was complete by day 11 pi, therefore cortison acetate both delayed the onset and reduced the rate of fluke loss considerably.

The change in position of flukes in the intestine which normally precedes expulsion did not occur in cortisone acetate-treated mice. On day 8 pi 3% of flukes were recovered 10-20 cm post pylorus, compared with 68% in controls. A maximum proportion of 8.3% of flukes were found in this region on day 11 pi in cortisone acetate-treated mice, after which the percentage proportion declined.

The lengths of flukes in cortisone acetate treated mice varied within very narrow limits during the course of infection (Table 2-4) and did not diminish in length. The flukes were larger than those recovered from control mice and of similar length to those recovered from day 4 of low level (50 metacercarial) infections (Table 2-2). Flukes recovered from 10-15 cm post pylorus were smaller than those recovered in the anterior five cm of small intestine in all mice. In cortisone treated mice there was a difference in length between flukes recovered 0-5 and 5-10 cm post pylorus, throughout infection.

A high proportion of flukes in cortisone acetate treated mice bore eggs (>69% up to day 19 pi) (Table 2-5). This proportion declined to 42.6% on day 25 pi, however as stated in chapter one, this is not a reliable criterion by which fecundity can be measured.

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Figure 2-3

The effect of cortisone acetate on the course of a 200 metacercarial primary infection

● = mice treated with cortisone acetate

O = untreated mice

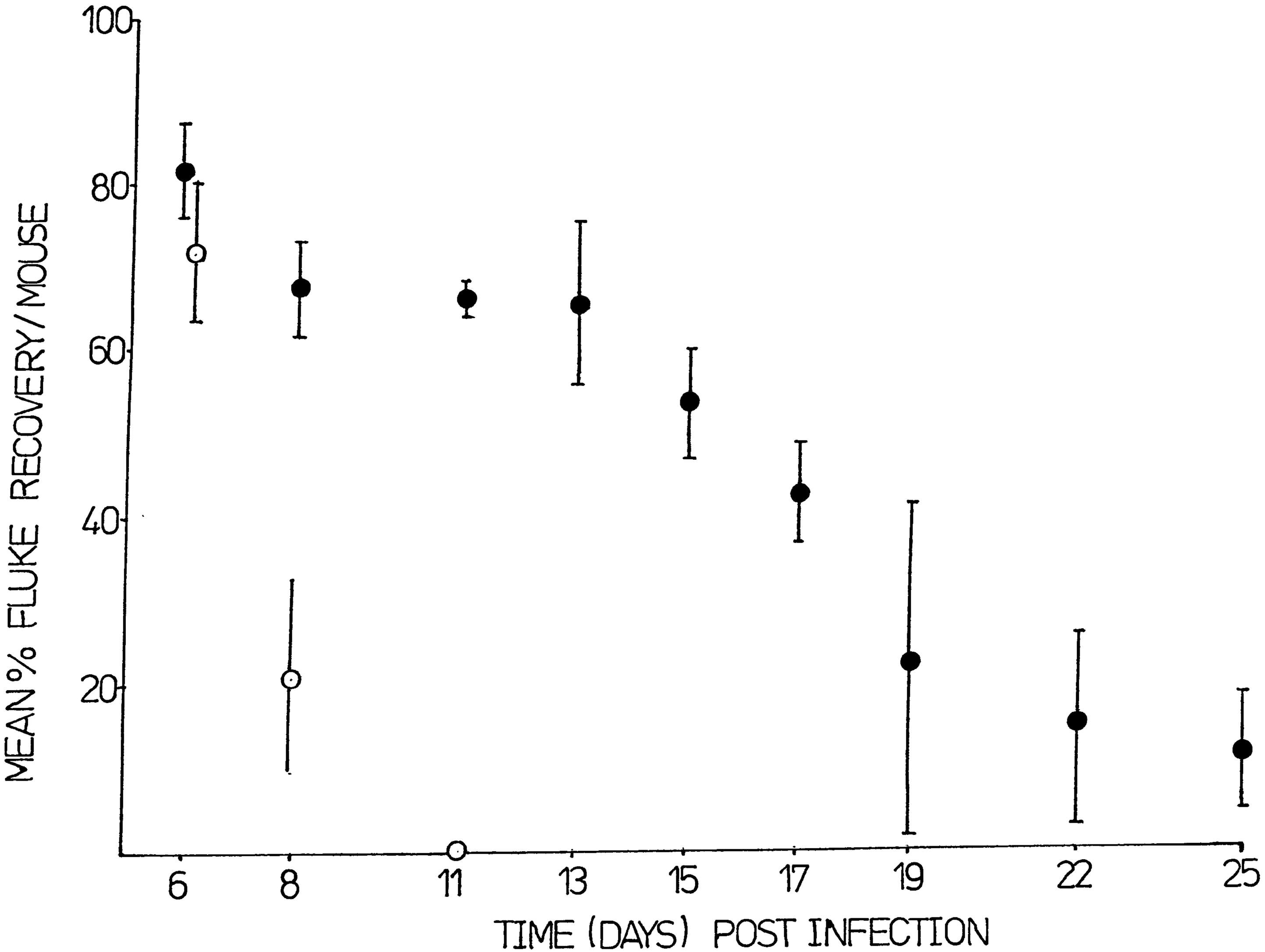
100
80
60
40
20
MEAN% FLUKE RECOVERY/MOUSE
6
8
11
13
15
17
19
22
25
TIME (DAYS) POST INFECTION

TABLE 2-4

Lengths of flukes recovered from infections in cortisone acetate treated and untreated mice

| Group | Day p.i. | No. mice | Flukes/ mouse meas. | Recovery position (cm p.p.) | Mean ± SD fluke length (µm) Posterior lobe | Total body length |
|---|---|---|---|---|---|---|
| Cort.rec. | 6 | 5 | 10 | 0 - 5 | 261 ± 27 | 624 ± 32 |
| | " | 6 | 10 | 5 - 10 | 224 29 | 576 53 |
| | " | 4 | 5 | 10 - 15 | 209 12 | 537 36 |
| Control | 6 | 5 | 10 | 0 - 5 | 197 ± 11 | 522 ± 26 |
| | " | 5 | 10 | 5 - 10 | 192 14 | 498 31 |
| | " | 4 | 7 | 10 - 15 | 165 9 | 458 9 |
| Cort.rec. | 8 | 5 | 10 | 0 - 5 | 282 ± 4 | 635 ± 47 |
| | " | 5 | 10 | 5 - 10 | 228 24 | 572 33 |
| | " | (pooled total 12) | | 10 - 15 | 207 (26) | 521 (47) |
| Control | 8 | 3 | 7 | 5 - 10 | 171 ± 24 | 488 ± 38 |
| | " | 5 | 10 | 10 - 15 | 170 12 | 473 24 |
| Cort.rec. | 13 | 5 | 10 | 0 - 5 | 254 ± 20 | 617 ± 39 |
| | " | 5 | 10 | 5 - 10 | 246 26 | 608 39 |
| | " | (pooled total 28) | | 10 - 15 | 207 (38) | 534 (66) |
| Cort.rec. | 19 | 4 | 10 | 0 - 5 | 263 ± 27 | 642 ± 69 |
| | " | 4 | 7 | 5 - 10 | 227 21 | 582 48 |
| Cort.rec. | 25 | 4 | 5-10 | 0 - 5 | 248 ± 15 | 624 ± 24 |
| | " | 3 | 7 | 5 - 10 | 252 8 | 618 29 |

TABLE 2-5

Proportions of egg-bearing flukes recovered from 200 metacercarial infections in cortisone acetate treated and control (untreated) mice

| Group | Day p.i. | % of recovered flukes bearing eggs (mean ± SD) |
|---|---|---|
| Cort.recip.s | 6 | 77·4 ± 10·6 |
| Control | " | 74·4 6 |
| Cort.recip.s | 8 | 67·5 ± 8·1 |
| Control | " | 53·5 8·4 |
| Cort.recip.s | 13 | 78·9 ± 6·2 |
| " " | 19 | 69·6 ± 11·6 |
| " " | 25 | 42·6 ± 15·1 |

TABLE 2-6

Survival of flukes from 20 metacercarial infections transplanted at six-day intervals

| No.of mice | Age of flukes (days) | Day of trans-plant. | Fluke recovery/mouse (mean ± SD) 0-5cm pp. | 5-10cm pp. | 10-15cm | Mean % recovery/ mouse |
|---|---|---|---|---|---|---|
| 15 | 6 | - | 11·5 ± 2·6 | 2·7 ± 1·6 | 0 | 70 ± 12 |
| 4 | 12 | - | 0·5 | 0·3 | 0 | 4 ± 5 |
| 9 | 12 | Day 6 | 7·7 ± 4·3 | 4·7 ± 3·3 | 0·6 | 65 ± 21 |
| 4 | 18 | Day 6 + " 12 | 4·8 ± 2·2 | 2·3 ± 1·9 | 0 | 35 ± 18 |

The use of serial transplantation to prolong the longevity of D.phoxini in the mouse intestine.

The results of an earlier experiment (Fig. 2-1) indicated that loss of flukes from a 20 metacercarial primary infection does not occur until after day 8 pi, therefore in order to minimise the exposure of parasites to the trauma associated with the recovery/transplantation procedure, and inflammatory changes in the duodenum following laparotomy, it was decided to use low level infections.

Infections of 20 flukes were transplanted into naive mice (NIH aged 8-10 weeks) at six day intervals. Infection levels were restored to twenty flukes at each transplantation.

Results are shown on Table 2-6. In the absence of a kill soon after transplantation it is difficult to assess losses of flukes due to infection procedure. Recoveries declined from 66% on day 12 to 35% on day 18, however both of these recoveries represent considerable increase in longevity of flukes, compared with those from a normal primary infection, which were almost completely expelled by day 12 pi.

There was no posterior shift in distribution of flukes associated with increasing age of flukes, suggesting that the normal process of fluke loss, (with which such a change in fluke distribution is usually associated) was not occurring at the time that any of the transplantations were performed.

Reversibility of changes in flukes associated with expulsion of primary infection.

Expulsion of a primary infection of 200 metacercariae is well advanced by day 8 pi. In order to observe whether flukes undergoing expulsion would successfully re-establish after transplantation into a naive host, 40 flukes (8-day-old) recovered from 200 metacercarial primary infections were transplanted to each of 16 naive recipients.

Oral infections of 40 metacercariae, followed by sham laparotomy, were adminstered to a further 12 mice.

Groups of between three and six mice were killed 3,6 and 11 days after infection by laparotomy or sham laparotomy, and their flukes recovered

Three donor mice were killed on day 11 pi and found to contain no flukes. Fluke recoveries were also checked in four donor mice on the day of transplantation. These mice were found to be harbouring 25% of the original infection (hence expulsion was underway).

Results are shown on figure 2-7.

Considerable losses of flukes occurred during transplantation. This is reflected in the means and large standard deviations of recoveries on day 3 after transplantation, and also on day 6 after transplantation at which stage further losses had not occurred compared with day 3. Expulsion began after day 6 and was completed by day 11 post transplantation. Flukes were found almost exclusively in the anterior 10 cm post pylorus, further indicating that the expulsion phase of infection had not yet begun on days 3 and 6 after transplantation.

A control (unoperated) was not included, to assess the effect of surgery upon the subsequent longevity and loss of a primary infection from the mouse, however the recoveries obtained from sham operated oral infections were similar to recoveries from normal oral infections in other experiments. Although laparotomy has been known to cause a one or two day delay in the expulsion of primary infections of some intestinal parasites, this effect would remain undetected in this experiment, due to the timing of the kills.

The results do indicate that flukes undergoing expulsion in primary infection are able to re-establish in naive hosts, where they undergo a time course of infection similar to that of a metacercarial infection. The level of infection used was too low to enable measurement of egg counts and thus effects of expulsion and transplantation on fecundity were not measured.

TABLE 2 - 7 Recovery of flukes after transplantation to naive mice

| DAY POST. TRANSPLANT. | GROUP | No. MICE /GROUP. | MEAN ± S.D. FLUKE RECOVERY / MOUSE 0-5cm p.p. | 5-10cm p.p. | 10-15cm p.p. | 15-20cm p.p. | MEAN± S.D. % RECOVERY/MOUSE. |
|---|---|---|---|---|---|---|---|
| DAY 0 | Donor infec. Day 8 pi | 4 | 3 ± 5.4 | 7.5 ± 12.5 | 32 ± 13.5 | 8.8 ± 7 | 25 |
| DAY 3 | Control | 4 | 23.3 ± 6.2 | 5.3 ± 3.5 | 0 | 0 | 71.3 ± 12 |
| | Transplanted (Day 8) | 5 | 20 ± 5 | 4.8 ± 2.7 | 0 | 0 | 55.1 ± 25 |
| | Donor infec. Day 11 pi | 3 | 0 | 0 | 0 | 0 | 0 |
| DAY 6 | Control | 4 | 22 ± 7.5 | 8.3 ± 4.4 | 0.3 | 0 | 77.5 ± 10.6 |
| | Transplanted ( Day 8) | 5 | 14.6 ± 7.1 | 4.8 ± 2.3 | 0 | 0 | 48.4 ± 15.6 |
| DAY 11 | Control | 4 | 0.8 | 0 | 0 | 0 | 0.8 |
| | Transplanted (Day 8) | 6 | 1.9 ± 1.6 | 0 | 0.1 | 0 | 5 ± 4.5 |

Controls given oral infection+ sham laparotomy

## Discussion

Results presented in this chapter suggest that parasite senescence does not cause loss of flukes from a primary infection of D.phoxini in the mouse. Both corticosteroid treatment and serial transplantation extended the longevity observed in primary infection. The fact that the expulsion phase of primary infection could be arrested by transplantation of flukes to a naive host provides further evidence that loss of flukes is host-mediated, as the process of senescence would be expected to continue unaffected by either the transplantation procedure or the immune status of the host.

The increase in longevity achieved by corticosteroid treatment was greater than that achieved by serial transplantation, however the relatively large loss of flukes during the latter experiment may be largely due to the trauma to which they are exposed during the recovery and transplantation procedures, including temperature fluctuation and disruption of nutrition.

The reason for loss of flukes which occurred in cortisone acetate-treated mice between days 6 and 8 pi is not understood. A similar effect was observed by Moqbel and Denham (1978) when studying the effects of Betamethasone (Betsolan, Glaxo) on a primary infection of S.ratti in the rat. Early loss of worms even when the drug was injected from day -2 of infection. This was thought to rule out the possibility of immunological stimulation occurring in the gut before the onset of treatment, however corticosteroids do not cause complete inhibition of all immunological components. This factor could be important in stimulating the loss of flukes from cortisone treated mice, after day 13 pi. There is no real evidence presented, to determine whether parasite loss at this stage was due to a delayed, suppressed immunological response, or to senescence. The size and position of flukes throughout this infection indicated that conditions in the gut were favourable for the parasite, perhaps favouring the theory that senescence was occurring. The change in position of flukes associated with the normal loss of flukes from a primary infection did not occur in surgically transplanted flukes and those from cortisone acetate-treated mice.

The fact that such a change in position was not stimulated suggests that the changes in the gut which precede and possibly cause expulsion, did not occur. A similar effect has been noted in other systems (Ogilvie 1971, Moqbel and Denham 1978, Kennedy 1980).

Blake (1974) found that the maturation rate of A.gracilis minor in Prednisolone treated ducklings was similar to that of a normal primary infection, but egg production was maintained in drug treated ducklings until treatment was terminated, whereas a decline in egg production occurred after day 8 pi in control ducklings. Unfortunately treatment was terminated too early to demonstrate increased maximum longevity of the parasite although results suggested that parasite rejection was delayed for the duration of drug treatment and that normal rejection of this parasite from the duckling is not due to senescence.

The slight increase in longevity of flukes which was achieved by reducing the size of the infection to only eight metacercariae was extremely small compared with that which was achieved by corticosteroid treatment and serial transplantation, furthermore, the expulsion curves for 400 metacercarial and eight metacercarial infections were similar apart from a four day displacement. The curves for eight and 20 metacercarial infections were almost superimposed.

Because the size of flukes varies little with the size of infection and because of the nature of the host-parasite interface in the D.phoxini -mouse system, the area of this interface (which in turn will determine the degree of mucosal damage and antigenic stimulation) will be approximately proportional to the size of the infection, hence the results obtained are compatible with the suggestion made by Wakelin (1973) that within defined limits antigenic stimulation of the gut is cumulative, i.e. the lower the level of stimulation, the longer the gut takes to respond. It is not possible here to evaluate any direct effects of increased parasite density upon parasite growth, as immunological and inflammatory effects may also be density dependant in their speed and intensity, as indicated above. Because of

the comparatively small size of the parasite and its interface, and its suspected immobility following establishment, and discrete method of nutrition, it seems that D.phoxini may be less susceptible to "interworm competition" or "crowding effects" than cestodes (Hopkins 1970, Hopkins et al 1977), particularly within the range of levels of infection used herein. There was no difference in size between day 2 flukes from 20 and 400 metacercarial infections. A slowing down (not cessation) of growth in a 400 fluke infection which was evident on day 4 might be the result of changes in the gut preceding the expulsion phase of infection, which was initiated rather early (day 5) in a 400 metacercarial primary infection. Further growth was evident in non-detached flukes in the anterior 10cm of the small intestine by day 6 whereas in a lighter (<200 metacercarial) infection, growth would be complete by day 3 pi.

Contrary to results obtained in other host-parasite systems (T.muris in the mouse (Wakelin 1973), N.brasiliensis in the rat (Hurley 1959), H.citelli in the mouse (Hopkins and Stallard 1974), there is no evidence to suggest the existance of a low threshold of infection, below which no spontaneous cure occurs. The results are more comparable with those of Wakelin and Lloyd (1976a) who found that infections of 10, 14 and 90 larvae of T.spiralis were all rejected from mice within 14 days pi even though the potential longevity of T.spiralis in the mouse is much greater (Kennedy, Wakelin and Wilson 1979).

Although the level of infection was too small to allow measurement of egg production, which at the moment is the only characteristic which is known to be affected by the factors causing expulsion of flukes from primary infection, it was found that flukes undergoing expulsion would re-establish when transplanted into naive recipients, where their loss occurred at a similar rate to that of a normal primary infection, and was not affected by their age at the time of transplantation. Similar results were obtained by Kennedy, Wakelin and Wilson (1979) when transplanting T.spiralis in NIH mice. Thus it appears that changes induced in D.phoxini

leading to expulsion are fully reversible unlike those observed in N.brasiliensis (Ogilvie and Hockley 1968, Jones and Ogilvie 1971). In view of the type of damage inflicted on parasites in other systems, described by the workers above, and the great nutritional requirements of egg production which ceases during expulsion, it is possible that "damage" to D.phoxini will be concentrated at the host parasite interface, and possibly in the intestinal caeca, where it might result in interference or disruption of nutritional processes occurring in these regions. Electron-microscopic examination of these areas of the parasite and of local changes in the gut during the course of infection would help to clarify the changes in the parasite which are associated with expulsion.

## Summary

Reduction of infection size from 400 to eight metacercariae resulted in a four day delay in expulsion of primary infection, which otherwise occurred normally. Some delay in growth of flukes in a 400 metacercarial infection may have been due to changes in the gut preceding expulsion.

Cortisone acetate treatment delayed the onset of main fluke loss, which occurred after day 13 pi, by which time a normal primary infection was completely removed. 11% of flukes still remained on day 25 pi in treated mice. It is suggested that this represents loss due to senescence, or to a delayed, reduced immunological response. Flukes in cortisone acetate-treated mice were large (comparable to those from low level infections) and remained in the anterior 10 or 15 cm of the small intestine. Serial transplantation also resulted in increased longevity of flukes but losses were greater, probably due to the trauma of recovery and transplantation.

Transplantation of flukes undergoing expulsion resulted in their re-establishment in naive donors, though once again losses occurred. Transplanted flukes then had a longevity similar to that of flukes in an oral primary infection.

The results indicate.

1. Expulsion of a primary infection is host-mediated.
2. D.phoxini is highly immunogenic as the expulsion of light infections (eight metacercariae) is similar to that of infections initiated by 400 metacercariae.
3. Damage to flukes is reversible, as shown by the establishment and survival of flukes in the process of expulsion, upon transfer to naive hosts.

# CHAPTER THREE

## Secondary infection and immunological memory

### Introduction

"The single most important experiment which indicates that rejection is immunologically mediated is the demonstration of memory". (Hopkins 1980). In the case of intestinal helminths, this involves the administration of a homologous secondary infection after the primary infection has been lost (or chemically abbreviated in the case of chronic infections), and preferably when lesions associated with the primary infection have had time to subside. Thus in host-intestinal parasite systems characterised by a strong immune response it is observed that anti-parasite effects are greater in homologous secondary and subsequent infections than in primary infections.

The following factors are most commonly measurably affected by the host immune response:

1. Growth, development and fecundity:

Decreased fecundity is often one of the first manifestations of parasite damage preceding spontaneous cure in primary infection, but in some cases particularly when the parasite maturation rate is slow, the rapid host response to homologous secondary infection may reduce the development rate and/or reduce the duration of infection to such an extent that challenge infections of some parasite spp may not achieve patency before rejection occurs e.g. S. ratti in the rat (Moqbel McLaren and Wakelin 1980), T. muris in the mouse (Wakelin 1973), N. brasiliensis in the rat (Ogilvie and Jones 1971) and during rapid expulsion of T. spiralis from rats and mice (Alizadeh and Wakelin 1982). Severe stunting of growth is also characteristic of secondary infections of Hymenolepis spp in rodent hosts (Hopkins, Subramanian and Stallard 1972, Howard 1976, Andreassen and Hopkins 1980, Roepstorff and Andreassen 1982).

2. Duration of Infection:

In intestinal nematode systems characterised by spontaneous cure of primary infection, an acceleration of both the onset and rate of parasite expulsion usually occurs in secondary infection (Ogilvie and Jones 1973, Wakelin 1973, Moqbel 1977, Wakelin and Lloyd 1976, Rothwell and Griffiths 1977). Similar results were obtained in mice given secondary infections of H. diminuta, orally or surgically (Befus 1975, Hopkins 1980, Roepstorff and Andreassen 1982). Although H. microstoma primary infections in mice and low level (5 cyst) H. diminuta infections in rats were not expelled, it was found that homologous secondary infections given a few days after chemical abbreviation of primary infection were expelled. (Howard 1975, Andreassen and Hopkins 1980). Accelerated expulsion of Echinostoma revolutum was also demonstrated in mice challenged eight days following elimination of a 20 day old primary infection of 11-15 worms by anthelmintic treatment (Sirag, Christensen, Frandsen, Monrad and Nansen 1980).

3. Ultrastructural changes:

These have been observed particularly in tissues of the parasite which are most exposed to material of host origin, usually the gut and the tegument or cuticle (Ogilvie and Hockley 1968, Lee 1969, Love, Ogilvie and McLaren 1976, Moqbel and McLaren 1980).

The effects of the host immune response may be reversible (Hopkins and Zajac 1976, Ogilvie and Hockley 1968, Jones and Ogilvie 1971, Moqbel, McLaren and Wakelin 1980, Kennedy and Bruce 1981), or permanent

The size of the immune response mounted against secondary infections may vary with the duration and/or intensity of antigenic stimulation of the immunizing infection. (The capacity of a host to respond immunologically to a given parasite will also vary with host species, and strain).

Parasite species vary in immunogenicity. Extremely low level infections of some species are sufficient to stimulate measurable or even full immunity to homologous secondary infection: immunity to reinfection is stimulated by as few as 10 T. spiralis larvae (Wakelin and Lloyd 1976), or as few as 10 T. muris eggs (Wakelin 1973), however in the latter case abbreviation of primary infection to two weeks duration reduces the level of immunity generated. Measurable immunity to challenge is elicited by a one or two cyst infection of Hymenolepis spp (Hopkins, Subramanian and Stallard 1972, Befus 1975, Howard 1976, Alghali 1981). Similarly an abbreviated infection of only 11-15 E. revolutum was sufficient to stimulate immunity to reinfection eight days after abbreviation. Interestingly, it has been shown in some systems that the level of primary infection required to stimulate immunity to reinfection is less than the threshold required to stimulate spontaneous cure of primary infection (Wakelin 1973, Howard 1975, Andreassen and Hopkins 1980).

The experiments in this chapter were performed to demonstrate the development in NIH mice of an anamnestic response to D phoxini and investigate its duration, and the amount of antigenic exposure (size and duration of primary infection) required forits stimulation and expression. Some visible effects of immunity in a secondary infection of D. phoxini and their reversibility were also investigated.

## Materials and Methods

Techniques of oral infection, transplantation, recovery and examination of flukes have been described (Chapter One). Oxyclozanide (Zanil, ICI) was administered in two doses of 125 mg/kg body weight (adjusted to 0.4 ml with water) given six hours apart.

## Results

1. Secondary Infection in male and female NIH mice

Groups of 20 male and 20 female NIH mice were infected with 200 metacercariae at approx 7 weeks of age. These mice together with age matched female naive control mice were infected with 200 metacercariae at 10 weeks of age. Four mice from each group were killed, 24, 43, 72, 88, 112 h post infection and the distribution and size of their fluke burdens determined.

No significant loss of flukes occurred from mice harbouring primary ($1^{o}$) infections (Fig. 3-1) (mean recovery / mouse remained > 80%). Rejection of secondary ($2^{o}$) infection from male and female mice had not begun by 48 h pi but was almost complete by 88 h pi.

Fluke distribution is shown on Fig. 3-2. During $1^{o}$ infection and the first two days of $2^{o}$ infection almost all flukes were recovered 0-10 cm. post pylones, but when fluke loss became detectable in mice harbouring $2^{o}$ infection, (72 h), a posterior shift in position of these flukes occurred.

No increase in body length of flukes in a $2^{o}$ infection was found after 24 h pi (secondary infection (Fig. 3-3). Whereas flukes in $1^{o}$ infection increased 40% in body length and 67% in posterior lobe length between 24 and 88 h pi.

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Figure 3 - 1

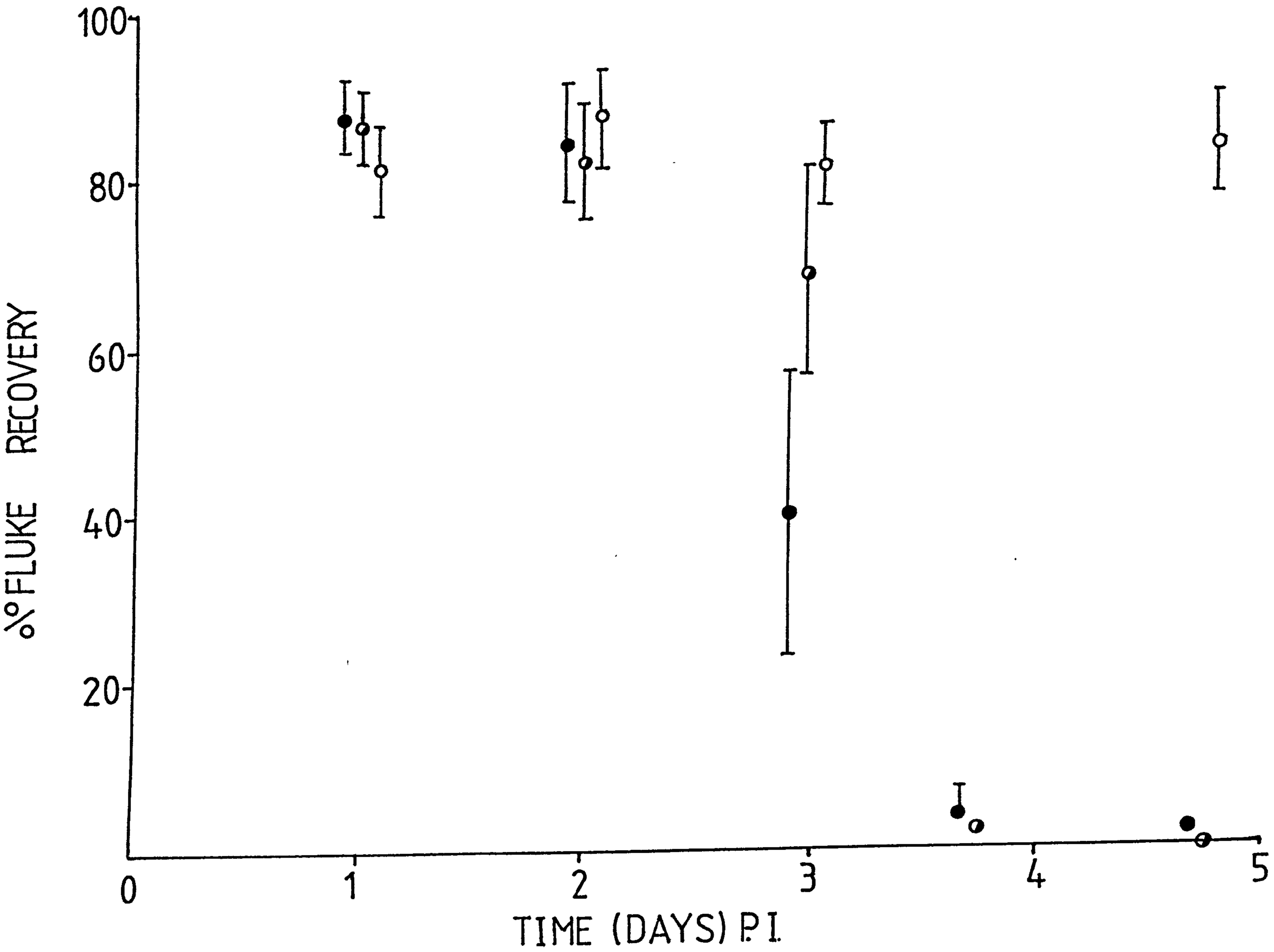
Course of a 200 metacercarial secondary infection in male and female NIH mice (immunized with a 200 metacercarial primary infection three weeks previously).

Mean % fluke recovery $\pm$ S.D.

● = secondary infection in male mice.

◑ = " " " female mice.

○ = primary infection in female mice.


100
80
60
40
20
% FLUKE RECOVERY
0
1
2
3
4
5
TIME (DAYS) P.I.

Facing page 67

Figure 3 - 2

Course of a 200 metacercarial secondary infection in male and female NIH mice (immunized with a 200 metacercarial primary infection three week previously)

Mean % of recovered flukes located in the anterior 10 cm of small intestine.

□ = primary infection. (♀)

▨ = secondary infection.(♀+♂except X)

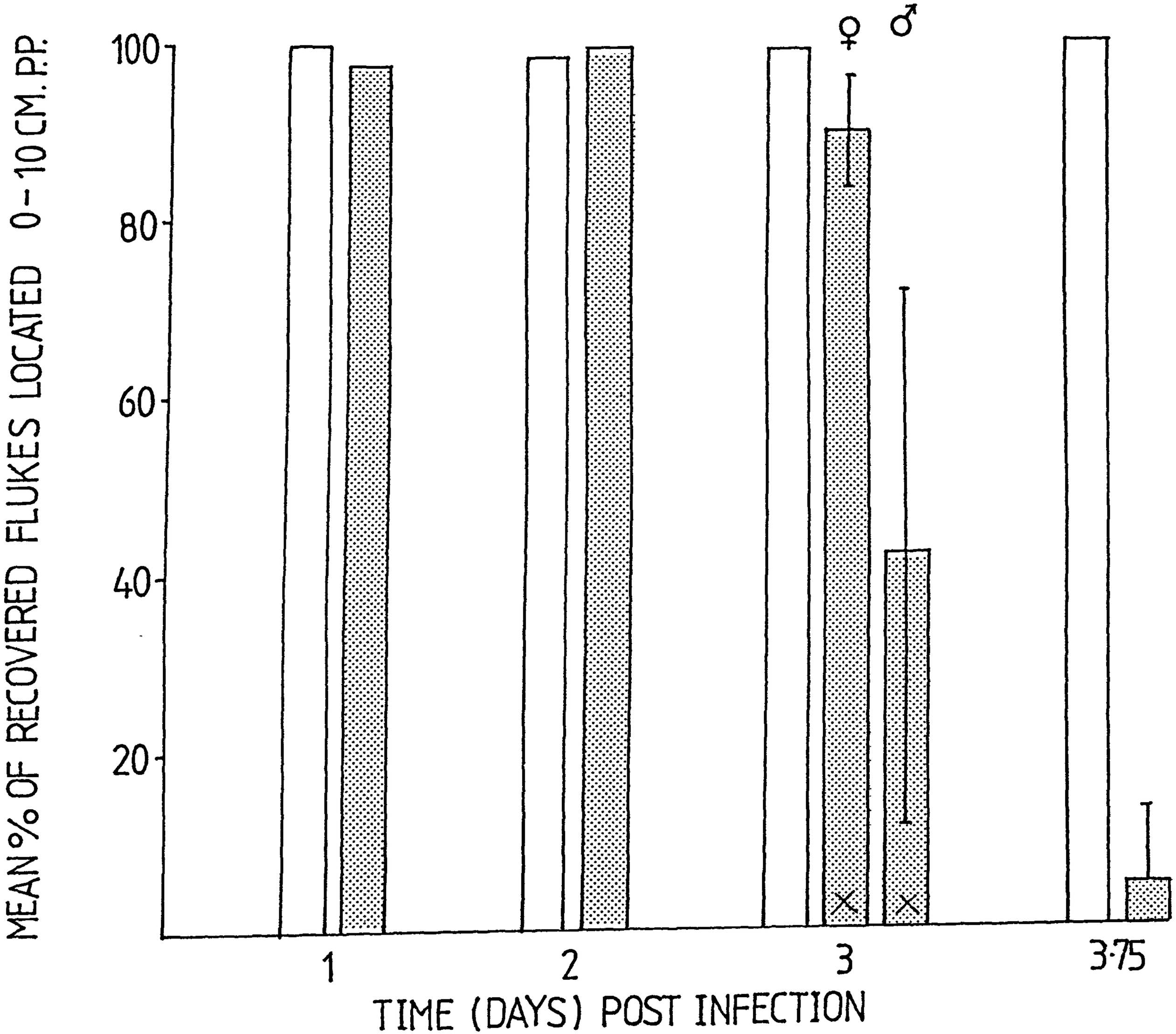

MEAN % OF RECOVERED FLUKE LOCATED 0–10 CM. P.P.
100
80
60
40
20
♀
♂
1
2
3
3·75
TIME (DAYS) POST INFECTION

TABLE 3-3

Lengths of flukes recovered from primary and secondary infections

| Age of flukes ( h.post infec.) | No.mice (♀except x) | Flukes/ mouse | Mean ± SD fluke length (μm) Body length | Post.lobe length |
|---|---|---|---|---|
| 24h.primary (I ) | 4 | 20 | 346 ± 8 | I02 ± 4 |
| " secondary (2) | 4 | 20 | 324 ± 4 | 94 ± I |
| 48h. I° | 4 | 20 | 4I6 ± 3 | I33 ± 6 |
| " 2° | 4 | 20 | 326 ± 8 | 90 ± 3 |
| 72h. I° | - | - | - | - |
| " 2° | 4 | 20 | 336 ± 9 | 89 ± I |
| 88h. I° | 4 | 20 | 485 ± 8 | I7I ± 7 |
| " 2° | x 8 | 3-I0 | 320 | 87 |

x denotes samples pooled (♀+♂)

2. Duration of Immunological Memory:

Female NIH mice (51) were infected with 200 metacercariae at 6½-7½ weeks of age. Groups of 8-12 mice, together with age matched naive controls, were subsequently challenged with 200 metacercariae : 4, 8, 16, 22 or 31 weeks after primary infection. Between four and six mice were killed, on days 2 and 5 after secondary (or control primary) infection, and their fluke burdens recorded. Flukes were measured and presence of eggs and vitellaria was noted.

Fluke recoveries : (Fig. 3-4)

Recoveries from all groups of mice killed on day 2 pi were fairly consistent (means ranging from 77% to 88% suggesting that "normal" establishment had occurred. The distributions (Fig 3-6) and recoveries (Fig 3-4) of flukes from mice killed five days after primary infection show that expulsion of flukes had unexpectedly begun in two of these five groups of control mice. Results obtained using older mice suggest that, at least in 15 week old mice, this was not due to ageing.

In all groups challenged 4-31 wk after primary infection, far fewer flukes were recovered on day 5 pi than from the corresponding control mice. Expulsion was complete or almost complete in all groups harbouring secondary infection, by this time. Although the results suggest that significantly more flukes were present in mice challenged 22 or 31 weeks after primary infection than in those challenged four weeks pi, the observed difference in recoveries is nevertheless small, and the long period of time lapsing between different kills means that other factors, seasonal or age-related, in addition to waning of immunological memory, might be significant.

Fluke lengths : (Figs. 3-5, 3-6)

The body lengths of flukes from mice within the age range tested (10½ to 37½ wk) recovered two days after primary infection, were consistent (mean range 386 to 414 µm ). The reduction in length of flukes recovered from all secondary infections was also consistent, varying from 10 to 14% of control length. The proportion of stunting which was directly

attributable to reduction in length of the posterior lobe in two day old flukes from secondary infections, varied from half to two thirds of the total reduction in body length. Flukes recovered from both primary and secondary infections administered 31 wk pi were slightly larger than those recovered four wk pi.

Almost all the flukes recovered on day 5 of secondary infections were in the 10-20 cm region post-pylones. To allow for the effect of site of infection on fluke length, worms recovered from challenge infections were compared with worms from the same region in the control mice (harbouring primary infection).

Day 5 flukes from control mice varied negligibly in length of body and posterior lobe, with age of mice (Fig. 3-6) Although results may seem to suggest that the degree of stunting observed in five day old flukes recovered from secondary infections administered eight and 16 weeks pi may be greater than in those from mice challenged 22 or 31 weeks pi, the number of flukes recovered (and the number of mice these represented) from the former two groups was too small to analyse. The reduction in body length of flukes recovered from mice given secondary infection 22 or 31 weeks pi was 15% and 14% respectively, and was statistically significant. The proportion of this stunting which was attributable to the reduction in growth of the posterior lobe was 68% and 59% respectively.

Egg production (Fig. 3-7)

Proportions of egg bearing flukes were low in control mice harbouring primary infection (means varying from 18% to 31%). With the exception of one mouse, it was found that all flukes in all groups of mice given secondary infection were devoid of eggs.

Vitelline development (Fig. 3-7)

This was significantly reduced in all groups of mice bearing secondary infections, in which between 71 and 100% (mean values) of flukes had no visible traces of vitelline development, and only 0-9% of

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Figure 3 - 4

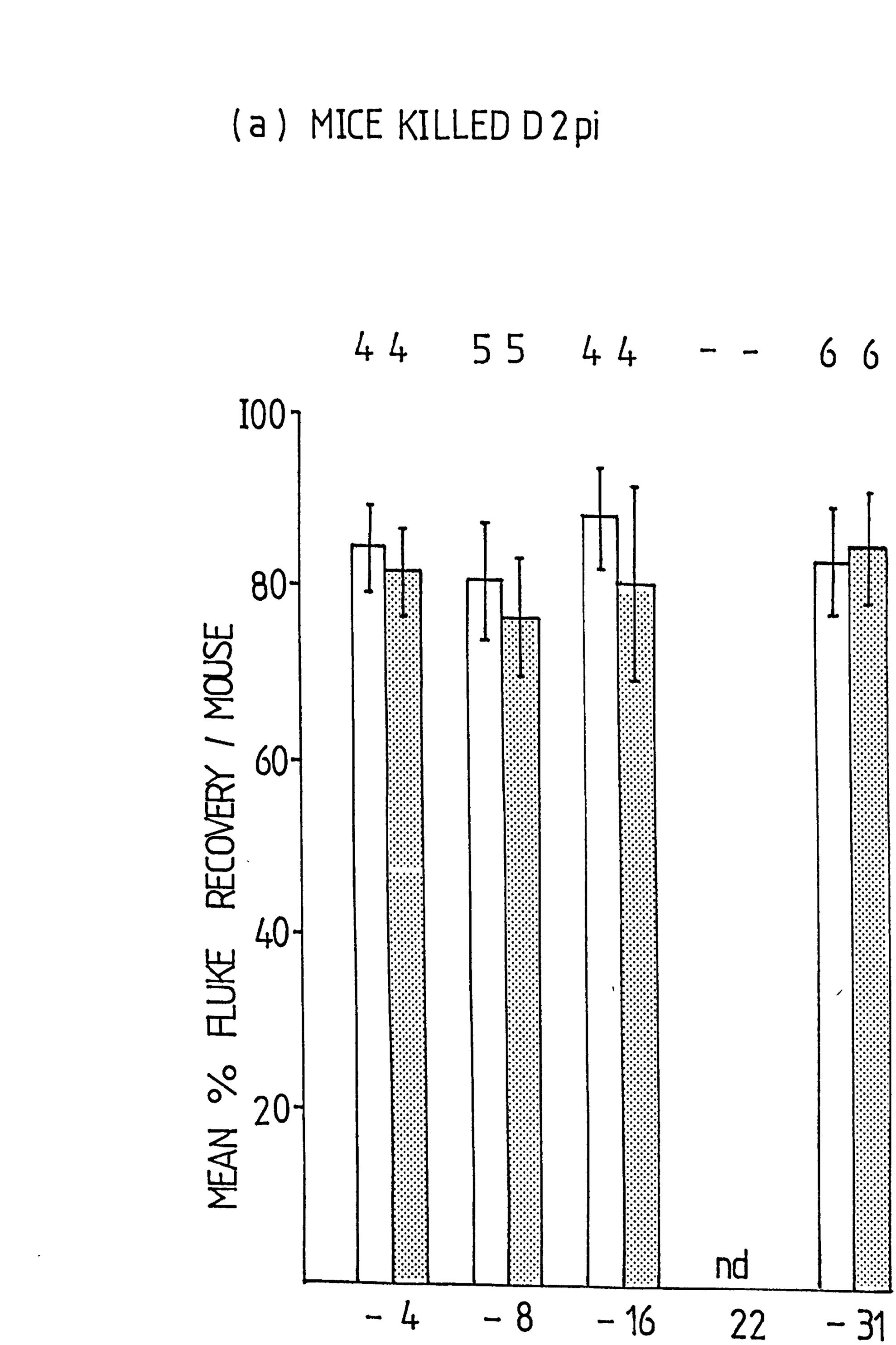
The effect of increasing the interval between primary and challenge infection, on the rate of expulsion of a 200 metacercarial secondary infection in NIH mice:

Mean ± S.D. % fluke recovery per mouse, day 2 and day 5 pi.

□ = primary infection (control).

▨ = secondary infection.

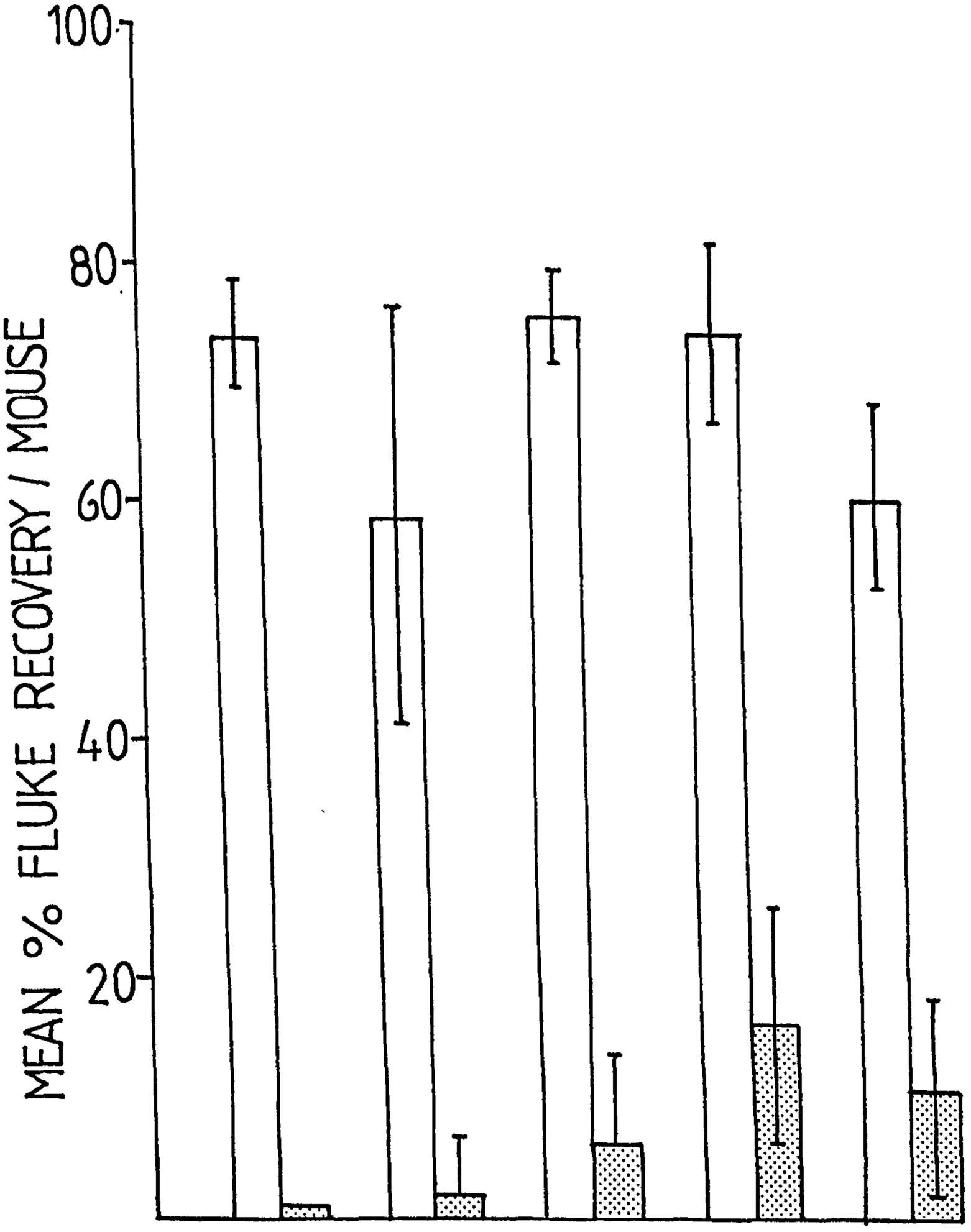
(a) MICE KILLED D 2 pi



b) MICE KILLED D 5 pi

97±9 64±3 83±14 93±6 60±25 [X]

6.6. 4.4. 6.6. 5.5. 5.6. MICE / GROUP




- 4 - 8 - 16 - 22 - 31

INTERVAL (WK) BETWEEN INFECTIONS

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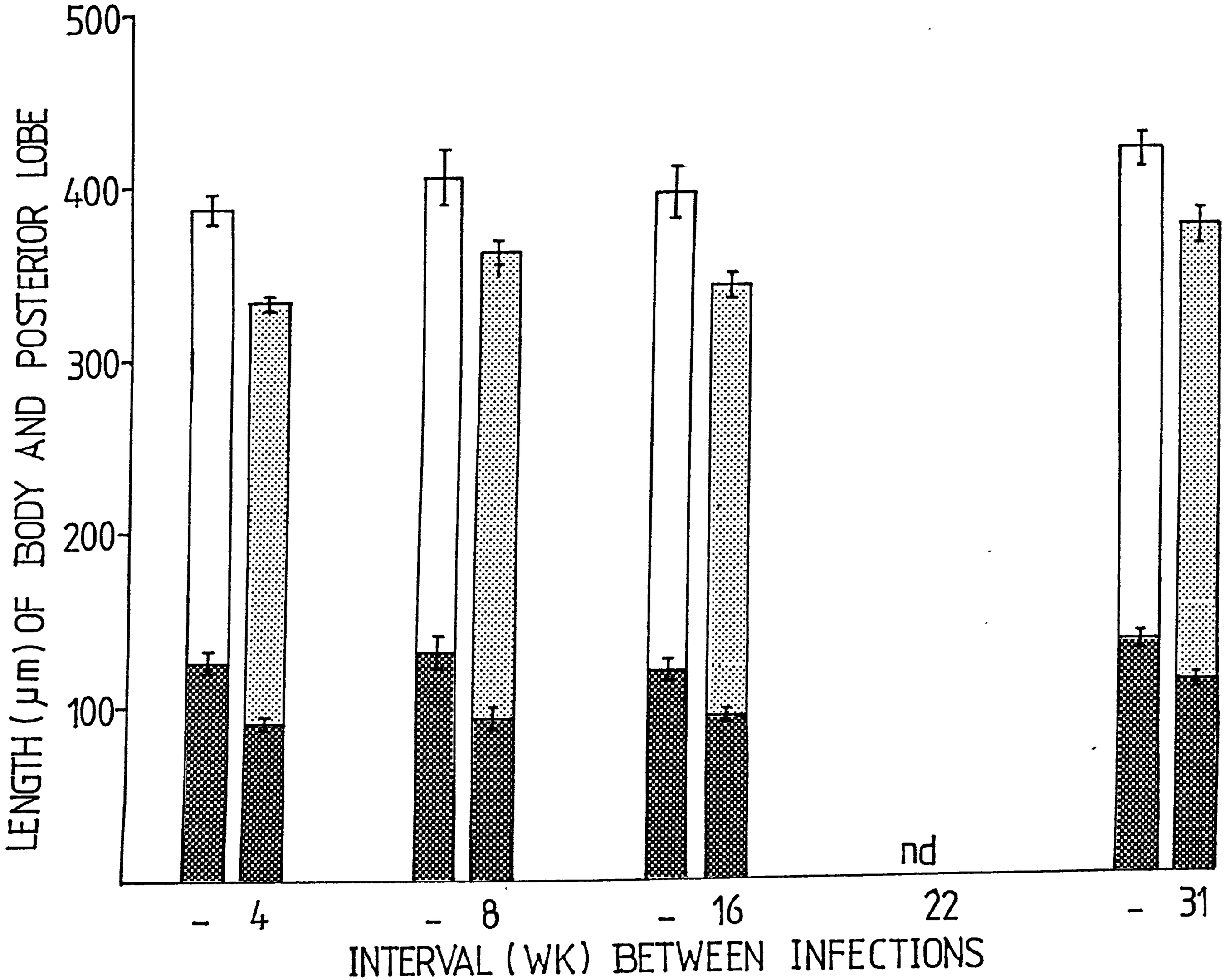
Figure 3 - 5

The effect of increasing the interval between primary and challenge infection, on thecourse of a 200 metacercarial secondary infection in NIH mice:

Mean ± S.D. body ( and posterior lobe ) lengths of flukes recovered from mice on day 2 after secondary infection.


500
400
300
200
100
LENGTH (μm) OF BODY AND POSTERIOR LOBE
nd
– 4
– 8
– 16
22
– 31
INTERVAL (WK) BETWEEN INFECTIONS

Facing page 74

Figure 3 - 6

The effect of increasing the interval between primary and challenge infection, on the course of a 200 metacercarial secondary infection in NIH mice

Mean ± S.D. body (and posterior lobe) lengths of flukes recovered from mice on day 5 after secondary infection.

□ = primary infection (control).
▒ = secondary infection.
} length of body
▓ = length of posterior lobe

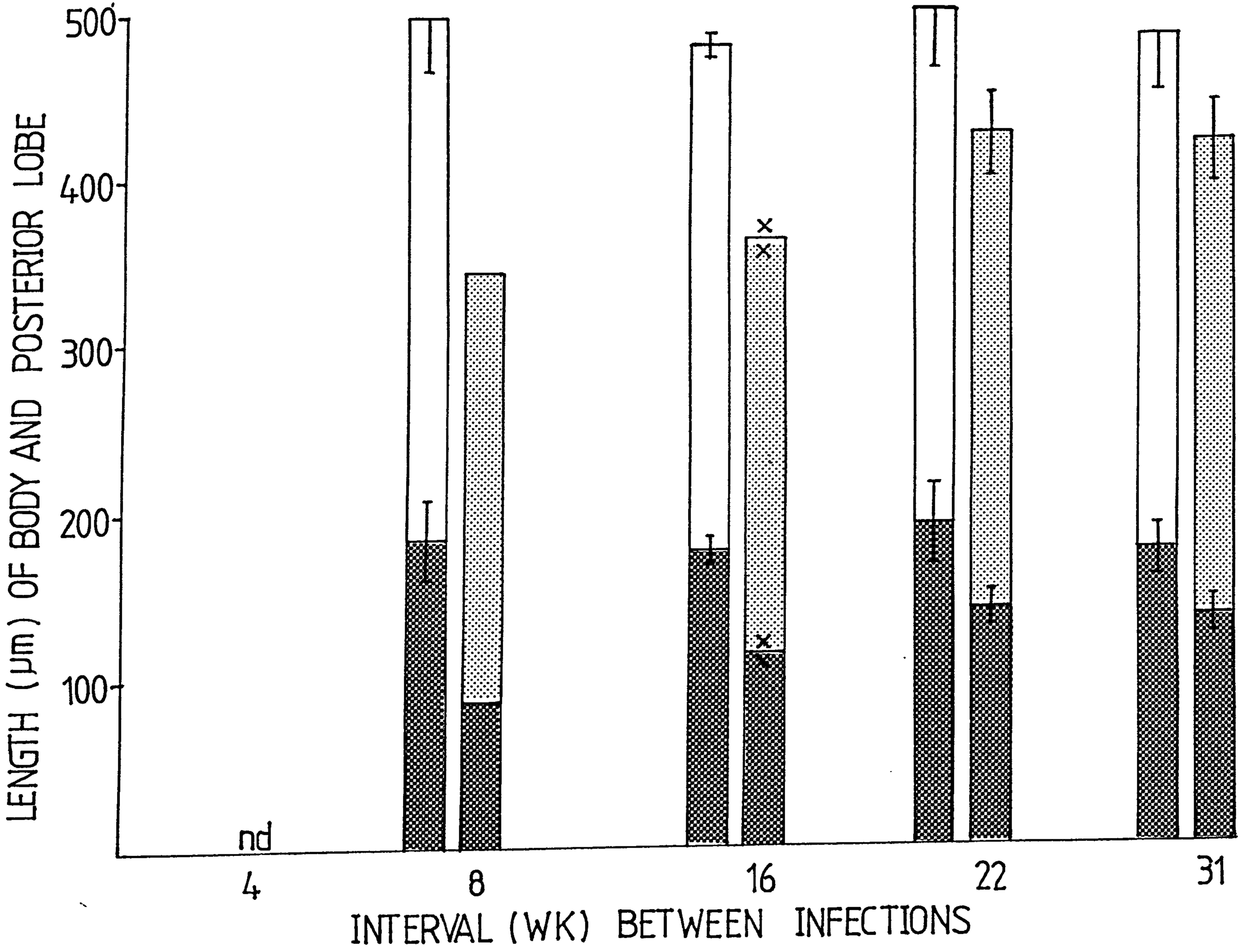

LENGTH (μm) OF BODY AND POSTERIOR LOBE
500
400
300
200
100
nd
4
8
16
22
31
INTERVAL (WK) BETWEEN INFECTIONS

TABLE 3-7

Duration of immunological memory: Vitelline development

| Interval between infections | Region of S.I. recovered | No.mice x flukes/mouse | % with eggs | % with normal vitell. | % with depleted vitell. |
|---|---|---|---|---|---|
| 8 wk | I0-20cm pp. | pooled | 0 | 0 | I00 |
| - control | 0-I0 " | 6 x I9-30 | 32 ± I3 | 80 ± I8 | I0 ± I4 |
| " | I0-20 " | 4 x 7 -30 | 24 ± I9 | 59 ± I0 | 35 ± I0 |
| I6 wk | I0-20cm pp. | 2 x 22,23 | 0 | 0 | 98 |
| - control | 0-I0 " | 3 x 30 | 28 ± I0 | 93 ± 5 | I ± 3 |
| " | I0-20 " | 5 x 20-30 | 3I ± 5 | 55 ± 3 | I0 ± 6 |
| 22 wk | I0-20cm pp. | 4 x I8-35 | 0 | 9 ± 5 | 7I ± 2I |
| - control | 0-20 " | 6 x I7-48 | 25 ± I4 | 89 ± 9 | 3 ± 3 |
| 3I wk | I0-20cm pp. | 4 x 20-30 | 0 | 4 ± 8 | 88 ± 9 |
| - control | 0-I0 " | 5 x 20 | I9 ± 7 | 74 ± I2 | I4 ± II |
| " | I0-20 " | 4 x 8-30 | 27 ± I8 | 59 ± 25 | I5 ± I0 |

flukes had "normal" vitellaria. Vitellaria were much better developed in control (primary) flukes. Only 1-14% of flukes recovered 0-10 cm. post pylorus and 10-35% of flukes recovered 10-20 cm post pylorus had no vitellaria. The percentages of the flukes in these populations having normal vitellaria were 74-94% and 55-59% respectively.

3. Size of primary infection necessary to induce immunological memory

Groups of eight female NIH mice, aged six to seven weeks, were infected with 5, 20 50 or 200 metacercariae. Three weeks later, all mice, plus eight control mice, were infected with 200 metacercariae. Four mice from each group were killed 92h and 116h pi, the number and distribution of flukes recorded, and samples of flukes were measured.

The results (Fig. 3-8) show that all secondary infections were expelled more rapidly than control primary infections. Both a reduction in parasite burden and a posterior shift in position of flukes in the gut was evident by 92h after secondary infection, whereas fluke burden and distribution did not alter in primary infection during the period of study. There is no evidence of a relationship between size of immunizing infection and the degree of acceleration of expulsion within the limits studied. The number of flukes remaining 92h pi in mice immunized with five metacercariae was not significantly greater than that measured in mice immunized with 200 metacercariae ($p=0.1$)

Stunting of growth was evident in all flukes recovered from previously immunized mice. This led to a 20% decrease in body length of flukes recovered from mice immunized with 20 or 200 met, compared with controls. The reduction in length of flukes from mice immunized with only five metacercariae was less marked (14%).

Results therefore suggest that very small immunizing infections may stimulate less depression of fluke growth in subsequent infections than larger (20-200 metacercariae) immunizing infections, but that the effect of all levels of immunizing infection studied on rejection of secondary infection is similar.

TABLE 3-8

Size of primary infection necessary to induce immunological memory:

| Immunizing infection | | Fluke recovery and distribution | | | |
|---|---|---|---|---|---|
| | | Mean ± SD% recovery/mouse | | Mean % of recovered flukes located IO-20cm pp. | |
| | | 92h.pi | II6h.pi | 92h.pi | II6h.pi |
| 5 | meta-cercaria | 37 ± I4 | 0 | 85 ± 8 | - |
| 20 | " | 26 ± I8 | I | 56 ± 30 | - |
| 50 | " | 3 ± 4 | 0 | I00 | - |
| 200 | " | I6 ± I5 | 2 | 7I ± 25 | - |
| - | control | 85 ± 9 | 8I ± 6 | 2 ± 2 | - |

TABLE 3-9 Fluke lengths (92h.pi, sample sizes:6-IO flukes /IOcm section /mouse.)

| Immunizing infection | | Mean ± SD fluke length (µm) | | | |
|---|---|---|---|---|---|
| | | Recovered 0-IOcm pp. | | Recovered IO-20cm pp. | |
| | | T. | P. | T. | P. |
| 5 | meta-cercaria | 396 ± 6 | I20 ± 7 | 4II ± IO | I35 ± 8 |
| 20 | " | 384 ± 8 | II4 ± 8 | 384 ± IO | IIO ± 7 |
| 200 | " | - | - | 380 ± 8 | I06 ± 3 |
| - | control | 479 ± I8 | I67 ± 6 | - | - |

T.= Total body length

P.= Length of posterior lobe.

4. Reversibility of the effects of the immune mouse gut on D phoxini and its effects on adult flukes.

Two groups of donor NIH mice aged 10-13 wk were infected with 200 metacercariae:

Group A had received an immunizing infection of 200 metacercariae, 21 days previously.

B were naive.

These mice were used as donors of three day old flukes, transplanted (30 per recipient) into the duodenum of the following groups of recipient mice:

C Naive mice, received three day old flukes from donor primary infection.

D Naive mice, received three day old flukes from donor secondary infection.

E Mice immunized with 200 metacercariae 21 days previously, received three day old flukes from donor primary infection.

A further group of mice (F), immunized with 200 metacercariae, 21 days previously, received 30 metacercariae by laparotomy.

Five mice from each recipient group were killed on day 3, 5, 9 post transplantation.

Five mice from group A were killed six days pi, Fluke recovery and distribution were noted. Fluke lengths were measured and presence of eggs and vitellaria recorded.

Results (Table 3-10) show that longevity of flukes derived from secondary infection was increased by transfer to a naive host. Whereas non-transplanted secondary infection was completely expelled by day 6 pi, transplanted flukes of the same age and origin persisted in the intestine of naive recipients, over half the inoculum being present three days after transplantation. (NB. the comparison is not strictly valid as secondary metacercarial infections were 200 metacercariae whereas all transplanted infections were of 30 Flukes). However, although longevity of flukes derived from secondary infections was thus increased, loss of

these flukes from naive recipients still preceded that of flukes of the same age derived from primary infection.

Flukes originating from secondary infection grew and developed rapidly after transplantation to naive mice (Table 3-11). Mean body length increased by 60% in the three days following transplantation.

Within three days of transplantation, 94% of flukes originating from secondary infection had detectable vitellaria and 19% carried eggs in utero.

Although on day 5 after transplantation the mean posterior lobe length of these flukes was still 17% shorter than that of primary flukes, the vitelline development and proportions of egg bearing flukes were similar in the two groups.

The immune gut profoundly affected the longevity of implanted metacercariae (Table 3-11) of which only 13% remained after five days, however such an effect was not apparent in flukes originating from primary infection and transplanted into immune mice. Although numbers of the latter were significantly reduced between days 3-5 post transplantation, a residual population of 20% remained on day 9, suggesting that adult flukes are relatively resistant to the effects of the immune gut. Although numbers of flukes examined from immune mice on days 5 and 9 post-transplantation were few, the data suggest a slight suppression of vitelline development in residual flukes, which may or may not have been representative of the population as a whole.

In a normal primary infection, little growth would be expected to occur after day 3 pi. In fact the body length of flukes from primary infection transplanted into naive mice increased by 45% in the three days following transplantation. Similar flukes transferred to immunized mice increased in length by only 20% in the same period of time. Throughout the period of observation, the length of the posterior lobe of primary flukes transplanted into immune mice was reduced by 20-32% compared with those transplanted to naive recipients.

TABLE 3-IO

The survival of 3-day-old flukes after transplantation to naive or immune recipients:

| Time of kill (Day after transplant.) | Mean ± SD % fluke recoveries / mouse | | | | |
|---|---|---|---|---|---|
| | Transplanted into naive recipients | | Non transpl. donor 2° infection | Transplanted into immune recipients | |
| | Day 3 I° flukes | Day 3 2° flukes | | Day 3 I° flukes | Metacer-cariae |
| 3 | 65 ± I0 | 5I ± 22 | 0 | 52 ± I3 | 56 ± II |
| 5 | 53 ± I6 | 37 ± 22 | - | 26 ± 8 | I2 ± I3 |
| 9 | I2 ± I0 | 0 | - | 20 ± 4 | 0 |

TABLE 3-II

The lengths and vitelline development of flukes after transplantation to naive or immune recipients:

| Donor infec | Recip -ient status | Time of kill (D.p-t) | Mean ± SD fluke length(µm) T. | Mean ± SD fluke length(µm) P. | Flukes /mouse | % of recovered flukes with: -ve vitell. | % of recovered flukes with: normal vitell. | % of recovered flukes with: eggs |
|---|---|---|---|---|---|---|---|---|
| I° | - | 0 | 455 ± 23 | I62 ± 9 | I0 | 7I ±I8 | 0 | 0 |
| 2° | - | 0 | 357 ± I4 | 96 ± 7 | I0 | I00 | 0 | 0 |
| I° | naive | 3 | 660 ± 32 | 267 ± 20 | I5 | 0 | 92 ± 3 | 62 ± II |
| 2° | " | 3 | 570 ± 44 | 2II ± I9 | I0 | 6 | 59 ± 6 | I9 ± I2 |
| I° | " | 5 | 674 ± 37 | 307 ± I5 | 9/I0 | 0 | 87 ± 6 | 42 ± I5 |
| 2° | " | 5 | 656 ± 30 | 256 ± 20 | 6-I2 | 0 | 80 ± 9 | 52 ± I0 |
| I° | " | 9 | 703 | 298 | pooled | 0 | 9I | 37 |
| I° | immune | 3 | 548 ± I3 | 209 ± II | I5 | 5 | 60 ± I3 | 48 ± 8 |
| I° | " | 5 | 533 ±(69) | 208 ±(37) | pooled | I3 | 46 | 35 |
| I° | " | 9 | 6I3 ±(I09) | 240 ±(47) | " | 0 | 34 | 37 |
| meta-cerc. | " | 5 | 4I0 ±(30) | II0 ±(I8) | " | 94 | 0 | 0 |

D.p-t = No. of days after transplantation
T. = fluke body length
P. = length of posterior lobe

## Discussion

A primary infection of D.phoxini in male or female NIH mice stimulated a high degree of immunity to reinfection, which caused accelerated expulsion and inhibition of growth and development of flukes in secondary infection, but did not affect establishment. No growth of challenge flukes was measured after 24h pi, the vitellaria did not mature and eggs were not produced. Vitellogenesis represents a late stage in fluke development (Bell and Hopkins, 1956) and is nutritionally very demanding, hence it is not surprising that both vitellogenesis and egg production should be so affected. Nevertheless, maturation of D.phoxini in the naive mouse is a rapid process, completed within four days, therefore the suppression of vitelline and egg production, and the early inhibition of growth (measured by body length) all emphasise the rapidity with which immunologically mediated degenerative changes in flukes occur in secondary infection. This contrasts with the A.gracilis minor/duck system in which the effect on secondary infection is less pronounced, and parasites achieve patency, maturing at the same rate as in primary infection but subsequent egg production is greatly reduced (Blake 1974)

A mucosal response to infection might be expected to affect directly, those functions dependent upon the integrity of the host-parasite interface, for instance attachment to the host, and parasite nutrition, of which extra-corporeal digestion of host tissues is thought to be a major component (Erasmus 1968,1969). Electron microscopic studies would facilitate a more useful assessment of the degenerative changes in the parasite, associated with secondary infection. Structural intestinal lesions have been noted in several intestinal parasite species, when these are damaged by host immunity: N. brasiliensis (Taliaferro and Sarles 1939, Ogilvie and Hockley 1968, Lee 1969, 1971), T.spiralis (Love, Ogilvie and McLaren 1976), S.ratti (Moqbel and McLaren 1980) and A.gracilis minor (Blake 1974). Collapse of the intestine and reduction and abnormal nature of intestinal contents of S.ratti from secondary infection (Moqbel and McLaren 1980) and absence of

intestinal contents in a secondary infection of N.brasiliensis undergoing rapid expulsion (Miller, Huntley and Wallace 1981) suggests that normal feeding was not occurring in these parasites. Membranous bodies observed in the gastrodermal cells of A.gracilis minor in Khaki Campbell drakelets appeared to be discharged into the caecal lumen in later stages of primary infection (after day 10 of a 200 metacercarial infection). By day 27 pi they almost occluded the lumen. It was suggested that similar structures in S.douthitti represent products of lipid metabolism (Shannon and Bogitsh 1969). Such membranous bodies appear to be associated with starvation in several helminth species, e.g. S.douthitti (Shannon and Bogitsh 1969) and S.ratti (Moqbel and McLaren 1980), however paradoxically, it was found that these structures were more pronounced in A.gracilis minor from thymectomized birds, than in those from intact birds, therefore the significance of these lesions remains obscure.

The effects of the host immune response on intestinal cestodes would similarly be expected to affect primarily the surface of the tegument which constitutes most of the host-parasite interface, and performs vital secretory and digestive-absorptive functions. Antibodies have been identified on the surface of H.diminuta and H.microstoma however their occurrence is not always associated with the occurrence of worm damage (Befus 1975, Hopkins 1980).

There is no evidence to suggest a waning of immunological memory when the interval between primary and secondary infections of D.phoxini was extended to seven months, by which time infammatory lesions associated with primary infection would almost certainly have subsided. The effect of previous immunization on the development of secondary infection was observed in the retardation of fluke growth (i.e. length) by day 2 pi. The timing of the kill on day 5 pi, although it maximized the difference in fluke recoveries between primary and secondary infections, led to insufficient recoveries of secondary flukes in some instances. This, together with the limited duration of the experiment, made it difficult to

demonstrate any real trends in the rate of expulsion, depression of fluke growth, vitelline development or egg production associated either with increasing age of mice or with increasing length of time between immunizing and secondary infections. Increasing age of mice did not affect the establishment of metacercarial infections.

Attempts have been made in several host-parasite systems, to establish thresholds of primary infection (i.e. antigenic exposure) which are necessary for the stimulation of immunological memory (a parameter which is affected greatly by host strain). Results show that no threshold was established below which immunity to reinfection was not stimulated. Expulsion rates of secondary infection in mice which had received an immunizing infection of five or 200 metacercariae were similar, however the degree of stunting observed in secondary flukes from mice immunized by five metacercariae was less than that observed in flukes from mice immunized with 200 metacercariae. Other investigators have found that NIH mice infected with as few as ten T.spiralis larvae (Wakelin and Lloyd 1975) or ten T.muris eggs (Wakelin 1973) show immunity to secondary infection. A primary infection of 11-15 E.revolutum, terminated after 20 days, immunized mice against reinfection 28 days after primary infection( Sirag et al 1980 ).

In nematode and trematode infections, within the limits examined, the quantity of functional antigen to which the host is exposed is probably proportional to the number of parasites present, however in the case of intestinal cestode infections the situation is often more complex. The size of the strobila may be very large and variable, and dependant upon the size of infection. There is much controversy regarding the relative contributions of strobila and scolex as sources of functional antigen (Andreassen, Hindsbo and Ruitenberg 1978, Christie 1979, Elowni 1980, Hopkins 1980), however, x-irradiated cysticercoids of H.diminuta, incapable of strobilar development, can stimulate measurable immunity to challenge of mice ( Elowni 1980 ) ( but not rats ( Hopkins and Barr 1982)). A primary infection of six H.diminuta cysts of only three days duration is sufficient to produce significant reduction in the growth of subsequent

challenge infections of one worm (Elowni 1980). A one-cysticercoid infection of H.microstoma removed after 15 days (but not five days) was sufficient to affect the growth of a six cysticercoid secondary infection (Howard 1976). A single cysticercoid infection of H.citelli allowed to persist for 21 days caused significant suppression of growth of a single cysticercoid secondary infection (Alghali 1980).

Interestingly, levels of primary infection which stimulate immunity to secondary infection in some of the above systems are less than those which are required to stimulate spontaneous cure of primary infection. This seems unlikely to be the case in the D.phoxini NIH mouse system in which as few as eight flukes in primary infection are rejected within 11-12 days pi, even though the longevity of flukes can be increased by cortisone acetate, or serial transplantation.

The finding that exposure of NIH mice to an abbreviated infection of D.phoxini for only 15h stimulates immunity to secondary infection contributes little more to the understanding of this system. Although exposure to infection was very short, the amount of antigen involved (for a 200 met. infection) would exceed that of a five metacercarial full term infection. Furthermore, the results of this experiment remain equivocal due to the fact that survival of very few flukes after anthelmintic treatment would be expected to stimulate full immunity to reinfection.

It has been shown that the observed effects of the immunized host on D.phoxini are reversible, vitelline development and body length returning to normal proportions after transplantation into naive recipients. Presence of eggs also reinforced the view that development to maturity had been completed, however the presence of eggs is not a quantitative parameter. Measurement of fecundity by faecal egg counts would have been a more useful criterion, but was not practicable with such low level infections. Similar reversibility of damage occurring in primary or secondary infections is observed in most other systems: T.spiralis in NIH mice (Kennedy and Bruce 1980), T.colubriformis in guinea pigs (Rothwell, Love, Adams, Love and

and McLaren 1980), S.ratti in rats (Moqbel, McLaren and Wakelin 1980) and H. diminuta in mice (Hopkins and Zajac 1976). (c.f. N.brasiliensis which in the terminal stages of primary infection in the rat, is irreversibly damaged, and is then rejected rapidly after surgical transplantation to a naive host (Jones and Ogilvie 1971)).

The survival of three day old flukes from primary infection transplanted into immune mice was considerably longer than that of metacercariae. Flukes would be expected to have almost reached maturityby day 3 pi therefore it is not surprising that egg production was initiated presumably before flukes were affected by the response of recipient mice. The basis of this apparent lack of susceptibility of mature flukes to the response of recipient mice is not known. As previously mentioned, measurement of fecundity, a parameter likely to be one of the first affected, was not practicable. Ultrastructural studies might be very rewarding in this context. Blake (1974) noted lesions in the vitellaria and testes of A.gracilis minor during the course of primary infection, though the ovaries were unaffected.

The strong and long lasting response generated by D.phoxini, a parasite characterised by acute infection, in the NIH mouse, an unnatural host, contrasts markedly with some better-adapted host-parasite systems characterised by chronicity of infection, and poor or short lived immunity to reinfection. For instance the poor ability of N.dubius to stimulate acquired immunity in the NIH mouse unless infective larvae are attenuated by Cobalt 60 irradiation (10-30 krad), preventing normal maturation and emergence of larvae and it is thought, resulting in increased exposure of the host to larval antigens (Hagan, Behnke and Parish 1981). N.dubius is thought to survive by immune suppression, which is evident in the impairment of expulsion of other nematode spp in mice concurrently infected with N.dubius (Jenkins and Behnke 1977, Behnke, Wakelin and Wilson 1978). H.diminuta evokes strong, long lasting immunity (>6 months) to challenge in the mouse (Hopkins, Subramanian and Stallard 1972, Hopkins 1980, 1982, Andreassen and Hopkins 1980), however memory wanes rapidly in the rat (Andreassen and Hopkins 1980) It

was suggested by these authors that the function of such rapidly waning memory might be "the limitation of the number of worms that can accumulate in the intestine in concurrent infection, not the prevention of reinfection after the loss of worms from previous infections."

## Summary

The characteristics of a 200 metacercarial secondary infection (administered three weeks after a 200 metacercarial primary infection) are described. After normal establishment, rejection occurred between two and four days post infection. Fluke development was impaired, functional vitellaria did not form and eggs were not produced. Growth stopped before day 2 pi. No waning of immunological memory occurred when the interval between primary and secondary infections was increased to seven months.

Reduction of the size of the immunizing infection to as few as five metacercariae resulted in no reduction in the effect of the immune gut on the rate of expulsion of secondary infection, although the inhibitory effect on fluke growth was less marked.

Abbreviation of a 200 metacercarial to 15h duration apparently did not diminish resistance of mice to subsequent reinfection.

The effects of the immune gut on the growth, development and longevity of D.phoxini were found to be reversible when flukes from a secondary infection were transplanted into a naive host.

The effect of the immunized gut on transplanted, almost mature (three day old) flukes from primary infection was less marked than the effect on metacercariae surgically implanted into the duodenum; survival of the three day old flukes in immune mice was almost as long as would be expected in primary infection, and development proceeded to completion.

# CHAPTER FOUR

Transfer of immunity to D phoxini using mesenteric lymph node cells (MLNC) or serum from previously infected mice.

## Introduction

The successful transfer of immunity by cells or serum from previously infected hosts is well established in many host-parasite systems. This technique coupled with the selective depletion/reconstitution of specific components of the donor and recipient immune systems, has enabled some evaluation of the possible roles of such components in protective immunity to be made.

For several years, efforts to transfer immunity with immune serum yielded inconsistent and disappointing results (Ogilvie and Jones 1971, Wakelin 1978), however the effects of factors such as host sex and strain (Dobson and Owen 1978), and immunizing schedules became apparent. Miller (1980) consistently conferred protection using serum removed from rats on day six of a primary infection of N. brasiliensis, or by using hyperimmune serum. The protective capacity of serum taken during primary infection was believed to support the hypothesis (Ogilvie and Jones 1968, Jones and Ogilvie 1971) that antibody-mediated damage occurs early in primary infection of N. brasiliensis, however the role of antibody in serum mediated effects in this system is not resolved (Miller 1980). It has been suggested that delay in production of effects of transferred serum on parasites might be due to an indirect role of serum (Behnke and Parish 1979, Miller 1980).

Hyperimmune serum protected NIH mice against T.muris well but inconsistently (Selby and Wakelin 1973). The effect of transferring serum (from Day 28 primary or secondary infection) on the faecal larval counts of S.ratti in mice was equivalent to that stimulated by previous infection. (Dawkins and Grove 1981).

In most cases the protection afforded by transfer of serum or cells is less than that which is generated by primary infection. in such cases

the effects of serum and cells are often either additive or synergistic (Love, Ogilvie and McLaren 1976, Wakelin and Lloyd 1976, Behnke and Parish 1980).

Large volumes of hyperimmune serum conferred a degree of protection (though not consistently) to guinea pigs challenged with T. colubriformis larvae (Connan 1972) but neither immune nor hyperimmune serum passively protected rats or mice against T. spiralis (Wakelin and Lloyd 1976[b], Crum et al 1977).

Immunity against a variety of intestinal nematodes can be transferred with IMLNC but similar attempts to transfer immunity adoptively in cestode/rodent systems have been largely unsuccessful (Bland 1976, Christie 1979) even though an increase in cellularity of the MLN is associated with infection.

The establishment of the technique of transferring MLNC in a number of host-parasite systems has demonstrated that a large number of factors may affect the protective capacity of transferred cells:

1. Source of cells. Some success has been achieved with cells from Peyers patches (Dineen, Ronai and Wagland 1968) and from peritoneal exudate (Larsh, Goulson and Weatherly 1964, Selby and Wakelin 1973).

Lymph drains from the small intestine via the MLN and TD, therefore it is not surprising that it is from these areas that sensibized lymphocyte populations responding to intestinally located antigens have been most successfully recovered. The involvement of the spleen in the immune response to enteric helminths is generally minor except in cases involving complex parenteral migrations. Measurable immunity to N. brasiliensis in rats and mice and S. ratti in rats has been transferred using spleen cells (Love 1974, Haddow, personal communication.

Immunity to a primary infection of S. ratti in rats is expressed enterally, and IMLNC taken late during primary infection are protective (Moqbel and Wakelin 1980), however immunity to secondary and tertiary infections is expressed parenterally, affecting worms before they reach

the gut. This may be associated with the inability of IMLNC from multiply infected donors to transfer immunity adoptively.

2. Number of cells administered. For cells of the same type and source (organ), optimum and minimum numbers of cells required for effective transfer of immunity vary considerably between different host species, however the range of effective inoculum for a given host species or strain varies within narrow limits and remains fairly consistent against a fairly wide range of intestinal nematode species. Thus $2.25 \times 10^7$ and $9 \times 10^7$ IMLNC are equally effective against T spiralis in the mouse (Wakelin and Lloyd 1976). Lower numbers of cells ($4 \times 10^5$, $4 \times 10^6$) may affect fecundity only (Wakelin and Wilson 1977). Similarly $2.5 \times 10^7$ MLNC affected fecundity of S. ratti in mice (Dawkins and Grove 1981). Larger numbers of cells are required in rat-nematode systems; $1 \times 10^8$ IMLNC affected length and fecundity of S. ratti in the rat, and $2 \times 10^8$ IMLNC effected early expulsion (Moqbel and Wakelin 1981). Although as few as $1 \times 10^7$ rat TDLC conferred measurable immunity to N. brasiliensis, the protective capacity was greater and dose dependant between $5 \times 10^7$ and $4 \times 10^8$ cells. (Nawa and Miller 1978). During infection, N. brasiliensis worms eventually become irreversibly damaged. Such worms are more susceptible to the effects of transferred TDLC than "normal" (younger) worms, thus "damaged" worms were affected by $1 \times 10^7$ TDLC whereas "normal" worms were only affected to the same extent by $1 \times 10^8$ TDLC. $Co^{50}$ attenuated N dubius in mice are similarly rendered susceptible to as few as $1 \times 10^7$ IMLNC (Behnke and Parish 1981).

Against T. colubriformis in the guinea pig, $8 \times 10^7$ IMLNC are effective but no protection is conferred by $1.2 \times 10^7$ IMLNC.

3. Time after infection at which donor cells are collected. Cells capable of transferring immunity appear to be present in the MLN/TDL in sufficient numbers for only a limited period after infection. The nature, size and speed of cellular changes in the MLN following primary, compared with subsequent infections, would be expected to differ, there-

fore it is not surprising that cells taken at different stages in primary and later infections differ in their ability to transfer immunity. Some attempts have been made to correlate the efficacy of transferred IMLNC with changes in MLN associated with donor infection, and with the kinetics of parasite loss (Rothwell and Dineen 1973, Grencis and Wakelin 1982). The ability of IMLNC from NIH mice to transfer immunity to T. spiralis is correlated with an increase mainly in T. lymphoblast activity in the MLN,which declines soon after the parasite expulsion phase begins (Grencis and Wakelin 1982). On the other hand, Moqbel and Wakelin (1980) found that rat IMLNC were ineffective against S. ratti until day 20 of donor primary infection when worm damage was apparent and MLN cellularity had already declined considerably.

It is known that different immunization regimes affect the efficacy of immune/hyperimmune IMLNC and serum, though the reasons for this are not fully understood. Guinea pigs immunized against T. colubriformis with two infections given 35 days apart were found to be a more useful source of IMLNC than those given four infections over four weeks (Adams and Rothwell 1977). TDLC from day 10 of primary infection are more effective than hyperimmune TDLC in transferring immunity to N. brasiliensis in rats (Nawa and Miller 1979).

4. Time required for transferred cells to express their effects.

For many systems, owing to the timing of observations, the period of time required by transferred cells to generate their anti-parasite effects is not known; indeed this is not a critical factor affecting the efficacy of cells associated with long term parasite infections, however in acute infections this factor becomes apparent. In the T. spiralis/NIH mouse system, IMLNC require six days to express their effect on the recipient infection, therefore because parasite expulsion begins eight days after infection, cells injected on the day of challenge were effective but those injected four or six days later were not (Wakelin and Wilson 1977).

IMLNC given to mice three or five days before challenge with N. dubius

were effective against irradiated worms by day 9, the earliest day of observation, however it seems probable that this observation is a result of earlier damage to the larval infection (Behnke and Parish 1981). Rat $^{T}$DLC transferred on the day of challenge of recipients with N. brasiliensis were effective by day 8 pi but IMLNC did not effect similar change in the parasite infection until two days later (Nawa and Miller 1978). Rat IMLNC require ten days or possibly less to affect a recipient infection of S. ratti (Moqbel and Wakelin 1980) and rat IMLNC are effective against T. spiralis within ten days of transfer.

All the examples quoted above have in common the fact that the duration of primary infection is sufficiently long to allow transferred cells to express measurable effects upon the recipient parasite infection. This may not be the case in much shorter-lived infections e.g. D. phoxini.

It is not known whether any of the required changes undergone by transferred cells can occur in the absence of antigenic stimulation. It is known that the transfer of cells well before recipients are challenged with the parasite does not reduce the efficacy of transferred cells (Wakelin and Wilson 1977, Wakelin, Grencis and Donachie 1982). It has been suggested by the latter authors that this would require the transformation of mediator cells (which in the T. spiralis/mouse system are short lived) into long term memory cells.

The separation of donor IMLNC into subpopulations has been valuable in aiding the characterization of mediator cells. It has been shown that adoptive transfer of immunity to T. spiralis in NIH mice is mediated by a short-lived population of T - lymphoblasts (Wakelin, Grencis and Donachie 1982). In the rat, IgA has been implicated. B-enriched or unseparated ITDLC transferred immunity more effectively to irradiated rats than T-enriched cells (Despommier, McGregor, Crum and Carter 1977). Crum, Despommier and McGregor (1977) suggested that failure of specific immune serum to immunize passively might be due to the failure of the plasma cells evoked by the parasite to mature and release Ig until after

leaving the circulation. Transfer of immunity to N. brasiliensis by rat TDL (from day 10 of primary infection) was successful only when the sIg-ve cell fraction was used (with slight contamination by sIg+ve cells) When hyperimmune TDLC were used, a small but significant effect of sIg+ve cells was measurable. It was suggested that the mediators in question were memory helper T cells (with some memory B cell activity accounting for the results obtained with hyperimmune serum) (Nawa, Parish and Miller 1978).

In this chapter a single attempt to transfer immunity with serum alone (and in conjunction with IMLNC) derived from mice infected with D. phoxini is described. Some factors affecting the efficacy of adoptive transfer of immunity in the D phoxini/mouse system are investigated and an attempt made to assess the relative importance of sIg+ve and sIg-ve IMLNC in the transfer of immunity.

## Materials and Methods:

### Animals:

Male and female mice of the inbred NIH strain, bred on the premises, were originally derived (1980) from mice obtained from Hacking and Churchill Ltd., Huntingdon. Mice of the same sex were used throughout each experiment unless stated otherwise.

### Infections:

All infections consisted of 200 metacercari of *D phoxini* administered in 0.15 ml of modified Hanks balanced salt solution, by the method described in chapter 1.

### Collection, storage and administration of serum:

Donor mice were heavily anaesthetised with chloroform and blood collected by cardiac puncture. After 1 hr. at room temperature, samples were ringed, then stored overnight at 4°c. Samples were centrifuged (2,000 *g* for 15 mins). Serum was collected by pipette and samples for each group of mice pooled, and either stored at -20°c until use, or used immediately. Serum was administered by intraperitoneal (I.P.) injection.

### Cell transfer:

Animals under ether anaesthesia were killed by cervical dislocation. The MLN were removed immediately and kept in modified Hanks BSS on ice until processed. After removal of surplus fat and connective tissue, nodes were cut into small pieces and pressed through a fine nylon sieve, into medium 199 (Gibco Europe Ltd.) containing 0.05 ml newborn calf serum and 10 iu of heparin per ml. Five minutes was allowed for the separation of fat and the sedimentation of cellular aggregates and connective tissue to occur. the cell suspension was removed by pipette and centrifuged for 5 minutes at 200 *g*. The cells were washed once, resuspended and counted using a haemocytometer. Viability was checked using the trypan blue dye exclusion test. Viability of MLNC was greater than 80% except where stated otherwise.

Administration of cells to recipients was by I.P. injection, in 0.2 - 0.4 ml modified medium 199. In one experiment, IMLNC were injected into

a lateral tail vein.

Cell labelling:

Dividing cells were labelled using the thymidine analogue ($^{125}I$) -UdR. MLNC were prepared and suspended in RPMI 1640 medium (Flow Laboratories Ltd) to which the following had been added (per 100 ml RPMI)

5 ml newborn calf serum

1 ml glutamine (200 m )

0.8 ml sterile 7.5% $NaHCO_3$ solution

0.1 ml Cristamycin (Glaxo)

4 mg heparin

Sterile plastic capped culture tubes containing the following were incubated for two hours at $37^0$C:

$2 \times 10^7$ MLNC in modified RPMI (total volume 0.5 ml)

4 ml modified RPMI

2 $\mu$ Ci of ($^{125}I$) - UdR, specific activity 5 Ci/mg (Amersham International Ltd) in 0.2 ml sterile 0.9% NaCl

The MLNC were washed three times in medium 199 and the activity of each sample measured by counting for one minute in a Packard Tricarb liquid scintillation spectrometer. Blast cell activity is expressed as a labelling index i.e. mean counts per minute of $2x10^7$ MLNC expressed as a percentage of the corresponding control count.

T and B cell separation:

A suspension of MLNC was prepared in HBSS containing 5% foetal calf serum and 4 mg heparin per 100 ml. The cell suspension was passed through a glass wool column (loosely packed, volume 10 cc). The suspension was centrifuged at 200g for 5 minutes and resuspended in 10-15 ml of medium. Cells were counted. The method for separating and collecting a non-adherent (T enriched) cell population using nylon wool is based on that of Julius, Simpson and Herzenberg(1973). The technique for recovery of the adherent (B enriched) population was developed by Handwerger and Schwartz (1974).

The cell suspension was layered onto nylon wool columns at $37^o$C, and incubated for 45 minutes at$37^o$C. Non adherent cells were slowly

drained from the columns with simultaneous addition of an equivalent volume of warm (37°C) medium. The T-enriched cell suspension was spun for 5 minutes at 200 g and the cells resuspended in fresh medium. Cell recovery, viability and % B cell contamination were estimated. Injection of these cells into recipients, or estimation of lymphoblast activity, was initiated as quickly as possible (within a few minutes).

The B-enriched fraction was recovered by teasing the nylon wool immersed in medium, for five minutes using fine forceps. The nylon wool was then placed in a syringe and compressed firmly to expel the medium and dislodged cells. The cell suspension was spun for five minutes at 200 g and cells washed once in medium 199. Cells were then resuspended at a concentration not exceeding 1 x $10^7$ per ml, in medium 199 containing Thy 1·2 F7D5 monoclonal IgM cytotoxic antibody (Olac 1970 Ltd) and allowed to stand at room temperature for 30 minutes with frequent agitation. The suspension was spun for five minutes at 200 g and cells resuspended in 10 ml of medium 199 to which 1 ml of unabsorbed guinea pig complement (Wellcome) was added, and the suspension incubated for 40 minutes at 37°C. Cells were washed twice in medium 199. Viability, total number of cells, and the percentage of recovered cells bearing Ig sIg+ve (i.e. B cells) were estimated.

Fluorescent labelling of cells:

The method used was described by Wakelin and Wilson (1979). One drop (0.05 ml) of cell suspension was added to a mixture of the following:

- Medium 199 0.4 ml
- 0.1% sodium azide 0.05 ml
- Fluorescein conjugated rabbit-anti-mouse Ig (Nordic immunological labs) 0.05 ml of 10% solution (in PBS pH 7.2).

The suspension was incubated for 30 minutes at 4°C in the dark. Cells were washed twice in modified medium 199 and resuspended in 0.1 ml of 50% Glycerol/Modified Hanks BSS. The percentage of labelled cells was estimated using a Leitz Ortholux 1 microscope fitted with a phloem incident-light fluorescent system.

Statistical treatment of results:

Statistical significance of differences between mean fluke recoveries, fluke lengths and proportions of egg bearing flukes was determined using Student's t test. A probability $< 0.05$ was considered to be significant.

Results

4a An early attempt to transfer immunity to D phoxini using MLNC. and serum collected on day 8 of primary infection.

Pools of serum were collected from the following 9-10 week old male NIH mice, and stored at -20°c until required,

1. 36 uninfected mice

2. 36 mice given a 200 metacercarial primary infection and killed on day 8 after infection.

A further 36 infected and 36 naive mice were used as donors of MLNC and serum. The serum collected from these mice was administered within 24 h and did not require freezing.

Recipient mice (male NIH 7-8 weeks old) in groups of four or six mice, received the following treatments:

| | | |
|---|---|---|
| Group | A | IMLNC (Day 8 primary) |
| | B | MLNC (Naive) |
| | C | IMLNC + serum (Day 8 primary) |
| | D | MLNC + serum (Naive) |
| | E | No cells or serum |
| | F | Serum (Day 8 primary) |
| | G | Serum (Naive) |
| | H | No cells or serum |

Administration of serum:

Recipients received 0.8 ml of thawed serum on the day of challenge and a further 0.8 ml of serum (collected from cell donors) on the day after challenge.

Administration of cells:

MLNC ($7 \times 10^7$) were injected into a lateral tail vein and mice were

then challenged on the same day with 200 metacercariae.

Control (naive) mice were challenged at the same time as cell/serum recipients. All mice were killed five days after challenge and their fluke burdens recovered.

Mice were not starved prior to infection in this experiment and this is reflected in the low and variable fluke recoveries, particularly noticeable in control groups (Fig 4 Ia). The anterior position of flukes (Fig 4 Ib) in these groups reinforces the view that low recoveries are not due to fluke expulsion having occurred (see Chapter 1).

Variability in recoveries within most groups was high.

None of the treatments produced a significant reduction in the number of flukes remaining in the gut, and in all groups over 90% of recovered flukes were located in the anterior 10 cm of the small intestine. Neither parameter, therefore, indicated that any protection could be transferred passively or adoptively.

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Figure 4 - Ia

An early attempt to transfer immunity to D phoxini using serum or cells collected on day 8 of primary infection:

Mean % (± SD) of flukes/mouse recovered from all groups of mice killed five days after challenge.

▨ recipients of MLNC from uninfected mice

▨ " " " " infected "

▨ recipients of serum from uninfected mice

▨ " " " " infected "

□ non-recipient controls

Figure 4 - Ib

Mean % (± SD) of recovered flukes which were located in the anterior 10 cm of small intestine

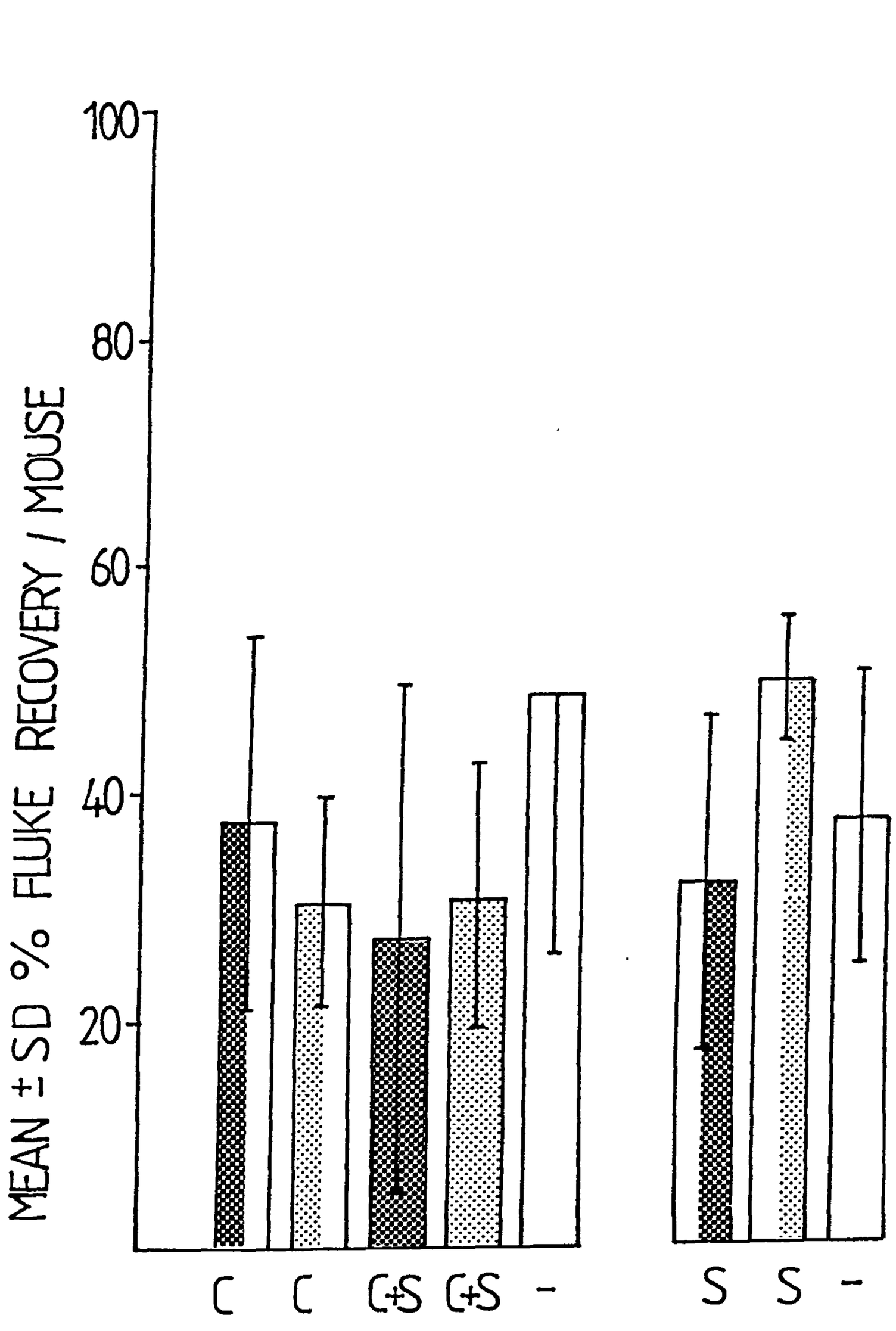
▨ recipients of MLNC from uninfected mice

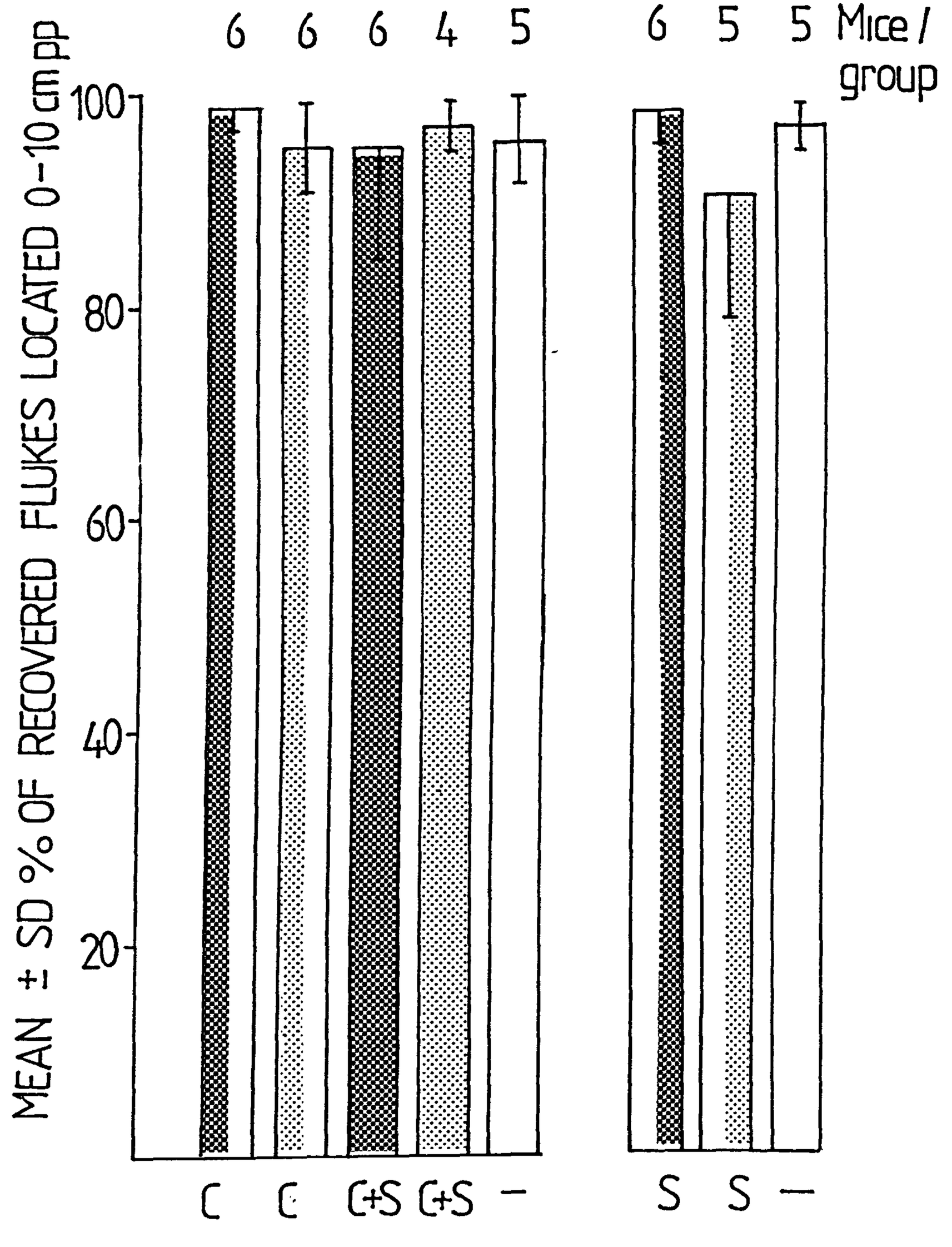
▨ " " " " infected "

▨ recipients of serum from uninfected mice

▨ " " " " infected "

□ non-recipient controls


MEAN ± SD % FLUKE RECOVERY / MOUSE
100
80
60
40
20
C
C
C+S
C+S
-
S
S
-


MEAN ± SD % OF RECOVERED FLUKES LOCATED 0-10 cm pp
100
80
60
40
20
6
6
6
4
5
6
5
5
Mice / group
C
C
C+S
C+S
-
S
S
-

4b. <u>An attempt to transfer immunity with IMLNC from donors harbouring primary infection for four days or a secondary infection for twelve hours, varying the time of cell transfer in relation to the challenge of recipients.</u>

Male NIH mice were used in this experiment.

Donors received the following treatments :

1. 20 mice infected at approx. seven weeks of age. MLNC were collected four days after challenge.

2. 16 age matched naive cell donors.

3. 20 mice infected at seven weeks and challenged at ten weeks of age. MLNC were collected 12h after challenge.

4. 16 age matched naive cell donors

Recipients (of 5 - 5.5x$10^7$ MLNC) were the following groups of 4-6 mice :

| | | |
|---|---|---|
| 1. Recipients of Day 4 primary IMLNC | 1 day before challenge | 7-8 wk old when challenged. |
| 2. " " " " | 2 days after challenge | |
| 3. " " 12h secondary " | 9 days before challenge | 8-9 wk old when challenged. |
| 4. " " " " | 1 day before challenge | |

Four corresponding control groups received naive MLNC.

Three further control groups received no cells.

Recipients and control mice were killed five days after challenge.

The position and number of flukes recovered from naive cell recipient and control mice (Figure 4-2a) indicated that expulsion had not begun in these mice.

No protection was conferred by day 4 IMLNC given to recipients two days after challenge. The slight reduction in %worm recovery in recipients of these cells on the day before challenge is significant ($p < 0.02$) if compared with the recovery from control (non-recipient) mice, but not ($p=0.1$) if compared with the recovery from naive cell recipients.

A highly significant reduction of 50% in fluke recovery was observed when recipients of 12h $2^o$ MLNC (given nine days before challenge of recipients) were compared with controls (Fig.4-2a). A marked posterior shift in position of the flukes recovered from these IMLNC recipients indicated that the

expulsion phase was underway (Fig.4-2b). These IMLNC had no effect when transferred one day before challenge of recipients; the recoveries and distributions of flukes from IMLNC recipients and untreated controls were almost identical.

There was no reduction in length of flukes observed in any groups of naive cell recipients, compared with controls (Table 4-2c).

D4 1° MLNC transferred into recipients two days after challenge, and 12h 2° MLNC transferred into recipients one day before challenge had no significant effect upon the lengths of flukes recovered from recipient mice; however flukes recovered from recipients of 12h 2° MLNC given nine days before challenge were stunted to a significant extent. A 12% reduction in overall length was evident, but stunting was most pronounced in the posterior lobe which was reduced in length by 19% compared with that of flukes from control mice.

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Figure 4-2a

Mean percentage recovery of flukes per mouse five days after challenge, following transfer of IMLNC. Vertical bars represent group standard deviation.

▩ = IMLNC recipients.

▩ = Naive MLNC recipients.

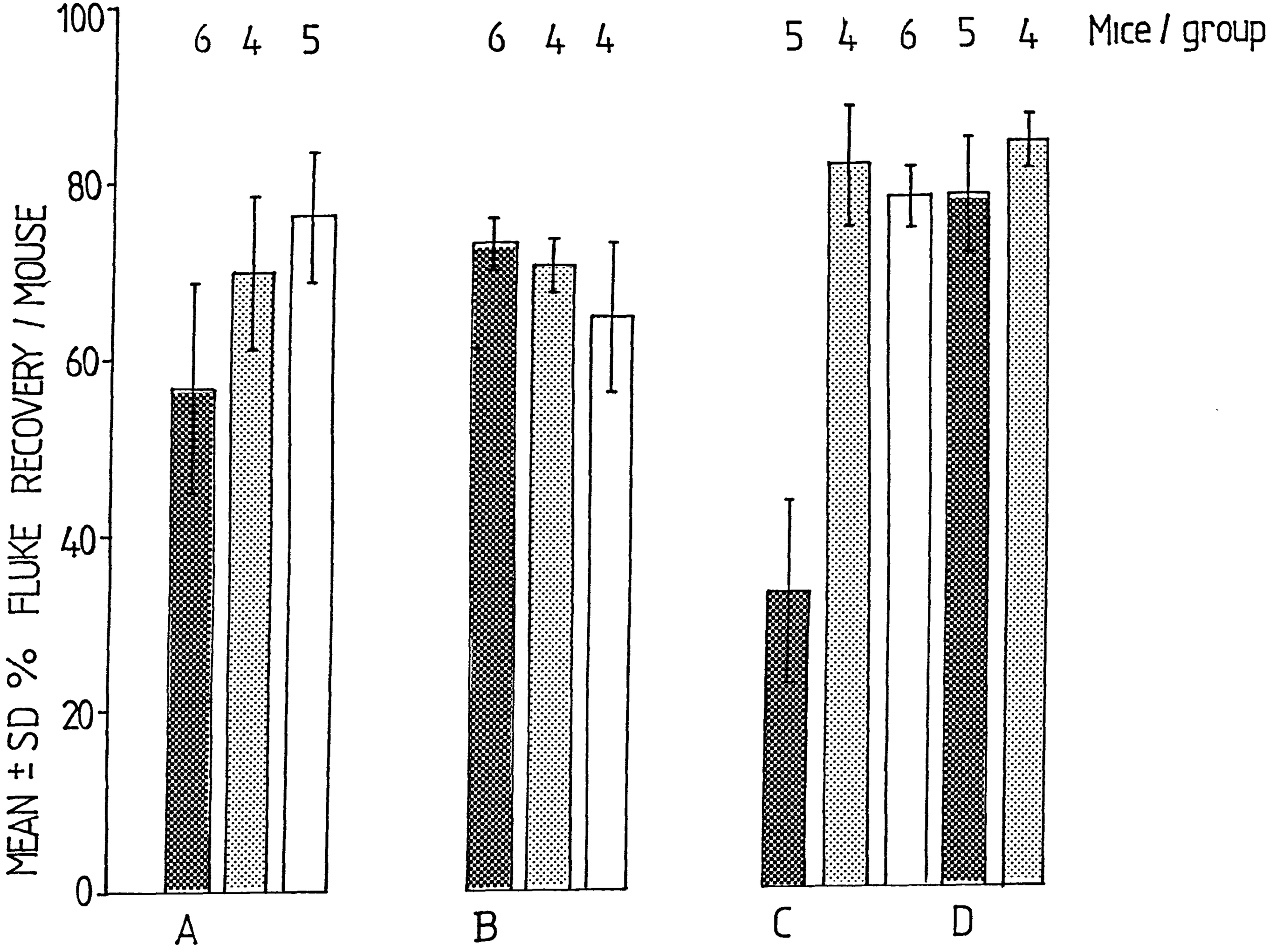
□ = non-recipient controls.

A = recipients of IMLNC recovered on day 4 of donor primary infection (recipients challenged one day after cell transfer.)

B = recipients of IMLNC recovered on day 4 of donor primary infection (recipients challenged two days before cell transfer).

C = recipients of IMLNC recovered twelve hours after donor secondary infection. (recipients challenged nine days after cell transfer).

D = recipients of IMLNC recovered twelve hours after donor secondary infection (recipients challenged one day after cell transfer).


Mice / group
6 4 5
6 4 4
5 4 6 5 4
MEAN ± SD % FLUKE RECOVERY / MOUSE
100
80
60
40
20
0
A
B
C
D

Facing Page 104

Figure 4-2b

Mean percentage of recovered flukes found in the anterior 10 cms of small intestine, five days after challenge following transfer of IMLNC.

▩ = IMLNC recipients.

▨ = naive MLNC recipients.

□ = non-recipient controls.

A = recipients of IMLNC recovered on day 4 of donor primary infection (recipients challenged one day after cell transfer).

B = recipients of IMLNC recovered on day 4 of donor primary infection (recipients challenged two days before cell transfer).

C = recipients of IMLNC recovered twelve hours after donor secondary infection (recipients challenged nine days after cell transfer).

D = recipients of IMLNC recovered twelve hours after donor secondary infection (recipients challenged one day after cell transfer).

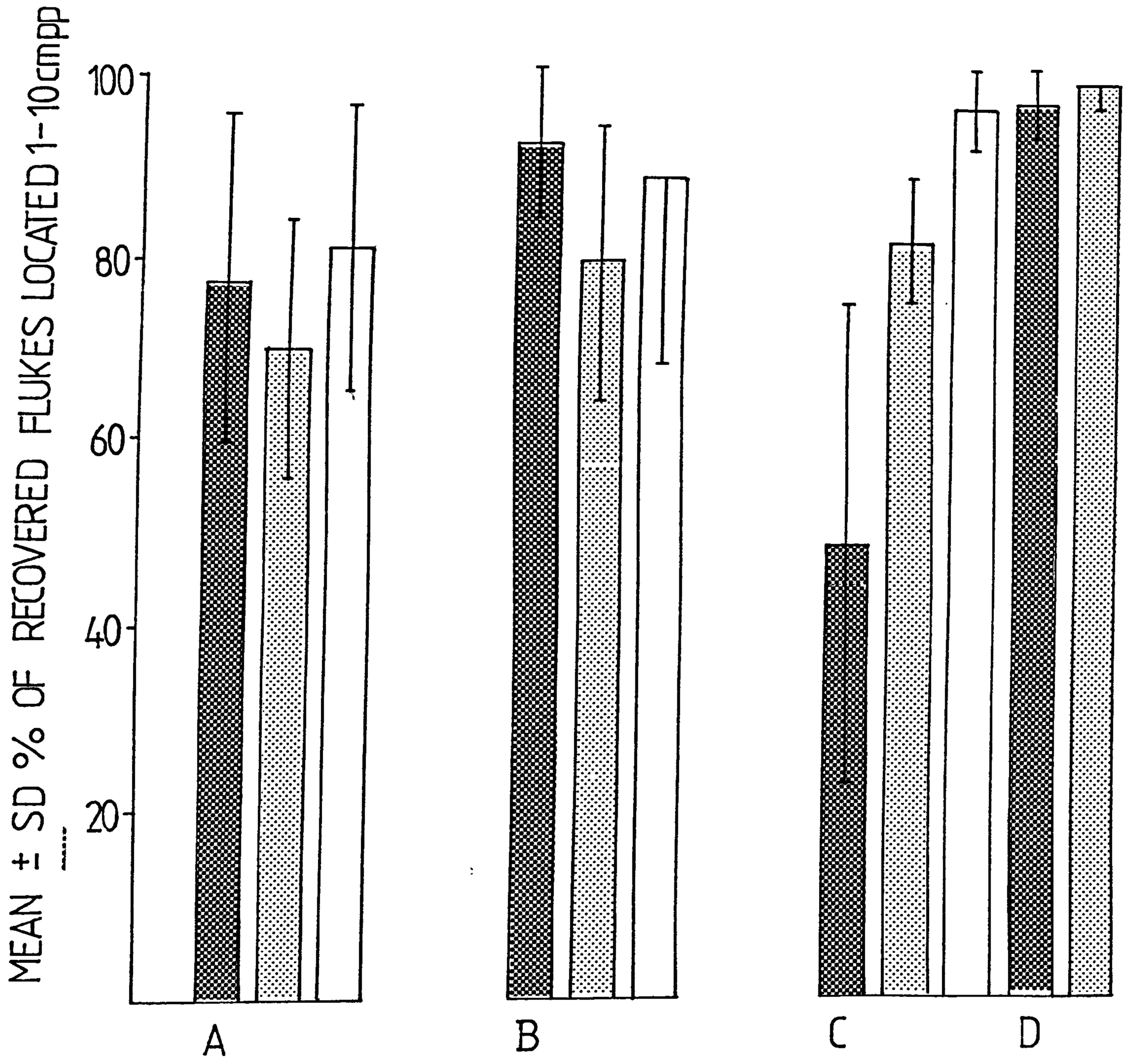

MEAN ± SD % OF RECOVERED FLUKES LOCATED 1-10cmpp
100
80
60
40
20
A
B
C
D

TABLE 4-2c

The effect of IMLNC transfer on the lengths (µm) of flukes recovered from recipient mice

| Cells given | Time of cell transfer | Site of fluke recovery | No. mice | Flukes /mouse | Mean ± SD fluke length (µm) Post.lobe | Total body |
|---|---|---|---|---|---|---|
| Day 4 primary | 2 days after recip. chall. | 0-10 cm (pp.) | 5 | 30 | 174 ± 11 | 492 ± 21 |
| | | 10-20 " | 4 | 6-12 | 144 ± 17 | 429 ± 23 |
| Uninfected | " | 0-10 " | 4 | 30 | 176 ± 29 | 492 ± 47 |
| | | 10-20 " | 3 | 16-18 | 158 ± 11 | 465 ± 20 |
| No cells transferred | | 0-10 " | 4 | 30 | 171 ± 6 | 485 ± 15 |
| 12h after secondary | 9 days before recip.chall. | 0-10 " | 4 | 17 | $^{x}$146 ± 14 | $^{x}$434 ± 24 |
| | | 10-20 " | 4 | 20 | $^{x}$140 ± 9 | $^{x}$426 ± 18 |
| Uninfected | " | 0-10 " | 4 | 30 | 185 ± 11 | 506 ± 17 |
| | | 10-20 " | 3 | 9-12 | 165 ± 17 | 468 ± 47 |
| 12h after secondary | 1 day before recip.chall. | 0-10 " | 5 | 20 | 195 ± 6 | 525 ± 17 |
| Uninfected | " | 0-10 " | 4 | 30 | 191 ± 9 | 507 ± 20 |
| No cells transferred | | 0-10 " | 5 | 20 | 185 ± 12 | 495 ± 23 |

$^{x}$ denotes mean significantly different to control mean ($P \leq 0.05$)

The Effect of transferring IMLNC taken at different stages in primary infection :

Results from the previous experiment raised the possibility that the successful adoptive transfer of immunity by cells derived from secondary infection might, within the experimental design, be determined by the delay between cell transfer and challenge of recipient mice, rather than the stage and type of infection from which the transferred cells were derived.

The following series of experiments began as an investigation of the effect of a nine day delay between cell transfer and recipient challenge on the efficacy of MLNC removed on day 6 of donor primary infection. It was then decided to define the period during donor primary infection when MLNC are capable of transferring measurable immunity. (The effect of varying length of time between cell transfer and recipient challenge on the efficacy of cell transfer is more fully investigated in a later experiment).

The efficacy of IMLNC from days 2, 4, 6, 8, 12 and 21 after donor primary infection was investigated. Because naive MLNC caused no measurable detrimental effect on challenge infection in recipients in the previous experiment, naive cell controls were not included in this, nor in subsequent experiments.

NIH donor and recipient mice within each experiment were of the same sex.

Male mice were used to test MLNC from days 2,4, and 8 of primary infection.

Female mice were used to test MLNC from days 6, 12 and 21 pi.

Donors were infected at 7-8 weeks of age, and groups of 5-8 recipient mice were aged 7-8 weeks when receiving cells.

Recipient mice were challenged nine days after receiving cells. Parasites were recovered 5.75 days after challenge, and their number, distribution and length recorded.

Fluke recovery and distribution :

All control groups retained more than 60% of their parasites (Fig 4-3a). There was no significant difference between fluke recoveries from control mice and recipients of IMLNC from day 8 and day 12 of donor primary infection. The anterior location of flukes in these groups of mice supported the view that expulsion had not begun.

The fluke burdens of recipients of IMLNC from days 2, 4 and 21 of donor infection were reduced by 60-68% compared with controls and only a negligible number of flukes remained in recipients of day 6 IMLNC. The posterior position of flukes recovered from these groups of recipients (Fig 4-3b) indicated that fluke expulsion was underway.

Fluke lengths (Table 4-3c)

Flukes from cell recipients were compared with those recovered from the corresponding region of the gut in control mice. Surprisingly, some flukes were found to be slightly reduced in size although their expulsion rate was not measurably affected by transferred IMLNC. Flukes occuring 0-10 cm post-pylorus in recipients of day 12 IMLNC were 5% smaller than those from control mice but no increase in vitelline depletion was measured in this group.

A reduction in length of flukes from recipients of day 2 and day 4 IMLNC was associated with the acceleration of expulsion of flukes from these groups. Reductions of 6% and 11% were observed, compared with controls, and over half of the flukes recovered from these recipients showed no signs of vitelline development compared with 10% in controls.

Flukes from recipients of day 21 IMLNC were similarly smaller than those from control mice (posterior lobe length reduced by 8-15% and total body length by 6-11%).

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Figure 4-3a

IMLNC recovered at different stages in donor infection and given to recipient nine days before challenge :

Mean fluke recoveries 5.75 days after challenge of recipients with 200 metacercariae.

Vertical bars represent group standard deviation

= recipients of IMLNC

= (non-recipient) controls

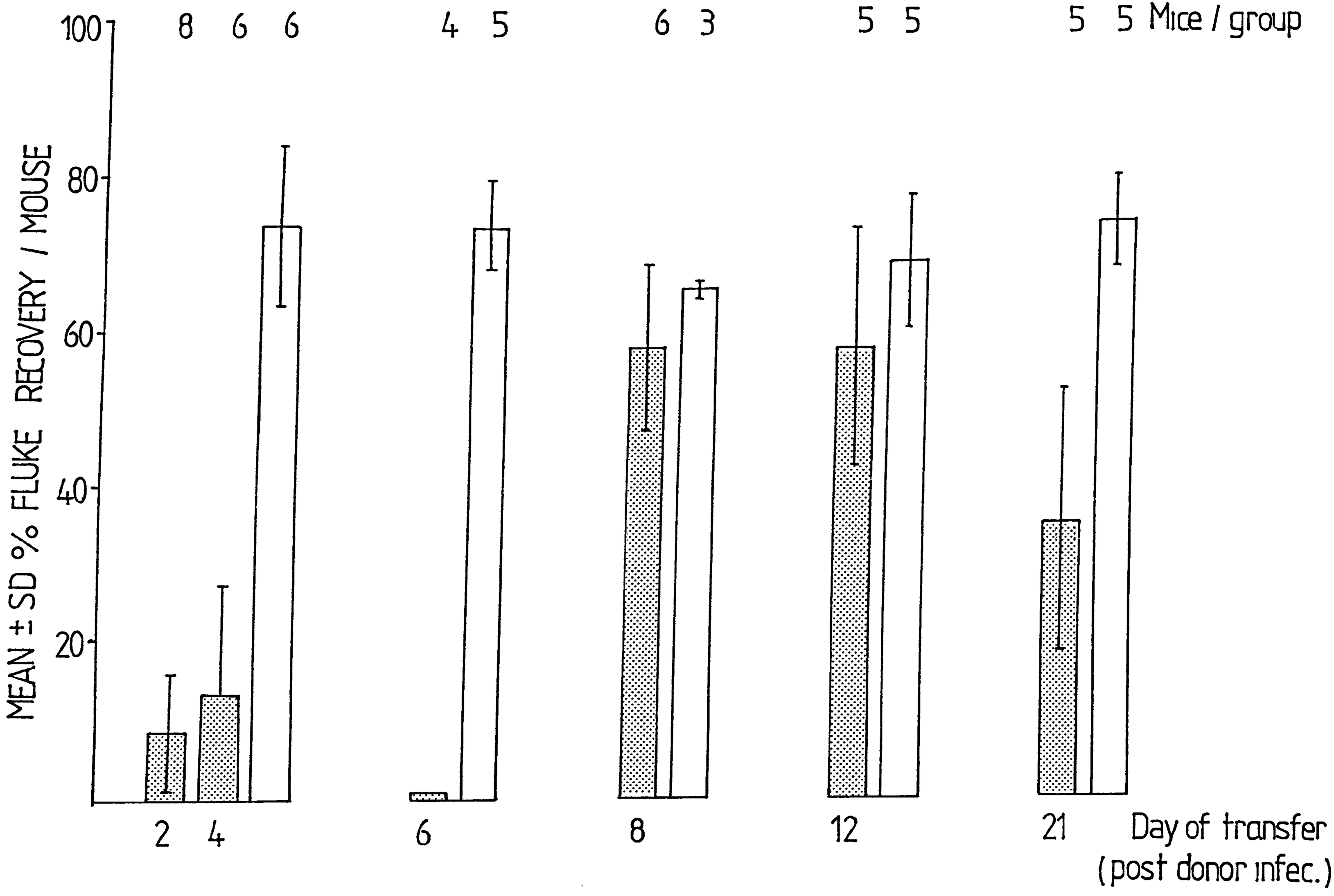
8 6 6
4 5
6 3
5 5
5 5 Mice / group
100
80
60
40
20
MEAN ± SD % FLUKE RECOVERY / MOUSE
2 4
6
8
12
21
Day of transfer
(post donor infec.)

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Figure 4-3b

IMLNC recovered at different stages in donor infection and given to recipient mice nine days before challenge : Mean % of recovered flukes located in an anterior position, 0-10 cm post-pylorus in the small intestine 5.75 days after challenge of cell recipients with 200 metacercariae.

Vertical bars represent group standard deviation.

▒ = recipients of IMLNC

□ = (non-recipient) controls

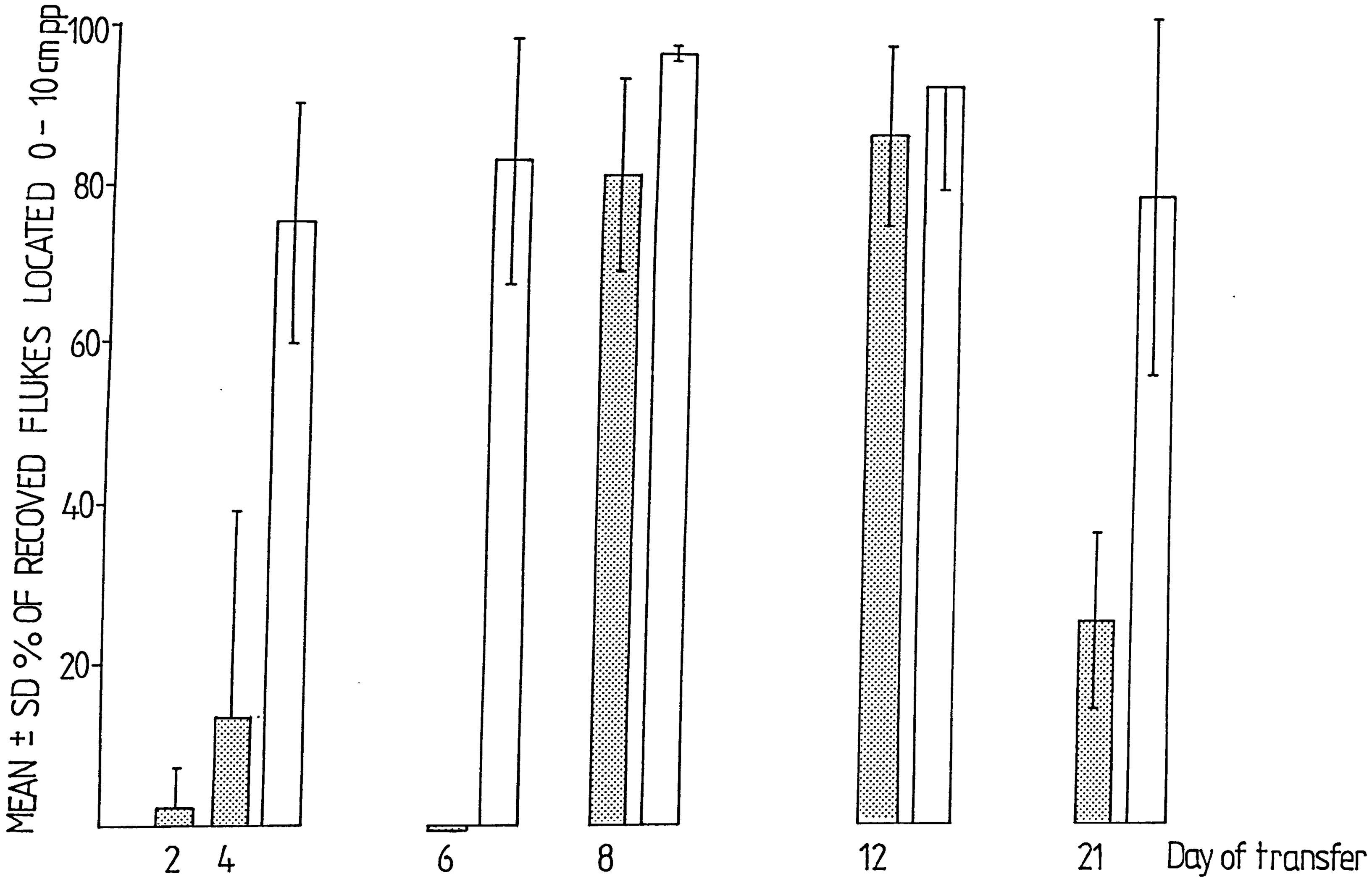
MEAN ± SD % OF RECOVED FLUKES LOCATED 0 - 10 cm pp
100
80
60
40
20
2
4
6
8
12
21
Day of transfer
( post donor infec. )

TABLE 4-3c

The effect of IMLNC transferred at different times during donor infection, on the body length and vitelline development of flukes recovered from recipient mice:

| Day of donor primary infection that cells transferred: | No.mice | Flukes /mouse | Site of fluke recovery (cm pp.) | Mean ± SD length Post. lobe(μm) | Mean ±SD length Body(μm) | Mean ±SD % flukes with deficient(or-ve) vitellaria |
|---|---|---|---|---|---|---|
| Day 4 | 5 | 9-10 | 10-20 | $^{x}$149 ± 26 | $^{x}$425 ± 48 | 76 ± 27 (60%) |
| Day 2 | 7 | 6-10 | 10-20 | $^{x}$162 ± 21 | $^{x}$449 ± 30 | 83 ± 24 (55%) |
| Control (no cells) | 6 | 10 | 0-10 | 207 ± 14 | 537 ± 27 | 9 ± 8 |
| | 6 | 6-10 | 10-20 | 183 ± 20 | 480 ± 32 | 25 ± 13 (10%) |
| Day 12 | 5 | 30 | 0-10 | 191 ± 18 | 512 ± 36 | 31 ± 17 |
| | 5 | 6 | 10-20 | 174 ± 17 | 490 ± 36 | 47 ± 24 |
| Control (no cells) | 5 | 30 | 0-10 | 210 ± 11 | 542 ± 24 | 16 ± 8 |
| | 3 | 12 | 10-20 | 185 ± 11 | 494 ± 17 | 36 ± 19 |
| Day 21 | 4 | 10 | 0-10 | $^{x}$177 ± 11 | $^{x}$483 ± 31 | n.d. |
| | 5 | 10 | 10-20 | 168 ± 12 | $^{x}$467 ± 18 | n.d. |
| Control (no cells) | 4 | 20 | 0-10 | 209 ± 6 | 542 ± 19 | n.d. |
| | 5 | 20 | 10-20 | 183 ± 14 | 499 ± 24 | n.d. |

$^{x}$denotes mean significantly different to corresponding control mean ($P = 0{\cdot}05$)

Too few flukes were recovered from recipients of Day 6 primary IMLNC to allow analysis.

The Effect of transferring IMLNC taken from different stages of donor secondary infection :

Donors (36 female NIH) were infected at 7-8 weeks of age. 24 were challenged 21 days later. The remaining 12 mice were donors of IMLNC taken 28 days after primary infection.

Recipients (19 female NIH) were given $8x10^7$ IMLNC at 7-8 weeks of age in the following groups.

5 mice received D28 1° IMLNC

8 mice received D6 2° IMLNC

6 mice received D12 2° IMLNC

7 controls received no cells.

Recipients and controls were challenged nine days after cell transfer and killed 5.75 days after challenge. The position, number and size of flukes recovered was noted.

Fluke recovery and distribution :

The %recoveries of flukes from recipients of IMLNC did not differ significantly from that of control mice (76%) (Figure 4-4a). Although substantial loss of flukes was not apparent, the distribution (figure 4-4b) of flukes in three of the groups, and their large standard deviation indicate that the onset of expulsion was imminent, when the mice were killed, particularly recipients of IMLNC from day 6 of secondary infection, in which only 49% ($\pm$ 24%) of recovered flukes remained in the anterior 10 cm of small intestine.

Fluke lengths and vitellaria : (Table 4-4c)

Flukes recovered from the small intestine of recipient mice did not differ significantly in length of body or posterior lobe from those recovered from control mice. There was no reduction in length of flukes from any cell recipients.

There was no significant increase in the proportion of recovered flukes with depleted vitellaria in cell recipients.

None of the cells transferred had any measurable effect on challenge infection in recipient mice

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Figure 4-4a

IMLNC transferred at different stages in donor secondary infections, and recipients challenged nine days after cell transfer :

Mean % recovery of flukes 5.75 days after challenge of recipient mice.

Vertical bars represent standard deviation.

Figure 4-4b

Mean % of recovered flukes located in an anterior position 0-10 cm post-pylorus in the small intestine.

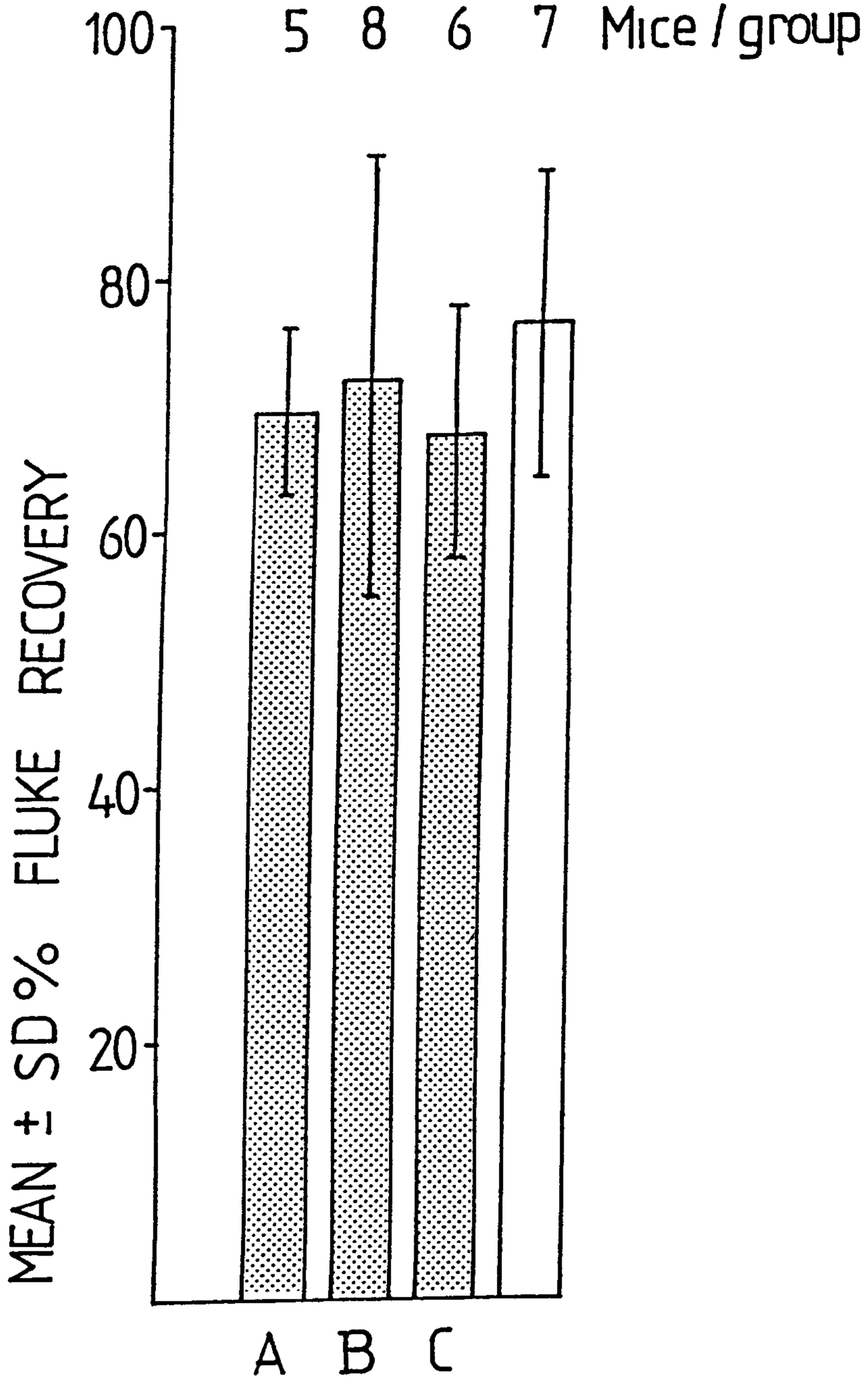
□ = non-recipient controls

▨ = recipients of IMLNC

A = recipients of IMLNC transferred on day 28 after donor $1^{o}$ infection

B = " " " " " Day 6 " " $2^{o}$ infection

C = " " " " " Day 12 " " $2^{o}$ infection


5 8 6 7 Mice / group
100
80
60
40
20
MEAN ± SD % FLUKE RECOVERY
A B C

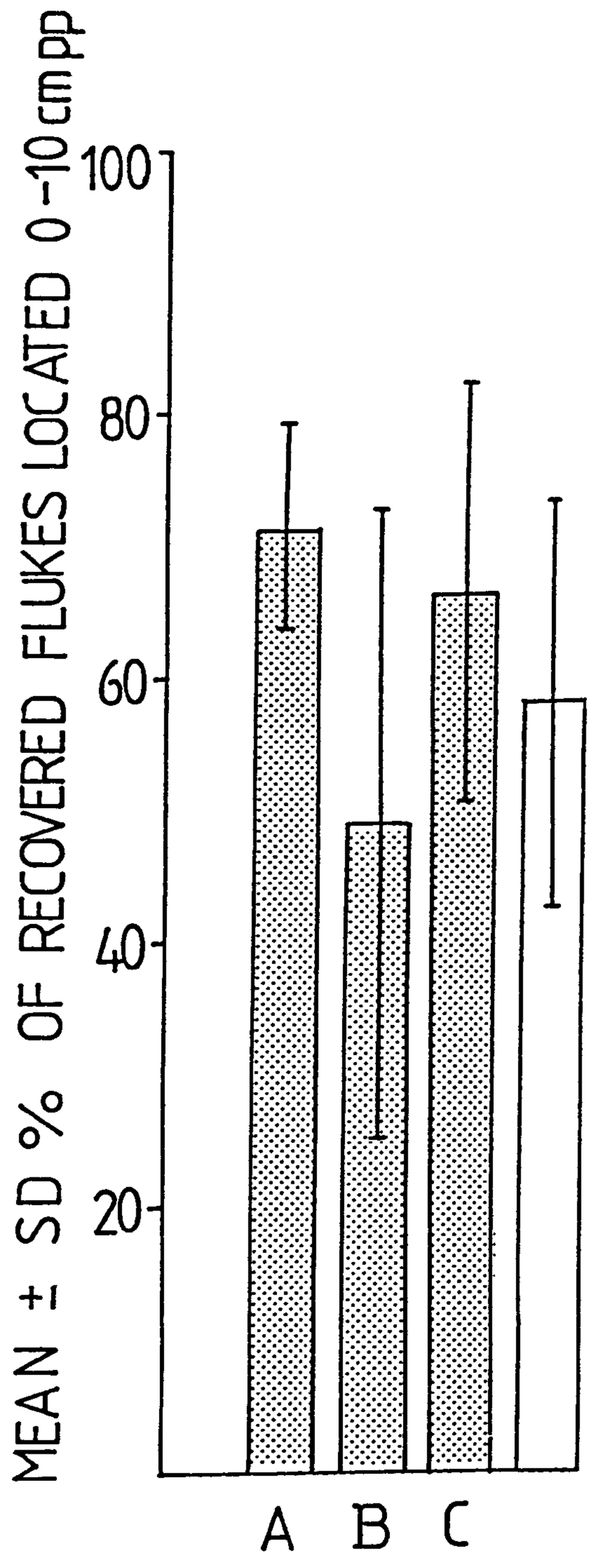

100
80
60
40
20
MEAN ± SD % OF RECOVERED FLUKES LOCATED 0-10 cm pp
A B C

TABLE 4-4c

The effect of IMLNC transferred at different times during donor secondary infection on the length and vitelline development of flukes recovered from recipient mice

| Day donor infection that cells transferred | No.mice | Flukes /mouse | Site of fluke recovery (cm pp.) | Mean ± SD (Post. lobe) | length (μm) (Body) | Mean ± SD % flukes with deficient vitellaria |
|---|---|---|---|---|---|---|
| Day 28 primary | 5 | 15 | 0-10 | 208 ± 12 | 530 ± 15 | 5 |
| | 5 | 20 | 10-20 | 205 ± 14 | 523 ± 15 | 13 ± 9 |
| Day 6 secondary | 7 | 10 | 0-10 | 204 ± 14 | 514 ± 17 | 6 ± 4 |
| | 8 | 10 | 10-20 | 191 ± 14 | 487 ± 25 | 22 ± 15 |
| Day 12 secondary | 5 | 20 | 0-10 | 205 ± 15 | 520 ± 31 | 12 ± 6 |
| | 5 | 20 | 10-20 | 179 ± 15 | 485 ± 31 | 36 ± 22 |
| No cells given | 6 | 20 | 0-10 | 209 ± 12 | 530 ± 23 | 7 |
| | 4 | 20 | 10-20 | 179 ± 27 | 480 ± 48 | 31 ± 19 |

The Effect of varying the number of MLNC transferred, on the efficacy of cell transfer.

This experiment was done to determine the minimum and optimum number of IMLNC required to transfer immunity adoptively.

Donors (16 female MH) were infected at $8\frac{1}{2}$-$9\frac{1}{2}$ weeks of age, and cells collected six days after infection. Recipients were given day 6 IMLNC at 7-8 weeks of age, and challenged nine days later.

| | | | |
|---|---|---|---|
| Recipient groups : | 6 mice | received | $1x10^7$ IMLNC |
| | 8 " | " | $2x10^7$ " |
| | 6 " | " | $4x10^7$ " |
| | 5 " | " | $8x10^7$ " |
| | 8 " | " | no cells |

Mice were killed 5.75 days after challenge and their flukes recovered.

Fluke recovery and distribution : (Figs 4-5a,and 6)

Expulsion had not begun in control mice which harboured 72% of their infection (76% of which were found 0-10 cm post-pylorus). Survivorship in recipients of $1x10^7$ IMLNC was very variable (SD± 18%). The mean % recovery was significantly lower than the control value. $2x10^7$ IMLNC were as effective as $8x10^7$ IMLNC in reducing survivorship of flukes at the time of kill. In all recipients a relatively low proportion of recovered flukes remained in the anterior 10 cm of small intestine : only 19% of flukes recovered from recipients of $1x10^7$ IMLNC were found in this region, and the number was negligible in recipients of $2\text{-}8x10^7$ IMLNC compared with 76% in control mice.

Fluke lengths and vitellaria:(Table 4-5c)

$1x10^7$ IMLNC had very little effect on fluke length. The length of flukes recovered 0-10 cm post pylorus was reduced by 7% compared with controls, and the posterior lobe length was reduced by 12% Fluke lengths from other cell recipients were significantly reduced in length by 10-11% and the posterior lobe length was significantly reduced by 14-17%.

$1x10^7$ IMLNC had no observable effect on vitelline development.

An increase in the percentage of flukes with depleted or negative vitellaria occurred in recipients of $2x10^7$ IMLNC but was far more consistent in recipients of $4x10^7$ and $8x10^7$ IMLNC.

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Figure 4-5a

The effect of varying the number of IMLNC transferred, on the efficacy of IMLNC from day 6 of donor primary infection, transferred nine days before challenge of recipients with 200 metacercariae :

Mean % fluke recoveries 5.75 days after challenge of recipients.

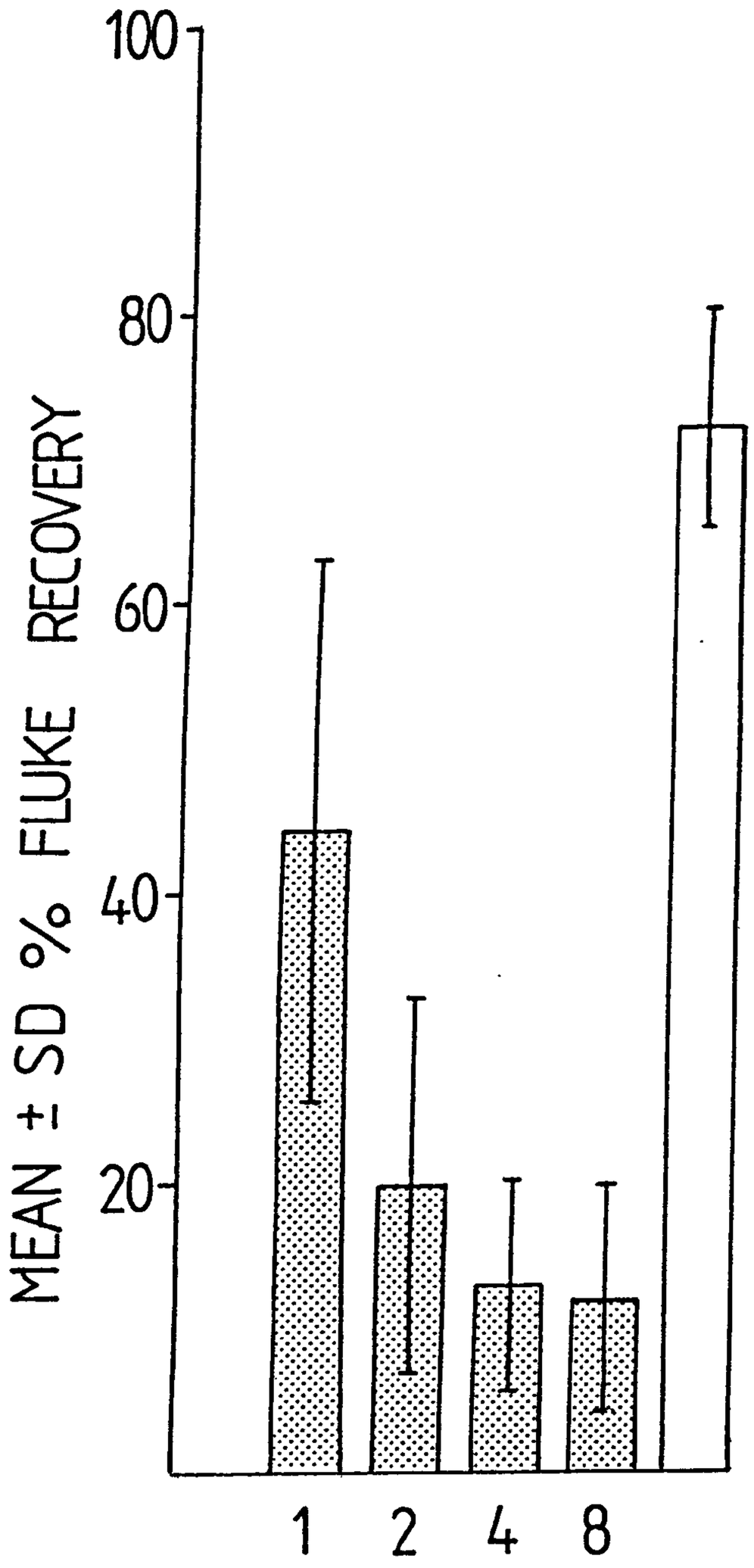
Vertical bars represent standard deviation.

Figure 4-5b

Mean percentage of recovered flukes located in an anterior position, 0-10 cm post pylorus, in the small intestine.

= non-recipient controls

= recipients of IMLNC


MEAN ± SD % FLUKE RECOVERY
100
80
60
40
20
1
2
4
8

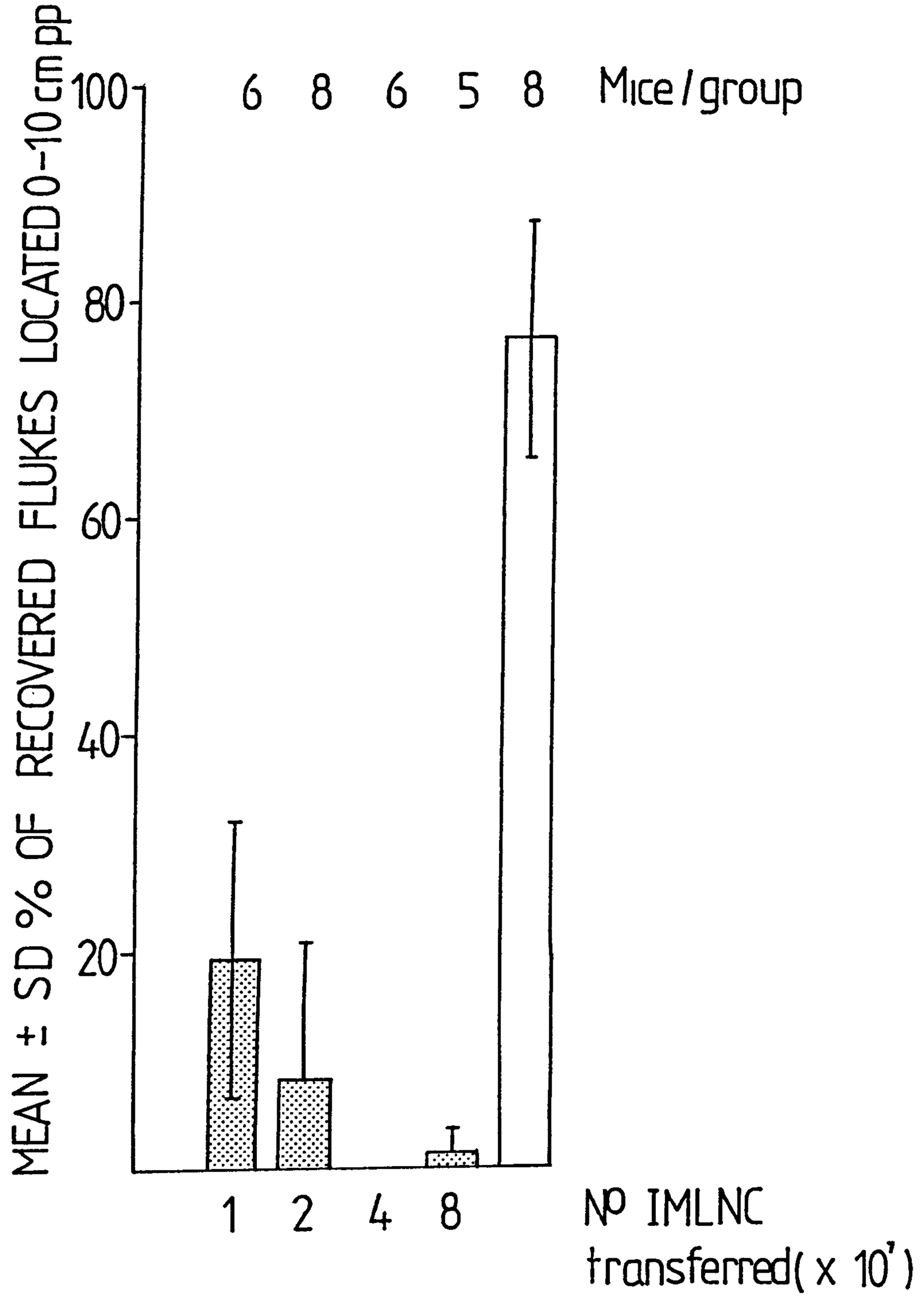

MEAN ± SD % OF RECOVERED FLUKES LOCATED 0-10 cm pp
100
80
60
40
20
6 8 6 5 8 Mice/group
1 2 4 8
Nº IMLNC transferred( x 10⁷)

TABLE 4-5c Effect of varying N$^{o}$ of IMLNC transferred, on lengths and vitellaria of flukes from recipient mice:

| No. IMLNC transferred | No. mice | Flukes /mouse | Site of fluke recovery (cm pp.) | Mean ± SD length (Post. lobe)(μm) | Mean ± SD length (Body) (μm) | Mean ± SD % flukes with deficient (or -ve) vitellaria |
|---|---|---|---|---|---|---|
| $1 \times 10^7$ | 4 | 14 | 0-10 | $^{x}198 \pm 11$ | $^{x}517 \pm 27$ | $18 \pm 14$ |
| | 6 | 24-30 | 10-20 | $191 \pm 11$ | $502 \pm 23$ | $34 \pm 22$ (5) |
| $2 \times 10^7$ | 8 | 13-18 | 10-20 | $^{x}167 \pm 21$ | $^{x}457 \pm 42$ | $^{x}77 \pm 24$ (29) |
| $4 \times 10^7$ | 5 | 16 | 10-20 | $^{x}168 \pm 15$ | $^{x}453 \pm 27$ | $^{x}60 \pm 9$ (32) |
| $8 \times 10^7$ | 5 | 7-14 | 10-20 | $^{x}162 \pm 8$ | $^{x}458 \pm 35$ | $^{x}68 \pm 9$ (25) |
| No cells | 8 | 30 | 0-10 | $224 \pm 12$ | $557 \pm 23$ | n.d. |
| | 8 | 9-15 | 10-20 | $195 \pm 17$ | $509 \pm 32$ | $36 \pm 19$ (10) |

$^{x}$ denotes mean significantly different to corresponding control mean. ($P = 0{\cdot}05$)

The Effect of varying the interval between cell transfer and challenge of recipient mice, on the efficacy of cell transfer.

Earlier experimental results suggested that a delay in recipient challenge, after cell transfer is necessary to produce measurable transfer of immunity in this system. In this experiment, the importance of the time at which cells were injected into the recipient mice, relative to the time the mice were challenged with fluke infections was further investigated.

Donors (four groups of nine female NIH) were infected at eight weeks of age. Groups of 5-6 recipients received $8x10^7$ IMLNC (recovered on day 6 of donor primary infection) at 7-8 weeks of age, and were challenged as follows :

Group 1. Challenged on day of cell transfer

2. " 2 days after cell transfer

3. " 4 " " " "

4. Controls - given no IMLNC

5. Challenged 19 days after cell transfer

6. Control - given no IMLNC

Mice were killed six days after challenge and their flukes recovered.

Fluke recovery and distribution : (Figures 4-6a, 4-6b).

Recipients of IMLNC at the time of challenge, and the corresponding control group, retained 68% and 69% of their original fluke burden, whereas fluke recovery was substantially lower in recipients of IMLNC given two days (46%) and four days (33%) pre-challenge. The fact that expulsion was occurring in these recipients (or imminent in the case of recipients of cells on the day of challenge) was reflected either by a reduction in the proportion of flukes remaining in the region of small intestine 0-10 cm post-pylorus, or by a large SD indicating variability in postion of flukes (Fig. 4-6b).

The efficacy of day 6 primary IMLNC transferred 19 days before challenge is obscured by the low recovery in the corresponding control group in which survivorship was only 45%. Of these flukes 76% ($\pm$ 18%) i.e. a large proportion remained in the anterior 10 cm of small intestine. This value would be

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Figure 4-6a

The effect of varying the time interval between cell transfer and challenge of recipient mice.

Mean % fluke recoveries 5.75 days after challenge of recipients with 200 metacercariae.

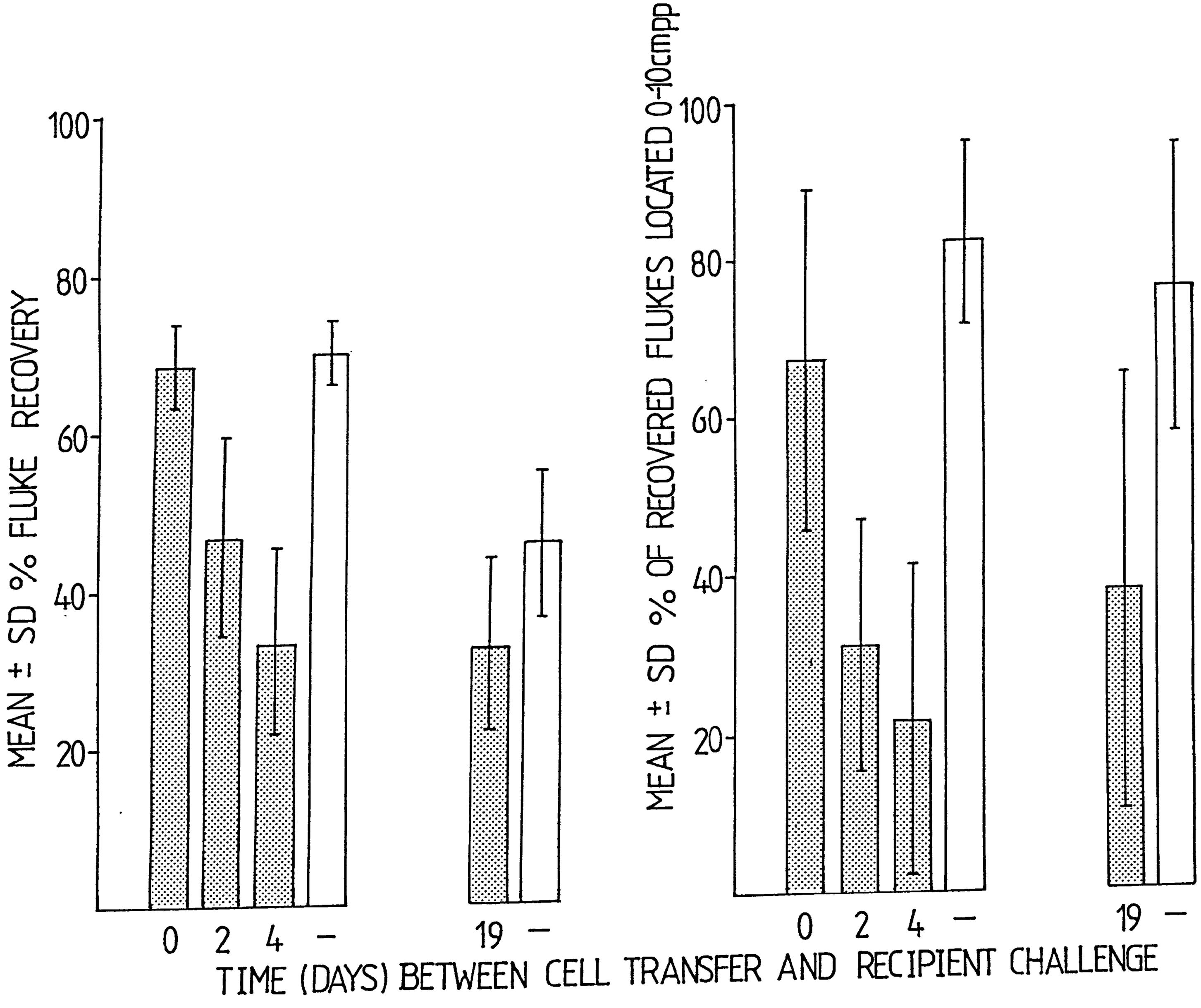
Vertical bars represent standard deviation.

Figure 4 - 6b

Mean % of recovered flukes located 0-10 cm post-pylorus.

□ = non-recipient controls

▨ = recipients of IMLNC


MEAN ± SD % FLUKE RECOVERY
100
80
60
40
20
0
2
4
–
19
–
MEAN ± SD % OF RECOVERED FLUKES LOCATED 0-10cmpp
100
80
60
40
20
0
2
4
–
19
–
TIME (DAYS) BETWEEN CELL TRANSFER AND RECIPIENT CHALLENGE

expected to be considerably lower if the low recovery in the control group was due entirely to loss by expulsion. The difference of 13% in fluke recovery between this group of recipients and controls is not significant ($P > 0.1$). The large variation in position of flukes in these recipients with a mean of only 37% found 0-10 cm post pylorus, suggests that expulsion was occurring in these mice.

Fluke lengths : (Table 4-6c)

The effect of transferred IMLNC on fluke length is very small. The only significant reduction in length was observed in flukes recovered from recipient mice given IMLNC on the day of challenge, in which the posterior lobe was 6% shorter than that of control flukes.

Vitelline development:(Table 4-6c)

Any effect of IMLNC transferred 19 days before challenge of recipients, on vitelline development, was observed by the unusually large degree of vitelline depletion observed in flukes recovered from control mice.

In recipients of IMLNC two or four days before, or on the day of challenge, a significant increase in vitelline depletion was recorded only in flukes recovered 10-20 cm post-pylorus.

TABLE 4-6c

The effect of varying the time interval between cell transfer and challenge of recipients,on the lengths and vitelline development of flukes recovered from recipient mice:

| Time of cell transfer | | Mn.length ± SD(µm) A | Mn.length ± SD(µm) B | No. mice x flukes/mouse A | No. mice x flukes/mouse B | Mn ± SD % flukes with defic.vitell. (-ve " ) A | Mn ± SD % flukes with defic.vitell. (-ve " ) B |
|---|---|---|---|---|---|---|---|
| Day of challenge | P. | I95 ± 9 | I83 ± I2 | 5 x 30 | 4 x I0 | I2 ± 6 | 28 ± I4 |
| | T. | 522 ± 23 | 480 ± 23 | | I x 9 | | (9 9) |
| 2 days prechall. | P. | I92 ± 25 | I65 ± I2 | 4 x I5 | 4 x 30 | 23 ± I2 | 48 ± I7 |
| | T. | 507 ± 50 | 460 ± I4 | | | (4 3) | (I8 8) |
| 4 days prechall. | P. | 200 ± I4 | I92 ± I8 | 3 x I5 | 6 x 20 | 40 ± 37 | 50 ± 33 |
| | T. | 5I8 ± 2I | 503 ± 28 | I x 6 | | | (9 I0) |
| | | | | I x 7 | | | |
| No IMLNC given | P. | 206 ± 3 | I85 ± I8 | 4 x 30 | 4 x I0 | 9 ± 4 | I0 ± 8 |
| | T. | 540 ± 9 | 492 ± 38 | | | | |
| I9 days prechall. | P. | I68 ± I5 | I73 ± I0 | 3 x I0 | 5 x 20 | 70 ± 5 | 74 ± 20 |
| | T. | 459 ± I6 | 472 ± I9 | I x 9 | | (I6 I3) | (I9 I5) |
| No IMLNC given | P. | I83 ± 26 | I7I ± I0 | 5 x 20 | 3 x I8 | 32 ± 2I | 78 ± 6 |
| | T. | 483 ± 45 | 458 ± 22 | | | (4 4) | (24 8) |

P.= Length of posterior lobe

T.= Total body length

A = Flukes recovered 0-I0 cm pp.

B = Flukes recovered I0-20 cm pp.

denotes mean significantly different to control (ie Probability $P < 0.05$)

Changes in the MLN associated with infection and adoptive transfer of immunity :

As a preliminary attempt to quantify cellular changes in the MLN associated with infection, it was decided to pool the data obtained in earlier experiments, referring to mean MLNC per donor mouse recovered at different stages in primary and secondary infections. It should be noted that it is not usual procedure to compare independantly obtained data in this way, and that the method of MLNC recovery employed in cell transfer experiments is not an accurate quantitative technique.

Certain trends in MLN cellularity associated with infection are evident. All values were within the range $1x10^7$ - $1.32x10^8$ MLNC per mouse. A marked increase in cellularity of the MLN occurred in the eight days following a 200 metacercarial primary infection (Figure 4-7 ) . MLNC counts per mouse in excess of $1x10^8$ were recorded on days 4-12 pi, after which available data are limited to single points which suggest a decline in cellularity.

Values obtained for mice following 200 metacercarial secondary infection (Figure 4-7 ) unfortunately consist mainly of single points. No increase in MLN cell number was evident until day 4 pi. On day 12 pi cell numbers resembled those of infected mice.

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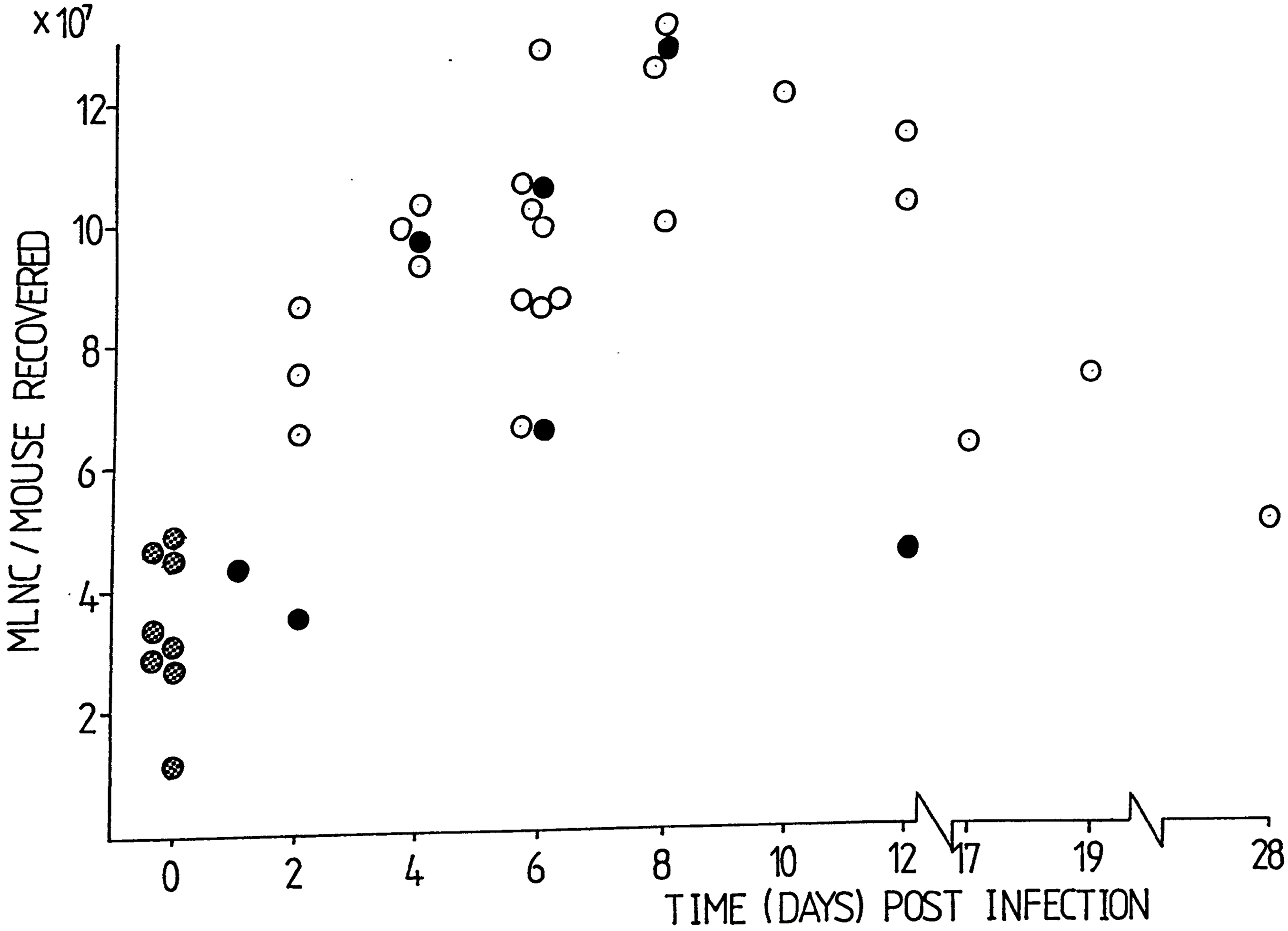
Figure 4 - 7

Mean number of MLNC/mouse recovered at different times after primary and secondary infections of D.phoxini, and from uninfected mice.

● = uninfected mice

○ = mice given primary infection of 200 D.phoxini metacercariae

● = " " secondary " " " " " "


x 10⁷
12
10
8
6
4
2
MLNC / MOUSE RECOVERED
0
2
4
6
8
10
12
17
19
28
TIME (DAYS) POST INFECTION

Changes in Lymphoblast activity and cellularity of MLN following primary and secondary infection :

This work was done to investigate the importance of cell division within the MLN, in the increase in cellularity occurring after infection, to establish the period after infection during which cell division was elevated, and in a later experiment, to initiate the identification of the cell populations involved. Blast activity was assessed by measuring the incorporation of $^{125}$IUdR by MLNC suspensions *in vitro*.

Donors were infected at seven weeks (primary) and ten weeks (secondary) of age. MLNC suspensions were prepared from groups of 4-5 mice on days 1,2,4,6 and 8 after secondary and days 2,4,6,8,10,12 and 17 after primary infection.

Both lymphoblast activity and MLNC/donor mouse are expressed as percentage of control (Uninfected) values.

Figure 4-8a shows that after primary infection a rapid increase in lymphoblast activity and cellularity occurs during the first four days pi. High blast activity (more than double that of controls) was maintained until day 8 pi but had declined markedly by day 10. The cellularity of MLN remained high ($>$ 3.5 x control value) until at least day 12 pi, before declining.

These responses occurred more slowly in response to secondary infection (Figure 4-8b). No increase in either parameter was measured until day 4 pi (when expulsion would be expected to be underway). Cellularity increased to a value $>$8.5 x control value on day 8 pi. Blast cell activity peaked on day 6 pi but remained $>$1.5 x control level on day 8 pi.

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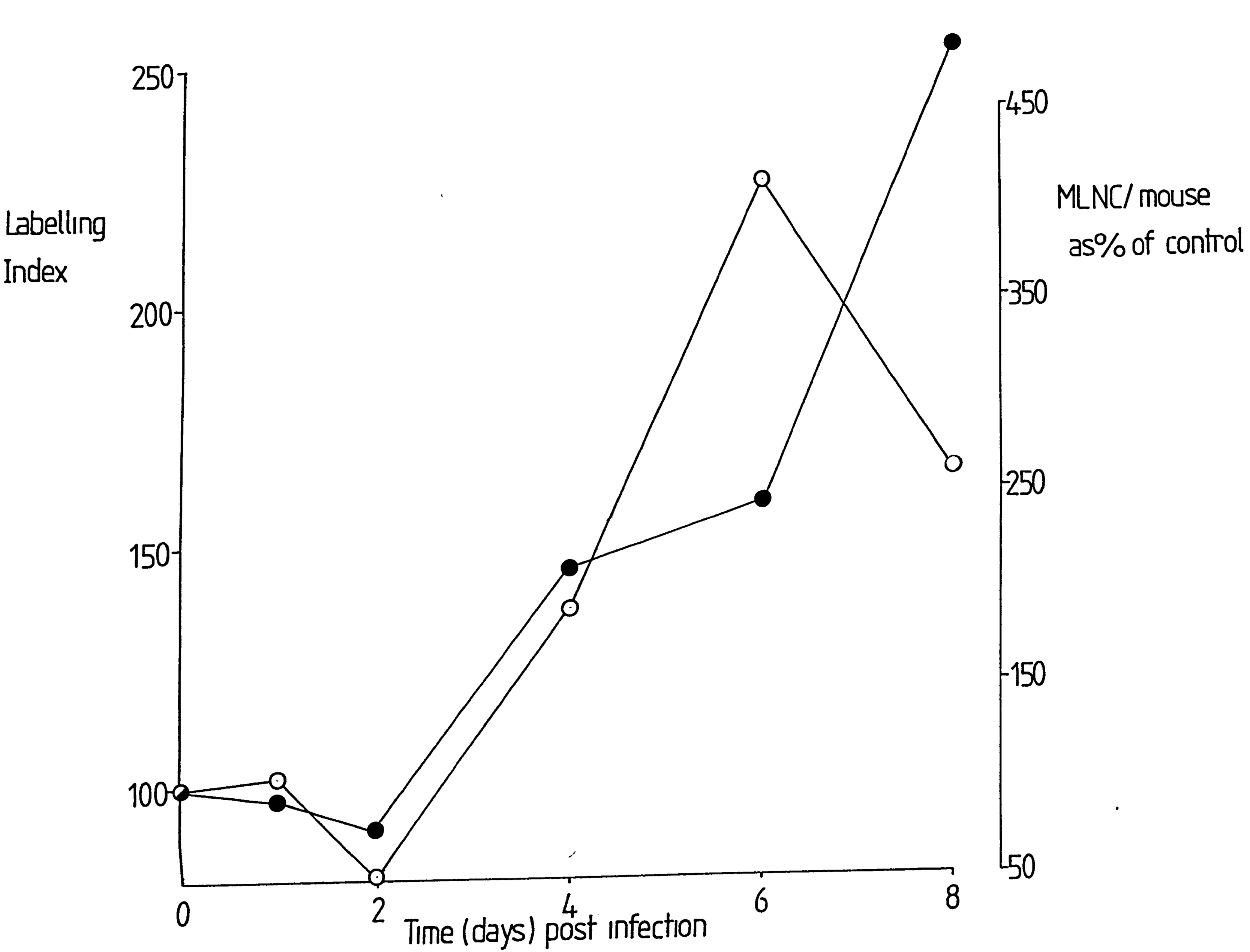
Figure 4-8a

Changes in labelling index and MLN cellularity (MLNC/mouse) following primary infection.

○ = labelling index as % of control value

● = Mean MLNC per mouse as % of control mean.


Labelling Index
250
200
150
100
MLNC/ mouse as% of control
450
350
250
150
50
0
2
4
6
8
Time (days) post infection

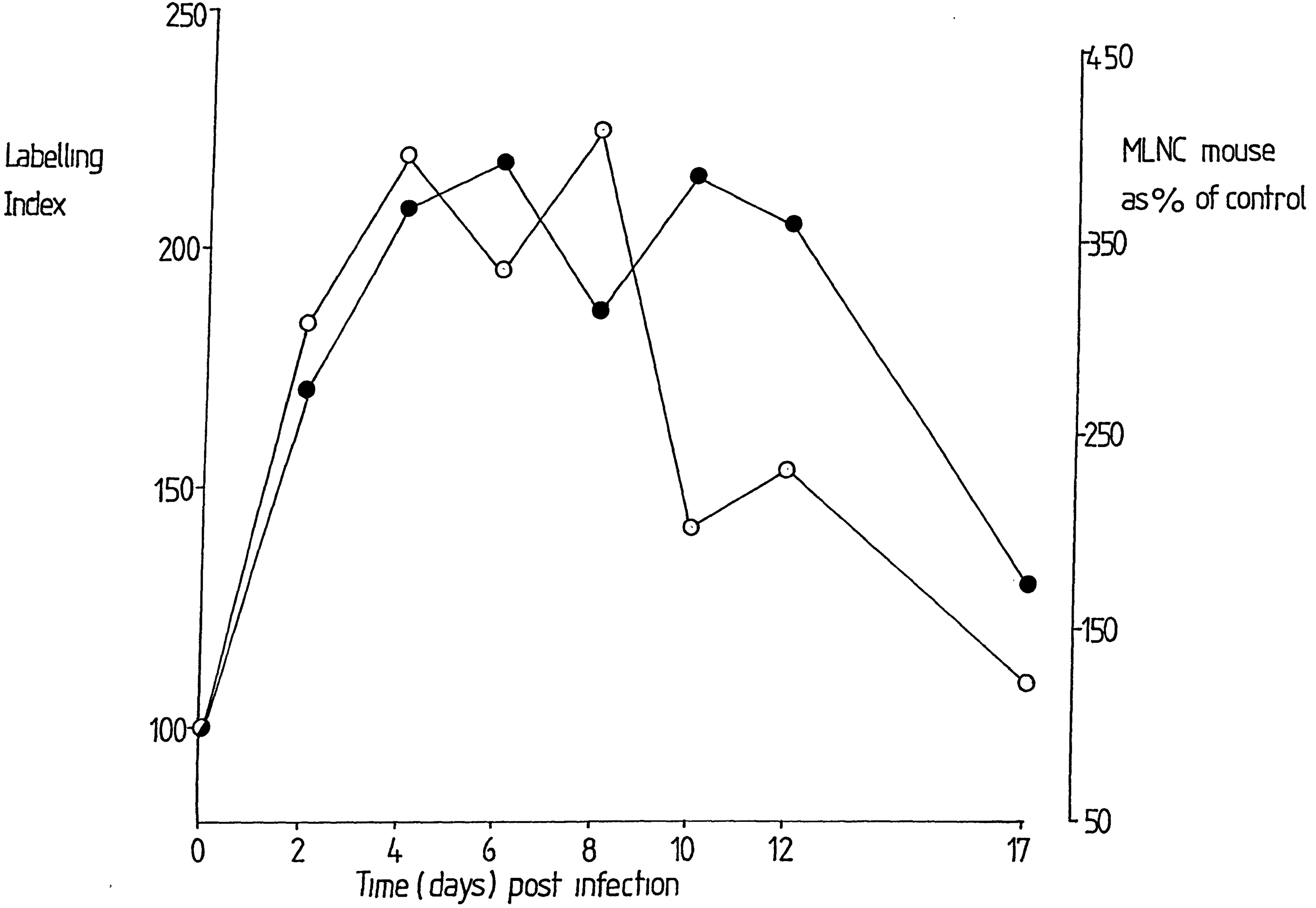
Facing page 126

Figure 4-8b

Changes in labelling index and MLN cellularity (MLNC/mouse) following secondary infection.

○ = labelling index as % of control value

● = mean MLNC/mouse as % of control mean


Labelling Index
250
200
150
100
450
350
250
150
50
MLNC mouse as % of control
0
2
4
6
8
10
12
17
Time (days) post infection

Lymphoblast activity in T cell and B cell fractions of MLNC during D.phoxini infection :

In the following expermiment an attempt is made to characterize more fully the MLN lymphoblast population responding to D.phoxini infection.

Ten male NIH mice were infected at seven to eight weeks of age, killed six days later and their MLNC and those of sixteen uninfected mice, collected. $8x10^7$ MLNC were removed from each suspension (infected and uninfected) of cells, and lymphoblast activity measured.

The remaining MLNC were separated into T and B enriched cell fractions. The T fraction was estimated to contain 4-11% B cell contamination and the B cell fraction after treatment with anti-Thy antiserum, and guinea pig complement, contained 87-94% sIg +ve cells. Viability in the T cell fraction was low (76% in infected mice). Estimates of lymphoblast activity in each sample were made using four aliquots of $2x10^7$ cells.

The experiment was repeated using MLNC from nine donors infected 12 days previously. MLNC from four control mice were not separated into T- and B- enriched fractions. The T- cell fraction from infected mice contained 8% sIg +ve cells, and the B fraction 88%

Results of these experiments are summarised in Tables 4-9a and b.

The lymphoblast activity in the T- enriched fraction of naive MLNC (table 4-9a) was low (only 1/3rd of unseparated cell count). Although the high activity of the B cell fraction is based on a single count, it is supported by comparison of counts for unseparated and T cell fractions.

The lymphoblast activity of unseparated day 12 IMLNC (table 4-9b) did not differ appreciably from that of controls (and a similar proportional activity to that occurring in uninfected day 6 controls was evident).

Available B cell samples were too few to show whether an appreciable increase in B cell activity had occurred at this stage after infection, compared with uninfected mice.

Compared with day 12 IMLNC, the distribution of lymphoblast activity

TABLE 4-9a

Lymphoblast activity in T cell and B cell enriched MLNC fractions 6 days after primary infection:

| Cell source | MLNC fraction | No. of aliquots | Mean ± SD counts /2 x $10^7$ MLNC/min. | Counts as % of unsep.cell count |
|---|---|---|---|---|
| Day 6 Primary inf. | B cell | 2 | I9375<br>I9263 - | 27 % |
| " " | T cell | 3 | 76435 ± 3708 | I09 |
| " " | Unsep. IMLNC | 4 | 70334 ± 4708 | I00 |
| Uninfected | B cell | I | 25006 - | 80 |
| " " | T cell | 4 | 9646 ± 832 | 3I |
| " " | Unsep. | 4 | 3I452 ± 3033 | I00 |

Back ground count = I35-I39

TABLE 4-9b

Lymphoblast activity in T cell and B cell enriched MLNC fractions I2 days after primary infection:

| Cell source | MLNC fraction | No. of aliquots | Mean ± SD counts /2 x $10^7$ MLNC/min. | Counts as % of unsep.cell count |
|---|---|---|---|---|
| Day I2 Primary inf. | B cell | 2 | I33I8 I34I9 | - | I24 % |
| " " | T cell | 4 | 3455 ± 355 | 32 |
| " " | Unsep. IMLNC | 4 | I0755 ± 374 | I00 |
| Uninfected | Unsep. MLNC | 4 | 9966 ± 858 | - |

Background count = I35

between the two fractions of IMLNC was reversed in mice which had been infected for six days.

Activity of the B cell fraction was only 27% (compared with 80% in controls). The activity of the T cell fraction was 109% of the unseparated cell value (cf 31% in controls).

<u>The Effect of T and B cells in transfer of immunity with IMLNC transferred on day 6 of donor primary infection</u>.

(i) Donors (22 male NIH mice, seven/eight weeks old) were infected, and IMLNC transferred on day 6 pi. Five recipients received $4x10^7$ unseparated IMLNC. The remaining cells were separated into T and B fractions. Groups of four or five mice received either $4x10^7$ T cells or $4x10^7$ B cells. A further group received no cells. These recipients and controls were challenged nine days after cell transfer and killed 5.75 days after challenge, and their fluke burdens recovered.

(ii) The experiment was repeated using female mice.

<u>Fluke recovery and distribution</u> : (Fig.4-10a and b)

Experiment (i)

High recovery (76%) and anterior location of flukes in control mice showed that expulsion had not begun. T cell recipients harboured slightly (16%) but significantly fewer flukes, but a high proportion (82%) remained in the anterior 10cm of the small intestine. Recovery from B cell recipients was not significantly higher than that from unseparated cell recipients, but was more variable. Over 90% of flukes in these recipients occupied a more posterior position (10-20 cm post-pylorus), thus expulsion was advanced in these mice.

Experiment (ii)

Fluke recoveries and distributions from B cell and unseparated cell recipients were similar to those observed in experiment (i), however, the recoveries from control mice and T cell recipients were reduced by 33% and 43% Considering the distribution of flukes in these mice, it is conceivable that rejection might have begun in control mice, however the extremely high proportion of flukes from T cell recipients occurring in the anterior 10 cm of the small intestine is not characteristic of an infection in which expulsion is well advanced.

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Figure 4 - 10a

Mean % Fluke recovery per mouse 5¾ days after challenge with 200 metacercariae following transfer of T, B, or unseparated IMLNC (transferred day 6 of donor infection).

▒ = recipients of unseparated IMLNC

▓ = recipients of T-enriched IMLNC

▒ = recipients of B-enriched IMLNC

□ = non-recipient controls.

4 - 10b

Mean % of recovered flukes located 0 - 10 cm post-pylorus, 5¾ days after challenge with 200 metacercariae following transfer of T,B or unseparated IMLNC

(a)

MEAN ± SD % FLUKE RECOVERY

100
80
60
40
20

(b)

MEAN ± SD % OF RECOVERED FLUKES LOCATED 0–10cm PP

100
80
60
40
20

5 4 5 4 4 4 4 4 Mice / group

Fluke lengths and vitellaria :

Experiment (i)

Flukes recovered from B cell and unseparated cell recipients were significantly shorter (7% and 12% respectively) than those recovered from control mice. The length of the posterior lobe was also significantly reduced in unseparated cell recipients. This reduction in size was accompanied by a large increase in the proportion of flukes with absent and depleted vitellaria. Body length of flukes from T-cell recipients was slightly larger than that of flukes from control mice and the posterior lobe was also increased in length by 15% Only 11% of flukes in this group had depleted vitellaria, and this value did not differ significantly from that of control flukes.

A similar effect was noted in experiment (ii) in which the lengths of flukes recovered from B cell recipients were 13% shorter than controls. The proportion of flukes in B-cell recipients bearing depleted vitellaria was 67% (compared with 15% in controls) almost half of which had no trace of vitellaria.

Fluke lengths and vitellaria were not significantly affected by T cell transfer in experiment (ii).

TABLE 4-IOc

The effect of T and B enriched IMLNC fractions on length and vitelline development of flukes recovered from recipient mice:

| IMLNC Transferred | Mean Length ± SD(μm) | No.Mice x Flukes/Mouse | Mn ± SD % Flukes with Defic.Vitell. (or -ve Vitell.) |
|---|---|---|---|
| (A) | | | |
| Unseparated IMLNC | P. I6I ± 2I<br>T. 452 39 | 3 x 20<br>I x II | 68 ± II<br>(24 II) |
| B enriched IMLNC | P. I74 ± I4<br>T. 474 30 | 3 x 20<br>I x 7 | 74 ± I8<br>(39 I8) |
| T enriched IMLNC | P. 227 ± 27<br>T. 574 43 | 5 x 30 | II ± 9 |
| No cells (control) | P. I94 ± 4<br>T. 5I0 I3 | 3 x 30 | I2 ± 8 |
| (B) | | | |
| B enriched IMLNC | P. I66 ± 3<br>T. 465 I9 | 3 x I0<br>I x 7 | 67 ± I2<br>(3I 9) |
| T enriched IMLNC | P. 2I9 ± 3<br>T. 552 I7 | 3 x 30 | 6 ± 7 |
| No cells (control) | P. 206 ± I2<br>T. 537 24 | 4 x 30 | I6 ± II |

P.= Length of posterior lobe

T.= Total body length

denotes mean significantly different to control (ie Probability $P < 0.05$)

## Discussion

The failure to transfer immunity in a single serum transfer experiment cannot be interpreted as meaning that antibodies or other serum components play no part in the immune response against D.phoxini. In view of the number of factors affecting the ability of IMLNC to transfer protection it seems probable that many variations in experimental design may be necessary before the possibility of a role for serum can be properly assessed.

In cases where protection has been successfully transferred to mice, volumes of serum used have varied within fairly narrow limits. A single (1ml) dose stimulated immunity to S.ratti (Dawkins and Grove 1981), two 1ml doses were successful against T.muris (Selby and Wakelin 1973), and Behnke and Parish (1979) protected mice against N.dubius with 2-2.5 mls in four to six small doses.

Although Miller (1980) transferred protection against N.brasiliensis to rats with serum from day 6 of primary infection, it was found that hyperimmune serum gave better protection. In general, passive protection has been most successfully transferred using hyperimmune serum. Even with hyperimmune serum however, immunization schedules are probably an important factor in determining the efficacy of serum transfer (Behnke and Parish 1979, Miller 1980).

Although there has been considerable interest in IgG as a possible effector following serum transfer (Jones, Edwards and Ogilvie 1970, Behnke and Parish 1979, Miller 1980) a role for other serum components has not been dismissed. The length of time required for the generation of antiparasite effects following serum transfer compared with the short half life of serum Igs suggests that for most systems direct effects alone would be insufficient to generate the effects observed (Behnke and Parish 1979, Miller 1980). In the N.brasiliensis - rat system, interest is focused upon the mucosal changes, particularly in goblet cells and mast cells, which can be generated by serum transfer (Miller 1979, Miller and Nawa 1979).

The relative timing of serum transfer and challenge of recipients is probably important in most systems, though this may be due to several factors. A specific period of time may be required for the expression of the effects of serum on parasites to occur, particularly if these are indirect. Miller (1980) observed a 72 hour delay in the effect of serum on N.brasiliensis in rats. Expulsion was complete within four days of the onset of serum administration. Behnke and Parish (1979) observed an effect on N.dubius in serum recipients on day 9-11 pi (i.e. 8-10 days after the start of serum administration), and the effect on worm expulsion was extended over several weeks. In most systems the minimum time required for serum to be effective has not been investigated. In this respect the D.phoxini/mouse system at the 200 metacercarial level of infection is far from ideal, as expulsion begins within six days of primary infection.

There may also exist, for each system, a specific period of time after infection when the recipient is capable of responding immunologically to transferred serum, especially in the mediation of indirect effects. Behnke and Parish (1979) suggested that failure of serum to protect mice against N.dubius when given after day 6 pi may have been due to the onset of parasite-induced immunosuppression by this time in an infection.

It was thought that even if immune serum alone had no effect on the subsequent challenge infection of recipient mice with D.phoxini, it might measurably improve the efficacy of IMLNC in cell transfer (Figs. 4-1a and b). The two components have either an additive or a synergistic effect in several other host parasite systems (Lovel974, Love, Ogilvie and McLaren 1976, Wakelin and Lloyd 1976, Behnke and Parish 1981 ).

It was, however, later shown that IMLNC from day 8 of donor infection do not confer protection, therefore the negative result obtained is not surprising.

Although recoveries from the MLNC and serum transfer experiment were very variable (Fig. 4-1a), any effect upon fluke expulsion ought to have been apparent in the distribution of flukes in the small intestine (Fig. 4-1b).

The discovery that IMLNC from mice given a secondary infection 12 hours

previously, accelerated expulsion of primary infection in recipient mice given nine days but not one day later, enabled this adoptive transfer system to be more fully characterised.

The decision to subsequently delay kill of recipients until 5.75 days pi may not have been wise. It maximised the difference in recovery of flukes from control mice and all recipients, but increased the danger of expulsion beginning in controls before the mice were killed. When this did occur, the resulting abnormally low recovery was usually associated with the characteristic change in distribution of flukes which accompanies rejection. At least this indicated whether or not poor recovery was due to the onset of expulsion, or to poor establishment.

The results obtained show that in common with other host-parasite systems, the maximum observed effect of immunity transferred adoptively, as assessed by response to challenge, is considerably less than that which is stimulated by prior infection. The effect of transferred IMLNC on length of flukes is comparatively small but consistent. Reductions in body length associated with significant effects of IMLNC on expulsion ranged from 4% to 13%
Although a large proportion of flukes recovered from IMLNC recipients showed severe depletion or absence of vitellaria, and eggs were generally not observed in these mice, a proportion of these flukes reached maturity. It is not known when expulsion of challenge infection begins following successful IMLNC transfer, but the presence of a low and variable number of established flukes, 5.75 days pi, suggests that expulsion occurs at least one day later in cell recipients than in mice immunized by primary infection. These observations infer that the effect of IMLNC on challenge flukes in recipient mice is not apparent until three or four days post infection. There are several factors which might determine the potential level of protection conferred by IMLNC :

1. The absence of accessory components present in the responding donor, which contribute to the protective immune response. In several cases where IMLNC alone stimulate a low level of protective immunity to challenge, this

may be increased by the administration of immune serum (Love et al 1976, Wakelin and Lloyd 1976, Behnke and Parish 1980).

2. The requirement of specific minimum period of time following antigenic stimulation of the recipient, during which transferred cells may proliferate, differentiate or interact with components of the recipient immune system.

The results obtained give no indication of which of these factors might be involved. Further cell and serum transfers, and evaluation of the contribution of the recipient in adoptive transfer of immunity would clarify this point.

The fact that IMLNC transferred less than two days before challenge were ineffective (Figs. 4-2a,b, 4-6a,b) indicates that a minimum period of time is required by cells in order to respond to stimulation. The fact that early stages in this response necessarily occur without antigenic stimulation suggests that the six day pre-expulsion phase of primary infection is simply too short for transferred cells to express their effects on the parasite population. It is possible that the intra-peritoneal route of cell transfer might increase this time requirement. This has not been clarified but is thought to be unlikely. Wakelin and Wilson (1977) found that at least six days were required by IMLNC (given intravenously) in NIH recipients in order to produce changes in fecundity and expulsion of T.spiralis. The duration of primary infection of intestinal helminths in all other adoptive transfer systems is far longer than that of D.phoxini in the mouse. Consequently other workers have not found it necessary to investigate the effects of IMLNC prior to day 8 after challenge of cell recipients.

It has been demonstrated that as few as $1x10^7$ IMLNC can measurably affect expulsion in recipient mice, though size and vitelline development were not affected by transfer of this number of cells. A maximum level of protection was conferred by $2\text{-}8x10^7$ IMLNC. Wakelin and Wilson (1977) found that $1x10^7$ IMLNC affected expulsion and fecundity of T.spiralis on challenging recipient mice, but a slight depression of fecundity was demonstrable with as few as $1x15^5$ IMLNC. Faecal larval output from C57 BL6 mice infected with S.ratti was depressed by $6.25x10^6$ IMLNC (Dawkins and Grove

1981). Expulsion of radiation attenuated N.dubius by NIH mice was measurably increased by $1x10^6$ IMLNC, although $1x10^8$ cells were more effective (Behnke and Parish 1981). It seems therefore that the quantitative cellular requirements of the D.phoxini - mouse adoptive transfer system are similar to those of other mouse-intestinal helminth systems.

The quantitative cellular (IMLNC) requirement for the stimulation of morphological anti-parasite effects in this system does not differ greatly from, and is certainly not less than that which is required to accelerate D.phoxini expulsion. No evidence, however, is presented to show whether these effects are generated independantly, which has been demonstrated to be the case in the T.spiralis - NIH mouse system (Wakelin and Wilson 1979). Reduced size and fecundity of S.ratti are stimulated in recipient rats challenged after transfer of a lower number of IMLNC than that which is required to accelerate expulsion. Small inconsistencies in the relationship between stimulation of morphological changes and that of accelerated expulsion did occur: the transfer of day 12 IMLNC had no effect on expulsion but a small (5%) decrease in fluke length was recorded; also the transfer of day 6 IMLNC 2-4 days before challenge of recipients, affected expulsion rate but had a very small (4%) and inconsistant effect on fluke length.

During normal primary infection, even before expulsion, flukes occurring in the anterior 10 cm of small intestine are longer than those occupying a more posterior location. It was therefore decided to compare flukes from the same region of the small intestine, in cell recipients and controls, in an attempt to isolate and evaluate length variations due to the activity of IMLNC; however, this substantially reduced the observed differences and may have been misleading in cases where the larger proportions of infections in controls and cell recipients occurred in different regions of the gut.

It is well established in most cases which have been studied, that IMLNC have the ability to transfer immunity for a specific period of time following primary or subsequent infections (though some effect was produced by rat ITDL taken five weeks after tertiary N.brasiliensis infection (Miller, Nawa and Parish 1979). The inability

of IMLNC from donor rats harbouring secondary or subsequent infections to protect against S.ratti may be due to the parenteral expression of immunity to such infections of this parasite (Moqbel and Wakelin 1981). The ability of cells to transfer immunity to D.phoxini declines shortly after the onset of expulsion of a primary infection (Figs. 4-3a and b). This may also be the case in secondary infection, from which IMLNC are ineffective by day 6 pi (Figs. 4-4a and b) when the infection has been eliminated. A similar situation has been demonstrated in NIH mice infected with T.spiralis (Wakelin and Wilson 1977, Grencis and Wakelin 1982), although it seems that IMLNC from D.phoxini infected mice are effective much earlier after primary infection of donors (day 2 pi) than those from mice infected with T.spiralis (effective on day 4 pi). The finding that cells taken 12 h after secondary infection with D.phoxini are protective was unexpected; contrasting results have been found in other systems in which protective IMLNC can only be demonstrated at later stages in infection when worm expulsion or other anti-worm effects are already apparent. Cells obtained during secondary infections of T.colubriformis in the guinea pig are ineffective until day 7 pi, when expulsion is well under way (Adams and Rothwell 1977, Rothwell and Griffiths 1977). IMLNC from rats infected with N.brasiliensis are most effective when taken during the worm expulsion phase (Day 13-15 pi) though TDL are effective on day 10 (Kelly and Dineen 1972, Ogilvie et al 1977, Nawa and Miller 1978). Cells capable of transferring immunity to S.ratti did not appear in rat MLN until 20 days pi (Moqbel and Wakelin 1981) and MLNC from mice are protective against this parasite two and three weeks after infection (Dawkins and Grove 1981).

The ability of IMLNC from day 21 of donor primary infection to transfer immunity to D.phoxini does not fit the general pattern of other results, yet the immunity conferred was expressed in both accelerated expulsion and reduced fluke length. It is hoped that this result does not reflect inconsistencies inherent in the cell transfer system.

Although there is an enormous increase in MLN cellularity associated

with D.phoxini infection, this factor alone (as in other systems, Grencis and Wakelin 1982,) does not correlate with the ability of cells to transfer immunity. The efficacy of IMLNC from NIH mice in cell transfer declines while MLN cellularity remains high in T.spiralis- and D.phoxini-infected mice. Conversely, MLN cellularity in rats infected with S.ratti declines before IMLNC are capable of transferring immunity.

The results obtained also suggest that there is no direct correlation between the intense lymphoblast activity measured in responding IMLN and the ability of the cells to transfer immunity. In secondary infections, no increase in lymphoblast activity was evident early in infection when immunity was transferable, but high activity was measured on day 6 pi when transferred cells were ineffective. The decline in efficacy of IMLNC is not associated with a decline in lymphoblast activity. The increase in lymphoblast activity associated with infection was found to be confined to the nylon wool non-adherent (T cell) fraction of IMLNC. Activity in the B-cell fraction appeared to have declined compared with that of uninfected B cells. The inability of the T-lymphoblast population to transfer immunity, and the efficiency of the mainly non-dividing B cell fraction contrasts with the results obtained using NIH mice infected with T.spiralis. Grencis and Wakelin (1982) demonstrated a close temporal relationship between T-lymphoblast activity during infection and ability of IMLNC to transfer immunity, and it was shown that T-enriched IMLNC (and not B-enriched IMLNC) were effective. This was confirmed by Wakelin, Grencis and Donachie (1982), who identified the mediators as short lived T-lymphoblasts. It was suggested that these short lived cells differentiate into longer term memory cells after transfer, thus explaining the fact that when challenge was delayed, after cell transfer, IMLNC were still effective. It is known that challenge following transfer of IMLNC from D.phoxini infected mice can be delayed for 19 days without loss of efficacy, however, it is not known how long challenge may be delayed, before the efficacy of transferred cells declines.

It is clear that there are fundamental differences in the mechanisms by which immunity is adoptively transferred in different host-parasite systems, reflecting differences in the nature of the immune responses generated. In rats infected with T.spiralis there is no doubt that dividing B cells are capable of transferring immunity adoptively, though non-dividing T.cells are also effective (Crum et al 1977, Despommier et al 1977). The failure of specific immune serum to immunize passively inferred that the cells concerned might not be protective (i.e. secrete antibody?) until they left the circulation. Nawa, Parish and Miller (1977) identified the effector cells in rat TDL (taken ten days after N.brasiliensis infection) as sIg-ve cells. This was also true of hyperimmune TDL, however some protection was also conferred by sIg+ve cells at this time.

It cannot be speculated, at this stage, how transferred B cells effect the expulsion of D.phoxini in recipient mice. There is much interest in the ability of transferred cells and serum to stimulate mucosal changes in recipient hosts (Miller and Nawa 1979, Nawa and Miller 1979, Alizadeh 1981). It seems probable that some of these changes may be generated by more than one mechanism. The histopathological consequences of cell transfer are investigated in chapter five.

## Summary

Serum from mice infected with D.phoxini eight days previously failed to transfer immunity. Immunity transferred adoptively by IMLNC was manifested as an acceleration of expulsion, and a retardation of vitelline development and reduced growth of flukes in recipient mice compared with controls. As few as $1x10^7$ IMLNC affected expulsion. $2x10^7$ IMLNC affected body length and vitelline development also.

IMLNC taken from donor mice between days 2 and 6 of primary infection were most effective. After day 6, efficacy declined, however IMLNC taken from donor mice on day 21 after primary infection unexpectedly had some effect on recipient challenge infection. IMLNC taken 12 hours after secondary infection were effective but those from days 6 and 12 of secondary infection were not. IMLNC transferred less than two days before challenge of recipient mice did not transfer immunity.

T-lymphoblast activity was high and cellularity of the MLN increased following primary and secondary infections, but these changes were not correlated temporally with the efficacy of IMLNC. T-lymphoblasts were ineffective but a population of mainly non-dividing B cells was effective in transferring immunity adoptively.

# CHAPTER FIVE

Histopathological changes in the small intestine associated with D.phoxini infection :

Host responses to intestinal helminths are highly complex, involving immunologically specific and non-specific components. Although many components involved in the generation of host responses have been characterized, the terminal effectors responsible for parasite expulsion have not been identified.

In the past, attempts to evaluate mediator or effector roles of cellular or humoral components have takent the form :

1. Establishment of temporal relationship between component proliferation/ activity and parasite expulsion or antiparasite effects.
2. Investigation of the effect of ablation/reconstitution of the component on parasite infection.
3. Testing of proposed effectors isolated in-vitro.
4. Investigation of origin and modulation of the component in-vivo.

The immune response to intestinal helminths is T-dependant in all models sufficiently investigated (Mitchell 1979 ). An immunologically specific response to parasite antigen is responsible for generating non-specific components in the gut which effect parasite expulsion (Wakelin 1978). The mucosa could respond to parasite antigen in several ways, via T cell activity. Reaginic and delayed hypersensitivity both generate inflammatory lesions in associated tissues. Immunological induction of inflammation is characteristic of very many but not all intestinal helminth infections, and both the means of induction and possible antiparasite effects of inflammatory components have aroused much speculation. An increase in number of mucosal plasma cells may also be associated with helminth and roles for antibody have been postulated in several host parasite models (Wakelin 1978, Bienenstock 1981).

Some of the evidence for involvement of these components in immune expulsion of intestinal helminths is considered below :

1. Inflammatory changes.

Inflammatory lesions, in particular, cellular accumulations,

associated with intestinal helminth infections, have been shown to be thymus dependent. Their nature and severity, which show great variation, may be related to many factors :- host species and strain, the form and extent of chemical or mechanical trauma occurring at the host/parasite interface, the antigens presented by the parasite involved, the nature of the specific immune response generated, and the extent of modification of host immune/ infammatory responses by some parasite products.

Severe acute inflammation occurs during T.spiralis and N.brasiliensis infections in rodents (Taliaferro and Sarles 1939, Larsh and Race 1954). It was suggested (Larsh and Race 1975) that an immunologically specific D H reaction generated inflammatory changes which produced an environment detrimental to T.spiralis. The authors extended the hypothesis to the N.brasiliensis -rat and T.Colubriformis -guinea pig systems. Subsequent experiments have supported a role for inflammatory components in expulsion of these parasites, and in some cases there is evidence that immediate (reaginic) hypersensitivity may be important as an inducer of inflammatory lesions, particularly in response to secondary or subsequent infections (Rothwell, Love and Evans 1978, Askenase 1980, Wakelin and Wilson 1979, Lee and Ogilvie 1980, Alizadeh 1981, Miller 1981).

Inflammation is not pronounced in all intestinal helminth infections. Comparatively small increases in numbers of mast cells, globule leukocytes (GL) and eosinophils may be associated with avian and rodent cestode infections (Gray 1973, 1976, Andreassen, Hindsbo and Ruitenberg 1973, Hindsbo, Ruitenberg 1973, Hindsbo, Andreassen and Ruitenberg 1982) but expulsion of H.diminuta from nude mice may occur in absence of mast cells and GL. The GL response in mice infected with T.muris may be temporally related to parasite rejection in some but not all strains of mice (Lee 1982).

The study of heterologous concurrent and sequential infections has suggested that inflammatory changes generated in response to one parasite may non-specifically affect the growth and survival of another species present

in the gut simultaneously. Intestinal cestodes do not stimulate marked inflammation in the gut and did not affect concurrent T.spiralis infection in the mouse and rat. Conversely the effects of T.spiralis - induced inflammation on intestinal cestodes was profound :- Expulsion of H.microstoma from mice was induced if the scolex was exposed to inflammation before reaching the bile duct (Howard, Christie, Wakelin, Wilson and Behnke 1978). T.spiralis - induced inflammation caused premature destrobilation and loss of H.diminuta from mice (Behnke, Bland and Wakelin 1977). Similar destrobilation and loss could not be induced in the rat, however the severe stunting of growth was thought to be at least partially attributable to the effects of inflammation (Christie, Wakelin and Wilson 1979).

Similar, probably non-specific effects of inflammation in concurrent heterologous infections have been demonstrated in many other systems (Dineen Gregg, Windon, Donald and Kelly 1977, Kazacos 1975, Au and Ko 1979, Kennedy 1980, Sirag et al 1980). The susceptibility of hosts to sufficiently delayed (i.e. until inflammatory lesions associated with primary infection had subsided) sequential heterologous challenge in the above systems demonstrated the parasite-specific nature of the generation of the inflammatory responses involved. Specific cross immunity has been demonstrated in several nematode systems : T.colubriformis and T.vitrinis (Dineen et al 1977), S.ratti and T.spiralis (Moqbel and Wakelin 1979), T.spiralis and T.muris (Lee, Grencis and Wakelin 1982) and S.ratti and N.brasiliensis (Kazacos and Thorson 1975). This relationship has been demonstrated most commonly in parasite species which are closely related phylogenetically or physiologically. There is evidence that T.muris and T.spiralis may be present common functional antigens in mice. It is remarkable that two host responses, only one of which is characterized by gross inflammation, might share, in part, a common origin (Lee, Grencis and Wakelin 1982).

The requirement for myeloid bone marrow derived cells in the expulsion of N.brasiliensis from rats (Dineen and Kelly 1973) and T.spiralis from mice (Wakelin and Wilson 1977) is thought to reflect the involvement of B M derived

cells in inflammation.

Inflammation consists of a highly complex matrix of interactions which may be generated by several immunologically specific (and also non-immunological) pathways and is subject to complex homeostatic control. The relative contributions of non-specifically generated local coagulation and kinin-generated pathways to inflammation in intestinal helminth infections (and their suppression or direct stimulation by parasite products) - reviewed Leid and Williams 1979 - remain largely uninvestigated.

Investigation of the immune induction and function of inflammation in parasitic infection has inevitably focussed on individual components. Some cellular components are characteristic of immune responses to several intestinal helminths. The origin and possible function of these cells is discussed below :

A. Mast cells :

The origin, structure and function of these cells in the gut have been the subject of recent reviews (Askenase 1980, Miller 1981). Widespread proliferation of cells with mast cell properties occur in the gut in response to many intestinal helminths : N.brasiliensis in the rat (Taliaferro and Sarles 1939, Jarrett, Jarrett, Miller and Urquhart 1968, Miller and Jarrett 1971, Befus and Bienenstock 1979), T.spiralis in the rat and mouse (Ruitenberg and Elgersma 1976, Ruitenberg, Elgersma and Leyners 1979, Alizadeh 1981, Alizadeh and Wakelin 1982). T.muris in the mouse (Lee 1982). H.Diminuta in the rat and mouse (Andreassen, Hindsbo and Ruitenberg 1978, Hindsbo, Andreassen, and Ruitenberg 1982), Apatemon gracilis in the duck, (Blake 1974 ), and Raillietina cesticillus in the fowl (Gray 1976).

In the intestines of rats and mice there exist at least three distinct populations of cells with "mast cell" properties.

(i) Connective tissue mast cells (CTMC). These cells occur in the outer serosal layers and resemble those found in other body tissues. Precursors are thought to originate in mouse foetal liver or B M. Kitamura, Go, Shimada, Matsuda, Hatanaki and Seki (1979) demonstrated that CTMC of skin and mesentery are B M derived and that the development of these, and mast

and Gowans 1976, Mayrhofer 1977).

Befus et al(1979) confirmed that LPMC are highly resistant to degranulation by compound 48/80 and although small and similar levels of degranulation were induced by the non-specific compounds peptide 401 and ionophore A23 181, as well as by N.brasiliensis antigen, it was attributable to degranulation of CTMC contaminating the LPMC. Interestingly, the response of mesenteric CTMC to these degranulators was considerably less than that of peritoneal CTMC. Askenase (1980) suggested that the poor response of LPMC to "anaphylactic-type secretagogues" reflects a specialisation to respond predominantly to T-cell activation rather than conventional anaphylactic antibody-mediated mechanisms.

The origin of LPMC has not been conclusively identified. The possibility of a common origin of CTMC and LPMC has not been excluded. Miller (1971) observed the local differentiation of lymphoblast-like cells into LPMC in the rat intestine. MLNC suspension from N.brasiliensis infected rats contain LPMC precursors which proliferate and differentiate to form "mast cells" when stimulated by N.brasiliensis antigen or PHA in vitro (Befus, Denburg and Bienenstock 1979). It is known that the LPMC response to parasite antigen is T-dependant, however the exact role of T cells (as precursors or inducers) has not been conclusively demonstrated. Neonatal thymectomy diminished the LPMC response in rats infected with T.spiralis (Ruitenberg, Elgersma and Lamers 1979) but it was concluded that the observed proportionate increase in numbers of LPMC in response to T.spiralis infection is T-independant; a conclusion based on the view that the local proliferation of LPMC occurred from stainable mast cells present in the naive animal (the number of which was believed to be T-dependant). The LPMC response to N.brasiliensis infection is absent in adult thymectomized, irradiated and bone marrow reconstituted rats (Mayrhofer and Fisher 1979). In thymectomized and TDL depleted rats the abrogated LPMC response was restored by TDL cells (Mayrhofer 1979). The LPMC response to T.spiralis and S.ratti is eliminated by treatment with anti-thymocytic serum (Olsen and Schiller 1978,

Ruitenberg, Elgersma and Kruizinga 1979).

(iii) Intra epithelial globule leukocytes (G L.). The rapid proliferation of GL in the mouse intestine in response to helminth infection (in contrast to the predominantly LPMC response occurring in the rat) has generated much interest in the origin and function of this cell, neither of which has been resolved.

GL in the rat, resemble LPMC both ultrastructurally (Miller 1971), and functionally. They contain intracellular and surface IgE (Mayrhofer, Bazin and Gowans 1976, Ruitenberg, Elgersma and Larners 1979) and contain histamine, glycosaminoglycan and basic protein (Miller and Walshaw 1972) and small amounts of a protease distinct from that of CTMC (Woodbury, Gruzensky and Lagunoff 1978). GL share with LPMC and peritoneal CTMC a specific cell surface antigen (Ruitenberg, Elgersma and Larners 1979).

The relationship between GL and LPMC remains controversial. The similarities listed above, the relative depletion of GL granule contents and study of the kinetics and ultrastructure of GL and LPMC in rats infected with N.brasiliensis have led some workers to believe that GL represent mature or partially degranulated LPMC (Murray, Miller and Jarrett 1968, Miller and Jarrett 1971), however Ruitenberg and co-workers believe that LPMC and GL in rats and mice represent totally independent cell populations (Ruitenberg and Elgersma 1980, Ruitenberg, Elgersma and Kruizinga 1979). It was suggested that the origin of LPMC is T-dependent, whereas the proliferation, but not the origin of GL is T-dependent (Ruitenberg, Elgersma and Larners 1979). These conclusions regarding the origin of GL did, however, involve the attachment of great significance to very small changes in minute numbers of cells present in naive animals.

The fact that GL proliferation generally precedes that of LPMC in mice responding to intestinal helminths, suggests that GL are not derived from stainable (mature) LPMC, unless the existance of the latter is extremely transient, but the possibility of a common precursor has not been precluded.

A conflicting interpretation of the nature of thymus dependance of GL is that of Guy-Grand, Griscelli and Vassalli (1978) who suggested that GL in

cells from stomach and caecum is T-independent.CTMC do not respond markedly to acute intestinal helminth infections and have therefore received little attention in this respect. A slow increase in number of CTMC is associated with chronic inflammation (Askenase 1980). A gradual increase in mesenteric MC degranulation occurs prior to and during expulsion of a primary infection of S.ratti from rats (Moqbel 1980) which is also characterized by intestinal mucosal mastocytosis (Olson and Schiller 1978).

CTMC are heavily granulated, releasing a large number of chemical mediators by exocytosis (reviewed Wasserman 1979), many of which have been characterized in rat mast cells : Vasoactive and smooth muscle reactive mediators include histamine, 5-hydroxytryptamine (5-HT), slow reacting substance of anaphylaxis (SRS-A), platelet activating factors (PAF) and arachidonic acid metabolites (prostaglandin precursors). A wide range of chemotactic mediators occur : histamine, eosinophil chemotactic factors (ECFs) neutrophil chemotactic factors and a lipid chemotactic factor of broad specificity. Granule associated enzymes include chymase, kallikrein, arylsulphatase and $\beta$-exoglycosidase. The matrix contains heparin. Mast cell mediators possess the ability to induce both immediate and short term tissue responses, and also to mediate (via chemotaxis) a prolonged inflammatory response (Wasserman 1979, Tannenbaum, Oertel, Henderson and Kaliner 1980).

(ii) Subepithelial (lamina propria) mast cells (LPMC). These cells proliferate in the rat intestine in response to helminth infection whereas in the mouse, the increase in number of LPMC is comparatively minor (see below).

In the rat and mouse, LPMC differ in several ways from CTMC :

LPMC are smaller and more varied in shape, with greater development of endoplasmic reticulum and golgi apparatus (Miller 1971) implying synthetic and secretory activity. LPMC contain fewer granules, less monoam ne (Miller and Walshaw 1972) very little or no 5-HT (Guy-Grand, Griscelli and Vassalli 1978), a different chymotryptic enzyme (Woodbury, Gruzensky and Lagunoff 1978), different proteoglycan and no heparin (Tas and Berndsen 1977). IgE is found in the LPMC matrix as well as attached to surface receptors (Mayrhofer, Bazin

mice are derived from recirculating T-cells stimulated to divide in the Peyers patches, in which microenvironmental factors stimulate gut homing via the MLN and TD. Suspensions of intraepithelial lymphocytes (IEL) from normal and beige mice were examined for T cell and mast cell characteristics, 80-90% were T cells. In beige mice, approximately 80% of IEL were granulated. The granules stained with alcian blue, and incorporated $^{35}SO4$, indicating potential proteoglycan synthesis. About half of granulated cells contained histamine, however, the cells with most marked mast cell characteristics bore only weak, or no T-cell characteristics. Although this is consistent with the suggestion that T-cells are GL precursors, such an hypothesis has not been conclusively proven. It was suggested that the differentiation of homing T-cells is determined in the gut : granulated IEL formed in the naive gut may represent an intermediate stage in differentiation, which proceeds after antigenic stimulation of the gut to produce GL. The results of a further attempt to demonstrate derivation of GL from T cells in N.brasiliensis infected rats are similarly equivocal (Guy-Grand, Luffau, Griscelli and Vassalli quoted by Askenase, 1980).

The roles of Lamina propria mast cells and globule leukocytes in the immune response to parasite infection :

CTMC degranulation may be stimulated by a variety of agents (reviewed Wasserman 1979). Cell surface receptors exist for homocytotropic antibody (IgE, IgG) and anaphylotoxin (c3a, c5a). Degranulation may also be stimulated by non-immunological factors e.g. enzymes and ionophores. It has also been suggested that certain parasite products may also be effective degranulators (Unvas and Wold 1967, Thompson 1972, Tolone, Brai, Bonasera Bellavia and Pontieri (1972) Tolone, Bonasera, Brai, Ferina and Pontieri (1974) Moqbel (1980).)

Degranulation is a graded response, resulting in only partial liberation of granule contents, and doesnot cause destruction of the cell (Monger and Foreman 1979).

Relatively little is known of the functional surface characteristics of

LPMC and GL. Although both carry receptors for IgE, mixed populations were found to be relatively resistant anaphylactic type degranulation (Befus, Pearce, Gauldie, Horsewood, Goodacre, Cole, Heatley and Bienenstock 1979).

Antibody-dependant degranulation of MC is initiated by the bridging of cell-bound Ig by antigen. Unlike IgE, IgG remains bound to MC for only a few hours, is stable to heat, and may activate complement .

It has been suggested that MC precursors may be sensitized to IgE in regional lymph nodes (Gillon 1981), which are the principal sites of IgE production in response to antigenic challenge at the mucosal surface (Mayrhofer, Durkin, Bazin and Waksman 1981 Bazin and Gowans 1976). In rats, development of tracheal and intestinal sensitivity to N.brasiliensis allergens is associated with early synthesis of IgE in associated lymph nodes (Befus, Johnston, Berman and Bienenstock 1982). IgE production is thought to be stimulated by a lymphokine (Urban, Ishizaka and Bazin 1980).

IgE mediated hypersensitivity is characteristic of many intestinal helminth infections. The finding that neither IgE (Ogilvie and Parrott 1977) nor "mast cells" (Ogilvie, Love, Jarra and Brown 1977, Befus and Bienenstock 1979) is indispensible for parasite expulsion to occur even in system characterized by their proliferation, does not preclude a role for these factors in the normal expulsion process. The transfer of accelerated mucosal mastocytosis to N.brasiliensis -infected rats with immune serum (Befus and Bienenstock 1979, Miller 1979) is mediated by a heat-labile serum component which is thought to be IgE. Local IgE mediated anaphylactic reactions are thought to be involved in resistance of mice to T.spiralis (Gabriel and Justus 1979) and rats to N.brasiliensis (Murray, Miller, Sanford and Jarrett 1971). Infections of rats with N.brasiliensis stimulates production of both parasite-specific and non-specific IgE. Both responses, like LPMC and GL hyperplasia, are transferable with TDL from infected rats (Nawa, Miller, Hall, and Jarrett 1981), however measured IgE production often correlates poorly with both parasite expulsion and mast cell hyperplasia, however this may be

unimportant : mast cell IgE receptors are of high affinity, therefore very small local amounts of IgE may be sufficient for sensitization to occur. It is also possible that IgE levels may be affected by the sequestering of surface and intra-cellular IgE by large numbers of "mast cells" during infection.

A role for complement in immune responses to intestinal helminths has not been firmly established. Complement was observed on the tegument of H.diminuta in the mouse (Befus 1975) but is unlikely to have retained activity in the gut. Anaphylotoxin-mediated participation of MC is associated with tissue immune complex lesions, and complement and neutrophils are required for the degranulation of MC by IgG-antigen complex (Leid and Williams 1979). Gabriel and Justus (1979) suggested that $IgG_1$-mediated anaphylactic reactions may be involved in the response of mice to T.spiralis, however the occurrence and significance of complement mediated degranulation of LPMC and GL has not been established. Depletion of circulating complement levels in rats did not affect their response to N.brasiliensis infection (Jones and Ogilvie 1971).

Mast cells have been implicated in the generation of DH reactions. The interaction of antigen-activated T cells with mast cells is necessary in skin reactions, to facilitate the diapedesis of B.M. derived cells (Gershon, Askenase and Gershon 1975, Bursztajn, Askenase, Gershon and Gershon 1978). Larsh and Race (1975) suggested that D.H. is closely associated with expulsion of T.spiralis from mice and suggested its involvement in expulsion of N.brasiliensis from rats and T.colubriformis from guinea pigs.

Specific roles for mast cells and globule leukocytes in the immune response to intestinal helminths have not been conclusively demonstrated. The temporal relationship between proliferation of these cells and parasite expulsion often varies with host strain. This inconsistency and the fact that expulsion of primary infections may frequently occur before mature (stainable) MC and GL proliferation occurs, has led some workers to suggest that these cells may exert their effects, if any, during the later stages of expulsion (Alizadeh 1981, Miller1981, Lee 1982). Askenase (1980) suggested

a role for the basophil in the early stages of expulsion of intestinal helminths from rats.

Mast cells and globule leukocytes may be more involved in the expulsion of secondary and subsequent helminth infections, rather than primary infections. These cells exhibit a classical T-dependent anamnestic response to parasite infection (Mayrhofer 1979, Alizadeh 1981)and such a role isfrequently supported by the temporal relationship between mastocytosis and parasite expulsion. In addition, the persistance of elevated numbers of LPMC and GL after infection varies with parasite and host species and strain, and has been correlated with the persistence of the ability of rats and mice to mount a "rapid expulsion" response to homologous reinfection with T.spiralis (Alizadeh and Wakelin 1982).

Possible mechanisms of action:

(a) Direct effects of mediators on parasites :

Both LPMC, GL and basophils are potential sources of amines. Intestinal histamine and S-hydroxytryptamine (S-HT) levels in N.brasiliensis -infected rats are increased during expulsion (Befus and Bienenstock 1979). In vitro studies have demonstrated direct anti-parasite effects of amines (Rothwell, Prichard and Love 1974, Richards, Bryant, Kelly, Windon and Dineen 1977), however experiments involving administration of amines and amine antagonists in vivo have provided conflicting results (Murray 1972, Ogilvie and Love 1974, Moqbel 1975, Rothwell, Love and Goodrich 1977, Hopkins 1980) probably related to the methods of administration (often orally, or via injection into the gut lumen) and the complex side effects of amine antagonist drugs (Wakelin 1978, Hopkins 1980).

It has also been suggested that GL may release IgE into the gut (Mayrhofer, Bazin and Gowans 1976) although the occurrence and significance of this have not been established.

(b) Effect of mediators on mucosal permeability :

Mast cell degranulation and consequent mediator liberation increases both vascular and mucosal permeability for maeromolecules. It was suggested

(Urquhart, Molyneux, Eadie and Jennings 1965, Barth, Jarrett and Urquhart 1966) that this allowed antibody access to luminal parasites in N.brasiliensis -infected rats, or that it induced other detrimental changes in the parasite's environment, however Nawa (1979) showed that increased permeability of rat intestine to Evans Blue preceded mastocytosis and appeared to be correlated with N.brasiliensis worm numbers in the gut. It was suggested that worm products rather than LPMC were primarily responsible for the observed permeability changes.

(c) Chemotactic mediators :

CTMC are known to secrete several chemotactic mediators for which principal target cells are neutrophils and eosinophils. Accumulation of these cells is characteristic of inflammatory responses to intestinal helminths, but their role in enhancing or modulating the immune response has not been resolved and will be considered below.

(d) Relationship between mast cells and goblet cells :

Goblet cells and mast cells in the rat are closely associated in several respects: IgE can mediate the anaphylactic release of goblet cell mucus, and histamine can stimulate goblet cell secretion in vitro (Shelhamer and Kaliner 1979, Lake, Bloch, Sinclair and Walker 1980).

Mucosal mast cell protease (RMCP11) is present in goblet cell granules in the intestines of rats immunized against N.brasiliensis but not in naive rats (Woodbury and Miller 1982). The goblet cells in crypts in closest proximity to mast cells contained most RMCP11, and it was suggested that goblet cells obtain secreted protease from mast cells, and extrude it to the gut lumen. The function of mast cell protease in the immune response is not known. The role of mucus is discussed below.

(e) Effects on peristalsis :

Altered intestinal motility is associated with inflammation during some helminth infections, e.g. T.spiralis (Schanbacher, Castro and Weisbrodt 1975, Castro, Badial-Aceves, Smith, Dudrick and Weisbrodt 1976). Regional increase in motility occurred during primary infection, and was most

pronounced in regions which were inflamed and contained worms. Similar alteration in motility did not occur in secondary infection. Askenase (1980) speculated that smooth muscle mediators liberated by mast cells might aid in expulsion of parasites in this way, during the later stages of infection.

(B) Goblet Cells :

The protective properties of mucus have been recognised for many years (Frick and Ackert 1948, Florey 1955, Dobson 1967). Mucus release can be stimulated both non-specifically (Florey 1955) and immunologically (Walker, Wu, and Bloch, 1976, Lake Bloch, Sinclair and Walker 1980). The possibility that goblet cell hyperplasia and mucus production might be important components of the secondary response of rodents to T.spiralis, T.muris, and N.brasiliensis infection has been investigated (Wells 1963, Miller 1979, Miller and Nawa 1979, Lee and Ogilvie 1980, 1981, Alizadeh 1981, Miller Huntley and Dawson 1981, Miller, Huntley and Wallace 1981). In a study of the rapid expulsion of N.brasiliensis from rats, Miller, Huntley and Dawson (1981) found substantial numbers of worms which failed to penetrate villi, were trapped in mucus. Similar mucus trapping of T.spiralis was demonstrated by Lee and Ogilvie (1981). Elevated numbers of goblet cells and inoreased incorporation of D-(1 - $^{14}C$) glucosamine into at least one fraction of intestinal secretion suggest that mucin turnover is increased during N.brasiliensis infection. Specific proteins, or mucoproteins contained in mucus may augment the effects of physical entrapment and immobilisation (Lee and Ogilvie 1981). N.brasiliensis worms appear to ingest mucus during the early stages of infection only (Miller, Huntley and Dawson 1981). The protective capacity of mucus is also associated with high levels of RMCPll protease in LPMC and goblet cell mucins in the gut (Woodbury and Miller 1982).

(C) Eosinophils :

Eosinophilia in the intestine wall is characteristic of most intestinal helminth infections, although the temporal relationship between parasite expulsion and the increase in eosinophil numbers is variable. Eosinophilia is observed during N.brasiliensis infection in rats (Wells 1962, Kelly and

Ogilvie 1972), T.spiralis in rats (Race, Larsh, Martin and Weatherley 1974) and mice (Ruitenberg, Elgersma, Kruizinga and Leenstra 1977) and S.ratti in the rat (Moqbel 1980). Numbers of eosinophils also increase during H.diminuta infection in the rat, a response which is reduced and delayed by anti-thymocytic serum(Hindsbo, Andreassen and Ruitenberg 1981), however infection and rejection in mice is not associated with eosinophilia (Andreassen, Hindsbo and Ruitenberg 1978).

The eosinophilic response to intestinal helminths in rats and mice is T-dependent; however eosinophilia is not totally ablated in T cell deficient animals (Fine et al 1973, Rothwell and Love 1975, Phillips et al 1977, Andreasson, Hindsbo and Ruitenberg 1978, Ogilvie, Askenase and Rose 1980). The failure of nude mice to develop an eosinophil response to S.mansoni (Philips et al 1977), A.suum (Nidsen et al 1974), T.spiralis (Ruitenberg et al 1977, Ogilvie Askenase and Rose 1980) and N.brasiliensis (Ogilvie et al 1977), confirms T cell involvement in the augmentation of eosinophilia.

Specifically sensitized T cells are necessary for stimulation of eosinophilopoiesis by helminth antigens (Basten and Beeson 1970) and the production of the eosinophil chemokinetic and chemotactic lymphokine ESP (Colley 1973, Lewis, Carter and Colley 1977) which is associated with T.spiralis infection (Warren Karp, Pelly and Mahmoud 1976). Macrophages are also necessary for antigen induced eosinophil production (Greene and Colley 1976). IgE plasma cells and mast cells have been suggested as additional sources of eosinophilopoietins (Parish Luckhurst and Cowan 1977).

Although some helminths produce eosinophil attractants (Campbell 1942, Tanaka, Baba and Torisa 1979), Goetzl and Austen (1977) suggested that eosinophil chemotactic factors (ECFs) are the most important chemotactic factors for eosinophils, which is consistent with the observation (Weller and Goetzl 1979) that eosinophils usually appear at sites of helminth infection shortly after mast cells. Other mast cell products (histamine, kallikrein and lipid chemotactic factor) and split complement components

(c3a, c5a, c567) are also chemotactic for eosinophils and may be instrumental in attracting them to sites of infection.

Selective depletion studies have so far failed to demonstrate an effector role for eosinophils against intestinally located helminths, although there is much evidence to suggest an effect on tissue-invasive stages: anti-eosinophil serum increased susceptibility of guinea pigs to T.colubriformis larval infection and reduced the level of acquired immunity to challenge (Gleich, Olson and Herlich 1979). Similarly, the number of T.spiralis muscle larvae in mice increased, but anti-eosinophil serum had no effect on the expulsion of adult worms from the intestine (Grove, Mahmoud and Warren 1977). The resistance of guinea pig strains to T.colubriformis infection was associated with higher numbers of eosinophils in both naive and infected animals compared with susceptible strains (Handlinger and Rothwell 1981).

The ability of eosinophils to induce antibody or complement-dependent damage to larval tissue dwelling stages of certain parasites, particularly schistosomes, is well documented (Reviewed Butterworth 1981). Eosinophils bear surface receptors of IgG, c3b, c3d, and c4. Damage to antibody coated schistosomules in vitro is effected by major basic protein and cationic protein (Butterworth, Wassom, Gleich, Hoegering and David 1979). Eosinophils are also cytotoxin to larvae of T.spiralis (Kazura and Grove 1978, Perrudet-Badoux, Antennis, Dumitescru, and Binaghi 1978), N.brasiliensis (Ogilvie, Mackenzie and Love 1977, Mackenzie, Jungery, Taylor and Ogilvie 1980) and Wucheria bancrofti (Higashi and Chowdhury 1970).

A function for eosinophils in the response to intestinal helminths has been suggested on the basis that these cells, during inflammation, are a major source of phospholipase B, a potentially destructive enzyme which could augment inflammation or have direct antiparasite effects. This enzyme also causes the release of prostaglandin (PG) precursors to occur. This indirect effect may be important,however the involvement of prostaglandins in helminth expulsion is highly controversial. Close correlation between presense of parasites and elevated PLB levels at the same site in the inflamed

small intestine has been demonstrated in mice infected with H.nana Ottolenghi 1973) rats and mice infected with T.spiralis (Larsh et al 1974, 1975, Goven and Moore 1980) and rats infected with A.cantonensis Ottolenghi 1977) or N.brasiliensis (Goven 1979a, 1979b). The anamnestic nature of the elevated intestinal PLB and bone marrow eosinophilia in the response of rats to S.ratti and N.brasiliensis has been demonstrated (Goven 1979, Goudsen, Hilton, Ottolenghi, Larsh 1981 ). PLB activity is correlated with the degree of inflammation generated (Goven 1979).

Involvement of prostaglandins (which may also be generated by mast cells) in expulsion of N.brasiliensis from rats was originally described by Dineen and co-workers: Intraduodenal injection of synthetic PGE or extracts of acidified ram semen significantly affected worm expulsion (Dineen, Kelly, Goodrich and Smith 1974, Kelly Dineen, Goodrich and Smith 1974, Smith, Goodrich Kelly and Dineen 1974). PGE levels in infected tissues were greatly increased before the onset of expulsion (Dineen and Kelly 1976) and in-vitro worm damage was effected by PGE (Richards, Bryant, Kelly, Windon and Dineen 1977), however Kassai, Redl, Jecsai, Balla and Harangozo (1980) failed to demonstrate effects of prostaglandins on N.brasiliensis in vitro or in vivo and failed to demonstrate PG in worm tissues. Similarly, no antiworm effects of PG precursors could be demonstrated. Richards (1982) suggested that differing experimental conditions, particularly during worm incubation, might have been responsible for some of the lack of demonstrable effects of prostaglandins in the work of Kassai et al. Rothwell, Love and Goodrich (1977) found no effect of PG on expulsion of T.colubriformis from guinea pigs.

Eosinophils are involved in the homeostatic control of inflammation. Several mediators deactivate mast cell products : arylsulphatase inactivates SRS-A; platelet activating factors (PAFs), lysophospholipids and histamine are neutralized by phospholipase D, lysophospholipase and histaminase respectively; major basic protein attacks heparin. Baggiolini, Horisberger and Martin (1982) demonstrated the ability of eosinophils and neutrophils to

inactivate (by phagocytosis) mast cell granules released during anaphylaxis. PGE is also known to modulate mast cell mediator release (Fang, Broughton and Jacobsen 1977).

2. Immunoglobulins :

Increased levels of circulating and mucosal antibody may be associated with intestinal parasite infection, but the function of such antibody remains obscure. An added complication is the frequent production of large amounts of non-parasite specific immunoglobulin as a result of the involvement of bystander B cells in the immune response to infection.

Although circulating antibody levels often correlate poorly with infection, and immunity, resistance to some parasites has been transferred passively with serum which it is accepted, may contain other protective factors in addition to antibody (Behnke and Parish 1979, Miller 1980).

In most systems investigated, B or B-enriched cell fractions from TDL or MLN are much less effective than T cells in transferring immunity to intestinal parasites (Nawa, Parish and Miller 1978, Wakelin, Grencis and Donachie 1982). An exception occurs in the T.spiralis/rat system, in which B enriched TDL cells were found to transfer immunity slightly more effectively than a T cell enriched fraction (Despommier, McGregor, Crum and Carter 1977), however attempts to immunize passively with serum were unsuccessful in this system (Crum, Despommier and McGregor 1977). It was suggested that transfer of immunity involved activity of T-helper and IgA-B cells. A limited ability to transfer immunity to N.brasiliensis with sIg+ve cells collected from the TD of hyperimmune rats was explained on the basis of transfer of memory B cells (Nawa, Parish and Miller 1978).

There are two methods by which antibody might affect intestinal helminths:

1. By direct effects on intestinal or immature tissue invasive stages.
2. Indirectly, by affecting other components of the host immune/inflammatory system.

Morphological and cytopathological changes are evident in many intestinal helminths prior to their expulsion. "Damage" observed in N.brasiliensis during infection in the rat was thought to be effected by anti-worm antibody, constituting an essential preliminary stage in the immune response (Ogilvie and Jones 1973), however, this direct role of antibody was never proved.

Subsequent experiments showed that in-vitro culture conditions may themselves produce similar damage (Love, Ogilvie and McLaren 1975). Mice incapable of producing immunoglobulins can expel N.brasiliensis (Jacobsen, Reed and Manning 1977). It was shown by Nawa and Miller (1978) that expulsion of both"normal" and "damaged" worms from rats could be induced by TDL from infected rats, hence the traditional view of antibody involvement in the response to N.brasiliensis infection has been questioned. An early antibody mediated component was also postulated in the immune response of mice to T.muris (Wakelin 1975), and rats to T.spiralis (Love,Ogilvieand McLaren 1976).

The formation of "oral plugs" thought to be caused by host immunoglobulin complexing with worm products, has been observed in S.ratti and N.brasiliensis recovered from rats (Taliaferro and Sarles 1939, Ogilvie and Jones 1973, Moqbel and McLaren 1980). It has been suggested that these may adversely affect parasites by restricting food intake. There is evidence to suggest that nutrition is restricted in damaged worms (Moqbel 1980, Miller, Huntley and Wallace 1981) however this is not necessarily attributable to the action of antibody. Incubation of larval and adult T.spiralis in antiserum for 18 hours resulted in oral and cuticular precipitates (Kim and Ledbetter 1981). There is no evidence to suggest that immunoglobulin adsorbed onto the tegument of intestinal cestrodes exerts any deleterious effects on the parasites (reviewed Hopkins 1980) or that rejection of Raillietina cesticillus is any slower in bursectomised irradiated chickens than in intact, antibody producing birds (Elowni 1980).

There are many ways in which antibodies might interact with, or stimulate other components of the host immune/inflammatory system. The most prominent examples are anaphylactic hypersensitivity, complement activation and granulocyte focussing. Klaus, Pepys, Kitajina, and Askonas (1979), found that IgG2 activated mouse complement well in vitro. IgG, and lgA activated complement via the alternate pathway, and LgM was relatively inert, but activated c3 weakly.

Although granulocyte accumulation in the intesinal mucosa is characteristic

of intestinal parasite infections particularly those involving inflammation, granulocyte focussing antibody has been demonstrated predominantly in connection with tissue invasive parasite stages. S.mansoni schistosomulae (Butterworth 1981) and T.spiralis larvae (Kazura and Grove 1973, Mackenzie, Preston, and Ogilvie 1978) are vulnerable to IgG-dependent eosinophil-mediated damage. Eosinophils also may bind via antibody to N.brasiliensis (Mackenzie et al 1978). Similarly bound macrophages and neutrophils may have antiparasite effects (Mitchell 1979). IgA aggregates may stimulate phagocytosis (Spielberg et al 1974), and IgM is thought to be involved in granulocyte damage to D.viteae microfilariae in hamsters (Weiss and Tanner 1979).

IgG, has been suggested as a component in protection against several intestinal helminths: circulating levels are increased during N.brasiliensis infection in the rat (Jones, Edwards and Ogilvie 1970). The effect of IgG, on early migratory tissue larvae of S.ratti is thought to be responsible for the passive transfer of immunity with hyperimmune rat serum (Murrell 1981) IgG, may also have a limited role in the response of mice to T.spiralis (Gabriel and Justus 1979).

IgA secreting plasma cells and IgA immunoglobulin predominate in the mucosa and secretions of the intestine, but specific antiparasite activity has rarely been demonstrated. Some T cells and neutrophils bear IgA surface receptors (Van Epps, Reed and Williams 1978, Lum et al 1979), and IgA can activate complement by the alternate pathway, but these interactions remain largely hypothetical in the case of intestinal helminths. Mucous membrane-located immunoglobulins may prevent access of antigen or attachment of parasites to the mucosa. IgA-mediated blocking of entry of T.taeniaeformis larvae to the mouse intestine has been demonstrated (Lloyd and Soulsby 1978).

It is apparent that there is little evidence of direct of indirect effects of antibody on intestinally located parasites. D.phoxini is confined to the intestine in the final host, therefore antibody production would be expected to occur locally in the intestinal mucosa. This chapter contains

a preliminary assessment of changes in the number of mucosal plasma cells containing IgA, IgG, IgG2 and IgM, associated with D.phoxini infection. The role of inflammation in the immune response of the mouse to D.phoxini is assessed by measuring some characteristic cellular changes (i.e. numbers of eosinophils, goblet cells, mast cells and globule leukocytes) associated with infection, and the adoptive transfer of immunity.

## Materials and Methods.

Mice were killed by ether anaesthesia followed by cervical dislocation. Segments of proximal small intestine, each three or four cms in length were removed and immersed immediately in the appropriate fixative. The segments were cut longitudinally and agitated gently to dislodge gut contents. Each specimen, immersed in fixative, was loosely wrapped round a 3 cm section of a plastic 1 ml syringe plunger, (see Reilly and Kirsner 1965). The plunger was removed after the appropriate period of fixation at room temperature, and specimens were dehydrated, cleared in xylene and embedded in either paraffin wax (melting point 52°c or 54°c, BDH) or "Polywax" (melting point 57°c, Difco Laboratories). Sections of 5μ thickness were cut. Permanent preparations were mounted in DPX after staining, dehydration and clearing.

1. Mast cells and globule leucocytes :

(a) Fixation in carnoy solution :

| | | |
|---|---|---|
| glacial acetic acid | 10 ml. | fixation for three to six hours. |
| chloroform | 30 ml. | |
| ethanol | 60 ml. | |

After fixation, tissues were washed for three hours in ethanol to remove chloroform, before clearing and embedding. Alternatively, when the Histokinette tissue processor was used, tissues were partially rehydrated and stored in 70% alcohol before processing. This did not appear to impair the relevant staining properties of the tissues involved.

(b) Staining :

(i) Astra Blue /Safranin O :

After rehydration, sections were stained in 1% Astra Blue (Gurr) in 0.7N HCI (pH 0.3) for 30 minutes. After rinsing in 0.7N HCI for 10 minutes, sections were stained in 0.5% Safranin O (Searle) in 0.125N HCI (pH 1) for 5 minutes, then dehydrated, cleared and mounted.

(ii) Alcian Blue/Safranin O :

Alcian Blue was substituted when Astra Blue became unavailable. The above method was used, and the relevant staining properties of the two substances were indistinguishable.

2. Goblet Cells :

(a) Fixation in Bouin Solution :

| | | |
|---|---|---|
| picric acid saturated aq. | 75 ml. | Fixation time : two days. |
| formalin | 25 ml. | |
| glacial acetic acid | 5 ml. | |

Tissue was washed in 50% alcohol before dehydration.

(b) Staining: The periodic acid - Schiff method :

After rehydration, the following procedure was followed :

| | |
|---|---|
| 1% Alcian Blue in 3% acetic acid (pH 2.5) | 30 minutes |
| Water rinse | |
| 1% periodic acid | 5 " |
| Water rinse | |
| Schiff reagent (BDH) | 15 " |
| Rinse in 2 changes of sodium metabisulphite (0.5%) | |
| Rinse in water | 10 " |
| Weigert Haematoxylin | 2-3 " |
| Dehydrate, clear and mount | |

3. Eosinophils :

(a) Fixation in Bouin-Hollande solution :

| | | |
|---|---|---|
| Copper acetate | 2.5 g | Fixation occurred over three days. |
| picric acid | 4.0 g | |
| formalin | 10 ml | |
| distilled water | 100 ml | |
| glacial acetic acid | 1.5 ml | |

After fixation, specimens were washed for three to four hours in several changes of water, before dehydration.

(b) Staining with Orange G and Eosin Y (Dominici 1963) :

After rehydration, tissues were stained using the following procedure. Staining solutions and rinse contained 0.1M phosphate buffer (pH 6.2), prepared by mixing the following two solutions :

$Na_2 HPO_4$ 2.8392g dissolved in 200 ml distilled water

$KH_2 PO_4$ 10.8872g " " 800 ml " "

Sections were stained in a 1:1 mixture of 0.5% $^{w/v}$ Orange G and 0.5% $^{w/v}$ EosinY in buffer, for five minutes. After rinsing in buffer sections were stained for one minute in 0.3% Toluidine Blue in buffer. Specimens were then dehydrated, cleared and mounted.

(c) Staining with Haematoxylin and Eosin :

This method was used when Bouin-Hollande fixed tissue was not available. After rehydration, sections were stained in Mayer Haematoxylin (Mallory 1944) for 10 minutes, then washed for three minutes in each of, (i) Scotts tap water, (ii) running tap water. Sections were counterstained in Eosin (0.05% w/v in 70% alcohol containing 5% glacial acetic acid.

4. Plasma Cells :

The indirect immunofluorescence technique described by Dorsett and Ioachim (1978) was used to detect sIg+ve cells in the lamina propria of the mouse intestine.

Tissue was fixed in Bouin solution, dehydrated, cleared and embedded. Sections 5$\mu$ thick were mounted using egg albumen (1 drop/25 ml water), and deparaffinized in two changes of Xylene. After washingin absolute alcohol, tissue was rehydrated via 95% and 80% alcohol, followed by PBS (pH 7.2). Sections were immersed for two hours in stirred PBS containing 2% bovine serum albumin (BSA). Slides were then overlaid with goat-anti-mouse IgG (Gibco) and left for one hour at 37$^{o}$c in a dark, humid chamber. Unattached antibody was removed by washing in three changes of PBS for 30 minutes each, with constant stirring. Sections were then overlaid with 10% rabbit-anti- goat FITC - conjugated antiserum in PBS containing 5% BSA and incubated, as previously for one hour. Slides were then rinsed in water, and coverslipped with 1:1

PBS and glycerol. Preparations were stored at 4°c in darkness prior to viewing (usually within one hour).

The number of fluorescing cells per vcu was recorded. Where possible at least 10 vcu were examined in each section.

In each batch of slides, one of each of the following was included :

(1) One slide, addition of goat-anti-mouse IgG to which was omitted

(2) " " " " rabbit-anti-goat-FITC " " " "

however, the Ig class specificity of binding properties of goat-anti-mouse IgG was not confirmed by any of these procedures.

Replication experiments were not performed.

## Results

1. Proliferation of globule leukocytes (GL) and mast cells (MC) in CFLP mice in response to D.phoxini infection, and the effect of cortisone acetate on this parameter.

Male CFLP mice (104) were infected with 200 metacercariae at seven-eight weeks of age. Four groups of mice received the following treatments :

Group A : 33 mice of which three were killed on days 2,4,6,8,10,12, 19,26 pi and sections of gut (0-5 cms post-pylorus) removed, fixed and stained to reveal mast cells and globule leukocytes. Further groups of three mice were killed on days 2,6,12 pi and their fluke burdens measured.

Group B : Cortisone acetate (Cortistab, Boots Ltd.) was administered to 24 mice (0.04 mls on alternate days beginning on day of infection) until the mice were killed. The timing of kills corresponded to that of group A except that the kill on day 26 pi and the fluke survivorship checks on days 2 and 6 pi were omitted.

Group C : 24 mice were given a secondary infection of 200 metacercariae 19 days after primary infection. Groups of these mice were killed and sections of intestine were processed on days 4,6,8,10,12,19 pi. Fluke burdens were checked on days 2 and 6 pi.

Group D : 21 mice were infected as for Group C but received cortisone acetate treatment following secondary infection, until killed. Timing of kills corresponded to that of Group C but the survivorship check on day 2 pi was omitted.

Age matched uninfected mice were killed on the day of primary infection, and 19 days later, and corresponding sections of the intestine removed and processed.

Figs. 5-1 and 5-2 show that establishment was low and variable in both infections. Although the precise kinetics of fluke loss are not known, the day 2 recovery probably indicates the % establishment (see Chapter 1). Flukes in primary infection (Fig.5-1) in untreated mice were expelled by day 12 pi, whereas mice treated with cortisone acetate still retained 29%

of the administered infection (or about 60% of day 2 recovery). Similarly, flukes were expelled by day 6 after secondary infection but mice treated with cortisone acetate retained 13% of their administered infection (or 37% of day 2 level).

Numbers of mast cells (MC) plus globule leukocytes (GL) were low in both groups of uninfected mice, with mean values corresponding to six (day 0) and 2.5 (day 19) per vcu. The rapid, almost 20-fold increase in MC + GL following primary infection (Fig. 5-1) occurred between days 6 and 10 pi. The termination of this increase phase was associated with the final stages of fluke expulsion. A decline in number of these cells was evident by day 12 pi, and became more consistent by day 19 pi. On day 26 pi, cell numbers were still significantly higher than those observed in uninfected mice killed at the time of infection.

This cellular response was greatly suppressed by cortisone acetate treatment. Only minor fluctuations in cell numbers occurred in the first 12 days of infection, however a significant increase in number of MC + GL was evident on day 19 pi, suggesting that the response, although greatly delayed, was not completely inhibited.

The mastocytosis associated with secondary infection (Fig.5-2) was clearly evident by day 4 and was maintained until day 10 pi, although parasite expulsion was complete by day 6 pi. The apparent transient significant reduction in the numbers of these cells on day 6 pi is not evident in other results (Fig. 5-5) and it is thought that it does not reflect degranulation associated with the final stages of parasite expulsion. It is possible that infection levels were low in the mice concerned.

This cellular response therefore occurred more rapidly in response to secondary than to primary infection, however, the maximum magnitude of the primary response was greater than that associated with secondary infection.

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Figure 5 - 1

The change in numbers of intestinal globule leukocytes (GL) and lamina propria mast cells (LPMC) associated with a 200 metacercarial primary infection of D.phoxini, and the effect of cortisone acetate given on alternate days throughout infection.

◍ = uninfected mice

○ = recipients of 200 metacercariae

● = " " " + given cortisone acetate

52±12 nd

32±25 nd

1 untreated
29±14 cort acetate treated
} %survival (recovery)

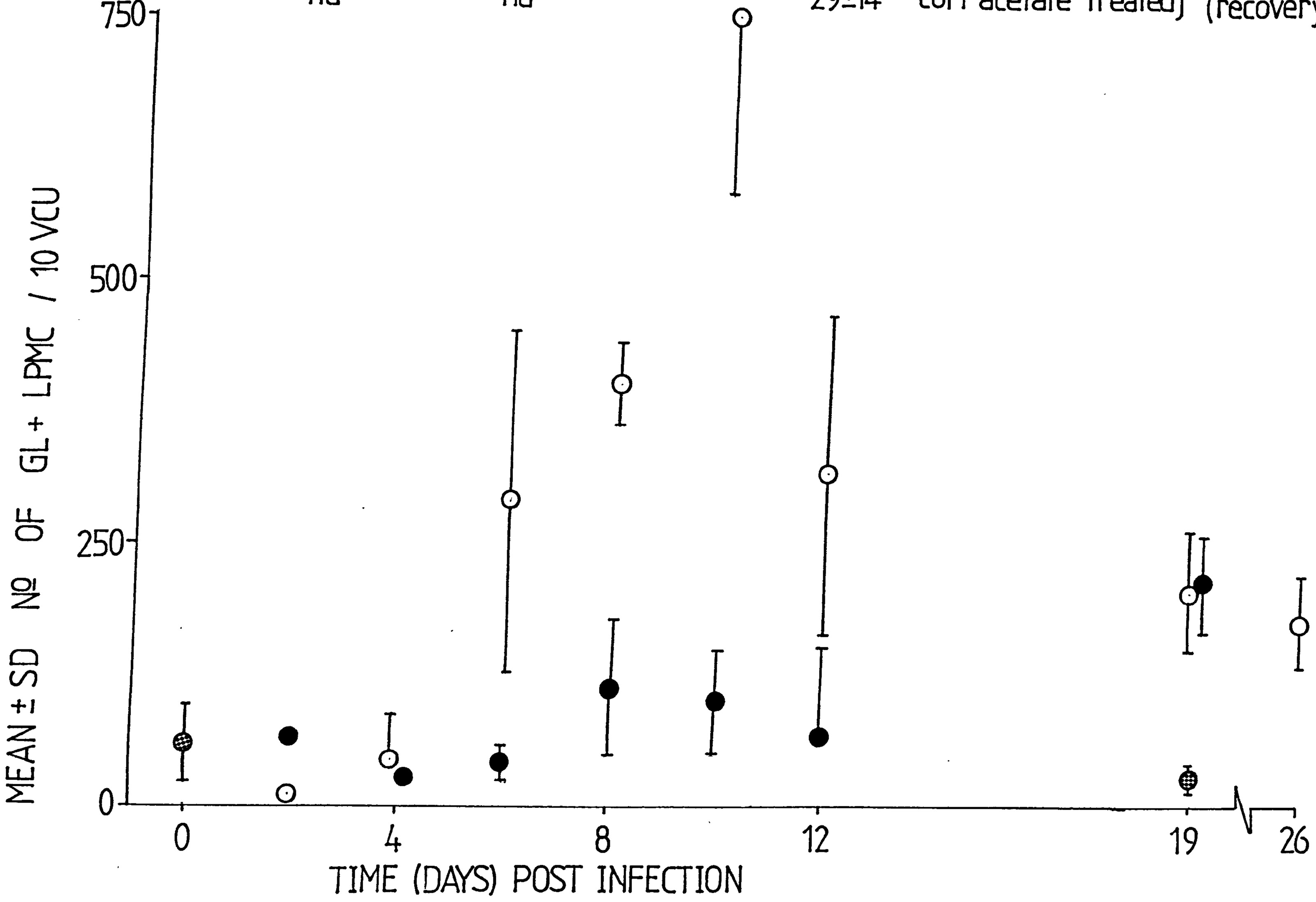

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Figure 5 - 2

The change in numbers of intestinal globule leukocytes (GL) and lamina propria mast cells (LPMC) associated with a 200 metacercarial secondary infection of D. phoxini, and the effect of cortisone acetate given on alternate days throughout infection.

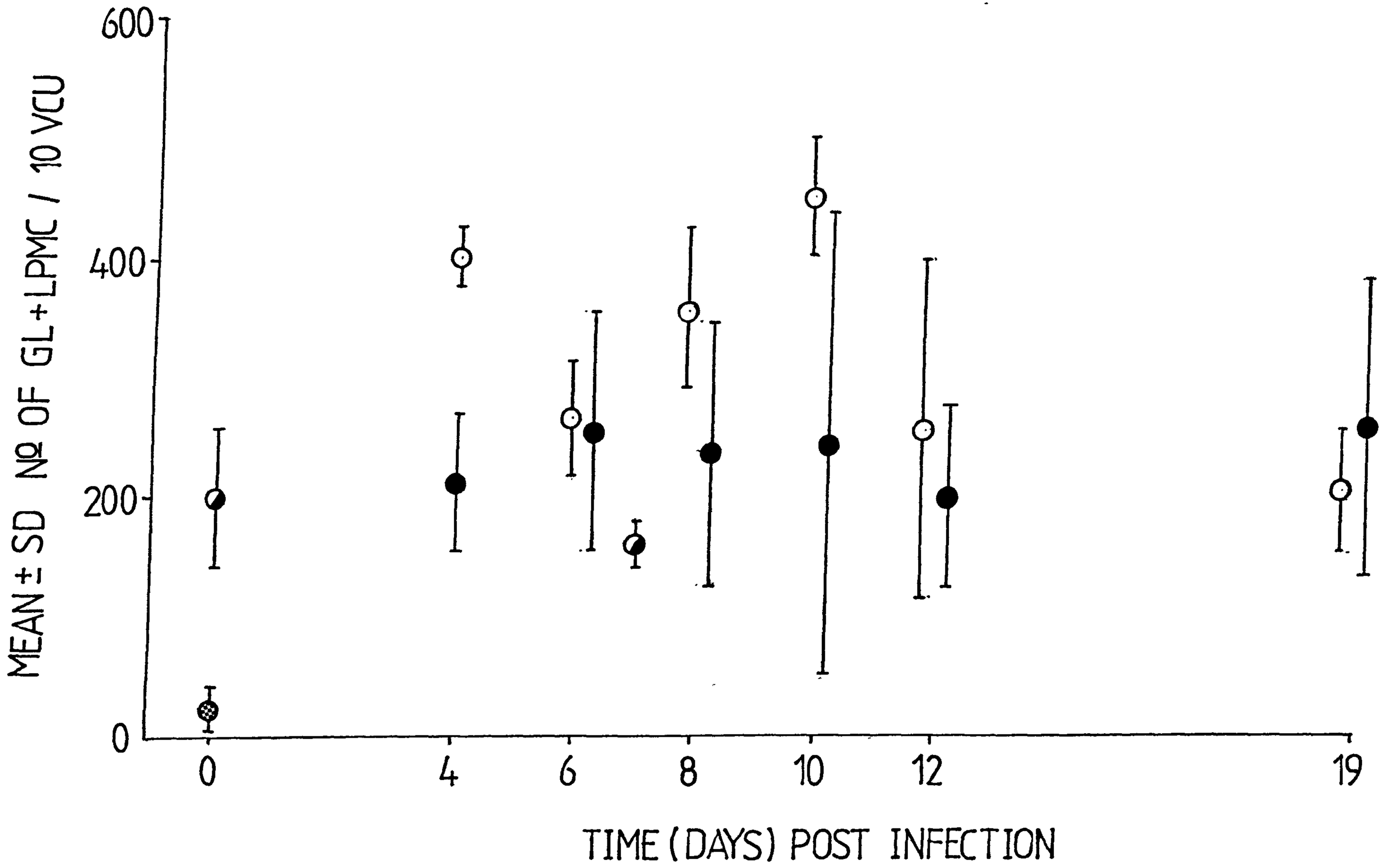
◐ = mice given 200 met. primary infection on day - 19

◍ = uninfected mice

○ = recipients of 200 met. secondary infection

● = " " " " " " + cortisone acetate.

| | | | |
|---|---|---|---|
| 41±16 | 3 | untreated | Mean% fluke recovery |
| 35±15 | 13±8 | cort.acetate treated | |



2. The cellular response of NIH mice to infection with D.phoxini :

The effect of primary and secondary infections in numbers of globule leukocytes, eosinophils, mast cells and goblet cells in the small intestine was measured.

NIH mice (61) were infected with 200 metacercariae at 7-8½ weeks of age. Three uninfected mice were killed and sections of proximal small intestine removed, fixed, sectioned and stained.

Infected mice received the following treatments :

Group A : Three mice received cortisone acetate (Cortistab, Boots Ltd., 0.04ml per dose) administered subcutaneously on days 1,3,5 pi, and were killed and sections of proximal small intestine removed and processed on day 6 pi.

Group B : 30 mice were killed in groups of three or four, and gut sections processed on days 2,4,5 (in error), 8,12,16,20,30 pi. Further groups of three mice were killed on days 5,8 and 11 pi and their fluke burdens measured.

Group C : 19 mice received a secondary infection of 200 metacercariae, 20 days after primary infection. Groups of four mice were killed on days, 2,4 and 6 pi and gut sections processed. Fluke burdens were checked in groups of three mice killed on days 2 and 6 pi.

Group D : 15 mice received a primary infection of 20 metacercariae at 7-8½ weeks of age. Groups of four mice were killed on days 4,6,9 pi and sections of proximal small intestine removed and processed. Fluke burdens were checked on day 8 pi.

(i) Mast cells and globule leukocytes : (Figs. 5-3, 5-4, 5-5).

In uninfected mice, lamina propria mast cells were very rare, and less than four GL per vcu were present.

A marked increase ( x 27) in GL numbers occurred between days 4 and 8 of 200 met. primary infection. (Fig. 5-3). A decline in numbers was evident by day 10 pi, and cell numbers had almost returned to control levels by day 20 pi. Fluke expulsion occurred after day 5 and was complete by day 11 pi.

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Figure 5 - 3

The effect of a 200 metacercarial primary infection of D.phoxini on the number of intestinal globule leukocytes (GL) and mast cells (LPMC) in the mouse intestine.

○ = mean No. of GL present in infected mice

◍ = " " " " " " " " given cortisone acetate day 1,3,5, pi.

● = mean LPMC present in infected mice

◐ = " " " " " " given cortisone acetate day 1,3,5, pi.

74±5 31±11 ×75±8 1

Mean% fluke recovery

[× = cortisone acetate treated]

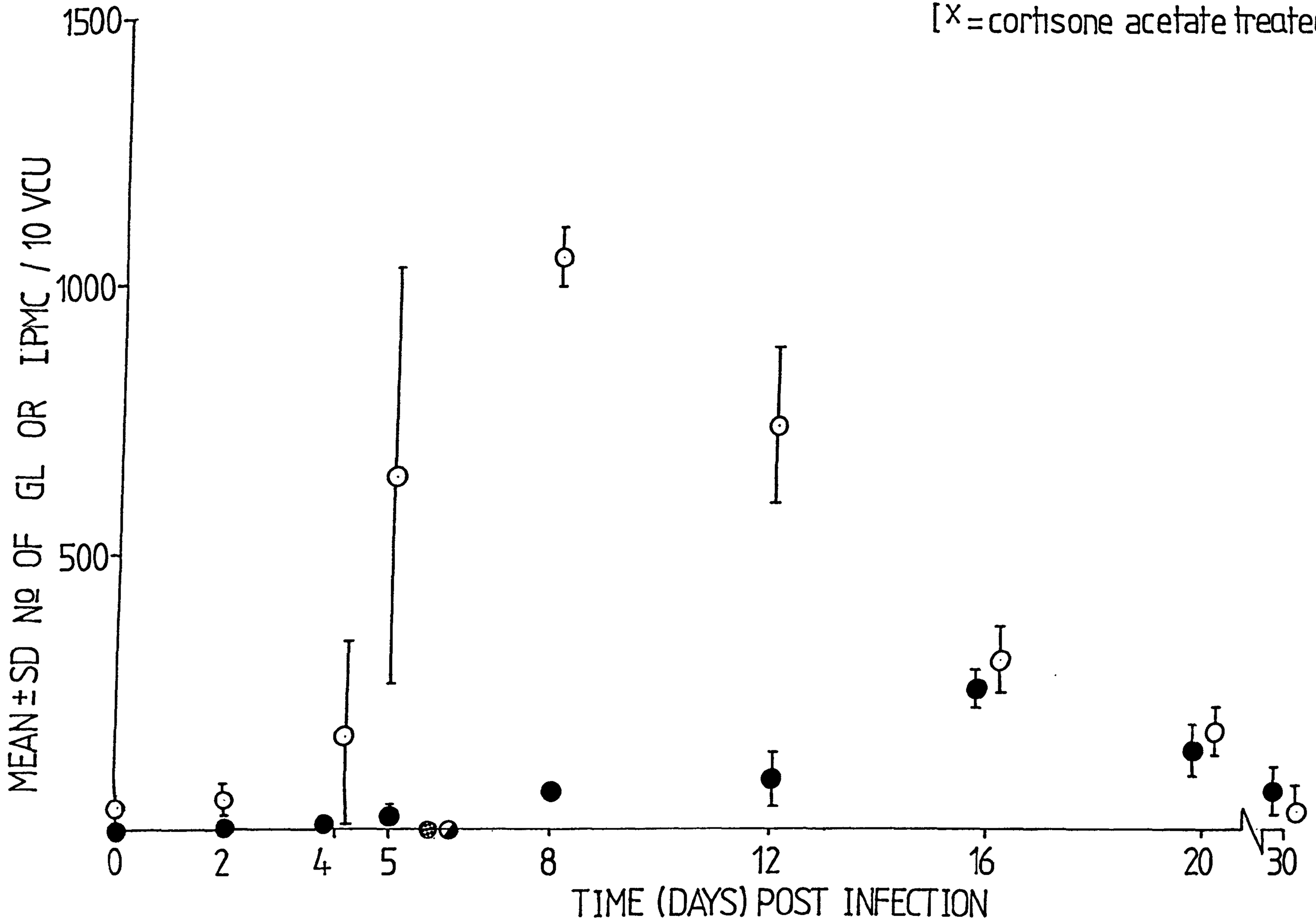

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Figure 5 - 4

The effect of a 20 metacercarial primary infection on numbers of globule leukocytes (GL) and mast cells (LPMC) in the mouse intestine.

● = mean number of LPMC/10 vcu

○ = " " " GL/10 vcu

| | | | | |
|---|---|---|---|---|
| 3 | 4 | 4 | 4 | Mice / group |
| | | | 63±8 | Mean ± SD fluke recovery (%) |

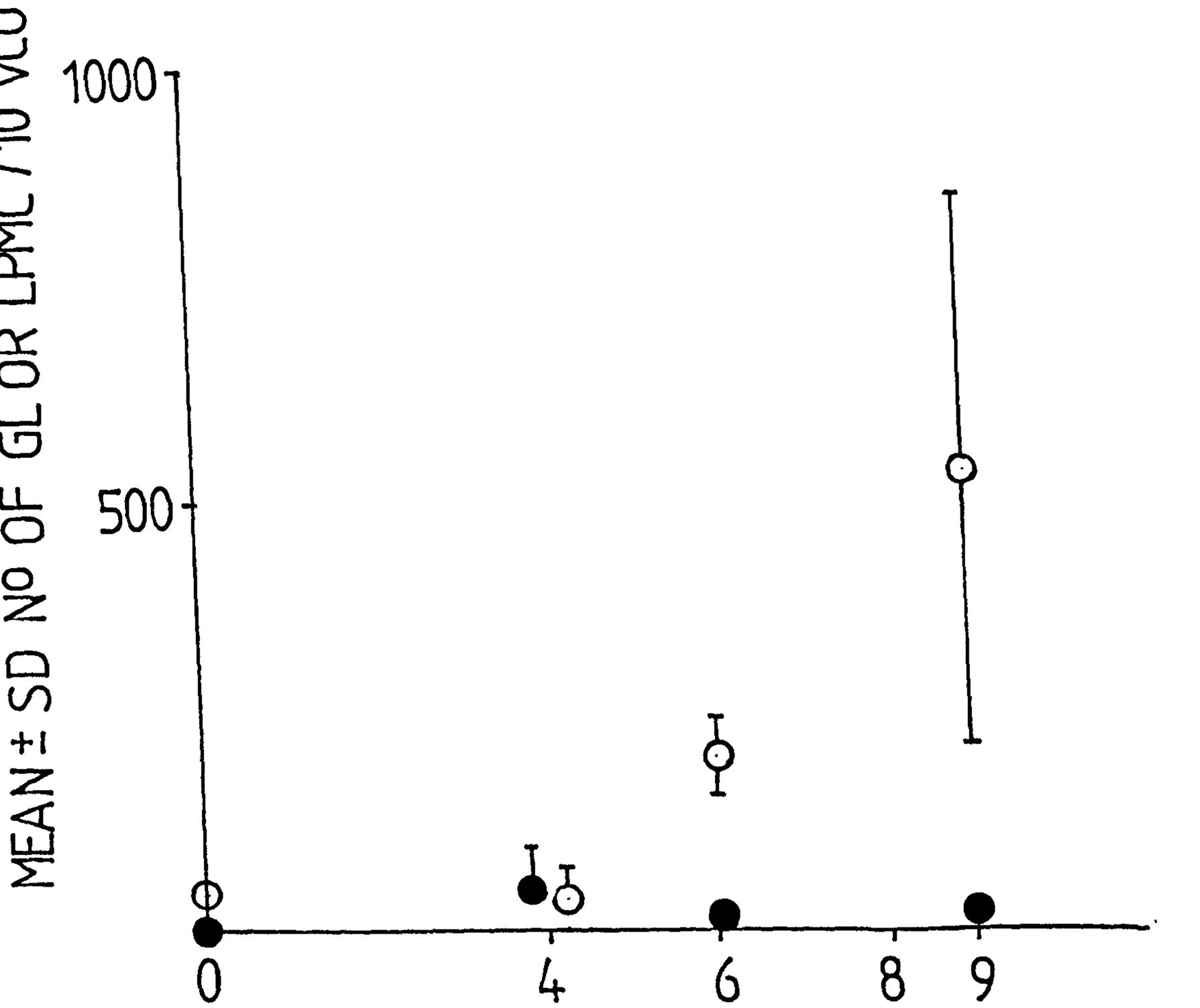

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Figure 5 - 5

The effect of a 200 metacercarial secondary infection on numbers of globule leukocytes (GL) and mast cells (LPMC) in the mouse intestine.

● = mean number of LPMC/10 vcu

○ = " " " GL/10 vcu

| | | | | |
|---|---|---|---|---|
| 4 | 5 | 4 | 4 | Mice / group |
| | 79±5 | | 0 | Mean ± SD fluke recovery (%) |

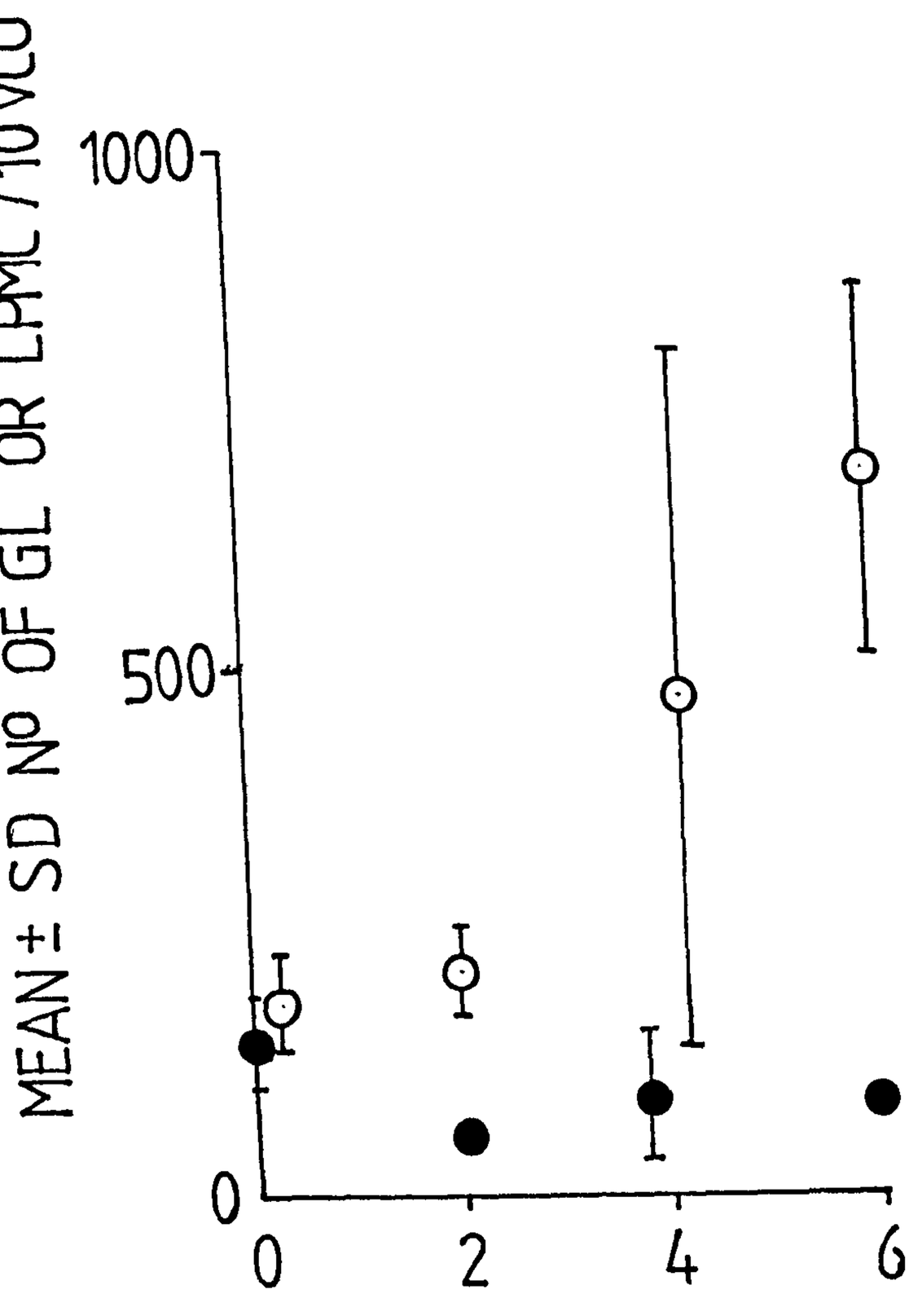

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Figure 5 - 6

The effect of 200 metacercarial primary and secondary infections on the number of eosinophils in the mouse intestine.

◍ = uninfected mice

○ = mice given 200 met. primary infection

▲ = " " " " and given cortisone acetate on days 1,3,5,pi.

● = " " " " followed by 200 met. secondary infection.

3 4 4 4 4 4 3 3 Mice / group

MEAN ± SD No OF EOSINOPHILS / 10 VCU

600

400

200

0

0 2 4 8 12 20 22 24

TIME (DAYS) POST INFECTION

Secondary infection given

hence the increase in GL preceded and included the rejection phase of infection, declining thereafter.

After low level (20 metacercariae) infection, fluke burdens were only checked on day 8 pi, when 63% of the administered parasites remained (Fig.4). This suggested that fluke expulsion had not begun in these mice. There was a two or three day delay in the GL response to a 20 metacercarial, compared with a 200 metacercarial infection.

When the secondary infection was given, GL numbers were already slightly elevated from primary infection given 20 days previously (Fig 5-5) A further increase in GL numbers in response to secondary infection was evident by day 4 pi. Fluke rejection occurred after day 2 and was complete by day 6 pi, therefore the proliferation of GL coincided with the rejection phase of secondary infection. The magnitude of the peak responses cannot be compared as observation of secondary infection was terminated on day 6 pi .

The effect of cortisone acetate on primary infection (Fig. 5-3), observed on day 6 pi was to completely suppress the increase in GL which was already apparent on day 5 pi in mice responding normally to infection.

(ii) Eosinophils :

Numbers of eosinophils present in uninfected mice (Fig.5-6) were comparatively few (equivalent to 32 per 10 vcu).

Four days after 200 metacercarial primary infection, a five-fold increase in number of eosinophils had occurred, however, this form of presentation of data fails to demonstrate the discontinuous "patchy" nature of the response at this early stage of infection, when eosinophils were located mainly in the tissues surrounding blood vessels. This level of eosinophilia was maintained on day 8 pi (with the exception of one mouse in which greatly elevated numbers of eosinophils were observed). A reduction in number of eosinophils was evident by day 12 pi and by day 20 numbers had returned to control levels.

The eosinophil response to primary infection therefore preceded and accompanied the expulsion phase of infection, during the latter stages of

which it declined.

Following secondary infection, eosinophil numbers had already increased by day 2 pi after which expulsion began. The elevation in cell numbers was maintained on day 4 pi.

Treatment of mice with cortisone acetate during primary infection resulted in an absence of eosinophils on day 6 pi whereas in mice undergoing normal infection, eosinophil numbers were elevated between days 4 and 8 pi.

(iii) Goblet Cells :

The results (Fig 5 - 7) suggest that there is no significant goblet cell hyperplasia associated with primary or secondary D.phoxini infection, however because of the very small number of uninfected mice included for comparison, it may be necessary to interpret the results obtained, with caution. This raises the question of whether the lower number of goblet cells present during the first five days of a 200 metacercarial primary infection (and first nine days of a 20 metacercarial infection), is real, or due to abnormally high numbers of these cells in uninfected mice. The goblet cell counts in uninfected mice are partly reinforced by the stability of cell numbers between days 16-30 following a 200 metacercarial infection which are higher than those obtained on days 2-5 (and 4-9 of a 20 metacercarial infection), and are approaching the values obtained for uninfected mice. Goblet cell emptying and villous atrophy could both contribute to a reduction in goblet cell counts following infection, however no significant change in goblet cell numbers was observed following secondary infection.

Goblet cell counts increased by 43% between days 4 and 12 of a 200 metacercarial primary infection, i.e. prior to and during fluke expulsion . No increase prior to expulsion was observed following 20 metacercarial primary infection.

Cortisone acetate treatment resulted in an 18% increase in goblet cell numbers on day 6 pi, compared with uninfected mice.

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Figure 5 - 7

The effect of primary and secondary D.phoxini infection on the number of goblet cells in the mouse intestine.

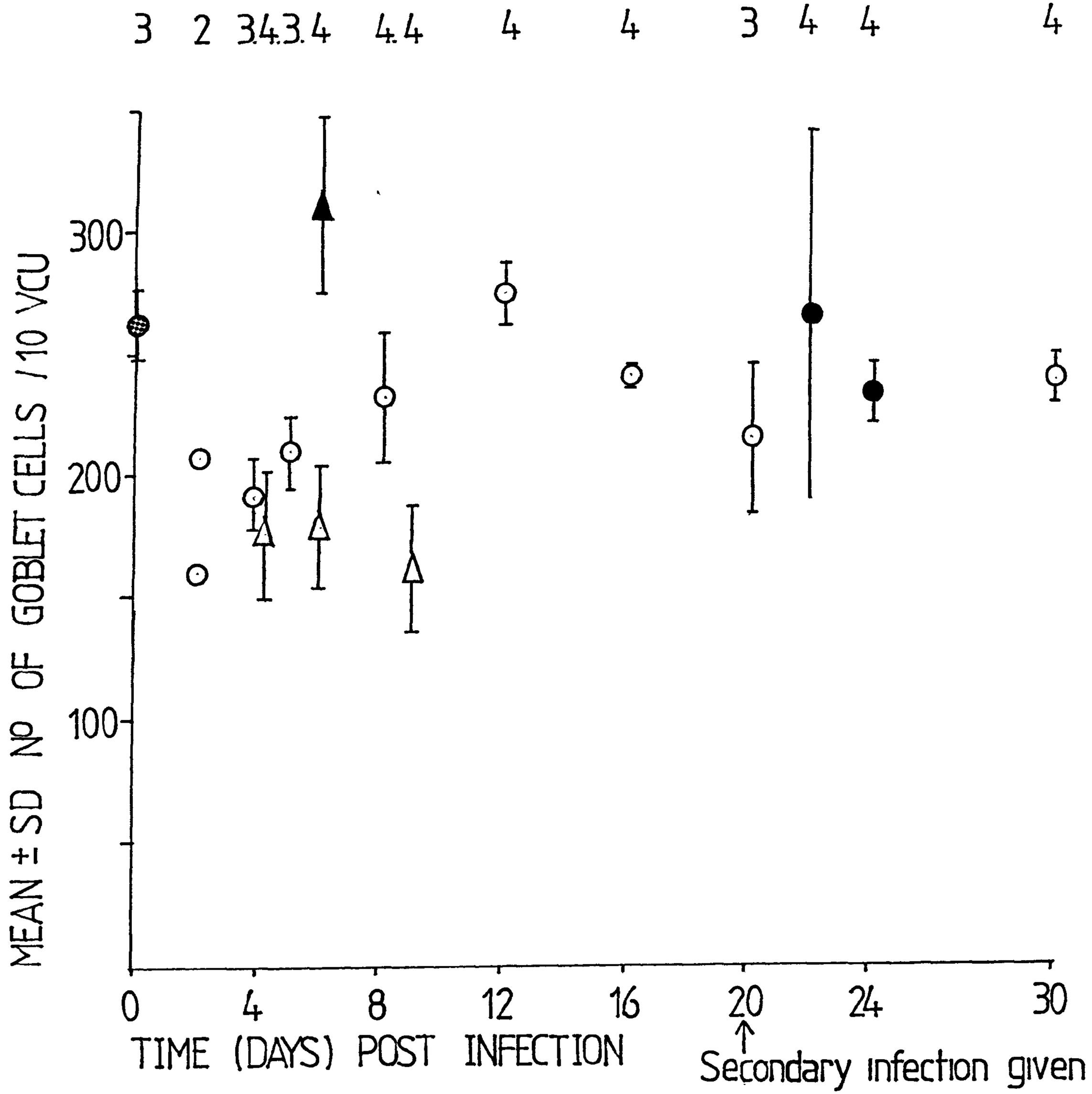
⊛ = uninfected mice

△ = recipients of a 20 met. primary infection

○ = recipients " " 200 " " "

▲ = " " " " " " and given cortisone acetate on days 1,3,5, pi.

● = " " " " " secondary infection.


3 2 3.4.3.4 4.4 4 4 3 4 4 4 Mice / group
MEAN ± SD Nº OF GOBLET CELLS /10 VCU
300
200
100
0
4
8
12
16
20
24
30
TIME (DAYS) POST INFECTION
Secondary infection given

The Histopathological effects of transferred IMLNC on the mouse small intestine and the response to infection :

Donor mice (34 NIH males aged 6½ to 8½ weeks) were infected with 200 metacercariae. On day 6 pi, donors were killed, IMLNC recovered and $8x10^7$ cells transferred into each of 25 recipients aged 7-8 weeks. Nine days later, five recipients and five control (naive) mice were killed and sections of proximal small intestine removed and processed. The remaining cell recipients and 18 control mice were infected with 200 metacercariae. Four or five mice from each group were killed on days 2,4 and 6 pi and gut sections processed. Fluke burdens were checked in the remaining mice on day 6 pi.

It was found that IMLNC recipients retained 10 $\pm$ 22% of their original infection, on day 6 pi, compared with 75 $\pm$ 8% in control mice. This suggests that transferred cells caused an acceleration of parasite expulsion in recipient mice, however an additional kill in the early stages of infection, to check establishment, would have been desirable to eliminate poor establishment as a factor affecting fluke recoveries.

(i) Effect of IMLNC transfer on globule leukocytes and mast cells in the small intestine :

Results show (Figs. 5-8, 5-9) that MC and GL counts in IMLNC recipient mice, nine days after cell transfer, did not differ from those of control mice, suggesting that transferred cells had no effect in the absence of further antigenic stimulation of recipients.

Pronounced GL hyperplasia was evident on day 4 after challenge of cell recipients, and numbers had doubled by day 6 pi (Fig.5-8). This cellular response appeared to be accelerated by at least two days in cell recipients, compared with normal mice undergoing primary infection.

The increase in mast cell numbers associated with infection (Fig.5-9) was less than 7% of the increase in GL in both groups of mice on day 6 pi. A small increase in mast cell numbers was observed on day 2 pi in cell recipients. Thereafter a steady ( 700%) increase occurred between days 2 and 6 pi, when

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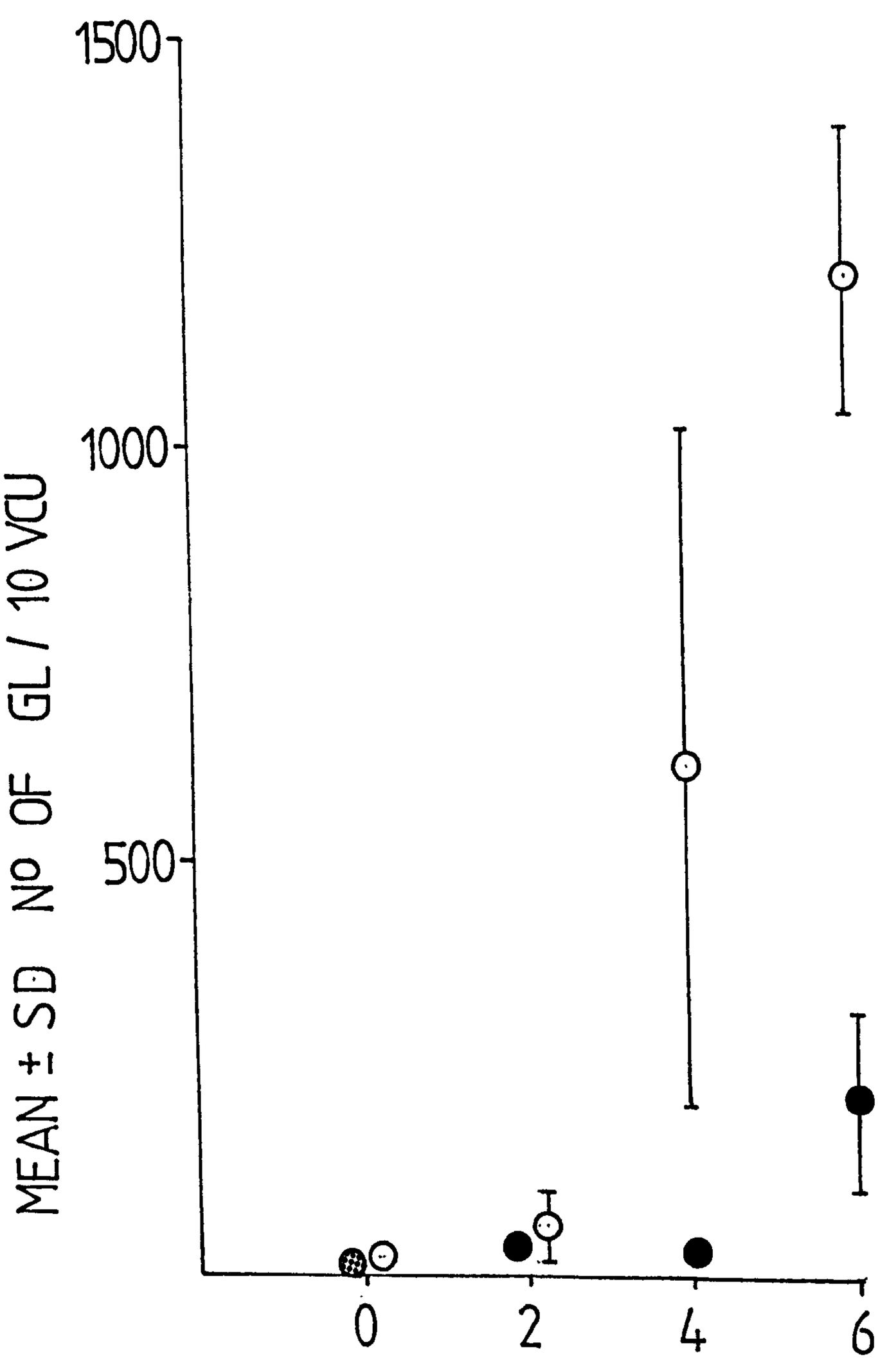
Figure 5 - 8

The effect of transferred IMLNC on the number of globule leukocytes (GL) in the intestine of recipient mice challenged with 200 metacercariae

⊛ = uninfected mice

○ = IMLNC recipients challenged with 200 metacercariae

● = recipients of 200 metacercariae.


1500
1000
500
MEAN ± SD No OF GL / 10 VCU
0
2
4
6
TIME (DAYS) POST INFECTION

Facing page 183

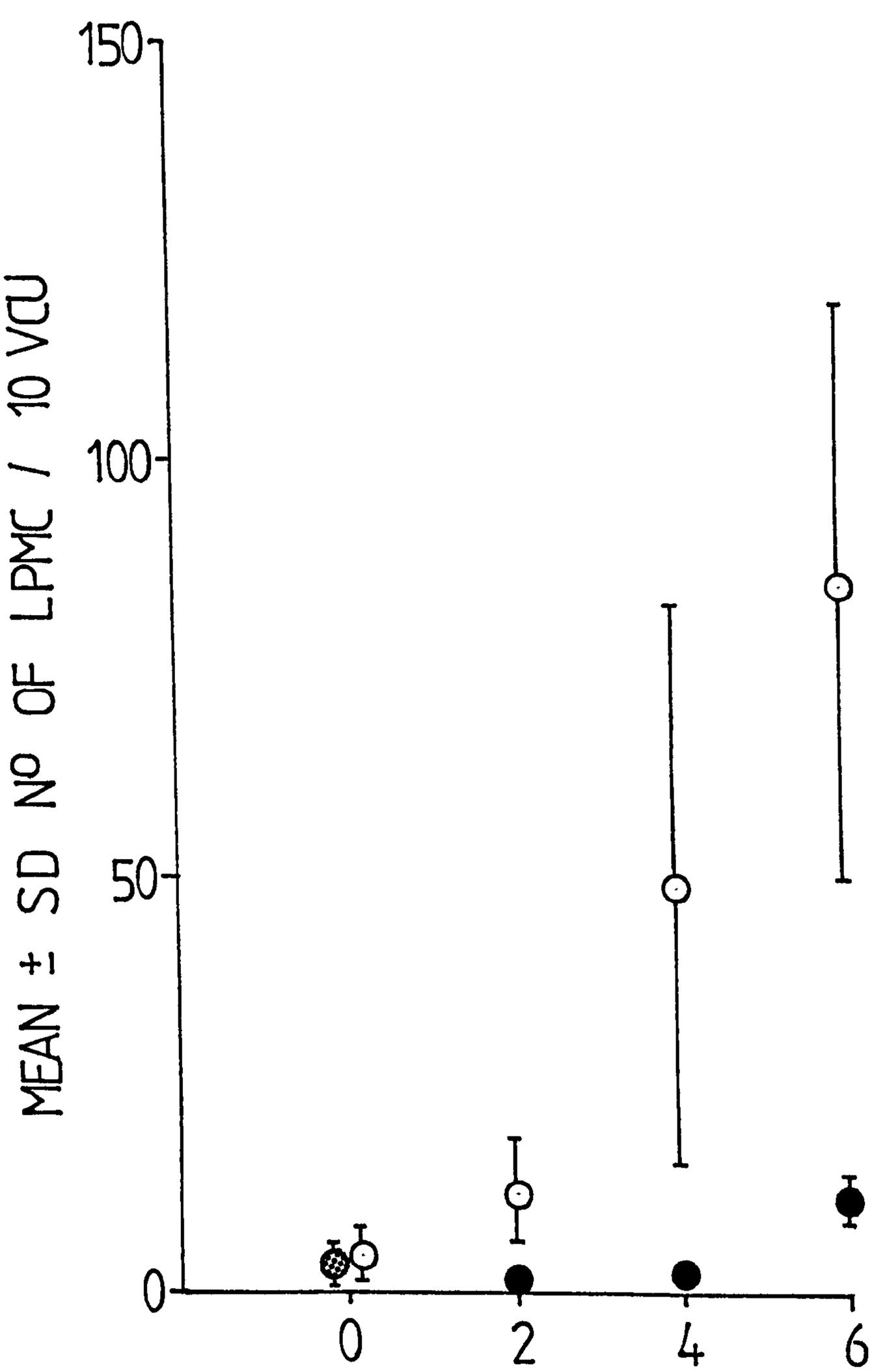
Figure 5 - 9

The effect of transferred IMLNC on the number of mast cells (LPMC) in the small intestine of recipient mice challenged with 200 metacercariae.

⊕ = uninfected mice

○ = recipients of IMLNC, challenged with 200 metacercariae

● = recipients of 200 metacercariae


MEAN ± SD Nº OF LPMC / 10 VCU
150
100
50
0
0
2
4
6
TIME (DAYS) POST INFECTION

approx. nine MC/vcu were present. Number of MC present on day 6 of primary infection were equivalent to approx. 1/vcu.

The duration of the experiment was too short to enable full quantitative comparison of these cellular responses to be made. It is unlikely that either response achieved a maximum by day 6 pi.

To summarize: Globule leukocyte and mast cell hyperplasia in cell recipients was advanced on day 4 and further increased by day 6 pi by which time fluke expulsion was almost complete. This represents an acceleration in these cellular responses, compared with those occurring in mice undergoing normal primary infection.

(ii) Effect of IMLNC transfer on goblet cells in the small intestine :

Fluctuations in goblet cell numbers (Fig. 5-10) were small throughout the experiment, maximum variation in means being equivalent to a difference of less than 8 goblet cells per vcu. Mean goblet cell numbers in all infected mice remained within the range 536-587 goblet cells per vcu between days 0 and 6 pi. Transferred cells did not affect the number of goblet cells present during infection.

Goblet cell numbers in control mice were slightly but significantly lower than those in all other groups. This difference is small however, and conflicts with earlier results (Fig. 5-7) in which the number of goblet cells in uninfected mice was higher than in infected mice.

(iii) The Effect of IMLNC transfer on eosinophils in the small intestine :

An increase in eosinophil numbers (Fig. 5-11) was observed in cell recipients on day 2 pi. A further increase in cell numbers (and variability) occurred on day 6 pi. An increase in eosinophil numbers occurred at least two days later in normal primary infection, but by day 6 pi cell numbers were similar to those observed in cell recipients.

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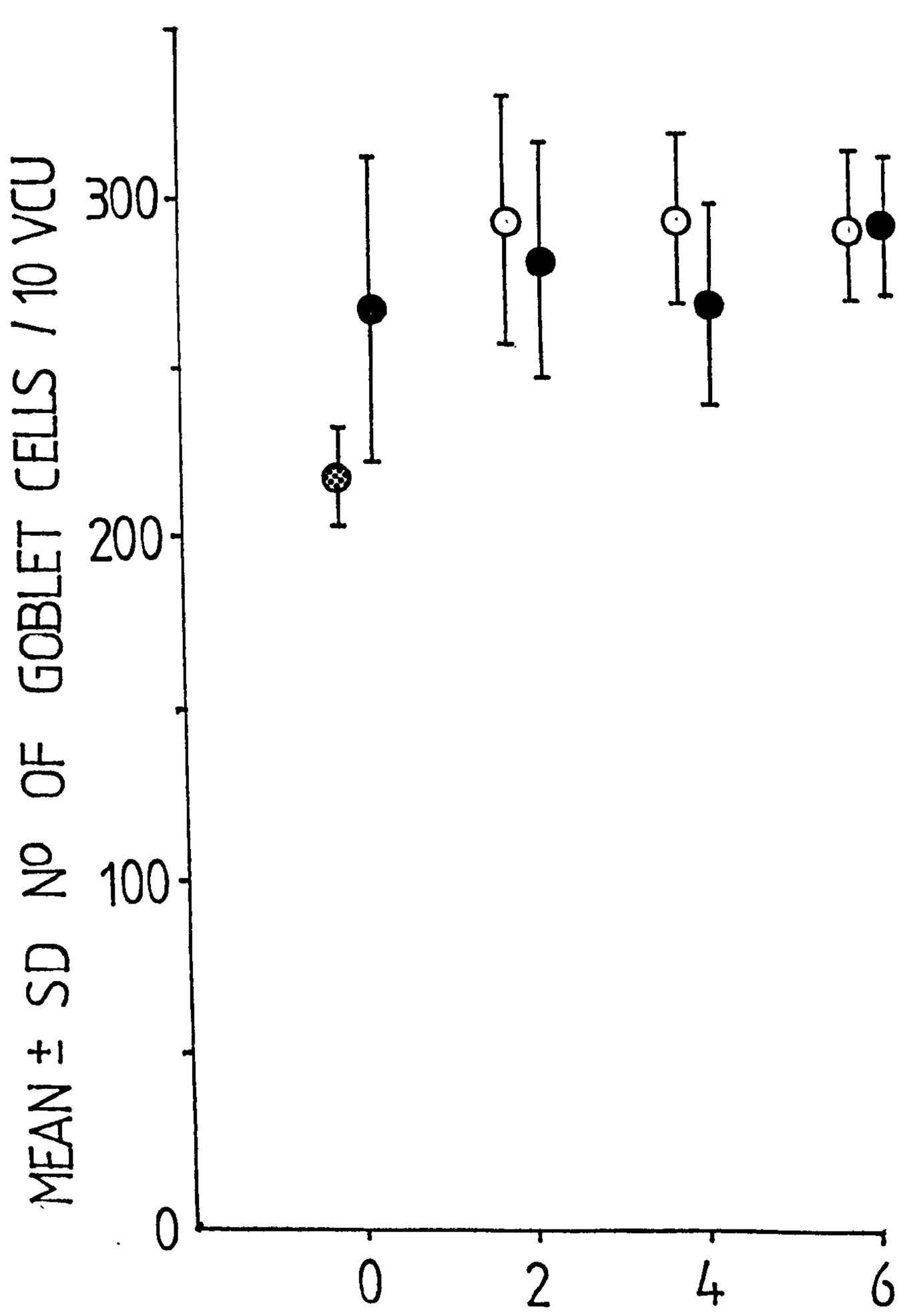
Figure 5 - 10

The effect of transferred IMLNC on the number of goblet cells in the small intestine of recipient mice challenged with 200 metacercariae.

◍ = uninfected mice

○ = recipients of IMLNC challenged with 200 metacercariae

● = recipients of 200 metacercariae


GOBLET CELLS / 10 VCU
MEAN ± SD Nº OF
300
200
100
0
0
2
4
6
TIME (DAYS) POST INFECTION

Facing page 186

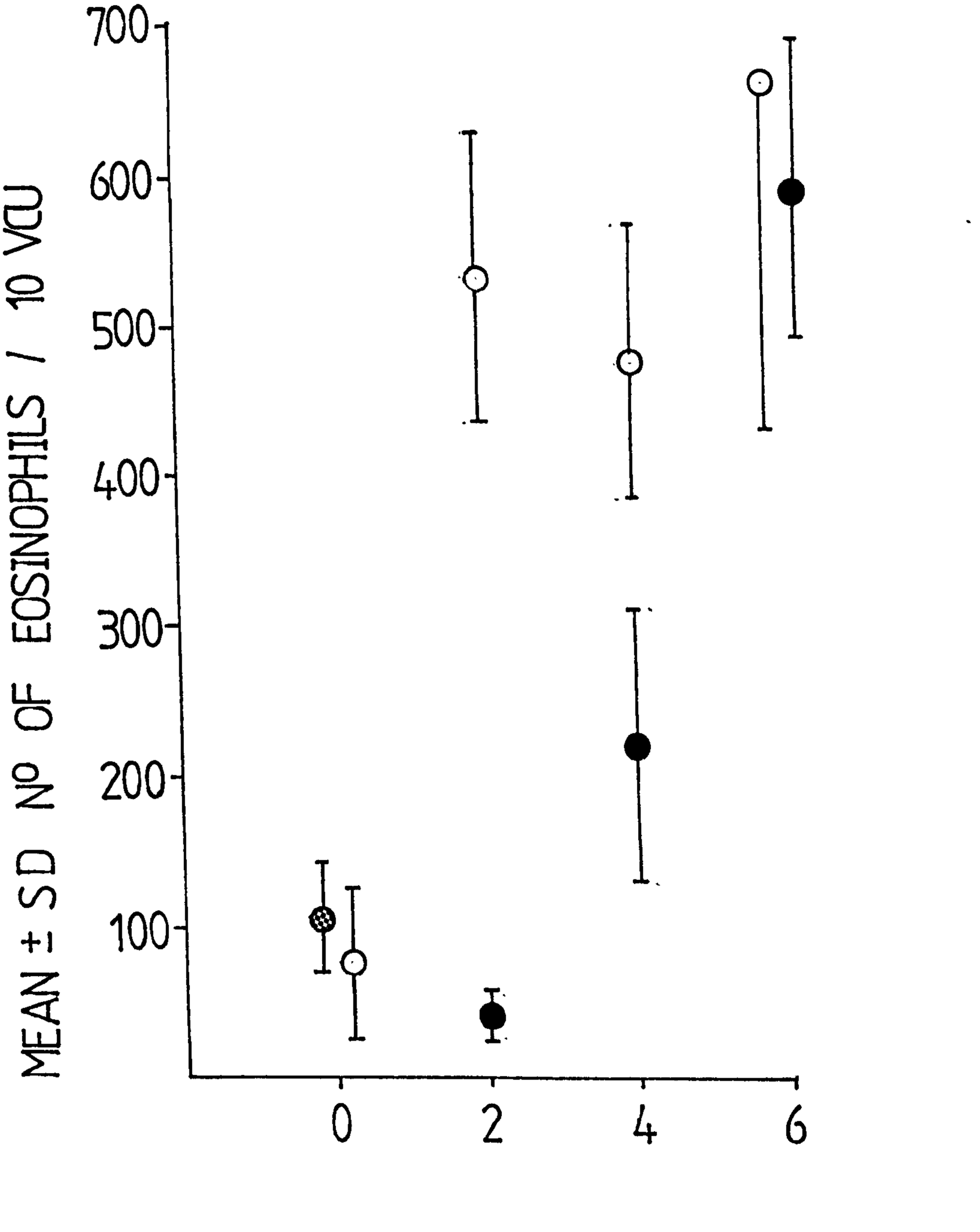
Figure 5 - 11

The effect of transferred IMLNC on the number of eosinophils in the small intestine of recipient mice challenged with 200 metacercariae.

● = uninfected mice

○ = recipients of IMLNC challenged with 200 metacercariae

● = recipients of 200 metacercariae


MEAN ± SD Nº OF EOSINOPHILS / 10 VDU
700
600
500
400
300
200
100
0
2
4
6
TIME (DAYS) POST INFECTION

The effect of B cell and T cell enriched fractions of IMLNC on the cellular changes occurring in response to D.phoxini infection :

This experiment was performed in conjunction with an experiment, the results of which suggested that B cells were more effective than T cells in transferring immunity to D.phoxini. (Chapter 4 P.131 ) An additional four mice were included in each group :

1. T cell recipients (received $4 \times 10^7$ IMLNC)
2. B " " " " "
3. Unseparated " " "
4. Controls - received no cells.

Mice were all killed four days after challenge, and gut sections processed.

1. Effect of transferred cells on the globule leukocyte response :

Results (Fig. 5-12(i) show that the number of GL was increased significantly in all groups of mice receiving IMLNC, however the numbers of GL present in T cell recipients was (>9/vcu) compared with the number (>50/vcu) in mice receiving unseparated cells.

Accurate counts of GL could only be made in two of four B cell recipients, due to tissue distortion. Both mice however contained considerably more GL than recipients of unseparated cells.

2. Mast cells :

Mast cell numbers were elevated (Fig. 5-12 (ii) in all cell recipient groups compared with normal primary infection, the elevation being greatest in recipients of B cells and unseparated cells, however the highest mean, that of B cell recipients, was still only equivalent to 3 MC/vcu.

3. Goblet cells :

There was no significant difference in goblet cell numbers (Fig. 5-12 (iii) between primary infection controls and T cell recipients. A small increase 117/20 vcu) was observed in unseparated cell recipients, but in view of the results obtained in the previous two experiments, and the size of the difference, it seems unlikely that it represents any functionally significant

stimulation of goblet cell differentiation; however a marked increase in goblet cell numbers (378/20 vcu) was observed in recipients of B cells. Such a large increase in goblet cell differentiation is not normally associated with the response to D.phoxini, judging by other experimental results herein.

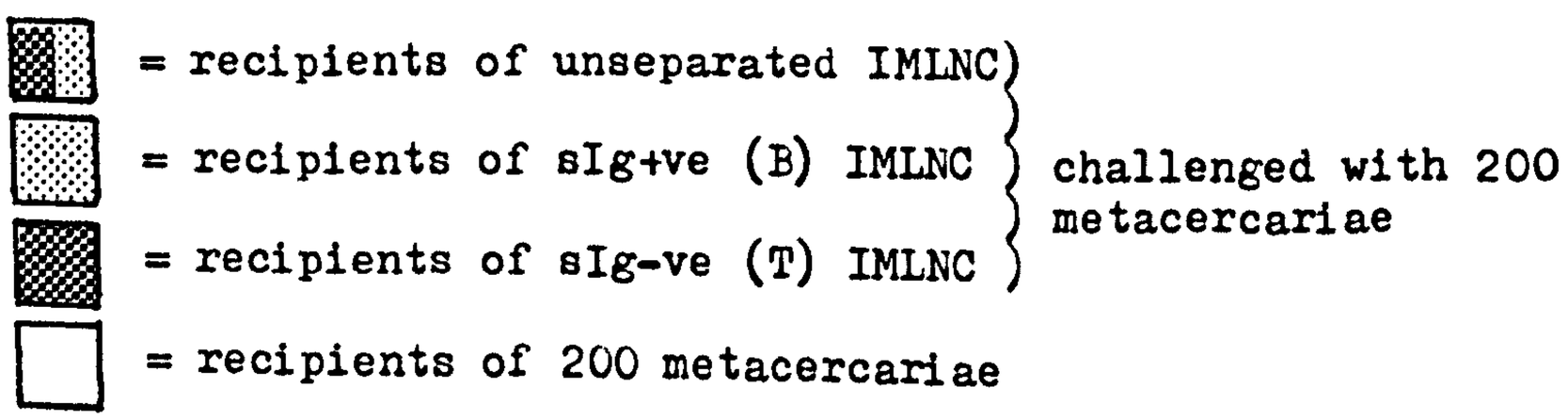
Eosinophils :

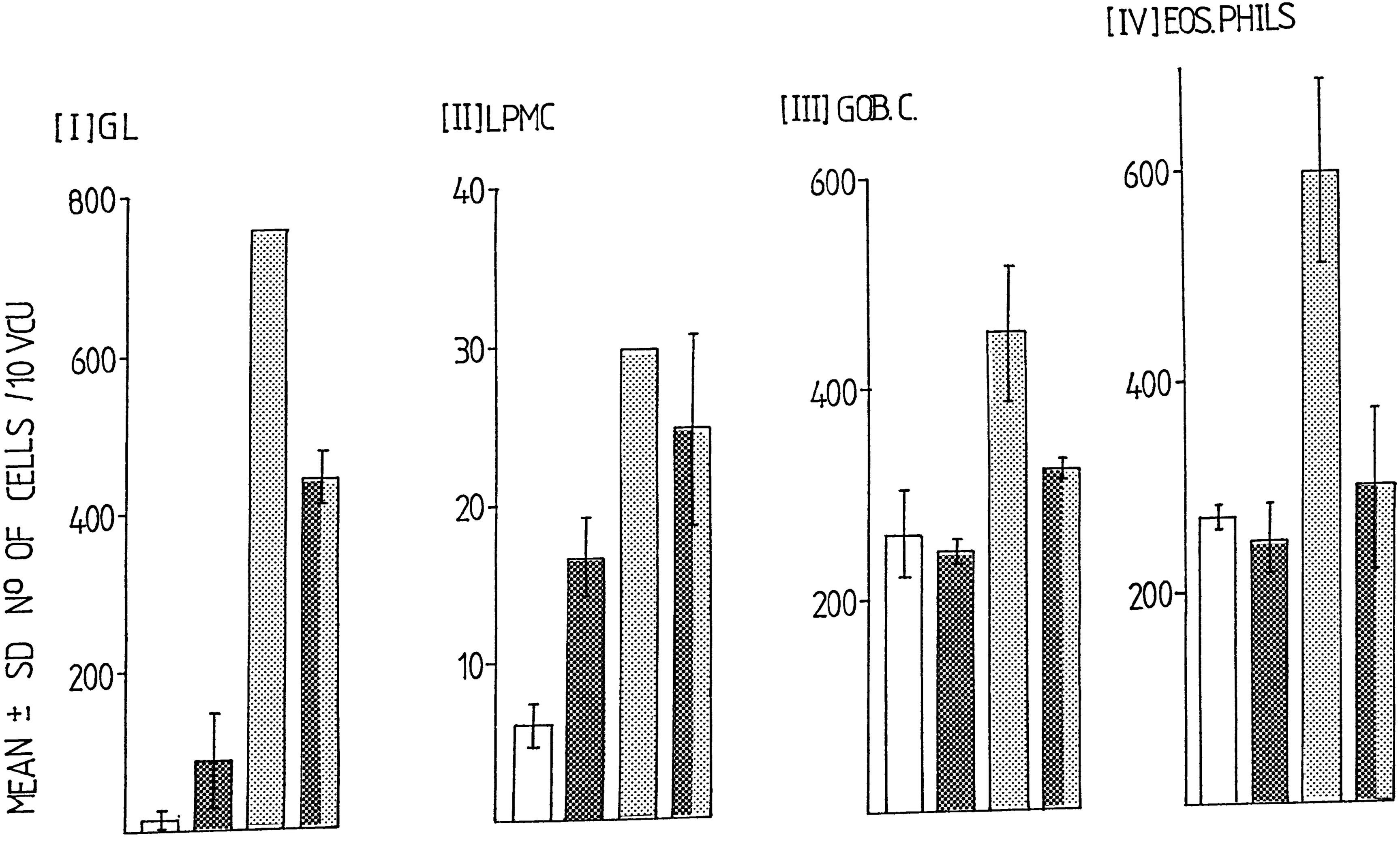
Similar numbers of eosinophils were observed in recipients of T cells and unseparated cells, and in primary infection controls (Fig. 5-12 (iv). The increase (>100%) in eosinophil numbers observed in B cell recipients compared with primary controls was similar in magnitude to that observed on day 4 pi in recipients of unseparated cells in the previous experiment (Fig. 5-10) therefore it is surprising that a similar response in such recipients was not observed in this experiment.

Facing page 189

Figure 5 - 12 I-IV

The effect of transferred B or T enriched fractions of IMLNC on the numbers of mast cells, globule leukocytes, eosinophils and goblet cells in the small intestine of recipient mice four days after challenge with 200 metacercariae.




[I]GL
[II]LPMC
[III] GOB. C.
[IV]EOS. PHILS
MEAN ± SD Nº OF CELLS / 10 VCU
800
600
400
200
40
30
20
10
600
400
200
600
400
200

The Effect of D.phoxini infection on the number of plasma cells present in the small intestine of the mouse :

This work formed part of the experiment described on page 173. Several aspects of the experimental design were unsatisfactory, especially the small size of groups and the inadequate number of uninfected controls used for comparison. This factor, coupled with a high degree of variation in sIg+ve cell numbers, severely limits the conclusions which may be drawn from the results obtained. No attempt was made to quantify the intensity of fluorescence produced which varied between preparations.

Unfortunately no estimation was made of the total number of sIg+ve cells per vcu. This would have provided some measure of the binding specificity of the samples of anti-mouse globulin used.

sIgA+ve cells : (Fig. 5-13).

The high variation in numbers of sIgA cells in naive mice virtually precludes drawing conclusions from the subsequent fluctuations in these cells associated with infection. There is some evidence to suggest a large increase in sIgA+ve cells between days 2 and 12 of primary infection, followed by a gradual decline in number of these cells, however no significant increase was observed in the initial four days of secondary infection, which incorporate the early stages of expulsion.

$sIgG_1$+ve cells (Fig. 5-14)

Variation in cell counts was high in all groups of mice after primary infection. Although an increase in $sIgG_1$+ve cells appears to be maintained for 30 days following primary infection, the small number of control mice used must be borne in mind. The large number of these cells measured on day 8 of a 20 met. infection (which would be expected to precede expulsion) was not borne out by results from a 200 metacercarial primary infection, however, elevated numbers of these cells were observed on days 2 and 4 after secondary infection.

$sIgG_2$+ve cells : (Fig.5-15)

A 70% increase in the number of these cells was associated with the

mid phase of rejection of primary infection (Day 8 pi). No increase in cell number was evident prior to rejection in either 20 or 200 metacercarial primary infections. Cell numbers were normal by day 20 pi but had increased by 60% on day 2 and 86% on day 4 of secondary infection.

sIgM+ve cells:(Fig.5-16)

Numbers of these cells remained stable prior to expulsion of primary infection (days 1-5) but increased by 350% by day 8 pi and thereafter declined to preinfection levels by day 16 pi.

The effect of cortisone acetate on sIg+ve cells :

Some depression of numbers of sIg+ve cells was evident in cortisone acetate treated animals during primary infection but sIgA+ve cells appeared to be unaffected. (Fig. 5-13).

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Figure 5 - 13

Changes in the number of mucosal IgA+ve cells associated with D.phoxini infection.

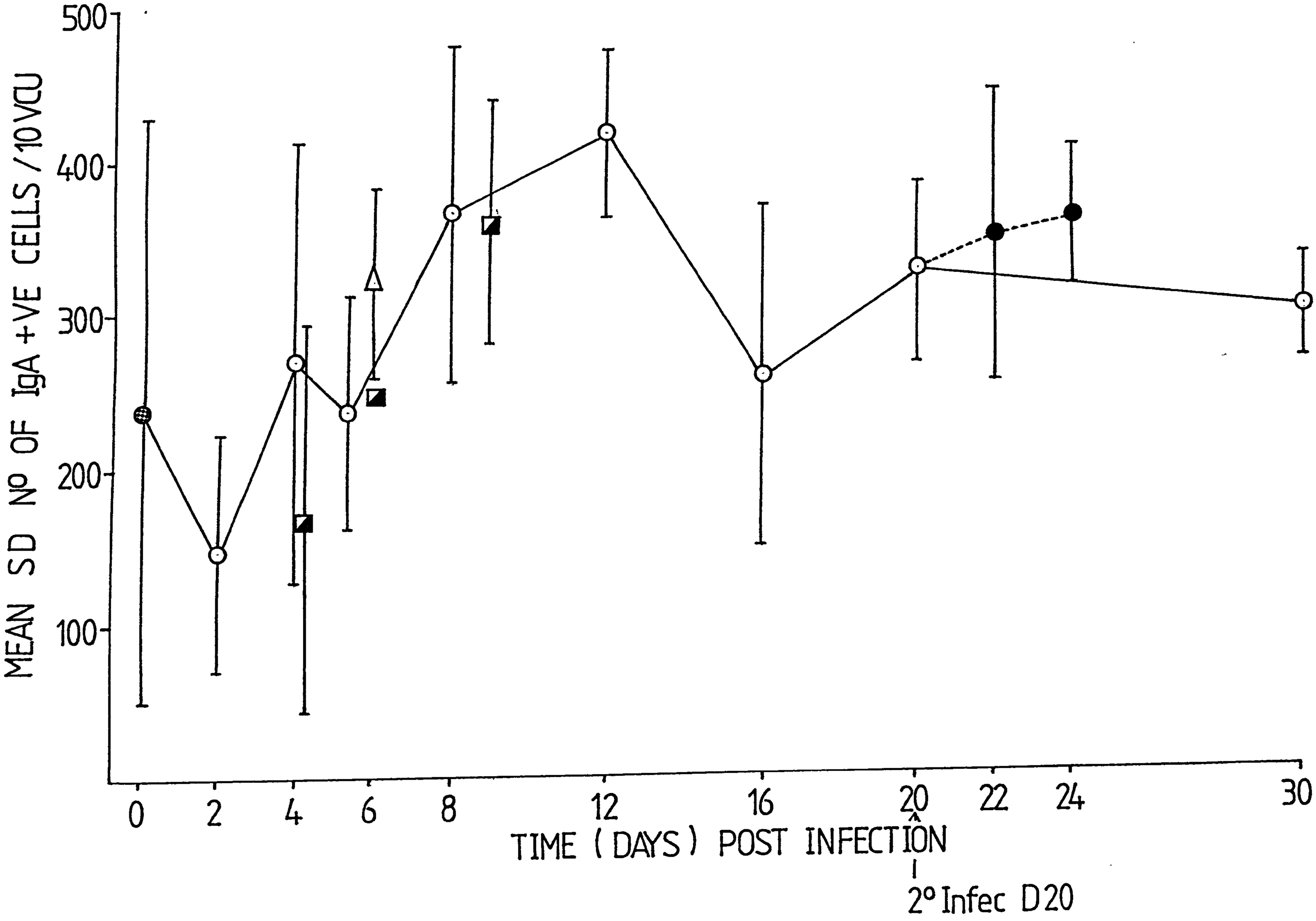
● = uninfected mice

○ = recipients of 200 met. primary infection

△ = " " " " " " given cortisone acetate days 1,3,5, pi.

◪ = recipients of 20 met. primary infection

● = recipients of 200 met. secondary infection


MEAN SD Nº OF IgA +VE CELLS / 10 VCU
500
400
300
200
100
0
2
4
6
8
12
16
20
22
24
30
TIME ( DAYS ) POST INFECTION
2º Infec D20

Facing page 193

Figure 5 - 14

Changes in the number of mucosal $IgG_1$+ve cells associated with D.phoxini infection.

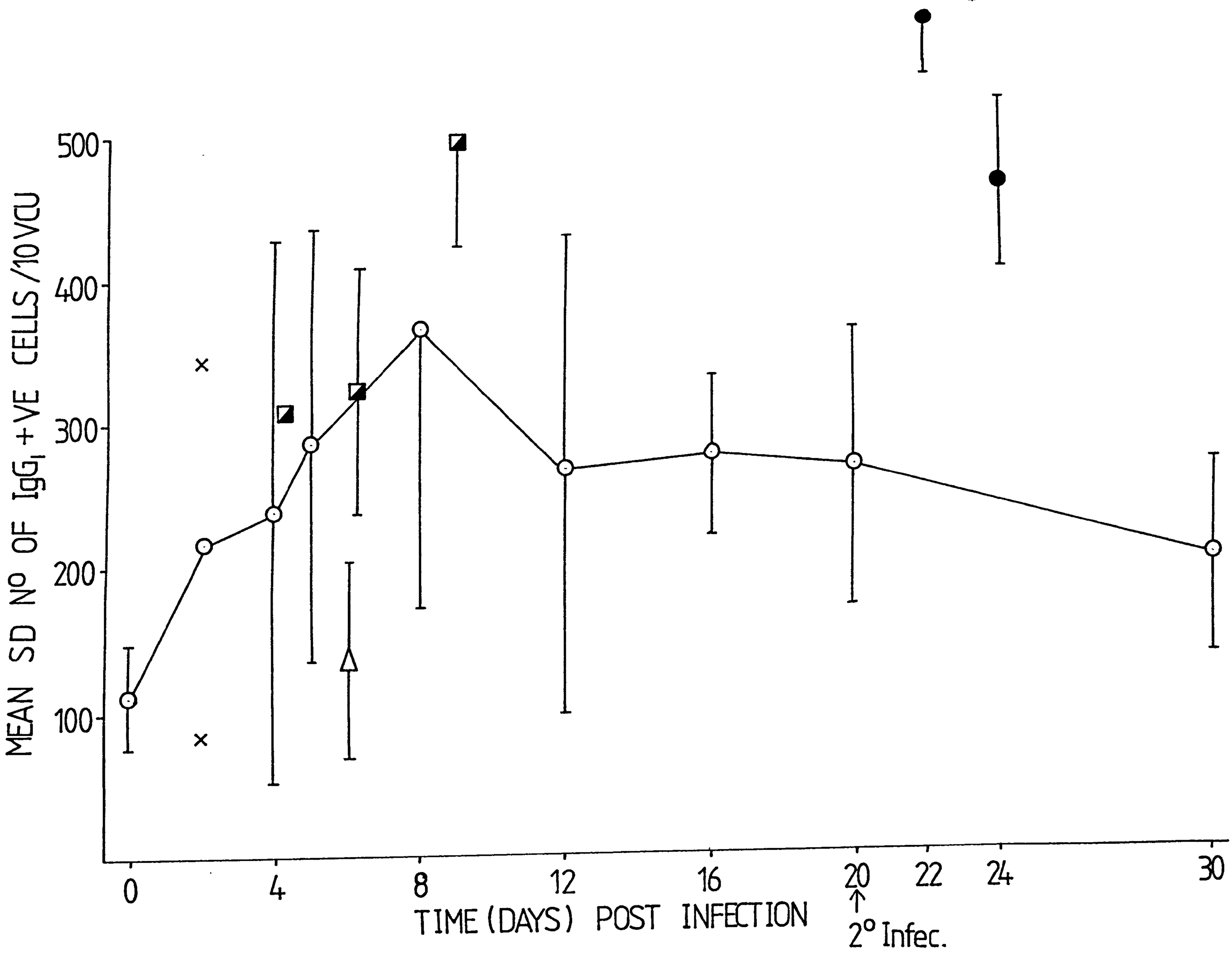
⊛ = uninfected mice

◪ = recipients of 20 met. primary infection

○ = recipients of 200 met. primary infection

△ = " " " " " " treated with cortisone acetate, days 1,3,5, pi.

● = recipients of 200 met. secondary infection


MEAN SD Nº OF IgG1 +VE CELLS /10 VCU
500
400
300
200
100
0
4
8
12
16
20
22
24
30
TIME (DAYS) POST INFECTION
2° Infec.

Facing page 194

Figure 5 - 15

Changes in the number of mucosal $IgG_2$+ve cells associated with D.phoxini infection.

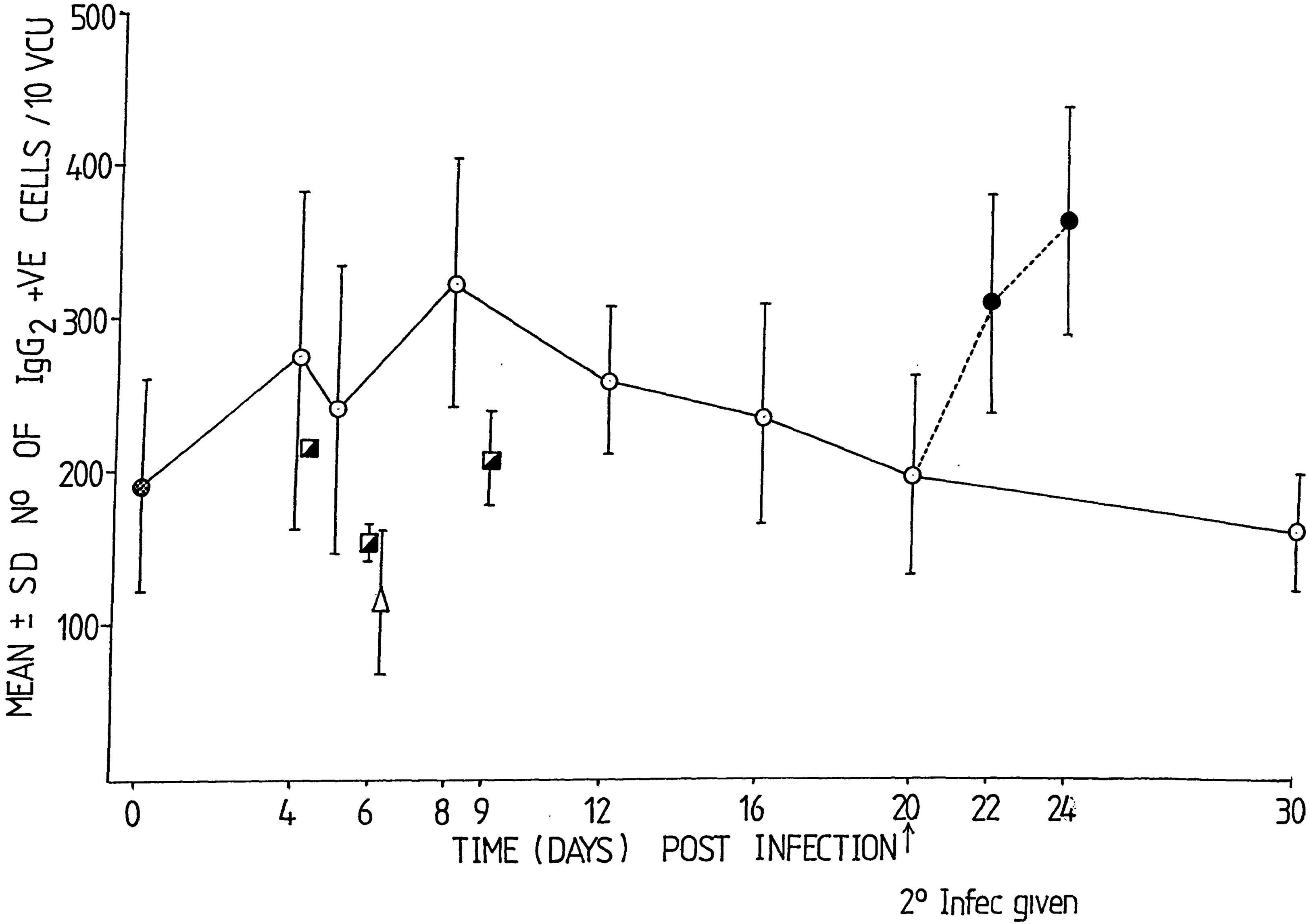
● = uninfected mice

◪ = recipients of 20 met. primary infection

○ = recipients of 200 met. primary infection

△ = " " " " " " treated with cortisone acetate, days 1,3,5 pi.

● = recipients of 200 met. secondary infection


MEAN ± SD Nº OF IgG2 +VE CELLS /10 VCU
500
400
300
200
100
0
4
6
8
9
12
16
20
22
24
30
TIME (DAYS) POST INFECTION
2º Infec given

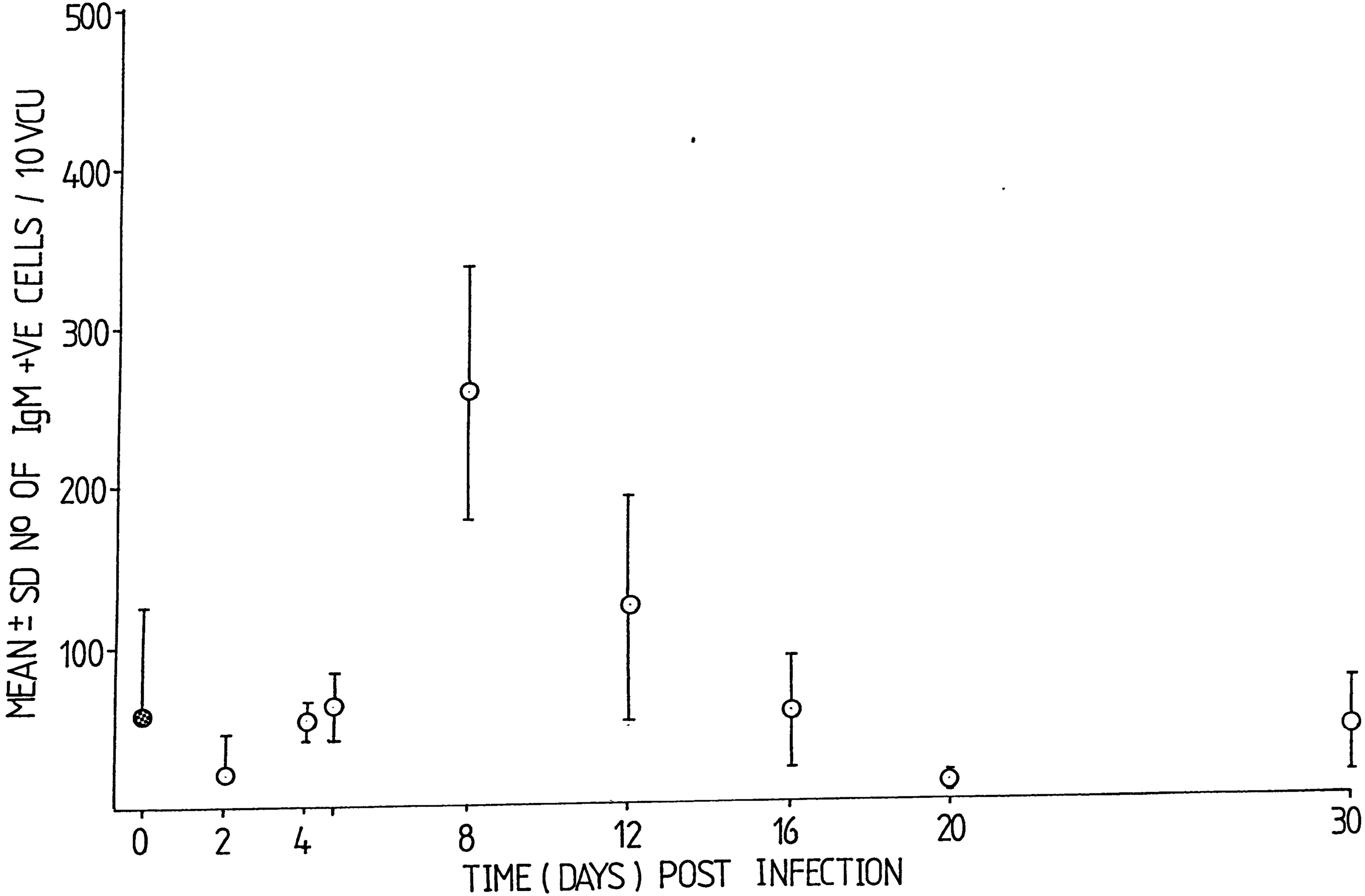
Facing page 195

Figure 5 - 16

Changes in the number of mucosal IgM+ve cells associated with *D.phoxini* infection

● = uninfected mice

○ = recipients of 200 met. primary infection


MEAN ± SD No OF IgM +VE CELLS / 10 VCU
500
400
300
200
100
0
2
4
8
12
16
20
30
TIME ( DAYS ) POST INFECTION

## Discussion

The experiments described in this chapter demonstrate some of the changes in the intestinal mucosa associated with D.phoxini infection, and allow assessment of the reproducibility of some of these characteristics to be made.

Rapid proliferation of globule leukocytes preceded expulsion of primary infection and declined after the termination of expulsion (Figs. 5-1, 5-3). The temporal relationship between onset of expulsion of secondary infection and globule leukocyte proliferation has not been adequately defined, however globule leukocyte proliferation is evident by day 4 pi when expulsion is underway (Figs. 5-2, 5-5). This cellular response has some of the characteristics of a T-dependent anamnestic response, but there is no evidence to suggest that the response is stronger in secondary than in primary infections. Globule leukocyte proliferation was both suppressed and retarded by corticosteroid treatment, which also delayed expulsion of the fluke infection. On balance, the results suggest that globule leukocytes might have some role in the expulsion of D.phoxini from the mouse intestine.

lamina propria mast cells do not appear to be involved in the normal response of the mouse to D.phoxini. Increases in numbers of these cells were very small, and delayed compared with the globule leukocyte response. The size of the response and its temporal relationship with expulsion of primary infection (Fig. 5-3) added to the absence of a response during expulsion of secondary infection (Fig. 5-5) suggests that these cells have little functional importance in the rejection of D.phoxini. These conclusions are consistent with those obtained for other mouse-helminth systems (Andreassen, Hindsbo and Ruitenberg 1978, Alizadeh 1981, Lee 1982).

The occurrence of eosinophilia in the intestine of infected mice also showed an anamnestic response to parasite infection (Fig.5-6), was inhibited by cortisone acetate (on the day tested) and was evident both before and during expulsion. There is no evidence of a lag between the proliferation of globule leukocytes and of eosinophils in primary and secondary infections;

indeed results superficially suggest that eosinophilia precedes globule leukocyte proliferation in secondary infection, however globule leukocyte numbers were elevated at the time of challenge, whereas eosinophilia had subsided. Mast cells are known to attract eosinophils to sites of inflammation. In view of the low and delayed response of lamina propria mast cells to infection it seems probable that globule leukocytes, which have many "mast cell" properties, may induce eosinophilia by chemotaxis, however other factors including T cell products, (which have been identified in T.spiralis infection) and complement components, may be chemotactic for eosinophils. There is no evidence to suggest that globule leukocytes are responsible for inducing the observed eosinophilia.

Interpretation of the response of goblet cells to infection is compromised by the lack of uninfected controls. The relatively small and inconsistent changes in goblet cell numbers associated with infection differ greatly from the rapid goblet cell proliferation associated with other helminth-rodent systems (Alizadeh 1981, Miller and Nawa 1979) however the results do not preclude increased mucin production by existing goblet cells. Although there is evidence to suggest a close relationship between lamina propria mast cells and goblet cells in the rat, it is possible that globule leukocytes and goblet cells represent mutually independent cell populations. Globule leukocytes are noticeably deficient in specific protease (RMCPll) particularly in rats infected with N.brasiliensis (Woodbury and Miller 1982). There is also evidence that lamina propria mast cells and globule leukocytes in mice are independent (Ruitenberg and Elgersma 1976, 1980). The kinetics of the two cellular responses to D.phoxini suggest that globule leukocytes, proliferation of which exceeds and precedes that of mast cells, are not derived from mature (Alcian Blue +ve) mast cells, but formation from a common precursor ( which might switch from differentiation to globule leukocytes in the latter stages of infection, to produce lamina propria mast cells instead) is possible.

Because globule leukocytes were indentified only on the basis of their alcian Blue -staining properties, it is possible that other cell types, for

instance intro=epithelial T cells, have been stained and counted as globule leukocytes, particularly in view of the results of Guy-Grand et al (1978).

The temporal relationship between fluke expulsion, globule leukocyte hyperplasia and eosinophilia in D.phoxini infection suggests that the role of inflammation in expulsion of flukes merits further investigation.

The immunological basis for these inflammatory changes was investigated using the techniques of cell separation and adoptive transfer of MLNC. The marked ability of sIg+ve (B) cells to transfer immunity to D.phoxini was unexpected, and contrary to results obtained in other rodent-helminth systems (Nawa and Miller 1979, Wakelin, Grencis and Donachie 1982, Lee 1982). Transfer of unseparated IMLNC (from mice harbouring a 200 metacercarial infection for six days) generated inflammatory changes some of which were variable: by day 4 pi recipients of unseparated IMLNC possessed marked elevation in numbers of globule leukocytes, but a very small (6 per vcu) increase in number of goblet cells. A major discrepancy lies in the number of eosinophils measured at this time. In one experiment, eosinophil numbers had increased in IMLNC recipients (compared with primary controls) as early as day 2 pi (Fig. 5-10), whereas in a subsequent experiment eosinophilia was not manifested by day 4 pi. This may have been due to a difference in the number of cells transferred, which was reduced from $8x10^7$ to $4x10^7$ in the second experiment (Fig. 5 - 12 IV), however this reduction in number of transferred cells would not be expected to reduce the effect of cell transfer on fluke expulsion (Chapter 4, Fig4-5)

In other systems it has been shown that inflammatory changes and anti-parasite immunity are transferred most effectively by sIg-ve IMLNC or TDL (Nava and Miller 1978, Nawa, Parish and Miller 1978, Nawa and Miller 1979, Wakelin, Grencis and Donachie 1982). In the present experiments, sIg-ve IMLNC failed to generate the globule leukocyte proliferation which was observed in recipients of unseparated IMLNC on day 4 pi, however the viability of separated and transferred T cells was markedly reduced.

It has been demonstrated (Chapter 4 Fig.4-9a) that blast cell activity is very high in sIg-ve and not sIg+ve IMLNC on day 6 of primary infection. The possibility that the selective depletion of certain T cells during this procedure is affecting the efficacy of T cells in subsequent cell transfer requires investigation.

In view of the nature of histopathological changes associated with D.phoxini infection, it is possible that the successful transfer of immunity with sIg+ve IMLNC may be attributable to the presence in infected MLN of activated B blasts or plasma cells responsible for the production of homocytotropic antibodies : IgE and perhaps IgG. It is possible that a period of migration, differentiation and/or proliferation of these sensitized cells occurs in the recipient without further antigenic stimulation. This is reflected in the necessity for a delay between cell administration and challenge of recipients, in order for measurable effects on parasite expulsion to occur (Chapter 4, Fig. 6a ). Migration of sensitized transferred MLN B cells to the recipient MLN or gut could be observed using cell labelling techniques. IgE production in MLN is associated with intestinal helminth infections in rats (Befus, Johnston, Berman and Bienenstock 1982). Although normal antigenic stimulation of the recipient immune system would occur after infection, the existence of a source of anti-parasite IgE at the time of infection would greatly accelerate the sensitization of globule leukocytes. The globule leukocyte represents a potential source of amine and other mediators which could initiate or amplify inflammatory changes in the recipient intestine.

The numbers of plasma cells producing other classes of immunoglobulin in the infected gut have not been measured adequately. It seems probable that increases in all classes of immunoglobulin producing cells are associated with infection, however the significance of this is not clear. Direct anti-parasite effects will only be assessed if antibody is identified bound to the parasite. Although interaction of antibody with complement, eosinophils and other components of the inflammatory response is theoretically possible, there is no evidence that this occurs.

## Summary

Histopathological changes in the mouse intestine associated with D.phoxini infection were characterized, and the effect of adoptively transferred immunity (via IMLNC) on these parameters was studied.

Infection was characterized by marked globule leukocyte proliferation and eosinophilia which preceded and accompanied the expulsion phase of infection. Both responses occurred more rapidly in secondary than in primary infection. By comparison, the response of lamina propria mast cells was delayed and very limited, and was not marked in secondary infection.

The response of goblet cells to infection was minor, and irregular during normal infection, however it is possible that mucus production by individual cells may be increased during infection.

Adoptive transfer of immunity led to an acceleration of all cellular responses. sIg+ve MLNC transferred immunity most effectively and generated a level of inflammation which was severe compared with normal infection, and was uncharacteristic as it involved increased goblet cell differentiation. The poor ability of T cells to transfer immunity might have been attributable to low viability and/or selective depletion during cell separation.

High variability was observed in the number of plasma cells in the intestine during infection. The most marked increases occurred in IgG, and IgM secreting plasma cells during primary infection, and IgG, during secondary infection.

## General Discussion

During the course of this study, it has become apparent that the D.phoxini mouse host parasite system is a potentially useful model for immunological studies: in addition to the ready availability of infective metacercariae, and the lack in the definitive host of immunological complications associated with parenteral juvenile stages, it has been found that a high, consistent level of establishment (> 80%) is attainable, allowing an accurate assessment of fluke losses during the course of infection.

The distribution of D.phoxini within the anterior region of the small intestine after oral infection is thought to reflect the attachment of the parasite to the first habitable site encountered in the mouse gut, however it is possible that D.phoxini is distributed over a wider area in the avian host. Ohman (1965) recovered D.spathaceum located in the mid region as well as the anterior of the small intestine of the gull. To investigate whether or not the distal regions of the small intestine presented an environment suitable for establishment and growth of D.phoxini, the survivorship and growth following implantation of metacercariae into different regions of the small intestine were compared. The results presented in chapter one (Fig.1-9 ) suggest that in general, establishment of implanted metacercariae was poorer than would be expected following oral infection. It was suggested that this might be due to cooling of the exposed gut during surgery, coupled with lack of pre-incubation, however if these were the only factors involved in poor retention, a mere delay in attachment of metacercariae might be expected to occur, resulting in a posterior displacement of fluke distribution. This does not seem to have occurred. Fig.1-7 shows that metacercariae that did establish remained initially close to the point of implantation. It is possible that non-attached (therefore probably non-feeding) D.phoxini metacercariae and adults have a very limited ability to survive conditions in the mouse gut. Posterior movement of established flukes from their site of initial attach-

ment was associated with reduced fluke growth or length, and only occurred over 10-15 cm before loss and presumably death of parasites occurred. It is difficult to evaluate any effects which detachment of flukes in itself might have on the organisms, because of the close association as in other systems of detachment and posterior drift with the immunological response and rejection of the parasite (Brambell 1965, Connan 1966, Blake 1974, Moqbel and Denham 1977, Kennedy 1980). In this context, close examination of flukes being lost from immunosuppressed mice would be of interest. In apparent contrast to D.phoxini, T.spiralis, although occupying an anterior location in the small intestine of most mouse strains, may be recovered alive from the large intestine during expulsion (Kennedy 1980).

Establishment of implanted metacercariae occurred very rarely in the posterior 40% of the small intestine (Fig.4-9) however the body lengths of recovered flukes were similar, irrespective of the positions of implantation, providing that flukes had not drifted in a posterior direction. This suggests that growth requirements are met in all regions of the small intestine. Conflicting with this, however, is the accelerated loss of flukes derived from metacercariae implanted in the posterior 70% of the small intestine.

The NIH mouse elicits a strong rapid immune response to primary D.phoxini infection and there is no evidence of tolerance of low level infections. The increase in longevity gained by serial transplantations and cortisone acetate treatment demonstrates that even low level (eight metacercariae) infections are expelled immunologically (after day 8 pi) (Chapter 2). The expulsion of heavier infections is accelerated loss of a 400 metacercarial infection beginning on about day 5 pi. Antigenic stimulation would be expected to increase with the size of infection, but in addition the host-parasite interface is a site of both chemical and mechanical trauma resulting in visible erosion or dissolution of the epithelium at the points of contact with the adhesive organ, lappets and ventral sucker (Ohman 1965, personal observation). It is therefore probable that inflammation generated non-specifically will augment immunologically

specifically generated responses (which almost certainly also include inflammatory components) especially in heavy infections.

The anamnestic response to homologous reinfection with D.phoxini was demonstrated in Chapter three, i.e. the immune response and its effects are accelerated in secondary infection: the parasite fails to mature, and is rejected by day 5-6 pi (Table 3-3, Fig. 3-1). Immunological memory is long lasting (at least seven months Fig. 3-4) and the protection stimulated by a five metacercarial infection is almost equivalent to the effect of a 200 metacercarial primary infection (Fig. 3-8).

The reversibility of suppression of growth, and development, (evident in suppression of vitelline development and achievement of patency) associated with secondary infection was demonstrated and expulsion of both primary and challenge flukes could be delayed, by transplantation to naive recipients (Fig. 2-6; 3-11). Such reversibility is characteristic of almost all rodent-gastro intestinal helminth systems investigated.

The successful adoptive transfer of immunity using IMLNC facilitates further analysis of the protective components of the immune response. In common with other systems, it was found that the level of immunity transferred is less than that which is stimulated by prior infection. An unusual and unexpected characteristic of this system was the failure of IMLNC given on the day of recipient challenge, to transfer immunity (Fig. 4-6). The necessity to inject IMLNC at least two days before recipient infection (at the 200 metacercariae level) probably reflects the minimum time required for the expression of immunity following cell transfer, for instance, six days are required for the expression of adoptively transferred immunity to T.spiralis in NIH mice (Wakelin and Wilson 1977a). In other more chronic parasite infections the need to investigate this parameter has not arisen, but it does seem likely that the six day pre-expulsion phase of (200 met.) D.phoxini infection does not allow sufficient time for transferred cells given on day of infection, to express immunity.

The use of recipient mice with low level infections, for instance

20 metacercariae, thus extending the duration of primary infection to at least eight days before rejection, would help to evaluate this factor. Interestingly it was also found that immunity to T.colubriformis was greater in guinea pigs which were challenged 28 days after IMLNC transfer than in recipients challenged on the day of cell transfer (Adams and Rothwell 1980).

It has generally been found that immunity transferred using IMLNC does not diminish when recipient challenge is delayed until several weeks after cell transfer. For instance challenge of CBA/ca mice with T.muris six weeks after cell transfer (Lee 1982) and challenge of NIH mice with T.spiralis 78 days after cell transfer (Wakelin, Grencis and Donachie 1982) occurred with no waning of the efficacy of transferred cells. Similar results were obtained with T.colubriformis (Adams and Rothwell 1980). It seems therefore that even in systems where transferred mediator cells are short-lived, they give rise to long-lived memory lymphocytes. Wakelin et al (1982) suggested that short-lived T-blasts gave rise to recirculating small lymphocytes which were stimulated into blast activity (and thereby regained mediator activity) upon recipient challenge. Transferred immunity to D.phoxini did not diminish when mice were challenged nineteen days after cell transfer. The ability of transferred cells to maintain their protective capacity for long periods is apparently not characteristic of cells from other sources in some systems, e.g. the efficacy of MLDC against T.colubriformis, waned after cell transfer , (Adams and Rothwell 1980).

The characteristics of cells capable of transferring immunity vary between host-parasite systems, and also vary with the time after donor infection that cells are transferred, for instance measurable protection was only transferred in B-TDLC from hyperimmune rats, long after infection, whereas B-cells taken during infection (Day 10 pi) were non-protective and T-cells were protective in both cases (Nawa Parish and Miller 1978). Preliminary results (Fig. 4-3) suggest that although the protective capacity of IMLNC against D.phoxini wanes after day 6 of primary infection,

it is later restored and day 21 IMLNC are protective. Such a regeneration of protective ability has not been demonstrated in other systems. IMLNC from T.spiralis - infected mice are no longer protective at this time, and although some protection was transferred with S C taken long after infection (Grencis 1983) it was suggested that this was due to presence of memory cells either in the spleen or the recirculating lymphocyte pool, which were not available in the MLN. From results obtained in other systems it does seem unlikely that long term protective memory cells in MLN could transfer immunity indefinitely. Indeed results (Fig. 4-4) suggest that the protective capacity of day 21 IMLNC is a transient phenomenon.

The association of successful adoptive transfer of immunity to T.spiralis in NIH mice with the availability of T.lymphoblasts in the MLN did not strictly apply to the D.phoxini - mouse system, in which lymphoblast activity remained high after the efficacy of IMLNC had diminished (Figs. 4-7, 4-9). Although the changes in the MLN equalled an increase apparently almost exclusively in T-lymphoblast activity, and in cellularity, - and indeed the inflammatory changes in the intestinal mucosa - are apparently very similar in the two systems, and attempt to transfer immunity against D.phoxini with the nylon wool non-adherent (T) fraction of IMLNC was unsuccessful, however viability of the cells concerned was low, and a further recent attempt to transfer immunity with T-IMLNC has been successful (Bowen, unpublished). The total lack of ability to transfer immunity and characteristic thymus dependent inflammatory changes in the mucosa, with T-IMLNC would conflict with the findings in every other gastro intestinal helminth system in which these parameters have been examined. Even in cases where B enriched TDL has been found in rats to be more effective at transferring immunity to T.spiralis, some protection was transferred with T cells, and neither fraction transferred immunity as effectively as unseparated cells (Crum, Despommier, McGregor 1977). Neither immunity to T.spiralis nor augmented inflammation were transferable to NIH mice with B-IMLNC (Alizadeh 1982).

In no host-intestinal parasite system characterised by intestinal inflammation has transfer of an enhanced inflammatory response by B-IMLNC been demonstrated, however there are instances in such systems ~~a~~ T.spiralis and N.brasiliensis in the rat - where protection has been transferred with B cells, furthermore it has been shown that some components of the inflammatory response to N.brasiliensis, principally LPMC and GC hyperplasia, can be transferred by immune serum as well as by TDL, and may therefore be mediated by several different pathways (Miller 1979, Befus and Bienenstock 1979, Miller and Nawa 1979). Neither of these cell types appears to be significantly involved in the inflammatory response to D.phoxini which, so far, has always been associated with rejection of this parasite from the mouse. The LPMC response to infection occurs long after rejection has occurred (Figs. 5-3, 5-5). As in most other mouse-intestinal helminth systems a predominantly globule leukocyte response occurred (although there are some exceptions to this generalisation, e.g. Mitchell, Westcott and Perryman 1983).

The goblet cell hyperplasia characteristic of the inflammatory response to many parasites, such as N.brasiliensis (Miller and Nawa 1979) and T.spiralis (Alizadeh 1982) was not observed (Fig. 5-7). A similar lack of goblet cell hyperplasia in the presence of a globule leukocyte response occurs in T.muris infection in mice, which is not characterized by gross inflammation (Lee 1982). A similar absence of goblet cell response was observed in rats infected with S.ratti in which a localised intestinal mastocytosis occurred (Mimori et al 1982). It seems therefore that although GC and MC responses may be stimulated by common factors (Walker, Wu, and Bloch, 1976, 1977, Lake, Bloch Neutra and Walker 1979), and MC mediators may also stimulate a GC response (Kownatzki et al 1977). The two may function independently, or weakly granulated GL may not have the stimulatory effect on GC which has been postulated for LPMC Eosinophilia occurred during D.phoxini infection (Fig. 5-6) and a large infiltrate thought to consist largely of neutrophils was also evident.

Hypothetically, mechanisms do exist whereby an inflammatory regime could be augmented by transferred B-IMLNC, though there is no evidence to suggest which, if any, do occur. Helminth infections are potent stimulators of parasite-specific IgE production, and also potentiate the production of IgE of other specificities. The possibility of a central role for IgE in the host response is indicated by the number of cell types bearing IgE receptors: mast cells, GL, eosinophils, macrophages and some lymphocytes. (The same may be said of complement). In N.brasiliensis - infected rats the MLN, draining the site of antigenic stimulation in the intestine, was described as the major site of IgE response (Ishizaka, Urbari and Ishizake 1976, Mayrhofer, Bazin, and Gowans 1976). The presence of a source of parasite - specific IgE would be expected to accelerate the occurrence of IgE mediated (Type 1) hypersensitivity in recipient mice on challenge, if indeed this mechanism is an important component of the response. The efficacy of immune serum transferred to rats challenged with N.brasiliensis was reduced (but not abrogated) by heating to 56° c, suggesting that IgE may be at least partly responsible for the immunity transferred, however IgE might be expected to stimulate activity or proliferation of goblet cells (Lake, Bloch, Sinclair and Walker 1980), a component which is not characteristic of the response to D.phoxini. Adoptive transfer of the parasite specific IgE response to N.brasiliensis in rats with TDL does not correlate well with the transfer of protective immunity or the transfer of an augmented LPMC response, and furthermore, it was not transferred with B or T cell fractions alone (Nawa, Miller, Hall and Jarrett 1981). Gabriel and Justus (1979) found that mice passively sensitized with serum containing both IgG and IgE antibodies showed accelerated expulsion of T.spiralis, and suggested that local anaphylactic reactions are an important component in host resistance. Mucosal anaphylaxis is thought to play a role in protecting "immune" hosts from further larval challenge of some nematodes (Rothwell, Love and Evans 1978, Lee and Ogilvie 1980, Miller, Huntley and Dawson 1981) However Bell,

McGregor and Adams (1982) concluded that immediate hypersensitivity was not a terminal effector of rapid expulsion of T.spiralis in rats. Suppression of total IgE response in infected rats does cause an elevation in the number of muscle larvae present (Dessaint, Parker, James and David 1981).

Augmentation of the inflammatory response generated on challenge of recipient mice might also occur via the transfer of IgG - B cells in IMLNC. The activity of IgG in this context is closely associated with that of complement. The generation of anaphylotoxins in IgG - immune complex lesions triggers mast cell activity (Askenase 1980) but a similar effect on globule leucocytes has not been demonstrated. Similarly it has not been shown that IgG can bind weakly to GL in the same way as to CTMC. Whether or not GL possess the same ability to stimulate the chemotaxis of accessory cells, and other inflammatory changes, by mediator release, the activators of complement, per se, might fulfil such a role. Anaphylotoxins are chemotactic for neutrophils and eosinophils, the two cell types, which appear to predominate in the cellular response to D.phoxini, and also affect vascular permeability (reviewed Leid and Williams 1979). In view of the potential effects of immunologically activated complement, it is possible that IgM may also contribute to such a mechanism. Identification of the cells involved in adoptive transfer of immunity, on the basis of immunoglobulin class, might aid the analysis of the mechanism of transfer of immunity.

Attempts to characterize the changes in plasma cell numbers associated with D.phoxini infection were wholly inadequate: variability in cell counts was high, and the number of mice examined too small (and lack of duplication was also an error). However results obtained did suggest that an increase in sIgG and IgM positive cells in the intestine was associated with primary infection and that an anamnestic increase in IgG+ve cells occurred after secondary infection.

The cellular inflammatory response occurring during D.phoxini infection is fairly typical of that observed in other systems, but the role of the globule leukocyte in this response remains unknown. Its role in anaphylaxis

and the generation of inflammation may not be equivalent to that of CTMC or LPMC. It may be also possible for GL to release chemical mediators without the requirement for granule exocytosis, as has been demonstrated in LPMC (Miller, Woodbury, Huntley and Newlands, 1983 ). In the T.muris/ mouse system, not characterized by gross inflammation, it was suggested that GL and/or intraepithelical lymphocytes possessed T-cell cytotoxic activity and interacted directly with T.muris (Lee 1982).

The inherent drawback in the D.phoxini - mouse system which remains to be resolved is a lack of accurate measurement of fecundity. This parameter would be particularly useful as an indicator of a reduced or late host immune response affecting populations of mature flukes (but not necessarily causing accelerated expulsion) for which no other criteria for evaluating immunological "damage" have yet been developed. There are several observations which suggest that the low egg counts recorded (Table1-6) may indeed be caused by depressed fecundity in the mouse, the body size attained by D.phoxini in the mouse and the number of eggs in utero are reduced compared with D.phoxini in avian hosts (Berrie 1960). Results in Chapter one also indicated that maturation is delayed in the mouse. In addition, a reduction in fecundity commonly precedes the expulsion of intestinal helminths. Because expulsion of a 200 metacercarial primary infection begins on day 6 pi and egg production is not evident until day 4, it is possible that the effects of a suboptimal environment combined with deleterious effects of the host response (perhaps effective before the onset of oviposition) may combine to reduce fecundity from the outset, to such an extent that it is not accurately measurable. The contribution of the second of these components might be evaluated by studying egg production in corticosteroid-treated mice. Although reduction of the size of infection would result in an increase in the period of time between patency and rejection of flukes (Fig. 2-1) and therefore perhaps allow a transient increase in fecundity, the reduction in number of parasites would in itself reduce the number of eggs, and almost certainly render this approach

ineffective.

If fecundity does in the future prove to be immeasurable, it will be necessary to develop alternative criteria, more sensitive than measurement of rejection, in order to measure immunological effects on adult flukes. This would be particularly useful in the adoptive transfer system. Methods of ultrastructural study, although cumbersome, might ultimately be required in this context.

# REFERENCES

ADAMS D.B.& ROTHWELL T.L.W. 1977. Trichostrongylus colubriformis: Host factors influencing the transfer of immunity in guinea-pigs using mesenteric lymph node cells. Experimental Parasitology 42: 121-128.

ADAMS D.B.& ROTHWELL T.L.W. 1980. The role of lymphocytes in immunological memory for resistance to infection by Trichostrongylus colubriformis in guinea pigs. Cellular Immunology 55:1-11.

ALGHALI S.T.O. 1981. Intestinal immunity to tapeworms: The rejection of Hymenolepis citelli by mice and rats.

Ph.D. Thesis,University of Glasgow.

ALIZADEH H. 1982. Immunological and inflammatory responses in the intestines of mice infected with the parasitic nematode Trichinella spiralis.

Ph.D. Thesis,University of Glasgow.

ALIZADEH H.& WAKELIN D. 1981. Mechanisms of rapid expulsion of Trichinella spiralis from mice. In: Trichinellosis. Eds KIM C.W. & RUITENBERG E.J. pp.81-84. Reedbooks,Surrey,England.

ALIZADEH H.& WAKELIN D. 1982. Comparison of rapid expulsion of Trichinella spiralis in mice and rats. International Journal for Parasitology 12: 65-73.

ANDRE A. 1918.Notes de parasitologie. Bull.Soc.Vaud.Sci.Nat.52:12-13

ANDREASSEN J.,HINDSBO O.&RUITENBERG E.J. 1978. Hymenolepis diminuta infections in congenitally athymic (nude) mice: worm kinetics and intestinal histopathology. Immunology 34: 105-113.

ANDREASSEN J.& HOPKINS C.A. 1980. Immunologically mediated rejection of Hymenolepis diminuta by its normal host,the rat. Journal of Parasitology 66: 898-903.

ARVY L. 1954. Distomatose cérébro-rachidienne due à Diplostomulum phoxini (Faust) Hughes 1929, chez Phoxinus laevis. Annales de Parasitologie Humaine et Comparée 29: 510-520.

ARVY L. & BUTTNER A. 1954. Données sur le cycle évolutif de Diplostomulum phoxini (Faust 1918)(Trematoda, Diplostomatidae). C.r.Acad.Sci.Paris 239: 1085-1087.

ARVY L. & BUTTNER A. 1955. Cycle évolutif de Diplostomulum phoxini (Faust 1918)(Diplostomatidae). Bulletin Societe Zoologique Francaise 80: 104-105.

ASHWORTH J.H. & BANNERMAN J.C.W. 1927. On a tetracotyle in the brain of the minnow. Transactions of the Royal Society of Edinburgh 55: 159-173.

ASKENASE P.W. 1977. The role of basophils, mast cells and vasoamines in hypersensitivity reactions with a delayed time course. Progress in Allergy 23: 199-320.

ASKENASE P.W. 1980. Immunopathology of parasitic diseases: Involvement of basophils and mast cells. Springer Seminars in Immunopathology 2: 1 - 59

AU A.C.S. & KO R.C. 1979. Cross-resistance between Trichinella spiralis and Angiostrongylus cantonensis in laboratory rats. Zeitschrift fur Parasitenkunde 59: 161-168.

BACHA W.J.Jr. 1964. Effect of salt solutions on the establishment of infections of the trematode Zygocotyle lunata in white rats. Journal of Parasitology 50: 546-548.

BAGGIOLINI M.,HORISBERGER U. & MARTIN U. 1982. Phagocytosis of mast cell granules by mononuclear phagocytes,neutrophils and eosinophils during anaphylaxis. International Archives of Allergy and Applied Immunology 67: 219-226.

BARTH E.E.E.,JARRET W.F.A. & URQUHART G.M. 1966. Studies on the mechanism of the self-cure reaction in rats infected with Nippostrongylus brasiliensis. Immunology 10: 459-464.

BASCH P.F.,DICONZA J.J. & JOHNSON B.E. 1973. Strigeid trematodes (Cotylurus lutzi) cultured in vitro: production of normal eggs with continuance of life cycle. Journal of Parasitology 59: 319-332.

BASTEN A. & BEESON P.B. 1970. Mechanism of eosinophilia 11:Role of the lymphocyte. Journal of Experimental Medicine 131: 1288

BEFUS A.D. 1975. Intestinal immune responses of mice to the tapeworms Hymenolepis diminuta and Hymenolepis microstoma. Ph.D. thesis,University of Glasgow.

BEFUS A.D. 1977. Hymenolepis diminuta and Hymenolepis microstoma: Mouse immunoglobulins binding to the tegumental surface. Experimental Parasitology 41: 242-251.

BEFUS A.D. & BIENENSTOCK J. 1979. Immunologically mediated intestinal mastocytosis in Nippostrongylus brasiliensis-infected rats. Immunology 38: 95-101.

BEFUS A.D.,DENBURG J.& BIENENSTOCK J. 1979. Mechanisms of intestinal mastocytosis, in The mast cell:its role in health and disease. Eds Pepys J.& Edwards A.M. Pitman, London 115-122

BEFUS A.D.,JOHNSTON N.,BERMAN L.& BIENENSTOCK J. 1982. The relationship between tissue sensitization and IgE antibody production in rats infected with the nematode Nippostrongylus brasiliensis. International Archives of Allergy and Applied Immunology 67: 213-218.

BEFUS A.D.,PEARCE F.L.,GAULDIE J.,HORSEWOOD P.,GOODACRE R.L.,COLE F., HEATLEY R,V.,& BIENENSTOCK J. 1979. Isolation and characteristics of mast cells from the lamina propria of the small bowel. In: The mast cell,its role in health and disease, Eds PEPYS J.& EDWARDS A.M. Pitman, London. pp 702-709.

BEHNKE J.M. 1974. The biology of Aspiculuris tetraptera Schulz ( Nematoda, Oxyuroidea ).
Ph.D. thesis University of London

BEHNKE J.M.& PARISH H.A. 1979. Expulsion of Nematospiroides dubius from the intestine of mice treated with immune serum. Parasite Immunology 1: 13-26.

BEHNKE J.M.& PARISH H.A. 1981. Transfer of immunity to Nematospiroides dubius: Cooperation between lymphoid cells and antibodies in mediating worm expulsion. Parasite Immunology 3: 249- 259.

BEHNKE J.M.,WAKELIN D.& WILSON M.M. 1978. Trichinella spiralis: Delayed rejection in mice concurrently infected with Nematospiroides dubius. Experimental Parasitology 46: 121-130.

BELL E.J. 1956. The development of Diplostomum phoxini in vivo and in vitro. Ph.D.Thesis,University of Glasgow.

BELL E.J.&HOPKINS C.A. 1956. The development of Diplostomum phoxini (Strigeida,Trematoda).Annals of Tropical Medicine and Parasitology 50: 275-282.

BELL E.J.& SMYTH J.D. 1958. Cytological and histochemical criteria for evaluating development of trematodes and pseudophyllidean cestodes in vivo and in vitro. Parasitology 48: 131-148.

BELL R.G.,McGREGOR D.P.& ADAMS L.S. 1982. Studies on the inhibition of rapid expulsion of Trichinella spiralis in rats. International Archives of Allergy and Applied Immunology 69: 73-80.

BERRIE A.D. 1960. The influence of various definitive hosts on the development of Diplostomum phoxini (Strigeida, Trematoda). Journal of Helminthology 34: 205-210.

BIENENSTOCK J.,BEFUS A.D.,& McDERMOTT M. 1981. Mucosal immunity. In: The mucosal immune system. Ed. BOURNE pp5-27 Publ. Martinus Nijhoff.

BLAIR D. 1974. Life cycle studies on strigeoid trematodes. Ph.D. thesis, University of Glasgow.

BLAKE C.J. 1973. Evidence for a possible immune reaction against an intestinal trematode. Parasitology 67: XVII

BLAKE C.J. 1974. Studies on aspects of the host parasite relationship of Apatemon gracilis minor ( Trematoda, Strigeidae ). Ph.D. thesis, University of Cardiff.

BLAND P.W. 1976. Immunity to Hymenolepis diminuta: Unresponsiveness of the athymic (nude) mouse to infection. Parasitology 72: 93-97.

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

BURSZTAJN S.,ASKENASE P.W.,GERSHON R.K.& GERSHON M.D. 1978. Role of vasoactive amines during early stages of delayed type hypersensitivity skin reactions. Fed. Proc. 37: 590

BUTTERWORTH A.E. 1980. Eosinophils and immunity to parasites. Transactions of the Royal Society of Tropical Medicine and Hygiene 74: 38-43.

BUTTERWORTH A.E. 1981. Eosinophil Function. New England Journal of Medicine 304: 154

BUTTERWORTH A.E., WASSOM D.L., GLEICH G.J. LOEGERING D.A., & DAVID J.R. 1979. Damage to schistosomula of S.mansoni-induced directly by eosinophil major basic protein. Journal of Immunology 122: 221

CAMPBELL D.H. Experimental eosinophilia with keratin from Ascaris suum and other sources. Journal of Infectious Diseases 71: 270-276.

CAMPBELL W.C. 1963. Spontaneous cure in Trichuris muris infections in albino mice, and its suppression by cortisone. Journal of Parasitology 49: 628-632.

CASTRO G.A.,BADIAL-ACEVES F.,SMITH J.W., DUDRIC S.J. & WEISBRODT N.W. 1976. Altered small bowel propulsion associated aith parasitism Gastroenterology 71: 620

CHRISTENSEN N.O., NYDAL R., FRANDSEN F. & NANSEN P. 1981 Homologous immunotolerance and decreased resistance to Schistosoma mansoni in Echinostoma revolutum - infected mice. Journal of Parasitology 67: 164-166.

CHRISTIE P.R. 1979. The intestinal immune response of the mouse to the tapeworm Hymenolepis diminuta. Ph.D. thesis , University of Glasgow.

CHRISTIE P.R., WAKELIN D.,& WILSON M.M. 1979. The effect of the expulsion phase of Trichinella spiralis on Hymenolepis diminuta infection in rats. Parasitology 78: 323-330.

CLAMAN H.N. 1972. Corticosteroids and lymphoid cells. New England Journal of Medicine 287: 388-397.

CLAMAN H.N. 1975. How corticosteroids work. Journal of Allergy and Clinical Immunology 55: 145

COLLEY D.G. 1973. Eosinophils and immune mechanisms. Journal of Immunology 110: 1419

CONNAN R.M. 1966. Experiments with Trichostrongylus colubriformis (Giles 1892) in the guinea-pig 1: The effect of the host response on the distribution of the parasites in the gut. Parasitology 56: 521-530.

CONNAN R.M. 1972. Passive protection with homologous antiserum against Trichostrongylus colubriformis in the guinea-pig. Immunology 23: 647-650.

CRUM E.D., DESPOMMIER D.D.& McGREGOR D.D. 1977. Immunity to Trichinella spiralis 1: Transfer of resistance by two classes of lymphocytes. Immunology 33: 787-795.

DAVENPORT H.W. 1977. Physiology of the digestive tract. An introductory text. Chicago: Year Book Medical Publishers.

DAWKINS H.J.S. & GROVE D.I. 1981. Transfer by serum and cells of resistance to infection with Strongyloides ratti in mice. Immunology 43: 317-322.

DESPOMMIER D.D., McGREGOR D.D., CRUM E.D. & CARTER P.B. 1977. Immunity to Trichinella spiralis 11: Expression of immunity against adult worms. Immunology 33: 797-805.

DESSEIN A.J., PARKER W.L., JAMES S.L.& DAVID J.R. 1981. IgE antibody and resistance to infection I: Selective suppression of the IgE antibody response in rats diminishes the resistance and the eosinophil response to Trichinella spiralis infection. Journal of Experimental Medicine 153: 423-436.

DINEEN J.K., GREGG P., WINDON R.G., DONALD A.D.& KELLY J.D. 1977. The role of immunologically specific and non-specific components of resistance in cross-protection to intestinal nematodes. International Journal for Parasitology 7: 211-215.

DINEEN J.K. & KELLY J.D. 1973. Expulsion of Nippostrongylus brasiliensis from the intestine of rats : The role of a cellular component derived from bone marrow. International Archives of Allergy and Applied Immunology 45:504-512.

DINEEN J.K. & KELLY J.D. 1976. Levels of prostaglandins in the small intestine of rats during primary and secondary infection with Nippostrongylus brasiliensis. International Archives of Allergy and Applied Immunology 51:429-440.

DINEEN J.K., KELLY J.D., GOODRICH B.S.& SMITH I.D. 1974. Expulsion of Nippostrongylus brasiliensis from the small intestine of the rat by prostaglandin-like factors from ram semen. International Archives of Allergy and Applied Immunology 46:360-374.

DINEEN J.K., RONAI P.M. & WAGLAND B.M. 1968. The cellular transfer of immunity to Trichinella spiralis in an isogenic strain of guinea-pig IV: The localisation of immune lymphocytes in small intestine in infected and non-infected guinea-pigs. Immunology 15: 671-679.

DOBSON C. 1967. Changes in the protein content of the serum and intestinal mucus of sheep with reference to the histology of the gut and immunological response to Oesophagostomum columbianum infections. Parasitology 57: 201

DOBSON C. & OWEN M.E. 1978. Effect of host sex on passive immunity in mice infected with Nematospiroides dubius. International Journal for Parasitology 8: 359-364.

DONGES J. 1969. Diplostomum phoxini(Faust 1918)(Trematoda). Morphologie des miracidiums sowie beobachtungen an weiteren Entwicklungsstadien. Zeitschrift fur Parasitenkunde 32:120-127.

DORSETT B.H. & IOACHIM H.L. 1978. A method for the use of immunofluorescence on paraffin-embedded tissues. American Journal of Clinical Pathology 69: 66-72.

DRACOTT B.N.& SMITH C.E.T. 1979. Hydrocortisone and the antibody response in mice 1: Correlations between serum cortisol levels and cell numbers in thymus,spleen,marrow and lymph nodes. Immunology 38: 429

DUBOIS G. 1953. Systematique des strigeida (Trematoda). Memoires de la Societe Neuchateloise des Sciences Naturelle 6:1-535.

DUBOIS G. 1964. Du statut de quelques Strigeata La Rue 1926 (Trematoda). Bulletin de la Societe Neuchateloise des Sciences Naturelles87: 27-71.

DUBOIS G. 1970. Synopsis des Strigeidae et des Diplostomatidae (Trematoda). Memoires de la societe Neuchateloise des Sciences Naturelles 10: 259-727.

DURKIN H.G., BAZIN H. & WAKSMAN B.H. 1981. Origin and fate of IgE bearing lymphocytes 1: Peyers patches as differentiation site of cells simultaneously bearing IgA and IgE. Journal of Experimental Medicine 154: 640

ELOWNI E.E. 1980 Immunity to tapeworms: Vaccination against Hymenolepis diminuta and the role of the bursa of Fabricius in rejection of Raillietina cesticillus. Ph.D. thesis , University of Glasgow.

ELOWNI E.E. 1982. Hymenolepis diminuta: source of protective antigens as determined by irradiation and chemical elimination of immunizing worms. Experimental Parasitology 54: 1-6.

ERASMUS D.A. 1968. The host-parasite interface of Cyathocotyle-bushiensis Khan 1962 (Trematoda;Strigeoidea) 111: Electron microscope observations on non specific phosphatase activity. Parasitology 58: 371-375.

ERASMUS D.A. 1969. Studies on the host-parasite interface of strigeoid trematodes Vl: Ultrastructural observations on the lappets of Diplostomum phoxini Faust 1918. Zeitschrift fur Parasitenkunde 32: 48-58.

ERASMUS D.A. 1970. The host-parasite interface of strigeoid trematodes Vll: Ultrastructural observations on the adhesive organ of Diplostomum phoxini Faust 1918. Zeitschnift fur Parasitenkunde 33: 211-224.

ERASMUS D.A. & BENNETT L.J. 1965. A study of some of the factors affecting excystation in vitro of the metacercarial stages of Holostephanus luhei Szidat 1936 and Cyathocotyle bushiensis Khan 1962 (Strigeida;Trematoda). Journal of Helminthology 39: 185-196.

ERASMUS D.A.& OHMAN C. 1963. The structure and function of the adhesive organ in strigeid trematodes. Annals of the New York Academy of Sciences 113: 7-35.

FANG W.F., BROUGHTON A.& JACOBSEN E.D. 1977. Indomethacin-induced intestinal inflammation. American Journal of Digestive Diseases 22: 749

FAUCI A.S. 1975. Corticosteroids and circulating lymphocytes. Transplantation Proc. 7: 37.

FINE D.P.,BUCHANAN R.D.& COLLEY D.G. 1973. Schistosoma mansoni in mice depleted of thymus dependent lymphocytes.1:Eosinophilia and immunologic responses to a schistosomal egg preparation. American Journal of Pathology 71: 193.

FLOREY H. 1955. Mucin and the protection of the body.
Proceeds of the royal Society of London 143: 147-158.

FRICK L.P.& ACKERT J.E. 1948. Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism.
Journal of Parasitology 34: 192.

GABRIEL B.W.& JUSTUS D.E. 1979. Quantitation of immediate and delayed hypersensitivity responses in Trichinella spiralis-infected mice.
International Archives of Allergy and Applied Immunology 60:275-285.

GERSHON R.K.,ASKENASE P.W.& GERSHON M.D. 1975. Requirement for vasoactive amines for production of delayed type hypersensitivity skin reactions.
Journal of Experimental Medicine 143: 732-747.

GILLON J. 1981. Where do mucosal mast cells acquire IgE?
Immunology Today 2: 80-81.

GLEICH G.J.,OLSON G.M.& HERLICH H. 1979. The effect of antiserum to eosinophils on susceptibility and acquired immunity of the guinea-pig to Trichostrongylus colubriformis.
Immunology 37: 873-880.

GOETZL E.J.& AUSTEN K.F. 1977. Cellular characteristics of the eosinophil compatible with a dual role in host defence in parasite infections.
American Journal of Tropical Medicine and Hygiene 26:142.

GORMAN A. M. 1980. Studies on the biology of Plagiorchis elegans (Rudolphi 1802)(Trematoda,Digenea) in its mammalian and molluscan hosts.
Ph.D. Thesis, University of Leeds.

GOULSON H.T.,OTTOLENGHI A. & LARSH J.E. 1981.
Phospholipase B in non-sensitized and sensitized rats after challenge with Strongyloides ratti.
American Journal of Tropical Medicine and Hygiene 30: 350-357.

GOVEN A.J. 1979a. The phospholipase B content of intestines of sensitized rats infected with varied larval doses of Nippostrongylus brasiliensis.
International Journal for Parasitology 9: 193-198.

GOVEN A.J. 1979b. The phospholipase B content of intestines of sensitized rats challenged with varied larval doses of Nippostrongylus brasiliensis.
International Journal For Parasitology 9: 345-349.

GOVEN A.J.& MOORE G.W. 1980. Phospholipase B activity in congenitally athymic (nude) mice infected with Trichinella spiralis.
Zeitschrift fur Parasitenkunde 61: 265-269.

GRAY J.S. 1973. Studies on host resistance to secondary infections of Raillietina cesticillus in the fowl.
Parasitology 67: 375-382.

GRAY J.S. 1976. The cellular response of the fowl small intestine to primary and secondary infections of the cestode Raillietina cesticillus (Molin.).
Parasitology 73: 189-204.

GREENE B.& COLLEY D.G. 1976. Eosinopnils and immune mechanisms III: production of the lymphokine,eosinopnil stimulation promoter, by mouse T lympnocytes.
Journal of Immunology 116: 1078

GRENCIS R.K. 1983.

Immunity to Trichinella spiralis.

Ph.D. Thesis , University of Glasgow.

GRENCIS R.K.& WAKELIN D. 1982. Short-lived,dividing cells mediate adoptive transfer of immunity to Trichinella spiralis in mice. 1: Availability of cells in primary and secondary infections in relation to cellular changes in the mesenteric lymph node. Immunology 46: 443-450.

GROVE D.J., MAHMOUD A.A.F.& WARREN K.S. 1977. Eosinophils and resistance to Trichinella spiralis. Journal of Experimental Medicine 145: 755-759.

GUY-GRANDE D., GRISCELLI C.& VASSALLI P. 1974. The gut-associated lymphoid system: nature and properties of large dividing cells. European Journal of Immunology 4: 435-443.

GUY-GRANDE D., GRISCELLI C.& VASSALLI P. 1978. The mouse gut T lymphocyte; a novel type of T cell: Nature,origin and traffic in mice in normal and graft-versus-host conditions. Journal of Experimental Medicine 148: 1661-1677.

GUY-GRANDE D.,& VASSALLI P. 1981. Nature and function of gut granulated T lymphocytes. in : Recent Advances in mucosal Immunology. Eds STROBER &SELL . Raven Press, New York.

HAGAN P.,BEHNKE J.M.& PARISH H.A. 1981. Stimulation of immunity to Nematospiroides dubius in mice using larvae attenuated by Cobalt 60 irradiation. Parasite Immunology 3: 149-156.

HANDLINGER J.H. & ROTHWELL T.L.W. 1981. Studies of the responses of basophil and eosinophil leucocytes and mast cells to the nematode Trichostrongylus colubriformis: comparison of cell populations in parasite resistant and susceptible guinea-pigs. International Journal for Parasitology 11: 67-70.

HANDWERGER B.& SCHWARTZ R.H. 1974. Separation of murine lymphoid cells using nylon wool columns: Recovery of the B cell enriched population. Transplantation 18: 544-548.

HIGASHI G.I.& CHOWDHURY A.B. 1970 In vitro adhesion of eosinophils to infective larvae of Wuchereria bancrofti. Immunology 19: 65.

HINDSBO O.J.,ANDREASSEN J.& RUITENBERG E.J. 1982. Immunological and histopathological reactions of the rat against the tapeworm Hymenolepis diminuta and the effects of anti-thymocyte serum. Parasite Immunology 4: 59 -76.

HOPKINS C.A. 1970. Location-specificity in adult tapeworms with special reference to Hymenolepis diminuta. Second International Congress of Parasitology. Journal of Parasitology 56: 561-4

HOPKINS C.A. 1980. Immunity and Hymenolepis diminuta. in: Biology of the tapeworm Hymenolepis diminuta. Ed. ARAI H.P. pp 551-614. Academic Press. New York.

HOPKINS C.A. 1982. Immunological memory in mice to adult Hymenolepis diminuta. Journal of Parasitology 68: 32-38.

HOPKINS C.A.& BARR I.F. 1982. The source of antigen in an adult tapeworm. International Journal for Parasitology 12: 327-333.

HOPKINS C.A.,GOODALL R.I.& ZAJAC A. 1977. The longevity of Hymenolepis microstoma in mice and its lmmunological cross-reaction with Hymenolepis diminuta. Parasitology 74: 175-183.

HOPKINS C.A.& STALLARD H.E. 1974. Immunity to intestinal taoeworms: The rejection of Hymenolepis citelli by mice. Parasitology 69: 63-76.

HOPKINS C.A.&STALLARD H.E. 1976. The effect of cortisone on the survival of Hymenolepis diminuta in mice.
Rice University Studies 62: 145-159.

HOPKINS C.A.,SUBRAMANIAN G.& STALLARD H.E. 1972a. The development of Hymenolepis diminuta in primary and secondary infections in mice.
Parasitology 64: 401-412.

HOPKINS C.A.,SUBRAMANIAN G.& STALLARD H.E. 1972b. The effect of immunosuppressants on the development of Hymenolepis diminuta in mice.
Parasitology 65: 111-120.

HOPKINS C.A.& ZAJAC A. 1976. Transplantation of Hymenolepis diminuta into naive,immune and irradiated mice.
Parasitology 73: 73-81.

HOWARD R.J. 1976. The growth of secondary infections of Hymenolepis microstoma in mice: The effect of various primary infection regimes.
Parasitology 72: 317-323.

HOWARD R.J., CHRISTIE P.R., WAKELIN D., WILSON M.M.& BEHNKE J.M. 1978. The effect of concurrent infection with Trichinella spiralis on Hymenolepis microstoma in mice.
Parasitology 77: 273-279.

HURLEY F.J. 1959. Immunization threshold in laboratory rats given a small initial infection of Nippostrongylus muris.
Proceeds of the Helminthological Society of Washington 26: 91-96.

ISHIZAKA T.,URBAN J.& ISHIZAKA K. 1976. IgE formation in the rat following infection with Nippostrongylus brasiliensis 1: Proliferation and differentiation of IgE bearing cells.
Cellular Immunology 22: 248.

JACOBSEN R.H.,REED N.D.& MANNING D.D. 1977. Expulsion of Nippostrongylus brasiliensis from mice lacking antibody production potential.
Immunology 32: 867-874.

JARRETT W.F.H.,JARRETT E.E.E.,MILLER H.R.P.,& URQUHART G.M. 1968. Quantitative studies on the mechanism of self cure in Nippostrongylus brasiliensis infections.
Proceeds of the Third International Conference of the World Association for the Advancement of Veterinary Parasitology, in: The reaction of the host to parasitism. Ed. SOULSBY E.J. pp. 191-198.

JENKINS S.N.& BEHNKE J.M. 1977. Impairment of primary expulsion of Trichuris muris in mice concurrently infected with Nematospiroides dubius.
Parasitology 75: 71-78.

JOHNSON L.R. 1977.
in: Gastrointestinal Physiology. Ed. JOHNSON L.R.

JONES V.E.,EDWARDS A.J.& OGILVIE B.M. 1970. The circulating immunoglobulins involved in protective immunity to the intestinal stages of Nippostrongylus brasiliensis in the rat.
Immunology 18: 621-623.

JONES V.E.& OGILVIE B.M. 1971. Protective immunity to Nippostrongylus brasiliensis: The sequence of events which expels worms from the rat intestine.
Immunology 20: 546-561.

JULIUS M.H.,SIMPSON E.& HERZENBERG L.A. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes.
European Journal of Immunology 3: 645-649.

KANNANGARA D.W.W.& SMYTH J.D. 1974. In vitro cultivation of Diplostomum spathaceum and Diplostomum phoxini metacercariae. International Journal for Parasitology 4: 667-673.

KASSAI T.,REDL P.,JECSAI G.,BALLA E.& HARANGOZO E. 1980. Studies on the involvement of prostaglandins and their precursors in the rejection of Nippostrongylus brasiliensis from the rat. International Journal for Parasitology 10: 115-120.

KAZAKOS R.K. 1975. Increased resistance in the rat to Nippostrongylus brasiliensis following immunization against Trichinella spiralis. Veterinary Parasitology 1: 165-174.

KAZAKOS R.K.& THORSON R.E. 1975. Cross-resistance between Nippostrongylus brasiliensis and Strongyloides ratti in rats. Journal of Parasitology 61: 525-529.

KAZURA J.W.& GROVE D.I. 1978. Stage-specific, antibody-dependant, eosinophil-mediated destruction of Trichinella spiralis. Nature, London 274:588-589.

KELLY J.D.& DINEEN J.K. 1972. The cellular transfer of immunity to Nippostrongylus brasiliensis in inbred rats (Lewis strain). Immunology 22: 199-210.

KELLY J.D., DINEEN J.K., GOODRICH B.S.& SMITH I.D. 1974. Expulsion of Nippostrongylus brasiliensis from the intestine of rats: Role of prostaglandins and pharmacologically active amines (histamine, 5-hydroxytryptamine) in worm expulsion. International Archives of Allergy and Applied Immunology 47: pp. 458-465.

KELLY J.D.& OGILVIE B.M. 1972. Intestinal mast cell and eosinophil numbers during worm expulsion in nulliparous and lactating rats infected with Nippostrongylus brasiliensis.
International Archives of Allergy and Applied Immunology 43: 497-509.

KENNEDY M.W. 1976. Kinetics of establishment and rejection of the enteral phase of a primary infection of Trichinella spiralis in the NIH strain mouse.
Transactions of the Royal Society of Tropical Medicine and Hygiene 70: 285.

KENNEDY M.W. 1979. Effects of the host immune response on the intestinal phase of Trichinella spiralis in mice.
Ph.D. Thesis, University of Glasgow.

KENNEDY M.W. 1980a Effects of the host immune response on the longevity,fecundity and position in the intestine of Trichinella spiralis in mice.
Parasitology 80: 49-60.

KENNEDY M.W. 1980b Immunologically mediated, non-specific interactions between the intestinal phases of Trichinella spiralis and Nippostrongylus brasiliensis in the mouse.
Parasitology 80: 61-72.

KENNEDY M.W.& BRUCE R.G. 1981. Reversibility of the effects of the host immune response on the intestinal phase of Trichinella spiralis in the mouse, following transplantation to a new host.
Parasitology 82: 39-48.

KENNEDY M.W., WAKELIN D.& WILSON M.M. 1978. Transplantation of adult Trichinella spiralis between hosts: Worm survival and immunological characteristics of the host-parasite relationship.
Parasitology 78: 121-130.

KIM C.W.& LEDBETTER M.G. 1981. Detection of specific antigen-antibody precipitates on the surface of Trichinella spiralis by scanning electron microscopy. In: Trichinellosis, Eds KIM C.W. and RUITENBERG E.J. pp. 65-69. Reed Books Ltd. Surrey.

KITAMURA Y.,GO S.,SHIMADA M.,MATSUDA H.,HATANAKA K.& SEKI M. 1979. Distribution of mast cell precursors in hematopoietic and lymphopoietic tissues of mice.
Journal of Experimental Medicine 150: 482.

KLAUS G.G.B.,PEPYS M.B.,KITAJIMA K.& ASKONAS B.A. 1979. Activation of mouse complement by different classes of mouse antibody.
Immunology 38: 687.

KOWNATZKI,E.,TILL G.,GAGELMANN M.,TERWORT G.& GEMSA D. 1977. Histamine induces release of an eosinophil-immobilizing factor from mononuclear cells.
Nature,London 270: 67.

LAKE A.N.,BLOCH K.J.,SINCLAIR K.J.& WALKER W.A. 1980. Anaphylactic release of intestinal goblet cell mucus.
Immunology 39: 173.

LARSH J.E.,GOULSON H.J.,WEATHERLY N.F. 1964. Studies on delayed (cellular) hypersensitivity in mice infected with Trichinella spiralis 11: Transfer of peritoneal exudate cells.
Journal of Parasitology 50: 496-8.

LARSH J.E.,OTTOLENGHI A.& WEATHERLY N.F. 1974. Trichinella spiralis: Phospholipase in challenged mice and rats.
Experimental Parasitology 36: 299-306.

LARSH J.E.,OTTOLENGHI A.& WEATHERLY N.F. 1975. Trichinella spiralis: Phospholipase in sensitized mice after challenge.
Experimental Parasitology 37: 233-238.

LARSH J.R.& RACE G.J. 1954. A histopathological study of the anterior small intestine of immunized and non-immunized mice infected with Trichinella spiralis. Journal of Infectious Diseases 94: 262-272.

LARSH J.R.& RACE G.J. 1975. Allergic inflammation as an hypothesis for the expulsion of worms from tissues: a review. Experimental Parasitology 37: 251-266.

LEE D.L. 1962. Studies on the function of the pseudosuckers and holdfast organ of Diplostomum phoxini (Faust)(Strigeida Trematoda) Parasitology 52: 103-112.

LEE D.L. 1969. Changes in adult Nippostrongylus brasiliensis during the development of immunity to this nematode in rats 1: changes in ultrastructure. Parasitology 59: 29-39.

LEE D.L. 1972. Structural and biochemical changes in Nippostrongylus brasiliensis during development of immunity to this nematode in rats. In: Comparative Biochemistry of Parasites, Ed. VAN DEN BOSSCHE H. pp. 311-318 Amsterdam;North Holland publishing company.

LEE G.B.& OGILVIE B.M. 1981. The mucus layer in intestinal nematode infections; In: The mucosal immune system in health and disease: Proceeds of the 81st Ross Conference,Columbus pp. 175-187. Ross Laboratories .

LEE T.D.G. 1982. Immunological consequences of infection of the mouse colon with Trichuris muris. Ph.D. Thesis, University of Glasgow.

LEE T.D.G.,GRENCIS R.K.& WAKELIN D. 1982. Specific cross-immunity between Trichinella spiralis and Trichuris muris: Immunization with heterologous infections and antigens and transfer of

immunity with heterologous immune mesenteric lymph node cells. Parasitology 84 : 381 –

LEID R.W.Jr & WILLIAMS J.F. 1979. Helminth parasites and the host inflammatory system. Chemical Zoology 11: 229-271.

LEWIS F.A.,CARTER C.E.& COLLEY D.G.1977. Eosinophils and immune mechanisms V: Demonstration of mouse spleen cell derived chemotactic activities for eosinophils and mononuclear cells and comparisons with eosinophil stimulation promoter. Cellular Immunology 32: 86.

LLOYD S.& SOULSBY E.J.L. 1978. The role of IgA immunoglobulins in the passive transfer of protection to Taenia taeniaeformis in the mouse. Immunology 34: 939-945.

LOVE R.J. 1975. Nippostrongylus brasiliensis infections in mice: the immunological basis of worm expulsion. Parasitology 70: 11-18.

LOVE R.J.,OGILVIE B.M.& McLAREN D.J. 1975. Nippostrongylus brasiliensis: Further properties of antibody-damaged worms and induction of comparable damage by maintaining worms in vitro. Parasitology 71: 275-283.

LOVE R.J.,OGILVIE B.M.& McLAREN D.J. 1976. The immune mechanism which expels the intestinal stage of Trichinella spiralis from rats. Immunology 30: 7-15.

LUM L.G.,MUCHMORE A.V.,KEREN D.,DECKER J.,KOSKI I.,STROBER W, & BLAESE R.M. 1979. A receptor for IgA on human T lymphocytes. Journal of Immunology 122: 65.

McDANIEL J.S. 1966. Excystment of Cryptocotyle lingua metacercariae Biological Bulletin 130 369-377.

MACKENZIE C.D.,JUNGERY M.,TAYLOR P.M.& OGILVIE B.M. 1980. Activation of complement; the induction of antibodies to the surface of nematodes and the effect of these factors and cells on worm survival in vitro. European Journal of Immunology 10: 595-601.

MACKENZIE C.D.,PRESTON M.P.& OGILVIE B.M. 1978. Immunological properties of the surface of parasitic nematodes. Nature,London 276 826-828.

MALLORY F.B. 1944. In: Pathological Technique. SAUNDERS W.B. Philadelphia.

MANKAU S.& HAMILTON R. 1973. The effect of sex and sex hormones on the infection of rats with Trichinella spiralis. Canadian Journal of Zoology 50: 579-602.

MANTZOURANIS E.& BOREL Y. 1979. Different effects of cortisone on the humoral immune response to T-dependent and T-independent antigens. Cellular Immunology 43: 202.

MATARE FR. 1910. Uber eine neue Tetracotyle im hirn von Phoxinus laevis. Z. Wiss. Zool 94: 488-550.

MAYRHOFER G. 1977. Sites of synthesis and localization of IgE in rats infected with Nippostrongylus brasiliensis. Ciba Foundation Symposium 46: 155-182.

MAYRHOFER G. 1979a. The nature of the thymus dependency of mucosal mast cells 1: An adaptive secondary response to challenge with Nippostrongylus brasiliensis. Cellular Immunology 47: 304-311.

MAYRHOFER G. 1979b. The nature of the thymus dependency of mucosal mast cells 11: The effect of thymectomy and of depleting recirculating lymphocytes on the response to Nippostrongylus brasiliensis.
Cellular Immunology 47: 312-322.

MAYRHOFER G., BAZIN H.& GOWANS J.L. 1976. Nature of cells binding anti-IgE in rats immunized with Nippostrongylus brasiliensis: IgE synthesis in regional nodes and concentration in mucosal mast cells.
European Journal of Immunology 6: 537-545.

MAYRHOFER G.& FISHER R. 1979. Mast cells in severely T cell depleted rats and the response to infection with Nippostrongylus brasiliensis.
Immunology 37: 145- 155.

MILLER H.R.P. 1971. Immune reactions in mucous membranes 11: The differentiation of intestinal mast cells during helminth expulsion in the rat.
Laboratory investigation 24: 339-347.

MILLER H.R.P. 1979. Passive transfer of the mucosal mast cell response.Its relationship to goblet cell differentiation.
In: The mast cell,its role in health and disease. Eds. PEPYS J.& EDWARDS A.M. pp. 738-743. Pitman, London.

MILLER H.R.P. 1980. Expulsion of Nippostrongylus brasiliensis from rats protected with serum 1: The efficacy of sera from singly and multiply infected donors related to time of administration and volume of serum injected.
Immunology 40: 325-334.

MILLER H.R.P. 1980. The structure, origin and function of mucosal mast cells: A brief review. Biologie Cellulaire 39: 229-232.

MILLER H.R.P., HUNTLEY J.F.& DAWSON A.McL. 1981. Mucus secretion in the gut,its relationship to the immune response in Nippostrongylus-infected rats. In: The Mucosal Immune System. Ed. BOURNE E.J.

MILLER H.R.P., HUNTLEY J.F.& WALLACE G. 1981. Immune exclusion and mucus trapping during the rapid expulsion of Nippostrongylus brasiliensis from primed rats. Immunology 44: 419-429.

MILLER H.R.P.&JARRETT W.F.H. 1971. Immune reactions in mucous membranes 1: Intestinal mast cell response during helminth expulsion in the rat. Immunology 20: 277-288.

MILLER H.R.P.& NAWA Y. 1979. Nippostrongylus brasiliensis: Intestinal goblet cell response in adoptively immunized rats. Experimental Parasitology 47: 81-90.

MILLER H.R.P.& NAWA Y. 1979. Immune regulation of intestinal goblet cell differentiation. Specific induction of nonspecific protection against helminths? Nouvelle Revue Francaise d'Hematologie 21: 31-45.

MILLER H.R.P., NAWA Y.& PARISH C.R. 1979. Intestinal goblet cell differentiation in Nippostrongylus brasiliensis -infected rats after transfer of fractionated thoracic duct lymphocytes. International Archives of Allergy and Applied Immunology 59: 281-285.

MILLER H.R.P.& WALSHAW R. 1972. Immune reactions in mucous membranes 1V: Histochemistry of intestinal mast cells during helminth expulsion in the rat.
American Journal of Pathology 69: 195-206.

MILLER H.R.P.,WOODBURY R.G.,HUNTLEY J.F.& NEWLANDS G. 1983. Systemic release of mucosal mast cell protease in primed rats challenged with Nippostrongylus brasiliensis.
Immunology 49: 471-480.

MIMORI T.,NAWA Y.,KORENAGA M.& TADA I. 1982. Strongyloides ratti: mast cell and goblet cell responses in the small intestine of infected rats.
Experimental Parasitology 54: 366-370.

MITCHELL G.F. 1979. Effector cells, molecules and mechanisms in host-protective immunity to parasites.
Immunology 38: 209 -223.

MITCHELL G.F. 1979. Responses to infection with metazoan and protozoan parasites in mice.
Advances in Immunology 28: 451-511.

MITCHELL G.F.,WESTCOTT R.& PERRYMAN L. 1983. Kinetics of expulsion of the nematode Nippostrongylus brasiliensis in mast cell deficient $W/W^{V}$ mice.
Parasite Immunology 5: 1-12.

MONGAR J.L.& FOREMAN J.C. 1979. Comtrol of histamine secretion.
In: The mast cell,its role in health and disease.Eds PEPYS J. & EDWARDS A.M. Pitman, London pp.30-37

MOQBEL R.M. 1977. Studies on the host-parasite relationship of Strongyloides ratti in rats.
Ph.D. Thesis, University of London.

MOQBEL R.M. 1980. Histopathological changes following primary, secondary and repeated infections of rats with Strongyloides ratti with special reference to tissue eosinophils. Parasite Immunology 2: 11-27.

MOQBEL R.M.& DENHAM D. 1978. Strongyliodes ratti: The effect of Betamethasone on the course of infection in rats. Parasitology 76: 289-298.

MOQBEL R.M.& McLAREN D.J. 1980. Strongyloides ratti: Structural and functional characteristics of normal and immune-damaged worms. Experimental Parasitology 49: 139-152.

MOQBEL R.M.,McLAREN D.J.& WAKELIN D. 1980. Strongyloides ratti: Reversibility of immune damage to adult worms. Experimental Parasitology 49: 153-166.

MOQBEL R.M.& WAKELIN D. 1979. Trichinella spiralis and Strongyloides ratti: Immune interaction in adult rats. Experimental Parasitology 47: 65-72.

MOQBEL R.M.& WAKELIN D. 1981. Immunity to Strongyloides ratti in rats: 1: Adoptive transfer with mesenteric lymph node cells. Parasite Immunology 3: 181-189.

MOSS G.D. 1971. The nature of the immune response of the mouse to the bile duct cestode Hymenolepis microstoma. Parasitology 62: 285-294.

MULLER R. 1975. Worms and disease, a manual of medical helminthology. Heinemann,London.

MURRAY M. Immediate hypersensitivity effector mechanisms 11: In vivo reactions. In: Immunity to animal parasites. Ed. SOULSBY E.J.L. pp 155-190. Academic Press, New York.

MURRAY M.,MILLER H.R.P.& JARRETT W.F.H. 1968. The globule leukocyte and its derivation from the subepithelial intestinal mast cell. Laboratory Investigation 19: 222 - 234.

MURRAY M.,MILLER H.R.P.,SANFORD J.& JARRETT W.F.H. 1971. 5-hydroxytryptamine in intestinal immunological reactions.Its relationship to mast cell activity and worm expulsion in rats infected with Nippostrongylus brasiliensis. International Archives of Allergy and Applied Immunology 40:236-247.

MURRELL K. 1981. The protective role of IgG in immunity to Strongyloides ratti. Journal of Parasitology 67: 167-173.

NAWA Y. 1979. Increased permeability of gut mucosa in rats infected with Nippostrongylus brasiliensis. International Journal for Parasitology 9: 251-255.

NAWA Y.& MILLER H.R.P. 1978. Protection against Nippostrongylus brasiliensis by adoptive immunization with immune thoracic duct lymphocytes. Cellular Immunology 37: 51-60.

NAWA Y.& MILLER H.R.P. 1979. Adoptive transfer of the intestinal mast cell response in rats infected with Nippostrongylus brasiliensis. Cellular Immunology 42: 225-239.

NAWA Y.,MILLER H.R.P.,HALL E.& JARRETT E.E.E. 1981. Adoptive transfer of total and parasite-specific IgE response in rats infected with Nippostrongylus brasiliensis Immunology 44: 119 –123

NAWA Y.,PARISH C.R.& MILLER H.R.P. 1978. The protective capacity of fractionated immune thoracic duct lymphocytes against Nippostrongylus brasiliensis.
Cellular Immunology 37: 41-50.

NIELSON K.,FOGH L.& ANDERSON S. 1974. Eosinophil response to migrating Ascaris suum larvae in normal and congenitally thymus-less mice.
Acta Pathologica et Microbiologica Scandinavica 82: 919

NOVAK M.,COLLINS M.& EVANS W.S. 1980. The growth of Hymenolepis microstoma in intact and gonadectomized mice.
Zeitschrift fur Parasitenkunde 61: 243 -247.

OGILVIE B.M.,ASKENASE P.W.& ROSE M.E. 1980. Basophils and eosinophils in three strains of rats and in athymic(nude) rats following infection with the nematodes Nippostrongylus brasiliensis or Trichinella spiralis.
Immunology 39: 385-389.

OGILVIE B.M.& HOCKLEY D.J. 1968. Effects of immunity on Nippostrongylus brasiliensis adult worms: reversible and irreversible changes in infectivity,reproduction and morphology.
Journal of Parasitology 54: 1073-1084

OGILVIE B.M.& JONES V.E. 1968. Passive protection with cells or antiserum against Nippostrongylus brasiliensis in the rat.
Parasitology 58: 939-949.

OGILVIE B.M.& JONES V.E. 1971. Nippostrongylus brasiliensis: A review of immunity and the host-parasite relationsnip in the rat.
Experimental Parasitology 27: 138-177.

OGILVIE B.M.& JONES V.E. 1973. Immunity in the parasitic relationship between helminths and hosts.
Progress in Allergy 17: 95-144.

OGILVIE B.M.& LOVE R.J. 1974. Cooperation between antibodies and cells in immunity to a nematode parasite.
Transplantation Reviews 19: 147-168.

OGILVIE B.M.,LOVE R.J.,JARRA W.& BROWN K.N. 1977. Nippostrongylus brasiliensis infection in rats: The cellular requirement for worm expulsion.
Immunology 32: 521-528.

OGILVIE B.M.,MACKENZIE C.D.& LOVE R.J. 1977. Lymphocytes and eosinophils in the immune response of rats to initial and subsequent infection with Nippostrongylus brasiliensis.
American Journal of Tropical Medicine and Hygiene. 26: 61

OGILVIE B.M.& PARROTT D.M.V. 1977. The immunological consequences of nematode infection.
In: Immunology of the gut. Ciba Foundation Symposium 46: 183-201. Elsevier, North Holland, Amsterdam.

OHMAN C. 1965. The structure and function of the adhesive organ in strigeid trematodes,Part 11: Diplostomum spathaceum Braun 1893.
Parasitology 55: 481-502.

OHMAN C. 1966a. The structure and function of the adhesive organ in strigeid trematodes,Part 111: Apatemon gracilis minor Yamaguti 1933.
Parasitology 56: 209-226.

OHMAN C. 1966b. The structure and function of the adhesive organ in strigeid trematodes, 1V: Holostephanus luhei Szidat 1936.
Parasitology 56: 481-491.

OLSON C.E.& SCHILLER E.L. 1978. Strongyloides ratti infections in rats, 1: Immunopathology.
American Journal of Tropical Medicine and Hygiene 27: 521-526.

OTTOLENGHI A. 1973. High phospholipase content of intestines of mice infected with Hymenolepis nana. Lipids 8: 426-428.

OTTOLENGHI A.,WEATHERLY N.F.,ROCAN A.A.& LARSH J.E.Jr. 1977. Phospholipase in nonsensitized and sensitized rats after challenge. Infection and Immunity 15: 13-18.

PARISH W.E.,LUCKHURST E.& COWAN S.I. 1977. Eosinophilia V: Delayed hypersensitivity,blood and bone marrow eosinophilia induced in normal guinea-pigs by adoptive transfer of lymphocytes from syngeneic donors. Clinical and Experimental Immunology 29: 75

PERRUDET-BADOUX A.,ANTEUNIS A.,DUMITRESCU M.& BINAGHI R.A. 1978. Ultrastructural study of the immune interaction between peritoneal cells and larvae of Trichinella spiralis. Journal of the Reticuloendothelial Society 24: 311

PHILLIPS S.M.,DICONZA J.J.,GOLD J.A.& REID W.A. 1977. Schistosomiasis in the congenitally athymic (nude) mouse 1: Thymic dependency of eosinophilia,granuloma formation and host morbidity. Journal of Immunology 118: 594

POULAKOS L.& KENT T. 1973. Gastric emptying and small intestinal propulsion in fed and fasted rats. Gastroenterology 64: 963-967.

RACE G.J.,LARSH J.E.,MARTIN J.H.& WEATHERLY N.F. 1974. Light and electron microscopy of the intestinal tissue of mice parasitized by Trichinella spiralis. In: Trichinellosis, Proceedings of the Third National Conference on Trichinellosis. Ed. KIM C.W. pp. 75-100 Intext, New York.

RAISYTE (RAISHITE) D. 1968. Development of Apatemon gracilis (Rud.1819) Szidat 1928 (Strigeidae) in the final host. Trudy Gel mintologicheskoi Laboratorii. 19: 154-162. Akademiya nauk SSR. Moskva, Leningrad.

REDDINGTON J.J.,STEWART G.L.,KRAMAR G.W.& KRAMAR M.A. 1981. The effects of host sex and hormones on Trichinella spiralis in the mouse.
Journal of Parasitology 67: 548-555.

REES G. 1955. The adult and Diplostomulum stage(Diplostomulum phoxini (Faust)) of Diplostomum pelmatoides Dubois,and an experimental demonstration of part of the life cycle.
Parasitology 45: 295-312.

REES G. 1957. Cercaria Diplostomum phoxini(Faust),a furcocercaria which develops into Diplostomulum phoxini in the brain of the minnow.
Parasitology 47: 126-137.

REILLY R.W.& KIRSNER J.B. 1965. Runt intestinal disease.
Laboratory Investigations 14: 102-107.

RICHARDS A.J. 1982. Prostaglandins and Nippostrongylus.
International Journal for Parasitology 12: 101.

RICHARDS A.J.,BRYANT C.,KELLY J.D.,WINDON R.G.& DINEEN J.K. 1977. The metabolic lesion in Nippostrongylus brasiliensis induced by prostaglandin E in vitro.
International Journal for Parasitology 7: 153-158.

ROEPSTORFF A.& ANDREASSEN J. 1982. Course of heavy primary infections and earlier immunologically mediated rejection of secondary infections of Hymenolepis diminuta.
International Journal for Parasitology 12: 23-28.

ROTHWELL T.L.W.& DINEEN J.K. 1973. The response of the regional lymph node of guinea pigs to primary and challenge infection with the nematode Trichostrongylus colubriformis.
International Journal for Parasitology 3: 201-208.

ROTHWELL T.L.W. & GRIFFITHS D.A. 1977. Comparison of the kinetics of expulsion of Trichostrongylus colubriformis from previously uninfected,reinfected and vaccinated guinea pigs. Journal of Parasitology 63: 761-762.

ROTHWELL T.L.W.& LOVE R.J. 1975. Studies of the responses of basophil and eosinophil leucocytes and mast cells to the nematode Trichostrongylus colubriformis II: Changes in cell numbers following infection of thymectomised and adoptively or passively immunized guinea pigs. Journal of Pathology 116: 183-194.

ROTHWELL T.L.W.,LOVE R.J.,ADAMS D.B.,LOVE D.N.& McLAREN D.J. 1980. Immunity against Trichostrongylus colubriformis infection in guinea pigs and sheep: Some comparisons with Nippostrongylus brasiliensis infection in the rat. International Journal for Parasitology 10: 43-50.

ROTHWELL T.L.W.,LOVE R.J.&EVANS D.P. 1978. Studies on the role of histamine and 5-hydroxytryptamine in immunity against the nematode Trichostrongylus colubriformis IV: Inhibition of the expulsion of worms transplanted into the duodenum of immune guinea pigs. International Arcnives of Allergy and Applied Immunology 56:457-462.

ROTHWELL T.L.W.,LOVE R.J.& GOODRICH B.S. 1977. Failure to demonstrate involvement of prostaglandins in the immune expulsion of Trichostrongylus colubriformis from the intestine of guinea pigs. International Archives of Allergy and Applied Immunology 53:93-95.

ROTHWELL T.L.W.,PRICHARD R.K.& LOVE R.J. 1974. Studies on the role of histamine and 5-hydroxytryptamine in immunity against the nematode Trichostrongylus colubriformis 1: In vivo and in vitro effects of the amines.
International Archives of Allergy and Applied Immunology 46: 1-13.

RUITENBERG E.J.& ELGERSMA A. 1976. Absence of intestinal mast cell response in congenitally athymic mice during Trichinella spiralis infection. Nature 264: 258-260.

RUITENBERG E.J.& ELGERSMA A. 1979. Response of intestinal globule leukocytes in the mouse during a Trichinella spiralis infection and its independance of intestinal mast cells.
British Journal of Experimental Pathology 60: 246-251.

RUITENBERG E.J.& ELGERSMA A. 1980. Study on the kinetics of globule leukocytes in the intestinal epithelium of rats after single or double infection with Trichinella spiralis.
British Journal of Experimental Pathology 61: 285-290.

RUITENBERG E.J.,ELGERSMA A.& KRUIZINGA W. 1979. Intestinal mast cells and globule leucocytes: role of the thymus on their presence and proliferation during a Trichinella spiralis infection in the rat.
International Archives of Allergy and Applied Immunology 60:302-309.

RUITENBERG E.J.,ELGERSMA A.,KRUIZINGA W.& LEENSTRA F. 1977. Trichinella spiralis infection in congenitally athymic (nude) mice: Parasitological,serological and haematological studies with observations on intestinal pathology.
Immunology 33: 581-587.

RUITENBERG E.J.,ELGERSMA A.& LAMERS C.H.J. 1979. Kinetics and characteristics of intestinal mast cells and globule leukocytes: In:The mast cell;its role in health and disease. Eds PEPYS A.M.& EDWARDS A.M. pp.732-737. Pitman,London.

SADUN E. 1948. Relationship of the gonadal hormones to the natural resistance of chickens and the growth of Ascaridia galli. Journal of Parasitology 34: 18

SADUN E. 1951. Gonadal hormones in experimental Ascaridia galli infections in chickens. Experimental Parasitology 1: 70-82.

SELBY G.R.& WAKELIN D. 1973. Transferof immunity against Trichuris muris in the mouse by serum and cells. International Journal for Parasitology 3: 717-722.

SHANNON W.A.Jr.& BOGITSH B.J. 1969. Cytochemical and biochemical observations on the digestive tract of digenetic trematodes V: ultrastructure, Schistosomatum douthitti gut. Experimental Parasitology 26: 344

SHELHAMER J.& KALINER M. 1979. Mucus glycoprotein secretion by human lung cultures in vitro. Journal of Allergy and Clinical Immunology 63: 141

SIRAG S.B.,CHRISTENSEN N.O.,FRANDSEN F.,MONRAD J.& NANSEN P. 1980. Homologous and heterologous resistance in Echinostoma revolutum infections in mice. Parasitology 80: 479-486.

SITEPU P.& DOBSON C. 1982. Genetic control of resistance to infection with Nematospiroides dubius in mice: selection of high and low immune responder populations of mice. Parasitology 85: 73-84.

SOLOMON G.B. 1969. Host hormones and parasitic infections. International Review of Tropical Medicine 3: 101-158.

SPIEGELBERG H.L.,LAWRENCE D.A.& HENSON P. 1974. Cytophilic properties of IgA to human neutrophils. In: Advances in Experimental Medicine and Biology, The immunoglobulin A system. Eds MESTECKY J.& LAWTON A.R. pp 67-74. Plenum Press,London.

STONER R.D.& GODWIN J.T. 1953. The effects of ACTH and cortisone upon susceptibility to trichinosis in mice. American Journal of Pathology 29: 943-950.

STONER R.D.& GODWIN J.T. 1954. The effects of adrenocorticotrophic hormone and cortisone upon acquired immunity to trichinosis in mice. American Journal of Pathology 30:913-918.

TALIAFERRO W.H.& SARLES M.P. 1939. The cellular reactions in the skin,lungs and intestine of normal and immune rats after infection with Nippostrongylus muris. Journal of Infectious Diseases 64: 157-192.

TANAKA J.,BABA T.& TORISU M. 1979. Ascaris and eosinophil 11: isolation and characterization of eosinophil chemotactic factor and neutrophil chemotactic factor of parasite in Ascaris antigen. Journal of Immunology 122: 302

TANNENBAUM S., OERTEL H.,HENDERSON W.& KALINER M. 1980. The biologic activity of mast cell granules 1: Elicitation of inflammatory responses in rat skin. Journal of Immunology 125: 325-335.

TAS J.& BERNDSEN R.G. 1977. Does heparin occur in mucosal mast cells of the rat small intestine? Journal of Histochemistry and Cytochemistry 25: 1058

THOMPSON A.R. 1972. Isolation and characterization of a mast cell degranulating substance from Ascaris suum. Biochimica,Biophysica Acta 261: 245-257.

TOLONE G., BRAI M.,BONASERA L.,BELLAVIA A.& PONTIERI G.M. 1972. Role of mast cells and eosinophils in tissue injury caused by injection of Ascaris fluid in the rat. Pathologica et Microbiologica 38: 192-199.

TOLONE G.,BONASERA L.,BRAI M.,FERINA F.& PONTIERI G. 1974. Isolation of mast cell degranulators from the celomatic fluid of Parascaris equorum. Pathologica et Microbiologica 41: 41-50.

URBAN J.F.,ISHIZAKA K.& BAZIN H. 1980. IgE B cell-generating factor from lymph node cells of rats infected with Nippostrongylus brasiliensis III: Regulation of factor formation by anti-immunoglobulin. Journal of Immunology 124: 527-532.

URQUHART G.M.,MULLIGAN W.,EADIE R.M.& JENNINGS F.W. 1965. Immunological studies on Nippostrongylus brasiliensis infection in the rat; The role of local anaphylaxis. Experimental Parasitology 17: 210-217.

UVNAS B.& WOLD J.K. 1967. Isolation of a mast cell-degranulating polypeptide from Ascaris suis. Acta Physiologica Scandinavica 70: 269-276.

VAN EPPS D.E.,REED K.& WILLIAMS R.C. 1978. Suppression of human PMN bactericidal activity by human IgA paraproteins. Cellular Immunology 36: 363-376.

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

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

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

WAKELIN D.,GRENCIS R.K.& DONACHIE A.M. 1982. Short-lived dividing cells mediate adoptive transfer of immunity to Trichinella spiralis in mice 11: In vivo characteristics of the cells. Immunology 46: 451-457.

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

WAKELIN D.& LLOYD M. 1976b. Accelerated expulsion of adult Trichinella spiralis in mice given lymphoid cells and serum from infected donors. Parasitology 72: 307-315.

WAKELIN D.& SELBY G.R. 1976. Immune expulsion of Trichuris muris from resistant mice: suppression by irradiation and restoration by transfer of lymphoid cells. Parasitology 72: 41-50.

WAKELIN D.& WILSON M.M. 1977a. Transfer of immunity to Trichinella spiralis in the mouse with mesenteric lymph node cells: time of appearance of effective cells in donors and expression of immunity in recipients. Parasitology 74: 215-224.

WAKELIN D.& WILSON M.M. 1977b. Evidence for the involvement of a bone marrow-derived cell population in the immune expulsion of Trichinella spiralis. Parasitology 74: 225-234.

WAKELIN D.& WILSON M.M. 1979. Trichinella spiralis: immunity and inflammation in the expulsion of transplanted adult worms from mice. Experimental Parasitology 48: 305-312.

WAKELIN D.& WILSON M.M. 1979. T and B cells in the transfer of immunity against Trichinella spiralis in mice.
Immunology 37: 103-109

WALKER W.A.,ABEL S.N.,WU M.& BLOCH K.J. 1976. Intestinal uptake of macromolecules V: comparison of the uptake by rat small intestine of antigen-antibody complexes prepared in antibody or antigen excess.
Journal of Immunology 117: 1028

WALKER W.A.,WU M.& BLOCH K.J. 1977. Stimulation by immune complexes of mucus release from goblet cells of the rat small intestine.
Science 197: 370-372.

WARREN K.S.,KARP R.,PELLY R.P.& MAHMOUD A.A.F. 1976. The eosinophil stimulation promoter test in murine and numan Trichinella spiralis infection.
Journal of Infectious Diseases 134: 277

WASSERMAN S.I. 1979. The mast cell and the inflammatory response.
In: The mast cell: its role in health and disease. Eds PEPYS J. & EDWARDS A.M. Pitman, London. pp.9-20

WEISS N.& TANNER M. 1979. Studies on Dipetalonema viteae(Filaroidea) 111: Antibody-dependant cell mediated destruction of microfilariae in vivo.
Tropenmed. Parasitol. 30: 78.

WELLER P.F.& GOETZL .J. 1979. The regulatory and effector roles of eosinophils.
Advances in Immunology 27: 339-571.

WELLS P.D. 1962. Mast cell,eosinophil and histamine levels in Nippostrongylus brasiliensis-infected rats.
Experimental Parasitology 12:82-101.

WELLS P.D. 1963. Mucin secreting cells in rats infected with Nippostrongylus brasiliensis.

Experimental Parasitology 14: 15.

WILSON M.R. 1960. Cultivation of the strigeid trematodes of the genus Diplostomum.

Ph.D. Thesis, University of Glasgow.

WOODBURY R.G., GRUZENSKY G.M.& LAGUNOFF D. 1978.

Immunofluorescent localisation of a serine protease in rat small intestine.

Proceeds of the National Academy of Science, USA. 75: 2785

WOODBURY R.G.& MILLER H.R.P. 1982. Quantitative analysis of mucosal mast cell protease in the intestines of Nippostrongylus -infected rats.

Immunology 46:

WYLIE M.R.,WILLIAMS M.O.& HOPKINS C.A. 1960. The in vitro cultivation of strigeid trematodes 11: Replacement of a yolk medium.

Experimental Parasitology 10: 51-57.

ZATZ M.M. 1975. Effects of cortisone on lymphocyte homing.

Israel Journal of Medical Science 11: 1368

# ABBREVIATIONS

| | |
|---|---|
| BM | bone marrow |
| BSA | bovine serum albumin |
| CTMC | connective tissue mast cells |
| DH | delayed hypersensitivity |
| GC | goblet cells |
| GL | globule leucocytes |
| h. | hours |
| HBSS | modified Hanks balanced salt solution |
| 5-HT | 5-hydroxytryptamine |
| IEL | intra-epithelial lymphocytes |
| IS | serum from previously infected hosts (e.g.mice) |
| LPMC | lamina propria mast cells |
| met. | metacercaria(e) |
| MLDC | mesenteric lymph duct cells |
| MLNC | mesenteric lymph node cells |
| IMLNC | " " " " from previously infected hosts |
| PBS | phosphate buffered saline |
| PG | prostaglandin |
| PLB | phospholipase B |
| post. | posterior |
| p.p. | post-pylorus |
| SC | spleen cells |
| S.D. | standard deviation |
| S.I. | small intestine |
| SRS-A | slow releasing substance of anaphylaxis |
| TDL | thoracic duct lymphocytes |

| | |
|---|---|
| vitell. | vitellaria |
| $1^{o}$ | primary infection |
| $2^{o}$ | secondary infection |