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THE INTESTINAL IMMUNE RESPONSE OF THE MOUSE TO THE TAPEWORM *HYMENOLEPIS DIMINUTA*

THESIS

for the

Degree of Doctor of Philosophy

by

Peter Riddell Christie

Department of Zoology, University of Glasgow

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This thesis is dedicated to my wife Evelyn, whose love and selflessness are a constant inspiration to me.

SUMMARY

It is now conclusively evident that the intestinal tapeworm Hymenolepis diminuta in the mouse can establish successfully, but is thereafter rejected by an immunological mechanism that is thought to be thymus-dependent. The lack of damage to the small intestine in H. diminuta infection makes this an almost unique and potentially valuable model for the investigation of intestinal immune responses.

The work presented in this thesis was undertaken to more fully characterise this host-parasite system, and the nature of the immune response of the mouse to H. diminuta was investigated in four different aspects. Firstly, the thymus-dependency of the response was confirmed, and the role of antibody in the expulsion of H. diminuta investigated; secondly, the source of the antigens which stimulate the protective response was investigated; thirdly, a study was made of the effect of an intestinal response to an unrelated parasite on H. diminuta and H. microstoma in rats and mice; fourthly, lymphocytes from the mesenteric lymph nodes of infected mice were tested for their ability to transfer immunity between mice, and to migrate to the small intestine of infected mice.

The effects of pregnancy and lactation in the mouse were studied in relation to rejection of H. diminuta ; both phases of the reproductive cycle are known to cause depression of T effector lymphocytes, whereas antibody responses are unaffected or enhanced. It was shown that pregnant and lactating mice reject H. diminuta more slowly than nulliparous mice, and that growth of the worms is much enhanced in comparison with worms from nulliparous mice.

The delay in rejection caused by lactation was found to be greater than that observed during pregnancy, from which it can be concluded that immunodepression is more marked in lactating mice. The period of slowest rejection coincides with the period when greatest growth of the worms was noted (i.e. during mid-lactation), but the relative contributions of the immunodepression and the increased food intake of pregnant and lactating mice to the enhanced worm growth are far from clear.

It was demonstrated that the immunological defect operating during pregnancy and lactation lies in the effector arm of the response, as sensitisation against H. diminuta occurred normally in these animals, but the response to reinfection of previously immunised animals was shown to be depressed during pregnancy and lactation.

Experiments were carried out in which immunisation of mice with H. diminuta failed to provide any protection against H. diminuta infection in their offspring; antibody transferred in colostrum had no effect on either growth or survival of the worms in the young mice, which have little innate capacity to respond to the infection. From these and the above experiments, it was concluded that anti-worm antibody in the absence of effective T lymphocyte function has no measureable independent effect on H. diminuta.

The relative roles of the scolex and strobila of H. diminuta in stimulating the immune response of the mouse were investigated by transplantation of worms with large or small weights of strobila into the duodenum of mice. It was found that the larger worms were rejected more quickly by the mice, and it is argued that this difference was not due to the smaller worms being more resistant to immunological attack, or to a time-related exposure of protective antigens, but to differences in size or surface area. Confirmation

of this idea came from studies with stunted worms derived from heavily irradiated cysticercoids. At 35 krad and above, the worms excyst normally, but show little growth thereafter. These stunted worms, which cosist of a scolex and a stump of undifferentiated neck tissue, were rejected by mice via an immunological mechanism, but were shown to be poorly immunogenic; they are rejected slowly by mice, and stimulate poor immunological memory in comparison with normal worms. It was concluded that the protective antigen(s) arise from the tegument, but that the anterior end of the worm is more antigenic per unit weight than the posterior end. This theory is shown to fit many established observations, and brief discussion is made of the implications for vaccination studies, and of the possible nature of the protective antigens from the tegument of the worm.

A study was made of the effects of the inflammatory expulsion phase of an unrelated parasite, Trichinella spiralis, on growth and survival of hymenolepids not normally rejected by their hosts, namely H. microstoma in the mouse and H. diminuta in the rat. Growth of the cestodes in dual-infected animals was markedly depressed, and the severity of the stunting increased if the cestode was administered closer to the time of expulsion of T. spiralis. H. microstoma was found to be susceptible to the effects of inflammation during the early migratory phase in the lumen of the small intestine, but if the scolex was inside the bile duct at the time of inflammation, no subsequent loss or destrobilation occurred. H. diminuta in the rat was neither expelled nor caused to destrobilate by the inflammatory changes in the intestine, but establishment of the cestode may be adversely affected if cysticercoids are given during the period of severest inflammation caused by T. spiralis or

Nippostrongylus brasiliensis. It is suggested that the bile duct is an immunologically privileged site for H. microstoma, and the implications of the interaction work are discussed both for T. spiralis and H. diminuta.

Syngeneic transfer of lymphocytes from the mesenteric lymph node (MLN) was used to study transfer of immunity against H. diminuta between mice, and to investigate the attractiveness of the small intestine of infected animals to intravenously injected isotopically labelled lymphocytes. It was found that enhanced responsiveness to H. diminuta was not transferable with less than a 4:1 donor: recipient ratio (1.7×10^8 MLN cells), which limits the usefulness of the technique, and the results obtained are discussed in relation to the requirements for adoptive transfer of immunity against T. spiralis.

No difference was recorded in the amount of radioactivity recovered from the small intestines of mice, infected with H. diminuta or uninfected, after injection of isotopically labelled MLN cells. The limitations of the technique and label (L-(^{75}Se)-selenomethionine) used are discussed, and it is concluded that lymphocyte migration studies are potentially valuable in the study of H. diminuta in the mouse, only with much more sensitive techniques. Alternatives to the MLN as the source of the lymphocytes that cause expulsion of H. diminuta are discussed, and it is suggested that future work includes investigation of Peyer's patches.

Future lines of research are suggested, particularly with respect to the cellular events in the wall of the small intestine.

ABBREVIATIONS

B	Thymus-independent; antibody-producing
C	Control
CA	Cortisone acetate
CT	Cell transfer
GALT	Gut-associated lymphoid tissues
HBSS	Modified Hanks' balanced salt solution
Hd	*Hymenolepis diminuta*
I	Infected / immune
%ID	Radioactivity in an organ as percentage of the injected dose of isotope
Ig	Immunoglobulin
IMLNC	Mesenteric lymph node cells from infected / immune mice
i.p.	Intraperitoneal
i.v.	intravenous
K	Killed
MLN	Mesenteric lymph node
MLNC	Mesenteric lymph node cells
N	Naive (previously uninfected)
n	Number in a group
Nb	*Nippostrongylus brasiliensis*
ND	Not determined
NR	Nil recovery of worms $>$ 0.1 mg from a mouse
p	Probability
p.i.	post infection
R	Roentgen
RIT	Rosette inhibition titre

T	Thymus-dependent
%TR	Radioactivity in an organ as percentage of the total isotope recovered from small intestine, MLN, spleen & liver
Tsp	*Trichinella spiralis*
Z	Anthelmintic ('Zanil')

ADDENDUM

ACTH	Adrenocorticotrophic hormone
HCG	Human chorionic gonadotropin
^{125}IUdR	(^{125}I) iodo-deoxyuridine

I certify and confirm that Mr. P.R. Christie made the major contribution to the scientific content and composition of the joint paper entitled "The effect of the expulsion phase of Trichinella spiralis on Hymenolepis diminuta infection in rats" which was published in Parasitology vol. 78 and which is included in this thesis.

Mr. Christie also made a major contribution, equally with Dr. R.J. Howard, to the content and composition of the joint paper entitled "The effect of concurrent infection with Trichinella spiralis on Hymenolepis microstoma in mice" which was published in Parasitology vol. 77 and which is also included in this thesis.

Dr. D. Wakelin

GENERAL INTRODUCTION

1) Historical background

The adult stage of the cyclophyllidean tapeworm *Hymenolepis diminuta* will establish successfully in a number of mammalian hosts, mainly rodents; in the wild, rats are the usual definitive host, although it has been reported that mice, hamsters and even man may act as hosts for this parasite (Smyth, 1962). The host is infected by ingestion of the arthropod intermediate host (normally a beetle) harbouring the cysticercoid larvae of *H. diminuta*. The life expectancy of the worm may be potentially longer than the host's lifespan; Read (1967) kept worms alive for 14 years by surgical transfer into new rat hosts. Coupled to this is the fact that a rat can support a low-level (1-5 worm) infection for long periods of time without any loss of worms occurring; e.g. Harris & Turton (1973) found no loss from a five worm infection after 14 weeks.

This parasite in the definitive host is found exclusively in the small intestine. *H. diminuta*, unlike many other hymenolepids, has a completely unarmed scolex and therefore relies to a large extent on the four acetabula (suckers) for adhesion, to prevent the worm being swept out of the gut by peristalsis. The absence of hooks reduces the possibility of the worms causing physical damage to the small intestine mucosa; the possibility of localised mucosal damage is further reduced by the fact that the worm alters its point of attachment of the scolex as it grows, moving anteriorly in the intestine of the rat as the strobila lengthens (Braten & Hopkins, 1969). There is also a very marked circadian

migratory pattern (Read & Kilejian, 1969; Hopkins, 1970). The lack of physical damage to the small intestine is accompanied by a lack of gross physiological damage to the host; growth rate of the host rat under normal conditions is apparently unaffected by infection with H. diminuta (Insler & Roberts, 1976) and food intake is unaltered (Mettrick, 1971). Mettrick (1972, 1973) reports unpublished observations indicating a 20% decrease in growth rate and increased caloric intake per gram increase in body weight of parasitised reats, but in the absence of several important details this cannot be accepted as important evidence. Henderson (unpublished), using large numbers of specific pathogen-free (SPF) rats, observed a small, but not statistically significant, decrease in the growth of infected rats on isocaloric diets.

Turton (1968) found that H. diminuta does not damage the gut epithelial cells. This lack of intestinal pathology, reinforcing the view widely held before 1970 that an immunological reaction in the intestinal lumen "develops only if the mucosa of the host's intestine is invaded and antigenic substances are introduced" (Rees, 1967), perpetuated the opinion that H. diminuta is not immunogenic. In contrast, it was widely accepted that H. nana was immunogenic, by virtue of its invasive parenteral stage of development (reviewed by Rees, 1967). The original statement by Chandler (1939) that H. diminuta is not immunogenic remained unchallenged until Weinmann (1966) reported experiments demonstrating that the same parasite in the mouse evoked strong immunity against challenge infection with H. diminuta and H. nana, although the implications of these results were not fully appreciated. The use of intra-peritoneal injections in mice of tincture of opium (Read & Voge, 1954) or morphine (Weinmann, 1966) to slow intestinal

emptying time, and pre-treatment of cysticercoids with acid pepsin (Read, 1955), was shown by Turton (1971) to be unnecessary for successful establishment of H. diminuta in the mouse. Using well-defined SPF mice and improved techniques (not least, I suspect,those involved in searching for small worms), it was shown by Hopkins, Subramanian & Stallard (1972a) that 90-100% of administered cysticercoids established and grew; they also showed that infections with one or two worms were expelled between days 10 and 16 of infection (agreeing with Weinmann's (1966) observations), and that rejection was heralded by a slowing of growth of the worms after day 10 of infection. Rejection took the form of destrobilation of many of the worms (a loss of strobila behind the neck, leaving only the scolex and a small part of the neck region in the intestine, a process first described by Turton (1971) for H. diminuta in the mouse), and most worms had destrobilated by day 14 of infection. The popuiation of destrobilated worms was expelled slowly over the subsequent 4 to 5 weeks. The observation that worms in previously infected mice grew much more slowly than in previously uninfected controls and that fewer worms were recovered from the former group than the latter was strong evidence in favour of these authors' proposition that loss of H. diminuta from the mouse is immunologically mediated. This theory was substantiated by a subsequent paper (Hopkins et al., 1972b) demonstrating that rejection of H. diminuta could be impaired by immunosuppressive agents.

By the time that it was established by these authors that rejection of H. diminuta from the mouse was immunologically mediated, investigation of immune responses to intestinal nematodes was in a relatively advanced state (reviewed at this

time by Jarrett & Urquhart, 1971; Ogilvie & Jones, 1973), which allowed workers studying the immunological rejection of hymenolepids a correspondingly advanced view of the mechanisms involved in the rejection of intestinal parasites. Progress in investigation of the immune response of the mouse to H. diminuta has been fairly rapid since 1972, given that there were (and are) relatively few researchers working on the system; this latter aspect is probably due to the "greater economic and medical importance of other metazoans" (Weinmann, 1970), although one suspects that the view that adult cestodes are, at best, weakly immunogenic, still persists with many people.

There are a number of parameters that markedly affect the kinetics of rejection of H. diminuta from the mouse: it would be useful at this point to summarise the effects of varying these parameters.

2) Factors influencing rejection

a) Mouse host

Close to 100% establishment followed by rejection of H. diminuta has been found to be a characteristic of most strains of mouse tested thus far; CFLP and Porton (Hopkins et al., 1972a), Balb/c (Isaak, Jacobson & Reed, 1975), $B_{10}LP$ (Andreassen, Hindsbo & Ruitenberg, 1978a), C57, C3H, CBA (Hopkins, personal communication), CD-1 and NIH (personal observations) mouse strains all respond to infection with this parasite, although direct comparison between strains has rarely been attempted.

The sex of mouse used appears to have little effect

(Read & Voge, 1954; Christie, unpublished), but the age of the mouse at the time of infection appears to be very important. Befus & Featherston (1974) found that rejection of H. diminuta took, on average, four days longer in mice under five weeks old than in mice 5-7 weeks old. Growth of the worm in previously uninfected mice over 12 weeks old has been found to be often, though not invariably, much reduced (Hopkins, personal communication; personal observations);this is presumably due to the maturation of the gut-associated lymphoid tissues, the immunological history of the mice, and the presence of concurrent protozoal or viral infections, although Befus & Featherston (1974) failed to find any difference in the course of infection in mice of differing quality (i.e. carrying few to many pathogens).

b) Level of infection

In infections of the mouse with H. microstoma (Hopkins, Goodall & Zajac, 1977) and H. citelli (Hopkins & Stallard, 1974) there appears to be an antigenic threshold above which an immunological response against the worms is mounted resulting in loss of worms, and below which no response may be evident; in both of these species of worm, rejection of heavy burdens proceeds until a 'residual population' of 1-5 worms remains, and this low-level infection persists over long periods of time. With H. diminuta in the mouse it is known that there is no such threshold, a single worm being sufficient to elicit an immune response and stimulate immunological memory (Befus, 1975a). Increasing the level of infection with H. diminuta from one to six worms shortens the time taken for the response to take effect

and reduces variability in the results (Befus, 1975a). In practice, five-worm infections have been found by the present author to be sufficient to stimulate a strong, rapid response; increasing the number of worms administered to 12 or beyond decreases the time taken for rejection to commence (Andreassen, Jespersen & Roepstorff, 1978b), but the increased technical difficulties involved in such experiments and the effects of crowding on worm growth (Roberts & Mong, 1968) complicate the interpretation of results.

3) The nature of the immune response to H. diminuta

a) Thymus dependency

The weight of available evidence suggests that the rejection of H. diminuta by the mouse is a strongly thymus-dependent process. Hopkins et al. (1972b) showed that anti-thymocyte serum substantially reduced the ability of mice to reject H. diminuta. It has also been shown by Isaak et al.(1975), Bland (1976a), and Andreassen et al. (1978a) that the 'nude' mouse (a strain that is congenitally athymic and hairless, homozygous for the mutant nu/nu gene - see Pantelouris, 1971; Wortis, 1971) is slower to reject H. diminuta than heterozygous littermates posessing a thymus, and at low levels of infection nude mice may not reject the worms at all.

Adult thymectomised, lethally irradiated, bone marrow reconstituted mice apparently cannot reject H. diminuta (Bland, 1976b), although this observation is complicated by the fact that the capacity to respond was impaired in sham thymectomised, lethally irradiated, bone marrow reconstituted mice, indicating a requirement for an additional radiation-sensitive component

in the rejection process. Further evidence for T cell involvement is presented in this thesis.

b) Antibody involvement

Antibodies produced by the host against its intestinal parasites are thought to be important in a number of systems in causing rejection and/or damage to the worms; e.g. Nippostrongylus brasiliensis in the rat (Ogilvie & Jones, 1968), Trichostrongylus colubriformis in the guinea-pig (Connan, 1972b), Trichuris muris in the mouse (Selby & Wakelin, 1973), and Trichinella spiralis in the mouse (Wakelin & Lloyd, 1976b) have all been shown to elicit production of serum antibody that is capable of transferring resistance to immunologically naive animals. It is also well-known that immunity to many parenterally-situated larval cestodes is transferable with serum, or is antibody-dependent, e.g. Taenia taeniaeformis in the rat, Taenia pisiformis in the rabbit, and H. nana in the mouse (reviewed by Gemmell & MacNamara, 1972; Gemmell, 1976).

In the case of H. diminuta in the mouse, the evidence for antibody involvement in rejection rests largely on the work by Befus (1977), which showed that IgA, IgM, IgG_1 and IgG_2 appear on the surface of the worm in a sequential fashion, all classes of antibody being present by day 12 of infection. Appearance of these antibodies was hastened by increasing the worm burden (i.e. the 'antigenic load'). Befus was careful to point out, though, that this may not be specific antibody, the possibility existing that it was immunoglobulin adsorbed non-specifically onto the polyanionic glycocalyx of the tegument (Lumsden, 1975). Rejection

of transplanted worms from normal mouse donors (and hence probably coated with antibody) is no faster than for worms from immunosuppressed mice or rats (Hopkins & Zajac, 1976), in contrast to the rat-*N. brasiliensis* model, in which antibody-damaged worms are rapidly expelled (reviewed by Ogilvie & Jones, 1973; Ogilvie & Parrott, 1977). One possible reason for the lack of effect of the antibody coating is that the antibody may have a relatively short half-life on the rapidly metabolising tegument of the tapeworm (Oaks & Lumsden, 1971).

Serum antibodies to *H. diminuta* in the mouse have proved very difficult to detect (see Befus (1975b), who used immunodiffusion techniques), although Choromanski (1978) apparently found that serum antibody to *H. diminuta* can be detected in quantifiable titres. Serum antibody reacting with *H. diminuta* cysticercoids has been described by Andreassen *et al.* (1978a). Serum antibody to *H. diminuta* in the rat was first detected by Coleman, Carty & Graziodei (1968), an observation confirmed by Harris & Turton (1973) using relatively sensitive techniques; likewise, high levels of specific serum antibody have been detected in mice infected with *H. microstoma* (Moss, 1971; Goodall, 1973), although this cestode is not normally rejected from the mouse, and to a much lesser extent in infections with *H. nana* and *H. citelli* cysticercoids (Goodall, 1973). The low level of serum antibody in *H. diminuta* infection in mice may be at least partly due to the fact that entirely lumen-dwelling parasites which cause little or no damage tend not to elicit a strong serum antibody response (Radermecker, Bekhti, Poncelet & Salmon, 1974). Serum antibody may, of course, be a most unreliable indicator of the antibody directed against intestinal parasites (see Ogilvie & Jones, 1971). Transfer

of massive volumes of 'hyperimmune' serum has no deleterious effect on H. diminuta in the intestine of the mouse (Hopkins, personal communication; Isaak, 1976; Andreassen et al., 1978a); serum antibody can protect against H. nana in mice, probably because it attacks invading oncospheres from an egg infection as they penetrate the villi (Ito, 1977), and hence serum antibody to H. diminuta may not reach the worm because the intestine is not damaged. Further evidence against the involvement of antibody was presented by Isaak (1976), who found that incubation of cysticercoids with immune serum and complement had no effect on their viability (in contrast to the work of Herd (1976) with Echinococcus), and also made the important discovery that abrogation of antibody response with anti-IgM did not prevent rejection of H.diminuta.

c) Other immune-related changes

Small intestine villous atrophy is known to be mediated by T cells, and is a feature of many parasitic infections (reviewed by Ferguson & MacDonald, 1977), and Befus (1975b) reported preliminary results indicating that the small intestine of mice infected with H. diminuta may display partial villous atrophy; this was not confirmed in a study by Andreassen et al. (1978a). The latter authors, however, did find a significant increase in the number of jejunal mast cells and globule leukocytes in mice infected with H. diminuta, both cell types reaching peak numbers on day 10 of infection; however, they also found that their nude mice could reject H. diminuta without the appearance of either cell type.

d) The effect of the immune response on the worm

The immunological attack mounted by the mouse against H. diminuta manifests itself in a variety of different ways.

Firstly, worms under attack often have opaque dark areas on the scolex, neck, and/or strobila, of "variable size and position" (Befus & Threadgold, 1975); these increase in number until the worms are expelled and occur with greater frequency in six-worm than in one-worm infections. That these dark areas are sites of worm damage was confirmed by these authors with electron micrographs of distal cytoplasm showing abnormal mitochondria, lipid droplet accumulation, etc., and they postulated that this damage was the result of immune reactions by the mouse.

Associated with the same (i.e. pre-rejection) phase of the response, Bland (1976b) found that transport of methionine and sodium acetate across the tegument was depressed in worms under immunological stress compared with that in worms from immunodepressed mice. This may be associated with the second major effect of the immune response of the mouse, i.e. the slowing or stopping of worm growth. This effect (Hopkins et al., 1972a) is reversible with the use of immunosuppressants such as cortisone acetate (Hopkins & Stallard, 1976). Growth of H. diminuta in secondary infections is very much retarded (Hopkins et al., 1972a; Befus, 1975a), as is the case with the long-surviving H. microstoma (Howard, 1976). Indeed, stunting of growth is the major feature of secondary cysticercoid infections in the mouse, Befus having pointed out that the complete rejection of worms in a secondary infection does not occur at a faster rate than in a primary infection; he therefore advised that, as severely stunted worms

are often completely indistinguishable from destrobilated worms, total worm weight per group of mice (biomass) should be used as the criterion for assessing the effect of a secondary response.

The third and most dramatic feature of rejection is that after 10 days in a naive (previously uninfected) mouse, many worms destrobilate, leaving the scolex and part of the neck (0.5-1.0 mm long) in the small intestine, the rest of the strobila passing out in the faeces (Turton, 1971: Hopkins et al., 1972a). Destrobilation may occur earlier than this in multiple-worm infections (personal observations), but not normally before day eight of infection. Destrobilation may not, however, be a sine qua non of rejection; the present author has, on a few occasions, recovered worms from the caecum of the mouse with the scolex still attached.

The fourth and final feature of rejection is the expulsion of the scolex from the small intestine. Destrobilated worms frequently have dark areas at their posterior tip (Befus & Threadgold, 1975) and are apparently restrained from regrowing by the immune response of the mouse; treatment of the mouse with immunosuppressants (Hopkins & Stallard, 1976) or transplantation of the worm into an immunologically naive host (Hopkins et al., 1972a) will permit regrowth of the destrobilated worm. The time taken for expulsion of destrobilated worms appears to be very variable, and the technical difficulties involved in finding these worms make recovery results very variable and unreliable.

e) Evaluation of the immune response to H. diminuta

In single-worm infections, the situation is relatively simple; direct comparison of growth of the worms between two groups or individuals is possible, and rejection is a single event (i.e. the worm is either intact or is destrobilated/rejected). Multiple-worm infections make results less variable and hasten rejection (Befus, 1975a), but there are associated problems. Rejection of all the worms in a multiple-worm burden rarely takes place simultaneously in a mouse, and much more common is the loss of worms over a 2-4 day period. Comparison of weights of worms in different groups of mice harbouring at that moment in time different numbers of worms is difficult, because the worms are extremely susceptible to growth-limiting inter-worm competition (Roberts & Mong, 1968; Hesselberg & Andreassen, 1975), and rejection of a proportion of the worms will release the remaining worms from some of the crowding stress. H. diminuta can double in weight in about 24 hours, so mice in which 50% of the worms have been lost may well support a greater mass of worms than mice in which rejection is less advanced.

In contrast, destrobilation is a unique event, and the use of destrobilation as a discrete marker of the 'end-point' of rejection is technically very simple, assuming that one is dealing with worms that are sufficiently large to be easily distinguished from destrobilated worms. Consequently, the means of graphical display of rejection in this thesis is often the percentage recovery of strobilate worms plotted against age of infection on the horizontal axis.

As the data on worm burden weight per mouse is not usually

normally distributed, standard deviation of the mean is, strictly speaking, not a valid measure of anything. Mean weight of worm tissue per mouse is itself also an unsatisfactory quantity, as bimodally distributed results (such as one obtains during the course of rejection) give a mean which is well-distanced from any other actual result. Total biomass of worms per group of mice is considered the most satisfactory quantity by many workers, although this figure gives no information as to its constitution. For these reasons, I have decided to display many results as weights of worm tissue for each individual mouse; differences between two groups of mice are fairly easily seen, as is the extent of the difference. Mean weight of worm tissue per mouse is sometimes superimposed on this type of graph; although not a 'real' figure, its use carries some advantages. This mean (hereinafter referred to as 'mean worm weight per mouse') is the total biomass divided by the number of mice, and as such allows for different numbers of mice in the groups; a visual comparison of the mean with the individual results is easily made, and thus this type of display makes more information available to the reader. Mice from which no worms were recovered are also plotted as such on these graphs, which conveys a little extra information about the distribution of the worms in the percentage recovery figures.

The present study

The work presented in this thesis was undertaken to characterise more fully the nature of the immune responses of the mouse small intestine directed against H. diminuta. The work is divided into four parts, each of which investigates a different aspect of the response.

In the first Chapter, the immunological changes which occur during pregnancy and lactation were studied with respect to H. diminuta. Both stages in the reproductive cycle are known to depress T effector lymphocyte functions, while antibody induction and production remain unaltered or enhanced. This therefore provided an opportunity to study the effect of a good antibody response with poor T cell function on the worm. The effects of antibody alone were investigated in young, immunologically incompetent mice receiving milk from immunised mothers. The first Chapter forms the major part of the thesis.

In the second chapter, the source of the protective antigens of H. diminuta (i.e. scolex and/or strobila) was investigated by transplantation of worms, and by study of the immunological properties of worms stunted by irradiation (described here for the first time).

In the third chapter, the effect of intestinal inflammation initiated by unrelated parasites was studied with respect to two Hymenolepis host-parasite systems in an attempt to clarify the reasons why some species of tapeworm are not rejected by their hosts.

The final chapter is an investigation into the role of the lymph nodes associated with the intestine in the response to Hymenolepis diminuta by the mouse, by attempting to transfer resistance with lymphocytes, and by study of cell traffic to the intestine.

GENERAL MATERIALS & METHODS

1. Animals used and their maintenance

a) Mice and rats

Male and female mice were used, usually females; unless otherwise stated, mice were of the same age and sex in any one experiment. For the majority of experiments mice were purchased from commercial suppliers, but many experiments utilised mice bred at the Wellcome Laboratories. The strains of mouse employed were outbred CFLP, inbred NIH (Anglia Laboratory Animals, Huntingdon) and outbred CD-1 (Charles River Ltd.).

To minimise the effects of concurrent infection with other metazoan, protozoan and bacterial organisms, mice used were of the highest grade available. Mouse quality was assessed by reference to the grading system devised by the MRC (The Accreditation & Recognition Scheme for Suppliers of Laboratory Animals 1974). Mice were of 4* category (free of all intestinal protozoa and helminths) whenever possible, but on some occasions 3* or 2* mice (free of cestodes, including intermediate stages) were used; if infection with the pinworms Aspiculuris tetraptera or Syphacia obvelata was detected, the mice were treated with piperazine before starting the experiment (see 'Anthelmintics' in this section). On no occasion was any cestode found at autopsy other than those administered during the course of the experiments.

Rats used were of the outbred CFHB strain (Wistar-derived) bred at the Wellcome Laboratories.

In most experiments mice were caged in groups of four or

five in polypropylene cages 48 cm x 15 cm x 13 cm; in some larger experiments, mice were caged in groups of 10 in 45 cm x 28 cm x 13 cm cages (North Kent Plastics Ltd.). Sawdust or wood shavings were used as bedding; bedding was replaced at least twice per week. Animal rooms were maintained thermostatically at 20-22°C; the lighting was automatically maintained on a 12 hour cycle in winter, but followed day length in summer. Tap water, normally without additives, was available to the animals ad libitum, as was food (Rat and Mouse Breeding Diet, Grain Harvesters Ltd.).

b) Parasites

The strains of Hymenolepis diminuta and H. microstoma used were obtained from Rice University, Houston, Texas in 1963 and 1964 respectively, and since then have been maintained at the Wellcome Laboratories by repeated passage through flour beetles (Tribolium confusum) and rats (for H. diminuta) or mice (for H. microstoma).

The technique for infecting beetles with the tapeworm eggs involved recovering adult worms from the small intestine of the mouse or rat, placing mature gravid proglottids (maturity assessed by the appearance of the eggs which escape from punctured proglottids) in distilled water at room temperature, and homogenising the proglottids in a MSE blender for 90 seconds by turning the (uncalibrated) speed control through 180°. The resulting homogenate was placed in a round-bottomed crystallising dish and the eggs allowed to settle out. The supernatant containing unwanted homogenised tissue was discarded and the eggs resuspended in water; this process was then repeated. After washing, the eggs were dried to a moist

paste with strips of filter paper, and the eggs were presented on a circle of filter paper to beetles which had been starved for 3 to 5 days. After 24 hours, most of the eggs had been eaten, and the beetles were then given wholemeal flour.

Latterly, a slightly different method was used for collection of H. diminuta eggs for beetle infection. Faecal pellets of rats harbouring mature H. diminuta were collected in tap water over 24 hours. Gravid proglottids were then recovered and blotted to remove most of the surface water before being given directly to starved beetles. It was found that this latter method gave a higher level of infection of beetles (15-20 cysticercoids per beetle) than the former (about 8 cysticercoids per beetle).

Although cysticercoids of H. diminuta are theoretically mature 14 days after infecting the beetles, at least 21 days were allowed after infection before using the cysticercoids. The beetles were maintained in dark incubators at 25°C (latterly 28°C). Beetle larvae were removed monthly from infected stock to prevent dilution of the infected population.

The strain of Trichinella spiralis used in experiments in Chapters 3 and 4 was obtained from the London School of Hygeine and Tropical Medicine, and was maintained by passage through mice (usually CFLP strain). Infective larvae were obtained by digestion of stock mice which had been infected for at least 60 days. One or two stock mice were killed, minced, and digested for 2½ hours in 500 ml of 0.5% pepsin in 0.5% HCl at 37°C. After filtering off coarse undigested sediment, the larvae were collected by repeated washing and sedimentation in 0.9% NaCl. The larvae were collected and suspended in 0.2% agar to give a final concentration of approximately

2000 larvae/ml.

2. Infection procedures

a) Oral infection

When fewer than 50 cysticercoids were required, they were dissected out from infected beetles in HBSS (see 'Balanced salt solution' in this section) with mounted needles and fine forceps. When more than 50 cysticercoids were required, they were obtained by homogenisation (see Ridley & MacInnis, 1968); the appropriate number of beetles was placed in HBSS and homogenised in the MSE blender at room temperature for 30 seconds at the 180° speed setting. The homogenate was then poured into a 10 cm petri dish; swirling the contents effectively collected the cysticercoids in the centre of the dish.

Within one hour of preparing the cysticercoids (unless otherwise stated), mice were infected by stomach tube while under light ether anaesthesia, The stomach tube apparatus consisted of a 2.5 ml syringe connected to 30-40 cm of polythene tubing (Portex Ltd.) via a hypodermic needle of appropriate gauge. 1.27 mm OD tubing was used for infecting rats, 1.00 mm OD for large (over 25 g) mice, and 0.80 mm OD for small mice. Cysticercoids were flushed into the stomach with approximately 0.3 ml HBSS from the syringe.

Larvae of T. spiralis were administered orally with a syringe and blunted cannula in 0.1-0.2 ml of 0.2% agar.

Throughout the thesis, the day of infection is designated day 0 p.i. (post infection).

b) Surgical infection

In many experiments, mice were infected with H. diminuta by inserting strobilate worms (recovered from donor rats or mice treated with cortisone) directly into the duodenum of recipients. Recipient mice were anaesthetised with a mixture of 'Sagatal' (sodium pentabarbitone 60 mg/ml; May & Baker Ltd.), 95% ethanol and HBSS in the ratio 1:1.5:7.5 by volume. This solution was administered by intra-peritoneal injection of 0.01 ml per gram body weight up to 25g, thereafter 0.03 ml per 5 g body weight. Induction is rapid (normally within one minute) and the anaesthesia persists for 2-3 hours; surgery was usually carried out within one hour of administration of the anaesthetic.

When the anaesthetic had fully taken effect, the abdomen of the mouse was shaved and swabbed with 70% alcohol. In addition to sterilising the skin, the evaporation of the alcohol causes small superficial blood vessels to constrict, thereby making larger vessels more obvious visually. A small incision (less than 1 cm) was made with a scalpel just to the animal's right of the midline 1 cm below the edge of the ribcage. The body wall was then penetrated using very sharp fine forceps, the points of which were opened out after insertion to provide an opening through which the proximal duodenum was pulled, using a small rounded metal hook. To reduce adhesions between the skin and body wall, the body wall was opened at a point that did not underlie the skin incision. The duodenum was punctured with a hypodermic needle, and the worm was introduced with Portex tubing of diameter appropriate to the worm, the tubing being attached to a 1 ml syringe. The worm was drawn into the tube, scolex first, making sure that the worm was not

damaged in the process, so that on inserting the tube the worm was expelled posterior end first. The tube was inserted so that the worm was washed down the intestine (with 0.3 ml HBSS) rather than towards the stomach; the open end of the tube was cut obliquely to facilitate insertion. After withdrawing the tube, the puncture was sutured with one stitch (0.7 M Mersilk Mersuture, Ethicon Ltd.). The exposed intestine serosa was then sprayed with an aerosol mixture of bacitracin, polymyxin and neomycin (Rikospray, Riker Laboratories Ltd.). The loop of intestine was then replaced into the body cavity and the body wall was sutured with one stitch and sprayed with Rikospray. The skin was then sutured with two stitches (it being essential for healing that the internal surfaces of the skin are presented to each other) sprayed with Rikospray and sealed with aerosol plastic skin (Nobecutane, Astra Chemicals Ltd.).

Using these procedures, about 90% of the operations were conducted without any noticeable blood leakage. Mortality, usually attributable to the anaesthetic, was less than 5%. Mice were routinely given Terramycin in their drinking water post-surgically (see 'Immunosuppression & Antibiotics' in this section).

3. Anthelmintics

Colonies of mice found to be harbouring the pinworms A. tetraptera or S. obvelata were treated with piperazine citrate ('Citrazine', Loveridge Ltd.) in the drinking water at a concentration of 3 g/l.

To remove experimental cestodes chemically, the anthelmintic oxyclozanide ('Zanil', ICI Ltd.) was administered to mice by

stomach tube at a concentration of 250 mg/kg. The mean weight per mouse was roughly determined and the Zanil diluted with distilled water accordingly such that each mouse received 0.5 ml.

4. Immunosuppression and antibiotics

In some experiments the immunosuppressive drug cortisone acetate ('Cortistab', Boots Ltd.) was used; this was administered every second or third day (normally Monday, Wednesday and Friday) commencing on day 0. The dose for mice was 1.0-1.25 mg per subcutaneous injection (0.04-0.05 ml Cortistab).

To prevent the appearance of opportunistic infection with bacteria, cortisone-treated animals and animals that had undergone surgery were given the antibiotic oxytetracycline HCl ('Terramycin', Pfizer Ltd.) at a concentration of 165 mg/l oxytetracycline (3 g/l Terramycin). This concentration provides a dosage of about 30 mg/kg/day for mice and 20-25 mg/kg/day for rats. Although oxytetracycline has recently been found to have immunosuppressive properties (Thong & Ferrante, 1979), no effect on growth or survival of H. diminuta has been detected; nevertheless, relevant control groups were also given oxytetracycline in their drinking water.

5. Recovery of worms

H. diminuta was recovered from mice and rats by removing the small intestine and flushing the contents into a crystallising dish with up to 50 ml of HBSS; to accomplish this, a 50 ml syringe was fitted with a wide-bore blunt cannula which was inserted into

the anterior end of the small intestine. If fewer than 100% of the worms were recovered, the washings and the small intestine (split longitudinally) were inspected under x6 magnification with a binocular microscope using transmitted light.

In experiments where destrobilated or small worms (less than 0.1 mg dry weight) were to be recovered, the intestine was split longitudinally and cut transversely into four roughly equal segments, and incubated in 5 cm petri dishes at 37°C for 1½-4 hours. These dishes were inspected at intervals for the appearance of worms which had become detatched from the mucosa. Although time-consuming, this method was found to be much more efficient than a Baermann type apparatus.

All the worms over 2-3 mm long (i.e. over 0.1 mg dry weight) recovered from each mouse were blotted dry on filter paper and placed in aluminium foil cups in an oven at 90-100°C for a minimum of 24 hours. The dry weight of worm tissue from each mouse was then recorded to the nearest 0.1 mg.

Adult T. spiralis were recovered by removing the small intestine, splitting it longitudinally, and incubating it at 37°C for 2 hours in a simplified Baermann apparatus.

6. Statistical treatment of results

Unless otherwise stated, the statistical significance of differences in worm weights between two groups of mice was assessed by ranking the values of dry weight of worm tissue per mouse followed by application of the Wilcoxon test for non-paired samples as described by Snedecor & Cochran (1967). Values of p less than 0.05 were considered to indicate a statistically significant difference.

In Chapters 2 and 4, Student's t test was sometimes applied, but it is always clearly stated that this was the test used; standard deviations are given with data when this test is used.

7. Balanced salt solution (HBSS)

Modified Hanks' balanced salt solution (referred to throughout this thesis as HBSS) used was as described by Hopkins & Stallard (1974). Hanks' saline was modified by excluding glucose and $NaHCO_3$ and increasing the remaining salts pro rata to an osmotic pressure of 300 m-osmole.

Solution I	NaCl	168 g
	KCl	8 g
	KH_2PO_4	2 g
	Na_2HPO_4	4 g
	0.2% phenol red	200 ml
	made up to 2 litres with deionised water	
Solution II	$CaCl_2.2H_2O$	3.92 g
	$MgCl_2.6H_2O$	2.00 g
	made up to 2 litres with deionised water	

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

CHAPTER I

PREGNANCY, LACTATION, AND TRANSFER OF IMMUNITY TO OFFSPRING

INTRODUCTION

1) Pregnancy

The mammalian foetus is a natural allograft that, under normal immunological conditions, would quickly be recognised as having foreign (i.e. paternal) histocompatibility antigens and would be immunologically rejected. Despite the fact that antibodies are produced by the mother against paternal transplantation antigens (Ceppellini, 1971), rejection of the foetus does not normally occur, and this is a problem that has fascinated immunologists for many years (see Medawar, 1953). Various theories to explain the non-reactivity of the mother have been advanced; the uterus may be an immunologically privileged site (Kirby, Billington & James, 1966), or may form an immunologically inert barrier (Currie, Doorninck & Bagshave, 1968), or the trophoblast may be poorly antigenic (Palm, Heyner & Brinstler, 1971). In recent years, however, it has been found that pregnancy alters the immunological reactivity of the animal as a whole. The iliac lymph nodes (those draining the uterus) increase in weight during pregnancy (see McLean, Mosley & Gibbs, 1974), and exhibit cellular proliferation and enhanced trapping of lymphocytes passing through the nodes (Ansell, McDougall, Speedy & Inchley, 1978); however, the other lymph nodes of the body and the thymus are adversely affected by pregnancy. There is a large volume of published work on the thymic involution that occurs during pregnancy; This effect was probably first described by Fulci (1913). The main features are that thymus weight decreases dramatically during pregnancy (Millar, Mills & Baines, 1973; McLean et al., 1974)

and that this is associated with disruption of thymic architecture, the cortical region being most affected (Ito & Hoshino, 1962; millar et al., 1973).

As might be expected, the immunosuppressive effects of pregnancy are largely due to interference with T cell function. Depressed in vivo T-dependent processes include the response to oxazolone (Skowron-Cendrzak, Ptak, Bubak & Czarnik, 1975) and picryl chloride (Fabris, 1973); slower skin allograft rejection (Andresen & Munroe, 1962; Beer & Billingham, 1974) and reduced response to local graft-versus-host reaction (Skowron-Cendrzak et al., 1975). The total number of circulating T cells may decrease during pregnancy, at least in humans (Bulmer & Hancock, 1977), although this is disputed (see Sablovic & DeRomemont, 1978).

Correlating with this, in vitro responses of lymphocytes are depressed after addition of pregnancy serum; e.g. stimulation of cell division of T lymphocytes by mitogens (Leiken, 1972; Bjune, Duncan, Barnetson & Melsom, 1978) and mixed lymphocyte culture reactions (Kasakura, 1971). Lymphocytes from pregnant women also show reduced responsiveness to PHA, a T cell mitogen, in the absence of pregnancy serum (Purtilo, Hallgren & Yunis, 1972) and lymphocytes from pregnant mice have higher rosette inhibition titres (a measure of immunodepression - see Morton, Hegh & Clunie, 1974). The degree of immunosuppression caused by pregnancy is apparently largely related to the extent to which paternal and maternal transplantation antigens differ; syngeneic matings stimulate less immunosuppression in mice than do allogeneic matings (Skowron-Cendrzak et al., 1975).

Thymus-dependant functions thus appear to be depressed during pregnancy, but B cell function is apparently largely

unaltered or even enhanced; the number of circulating B cells remains unaltered during pregnancy (Bulmer & Hancock, 1977; Plum, Thiery & Sabbe, 1978). IgE production is unaffected by pregnancy (Vijay & Buttar, 1977) and antibody response to sheep erythrocytes is enhanced in pregnant mice (Fabris, 1973); as the antigens in these experiments were presented during pregnancy, the possibility must exist that 'helper' T cells, necessary for production of antibodies to T-dependent antigens (reviewed by Gisler, 1977), remain unaffected, at least in a suppressive sense, by pregnancy.

Other cell types that have been investigated include neutrophils and monocytes, which increase in number during pregnancy (Plum et al., 1978). Neutrophils display enhanced phagocytosis of immune complexes in the presence of pregnancy serum (Rosenthal, 1977), but pregnancy serum will also cause a decrease in the release of lysosomal enzymes from macrophages.

Again, many theories have been advanced to explain why pregnancy has this modulating effect on the non-uterine immune system, the most attractive hypotheses being based on the observed changes in concentrations of serum proteins or hormones. The non-hormonal theories include evidence for the immunosuppressive action of placental and hepatic transcortin (Werthamer, Govindaraj & Amaral, 1976), 'blocking' antibodies (Hellstrom, Hellstrom & Brawn, 1969), pregnancy zone protein (Von Shoultz, Stigbrand & Tarnvik, 1973), and alpha-foetoprotein (Yachnin, 1975; Gupta & Good, 1977). Steroid hormones have long been suspected as being the cause of the immunodepression, the state of pregnancy inducing radical changes in the pattern of hormone secretion; for example, the level of serum corticosterone in the mouse increases from a nulliparous level of 2.3 µg/100 ml plasma 60-fold to a level of

138 µg/100 ml plasma in the second half of pregnancy (Barlow, Morrison & Sullivan, 1974). Corticosteroids are known to be immunosuppressive, especially in the mouse, which is a steroid sensitive species (reviewed by Claman, 1975). Although plasma corticosterone during pregnancy is 98% protein-bound (Barlow et al., 1974), the carrier protein may also contribute to the immunodepression (Werthamer et al., 1976). Other hormones found to have an immunosuppressive effect in vitro include progesterone and derived steroids, estradiol, estrone, estriol, testosterone derivatives, hydrocortisone, and human chorionic gonadotropin (HCG) (Schiff, Mercier & Buckley, 1975; Rembiesa, Ptak & Bubak, 1974; Mendelsohn, Multer & Bernheim, 1977). Schiff et al. (1975) found that various gestational hormones could inhibit human lymphocyte DNA synthesis, but only at very high, supraphysiological concentrations; the possibility exists, however, that these hormones act synergistically or have a long-term effect. Progesterone is probably a more important factor than the oestrogens; in the mouse, the concentration of serum progesterone rises sharply between days two and six of pregnancy, and only begins to decline after day 17. The concentration of oestradiol drops dramatically on the first three days of pregnancy, and levels are roughly inversely proportional to progesterone during pregnancy (McCormack & Greenwald, 1974). Early reports of the strong immunosuppressive effect of HCG (Contractor & Davies, 1973; Adcock, Teasdale, August, Cox, Meschia, Battaglia & Naughton, 1973) have not been confirmed in experiments using highly purified HCG rather than crude commercial preparations (Muchmore & Blaese, 1977).

There has been relatively little work on the effect of pregnancy on the course of infection with parasitic organisms:

susceptibility to the effects of smallpox, poliomyelitis, rubella and varicella viruses is enhanced during pregnancy (Pickard, 1968; Mims, 1977), and exacerbation of malarial infections in pregnant women is common (Gilles, Lawson, Sibelas, Voller & Allen, 1969). One of the few reports of the effect of pregnancy on metazoan parasite infection is from Larsh (1949), who found that mice infected with 1000 'Hymenolepis' (=H. nana) eggs in the second week of pregnancy "harboured an average of about twice as many cysts as the controls (67.7 and 31.2)". He attributed this to anaemia in the pregnant mice, but the altered physical and physiological state of the small intestine in late pregnancy (Craft, 1970) may also have altered the "natural resistance to this parasite" in addition to any deficiency in immune responsiveness.

2) Lactation

In contrast to pregnancy, lactation is well-documented by parasitologists as depressing immunity to parasites of the intestine, but very little 'purely immunological' work has been published on the subject. The thymic involution of pregnancy stops at parturition and the thymus starts to regenerate; this regeneration, however, is retarded by lactation, being faster if lactation is prematurely terminated (Ito & Hoshino, 1962). As with pregnancy, induction and expression of antibody responses appear to be largely unaffected during lactation (Kelly & Ogilvie, 1972; Connan, 1973; Selby & Wakelin, 1975), so it appears that cell-mediated immunity is again the function that is depressed.

Increased susceptibility to intestinal nematode infection during lactation has been demonstrated in several host-parasite

systems (Table I:I). In experimental situations, most work on the immune mechanisms depressed during lactation has been on the Nippostrongylus brasiliensis - rat model. It has been found that although anti-worm antibody production is unaffected by lactation (Connan, 1973a) and that worms in lactating rats are damaged by this antibody (Kelly & Ogilvie, 1972), the expulsion of worms is impaired in primary and secondary infections (Connan, 1970, 1972a).

Mesenteric lymph node cells (MLNC) from lactating rats are capable of transferring immunity to N. brasiliensis to nulliparous recipients, suggesting that it is the final effector arm of the response that is impaired (Dineen & Kelly, 1972); although these authors also found that immune MLNC from nulliparous rats were functional in lactating recipients, Ngwenya (1976a), using Trichinella spiralis in the mouse found that transfer of immunity with cells between lactating and nulliparous animals was not possible in either direction. This may, however, be due to the experimental proceedure used by the latter author (immunisation with T. spiralis antigen and transfer of immunity with spleen cells). Eosinophilia and mast cell infiltration in the wall of the small intestine of rats infected with N. brasiliensis is delayed by lactation, but it is open to doubt whether these cell types are involved in the rejection process (Kelly & Ogilvie, 1972).

Not surprisingly, hormonal levels associated with lactation have been implicated in the lowered resistance to intestinal parasites. Levels of ACTH, adrenal corticoids and prolactin are all elevated during lactation (Meites & Nicholls, 1959). Prolactin appears to be the most favoured candidate, causing suppression of rejection of N. brasiliensis in the rat (Kelly & Dineen, 1973) and T. spiralis in the mouse (Ngwenya, 1976b); again, however, the

Table I-I

Some intestinal parasitic nematodes known to have enhanced establishment, fecundity, and/or survival in lactating hosts.

Host	Parasite	Reference
Ewe	*Haemonchus contortis*	a
	Nematodirus helvetianus	b
	Ostertagia spp.	c
	Trichostrongylus colubriformis	d
Sow	*Hyostrongylus rubidis*	e
Guinea-pig	*T. colubriformis*	f
Rat	*Nippostrongylus brasiliensis*	g
	Trichinella spiralis	h
Mouse	*T. spiralis*	i, j
	Trichuris muris	k

References

a) O'Sullivan & Donald, 1973
b) Zawadowesky & Zvjaguintzer, 1933
c) Dunsmore, 1965
d) O'Sullivan & Donald, 1970
e) Connan, 1971
f) O'Sullivan, 1974
g) Connan, 1970
h) Love, Ogilvie & McLaren, 1976
i) Duckett, Denham & Nelson, 1972
j) Ngwenya, 1976a
k) Selby & Wakelin, 1975

observed effect is probably due to a synergistic effect of various hormones, as levels of prolactin during the oestrus cycle frequently approach concentrations present during lactation without any obvious effect on immune status (reviewed by Connan, 1973b). It is also interesting to note that administration of prolactin causes progesterone secretion by mouse ovaries (Nicoll & Bern, 1972), and may therefore affect other hormone levels which in turn may have an effect on the functioning of the immune system. Whatever the hormonal basis, lactation is maintained by the suckling stimulus, and immune competence returns rapidly if litters are removed prematurely (Connan, 1970; Selby & Wakelin, 1975).

3) Transfer of maternal immunity to offspring

It is a feature of many mammals that immunity acquired by the mother can be transferred passively to the offspring, either by transplacental passage of immunoglobulin (Ig) or by secretion of Ig into the colostrum and milk. This latter Ig can be absorbed intact across the gut wall of the suckling neonate (reviewed by Hemmings, 1974).

Transfer of immunity to intestinal helminths by these methods has been described in detail for T. spiralis (Duckett, Denham & Nelson, 1972; Perry, 1974), Nematospiroides dubius (Chaicumpa, Jenkin & Rowley, 1976), and H. nana (Larsh, 1942). The presence of an infection in the mother at the time of pregnancy and lactation is not required for transfer of immunity; protection afforded by Ig transfer in utero appears to be of short duration (disappearing 7-9 days after birth), the major contribution being milk-borne Ig which gives protection for up to six weeks after birth. The major

Ig of colostrum is IgA, less than 6% of total colostral Ig being accounted for by IgG and IgM (Brandtzaeg, Fjellanger & Gjeruldsen, 1970).

The present study

Investigation of the effects of pregnancy, lactation and transfer of Ig from mother to offspring is therefore potentially very important in the study of the immunological mechanisms involved in the rejection of H. diminuta from the mouse. In particular, the roles of T cells and antibodies in rejection can be judged. Pregnant and lactating mice have impaired T cell function, and might prove to be a further way of demonstrating the thymus-dependency of the response against H. diminuta as well as giving an insight into the activity of the (unaltered or enhanced) antibody response. As mice younger than five weeks old have little capacity for rejection of H. diminuta (Befus & Featherston, 1974), transfer of maternal immunity with Ig offers an even better system for investigating the effects on the worm of antibody in isolation.

MATERIALS & METHODS

Female mice were always mated with males of the same strain. Two females were mixed with 2-3 males in a cage, and the females were inspected daily (normally in the morning) for the appearance of copulatory plugs; it should be noted that these plugs are often not externally obvious, but with close inspection this technique was about 90% efficient for detecting mated animals. The ratio of males to females in a cage was high in order to reduce the possibility of mixing females with poor breeders, and also the refractory period between matings may be over 24 hours for some strains of male mice (Rugh, 1968), although this has not been determined for the mice used in this study. Using this high male:female ratio, the pregnancy rate was about 15% per day. The day of appearance of mating plugs was taken to be day +1 of pregnancy. 1-4 days before parturition, pregnant mice were caged singly with shredded tissue as nesting material; parturition normally occurred on day 20 of pregnancy. Litters were normally redistributed within two days of parturition to give 10 pups per suckling female; mice with fewer than six in their original litter were discarded (see Selby & Wakelin, 1975).

RESULTS

1. Pregnancy

a) Effect of pregnancy on primary course of infection

In the first experiment, CFLP mice were infected with five cysticercoids. Pregnant mice were infected on day 4 of pregnancy, and pregnant and nulliparous (control) mice were autopsied on days 8, 10, 12, 14 & 16 post infection (p.i.).

Establishment of worms was over 80% in the control group and over 90% in the pregnant group (Fig. 1-1). In the control group, worm loss had commenced by day 10 p.i. and was complete by day 14 p.i. In the pregnant group, however, recovery was still 100% on day 10p.i., but worm loss proceeded steadily thereafter. On day 16 p.i. (the end of the experiment, also the day of parturition), 35% of the worms administered to the pregnant mice still remained. The delay in rejection caused by pregnancy in this experiment was of the order of two days.

The graph of weight of worms recovered from each mouse (Fig. 1-2) shows that pregnant mice consistently supported a higher biomass of worms than the controls; the difference is statistically significant on all the days tested ($p < 0.05$). The difference in weights was not wholly due to the higher percentage recovery from the pregnant mice; the difference is still very marked between the mean weights per surviving worm for the two groups (Fig. 1-3), although no statistical significance can be attached to these results as the worms were not weighed singly.

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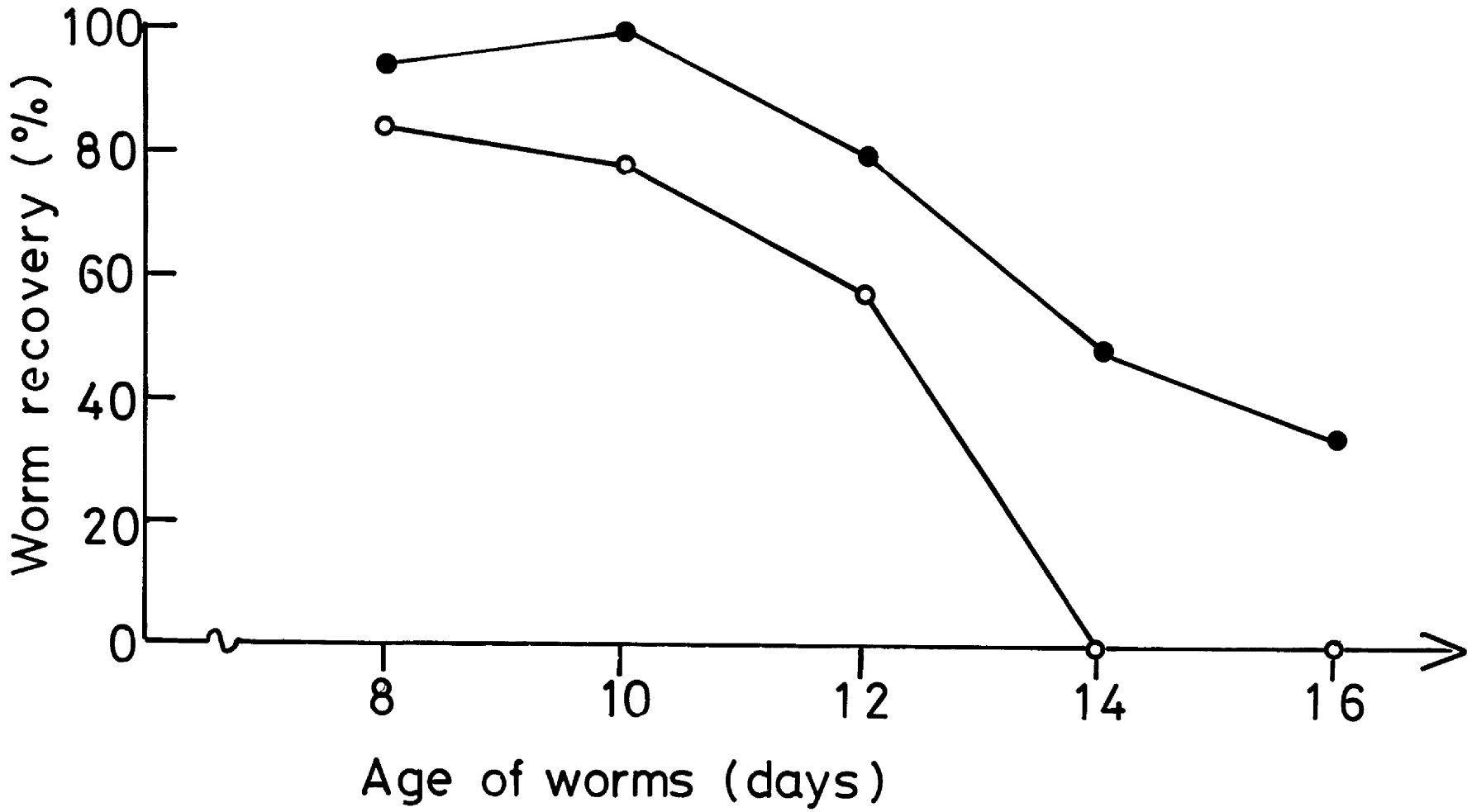
Figure I-1

Recovery (%) of H. diminuta from five-cysticercoid infections in pregnant (●) or nulliparous (o) CFLP mice; pregnant mice infected on day 4 of pregnancy.


100
80
60
40
20
0
Worm recovery (%)
8
10
12
14
16
Age of worms (days)

Facing page 36

Figure I-2

Dry weight of H. diminuta from five-cysticercoid infections in pregnant (⊙) or nulliparous (o) CFLP mice. Each point represents the total dry weight of worm tissue recovered from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.

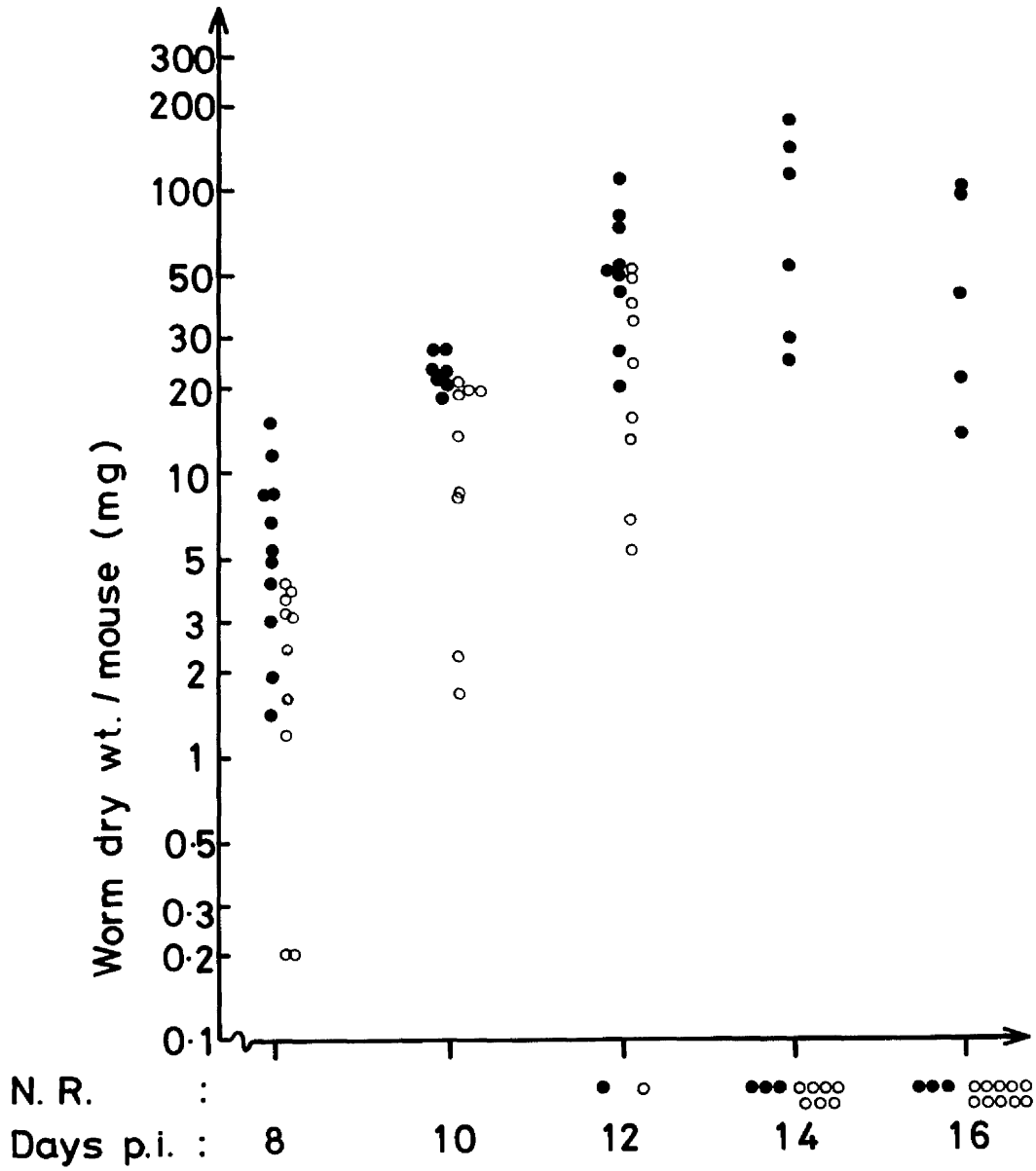

300
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Worm dry wt. / mouse (mg)
N. R. :
Days p.i. : 8 10 12 14 16

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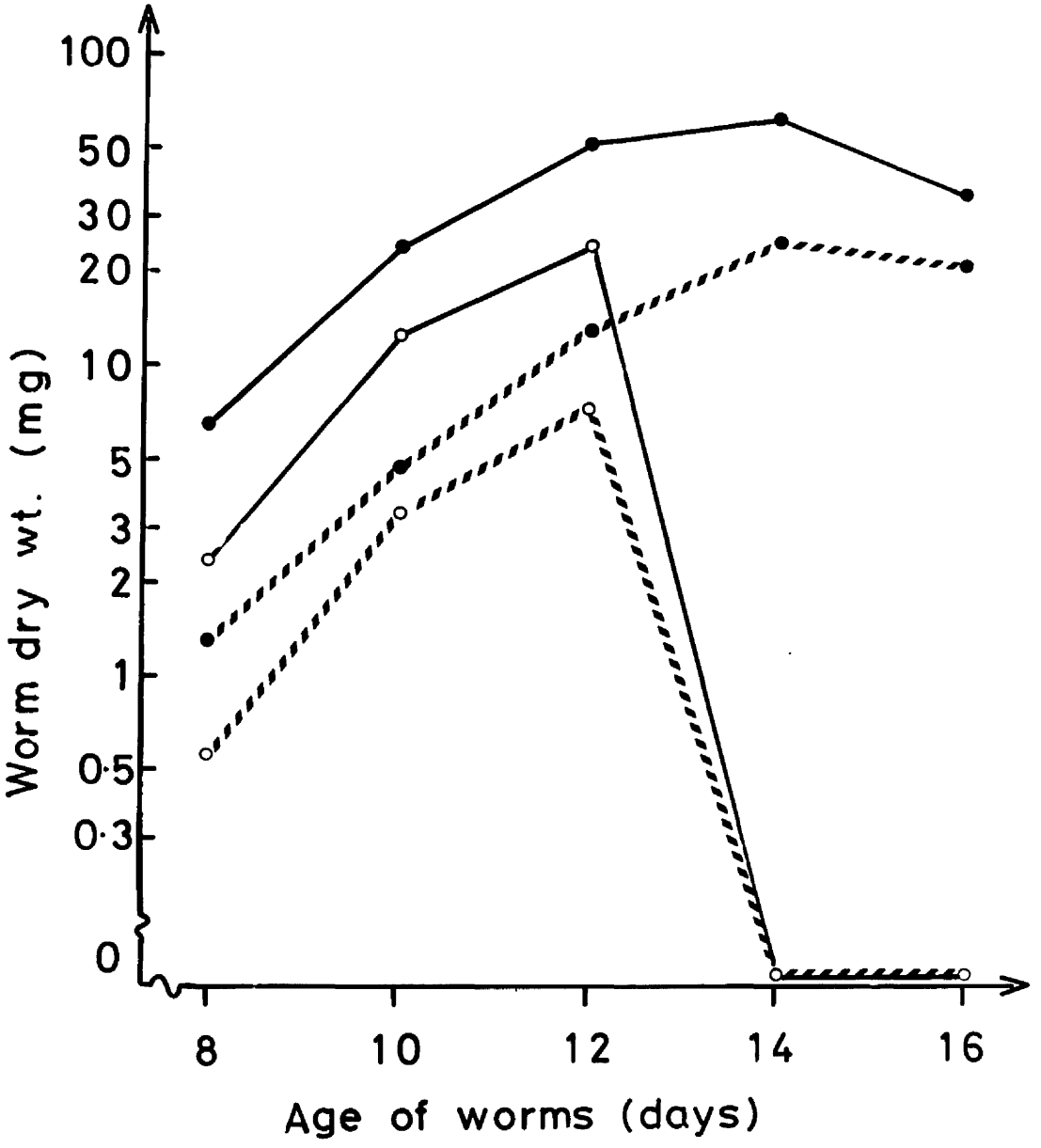
Figure I-3

Dry weight of *H. diminuta* from five-cysticercoid infections of pregnant (o) or nulliparous (o) CFLP mice.

———, mean dry weight per mouse

▰▰▰▰▰, mean dry weight per surviving worm


100
50
30
20
10
5
3
2
1
0·5
0·3
0
Worm dry wt. (mg)
8
10
12
14
16
Age of worms (days)

b) Effect of varying worm burden

Experiments were designed in which two different levels of infection were employed, the rationale being that rejection of H. diminuta from pregnant mice may be restored to control levels by increasing the 'antigenic load' to eight cysticercoids, and that by lowering the level of infection to three cysticercoids (and thus reducing the antigenic stimulus) the mice might display the immunodepressive effect of pregnancy more clearly and sensitively.

(i) Eight-cysticercoid infection

In this experiment pregnant and control (nulliparous) CD-1 mice were infected with eight cysticercoids. Pregnant mice (infected on day 5 of pregnancy) were autopsied on days 10, 12, 14 and 16 p.i., and control mice were autopsied on days 6, 10, 12 and 14 p.i. Owing to the low number of pregnant mice killed on day 10 p.i., the first day on which comparison with the controls can be made is day 12 p.i., on which day the pregnant mice supported heavier worms than the controls (Fig. 1-4), a statistically significant difference ($p < 0.01$). On days 14 and 16 p.i., no control mice harboured weighable worms, but 33% of the pregnant mice had recoverable worm burdens ranging from 5.7 mg to 154.4 mg.

The worm recovery results (Fig. 1-5) show that initial establishment was good (92.5%), and rejection by the control mice was very rapid, only 17.5% of the worms remaining on day 12 p.i., rejection being complete by day 14 p.i. As in the

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Figure I-4

Dry weight of H. diminuta from eight-cysticercoid infections of pregnant (◎) or nulliparous (o) CD-1 mice. Each point represents the total weight of worm tissue from a single mouse.
N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.

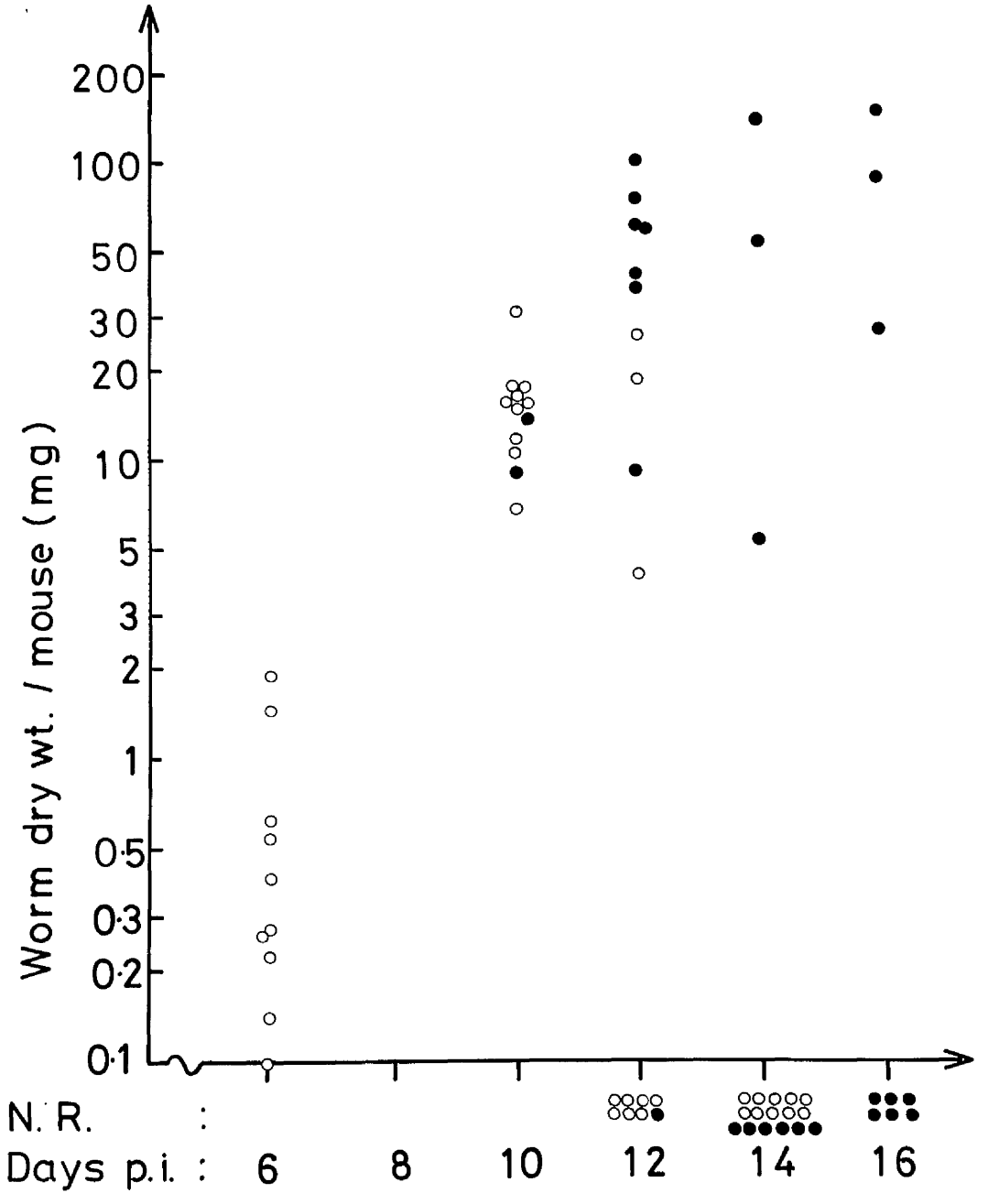
Worm dry wt. / mouse (mg)
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
N. R. :
Days p.i. :
6
8
10
12
14
16

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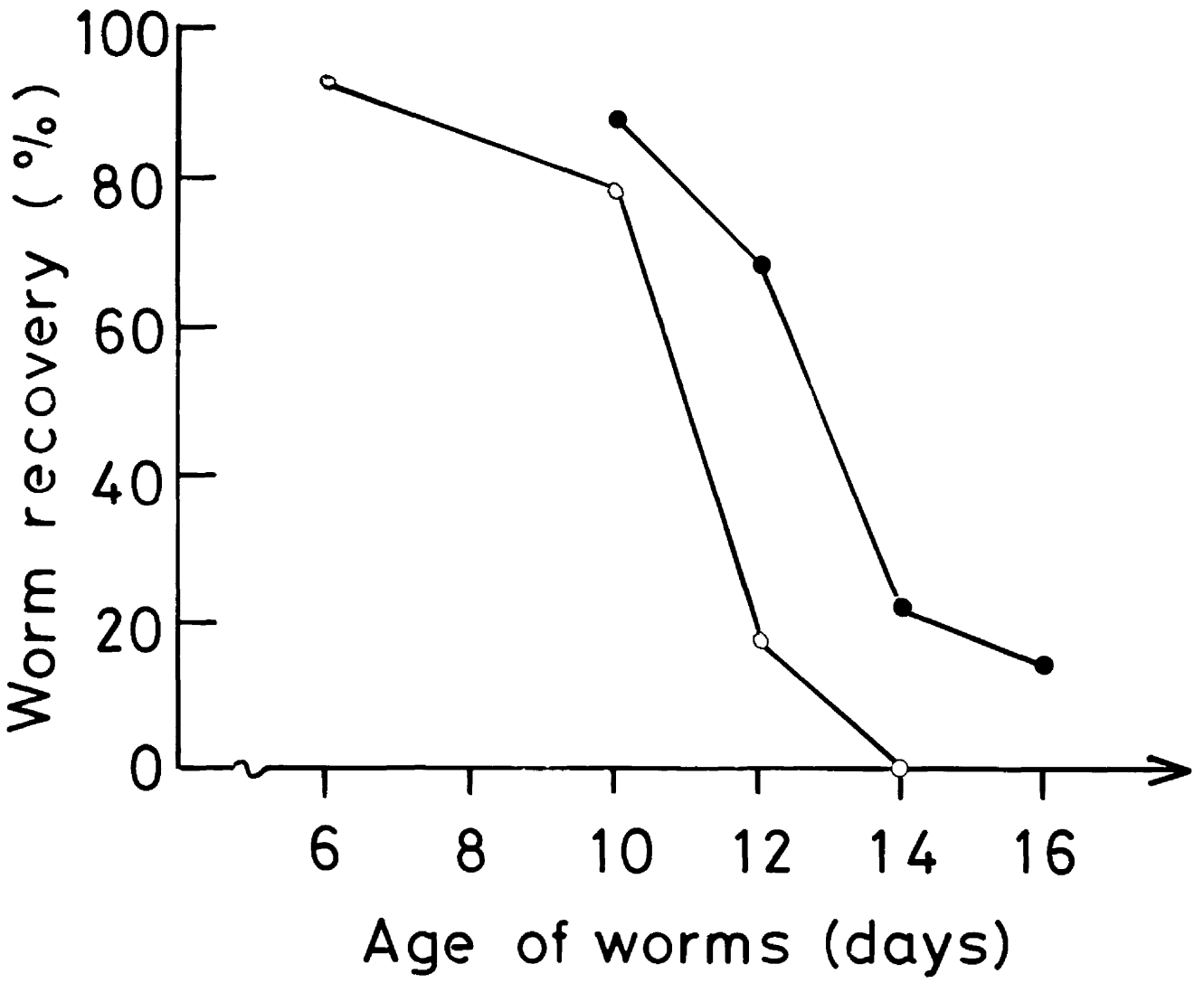
Figure I-5

Recovery (%) of H. diminuta from eight-cysticercoid infections of pregnant (●) or nulliparous (○) CD-1 mice; pregnant mice infected on day 5 of pregnancy.


100
80
60
40
20
0
Worm recovery (%)
6
8
10
12
14
16
Age of worms (days)

previous experiment, rejection of the worms by pregnant mice was consistently slower, the curves for the two groups being again about two days apart. As in the previous experiment, rejection was not complete by day 16 p.i. (day +1 of lactation in this experiment) in the pregnant mice.

(ii) Three-cysticercoid infection

Fig. 1-6 shows the combined results from two experiments using CFLP mice infected with three cysticercoids. Pregnant mice were infected 3-5 days after mating. In the first experiment, mice were killed on days 8, 9, 12 & 14 p.i., and on days 8, 9, 12, 14 & 17 p.i. in the second experiment. Again it can be seen that the pregnant mice support an increased weight of worm tissue ($p < 0.05$ on day 8 p.i.), and the percentage recoveries were always higher than in the controls (the recoveries from pregnant mice on days 12 and 17 are perhaps misleading, due to the low number of mice involved). It should be noted that rejection from the control mice was less efficient than usual, especially in the second experiment; 26% and 20% of the worms remained on days 14 and 17 respectively. This slow rejection was a feature of all the mice used at this time, and is not a reflection of lower antigenicity of three-worm infections. In these experiments, pregnancy may have acted synergistically with the slower response of the mice at this time, resulting in worm tissue weights per mouse of 260-465 mg on days 14 and 17 p.i.

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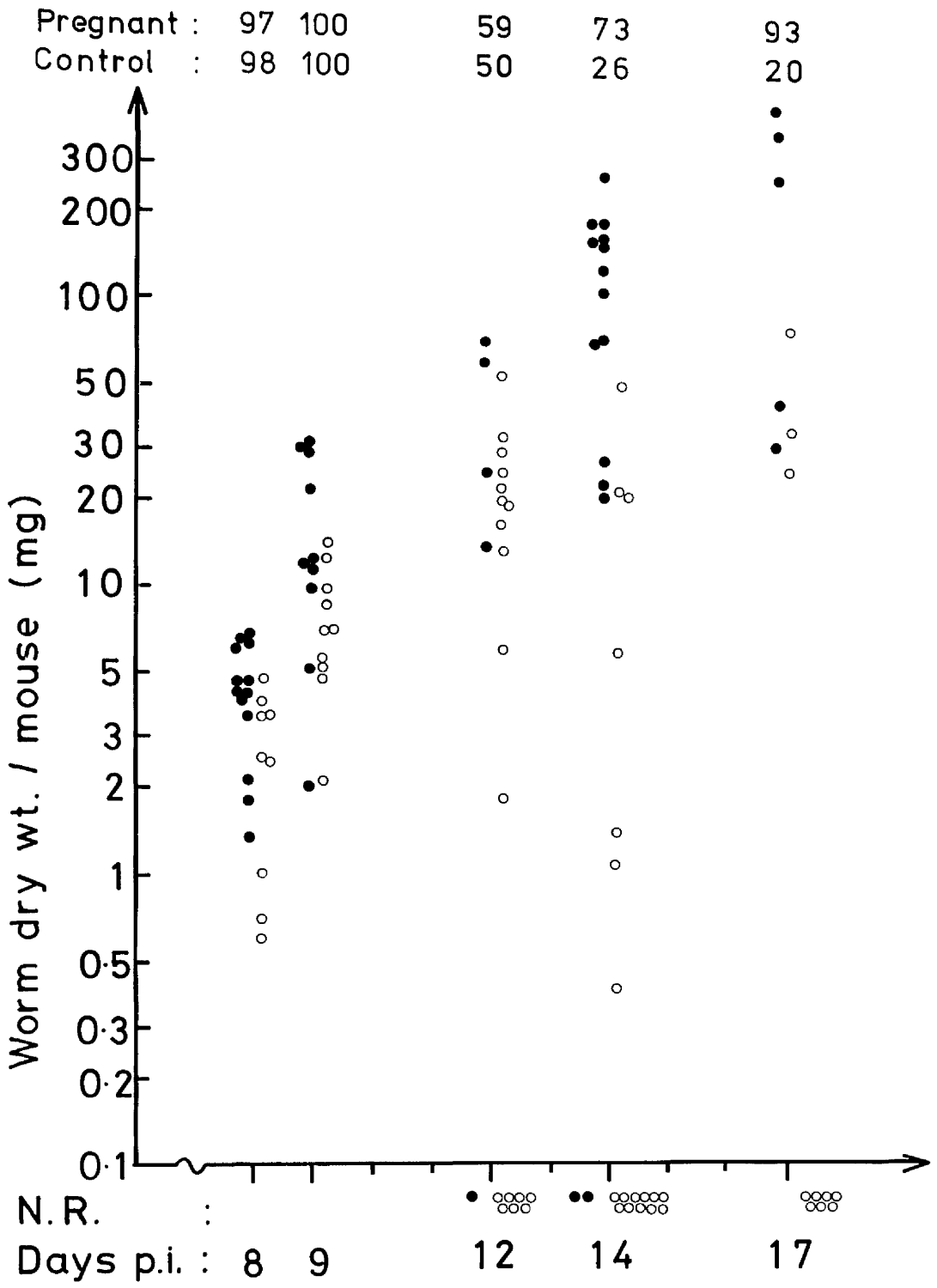
Figure I-6

Dry weight of H. diminuta from three-cysticercoid infections of pregnant (⊛) or nulliparous (o) CFLP mice. Each point represents the total dry weight of worm tissue recovered from a single mouse. The figures above the graph represent the recovery (%) of worms from the two groups. N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


Pregnant : 97 100 59 73 93
Control : 98 100 50 26 20
300
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Worm dry wt. / mouse (mg)
N.R. :
Days p.i. : 8 9 12 14 17

c) Effect of varying the time of infection

The design of the previous experiments in this chapter was such that rejection of H. diminuta from pregnant mice commenced in the last 4-6 days of pregnancy. Days 16-20 of pregnancy in the mouse is also the period when plasma corticosterone levels drop (Barlow et al., 1974), progesterone levels fall, and oestrogen levels rise (McCormack & Greenwald, 1974). This raises the possibility that rejection may occur during a period of relatively increased immunological responsiveness at parturition as these hormone levels return to nulliparous levels.

Although immunosupression during pregnancy has mainly been associated with the latter half of gestation (e.g. Fabris, 1973), there is evidence for an immunosuppressive factor appearing very early in pregnancy (Clarke, Morton & Clunie, 1978), so in addition to the experiment investigating the effect of parturition, an experiment to study the effect of early pregnancy response to H. diminuta was performed.

(i) Varying the time of infection during pregnancy

CD-1 mice were infected with eight cysticercoids on days 2, 5 or 13 of pregnancy to ascertain if the relative timing of parturition had any effect on the course of infection. mice were killed on the days shown in Table 1-2. Control (unmated) mice were killed on days 12 & 14 p.i. to determine whether the worms were rejected within the normal time-course, i.e. complete rejection by day 14 p.i.

The percentage recoveries plotted against the day of

Table I-2

Day of pregnancy at infection	Day of autopsy (post infection)	n
- (control)	12, 14	10, 9
2	14, 16, 18	8, 11, 5
5	13, 15, 17	8, 9, 10
13	7, 9, 14, 17	7, 4, 7, 8

pregnancy (Fig I-7) in the two groups infected on days 2 and 5 of pregnancy show that worm loss occurred in these two groups, apparently at the same rate, 2-4 days later from the day 5 pregnant group than from the day 2 pregnant group. That these mice were responding identically regardless of the timing of parturition is confirmed by the fact that the recoveries from these two groups plotted against day of infection (the lower graph in Fig. I-7) are almost identical. The total worm biomass supported by these two groups is also virtually identical (Fig. I-8), indicating that growth and rejection of worms in these two groups was largely unaffected by the relative timing of parturition. If parturition were a time when the immune response recovers briefly, one would expect that the mice infected on day 5 of pregnancy would expel expel their worms in a shorter time than those infected on day 2 of pregnancy; the present results therefore support the hypothesis that pregnancy has a depressive effect rather than a suppressive effect and that immune responsiveness is largely unaffected by parturition, at least not increased to a point at which a response in progress is accelerated.

The third group in this experiment (infected on day 13 of pregnancy) exhibited a degree of loss of worms at day 14 p.i.

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Figure I-7

Recovery (%) of H. diminuta from eight-cysticercoid infections of nulliparous mice (o) or mice infected on day 2 (□), 5 (●), or 13 (▲) of pregnancy, plotted against day of pregnancy and day of infection. Vertical dotted line indicates day of parturition.

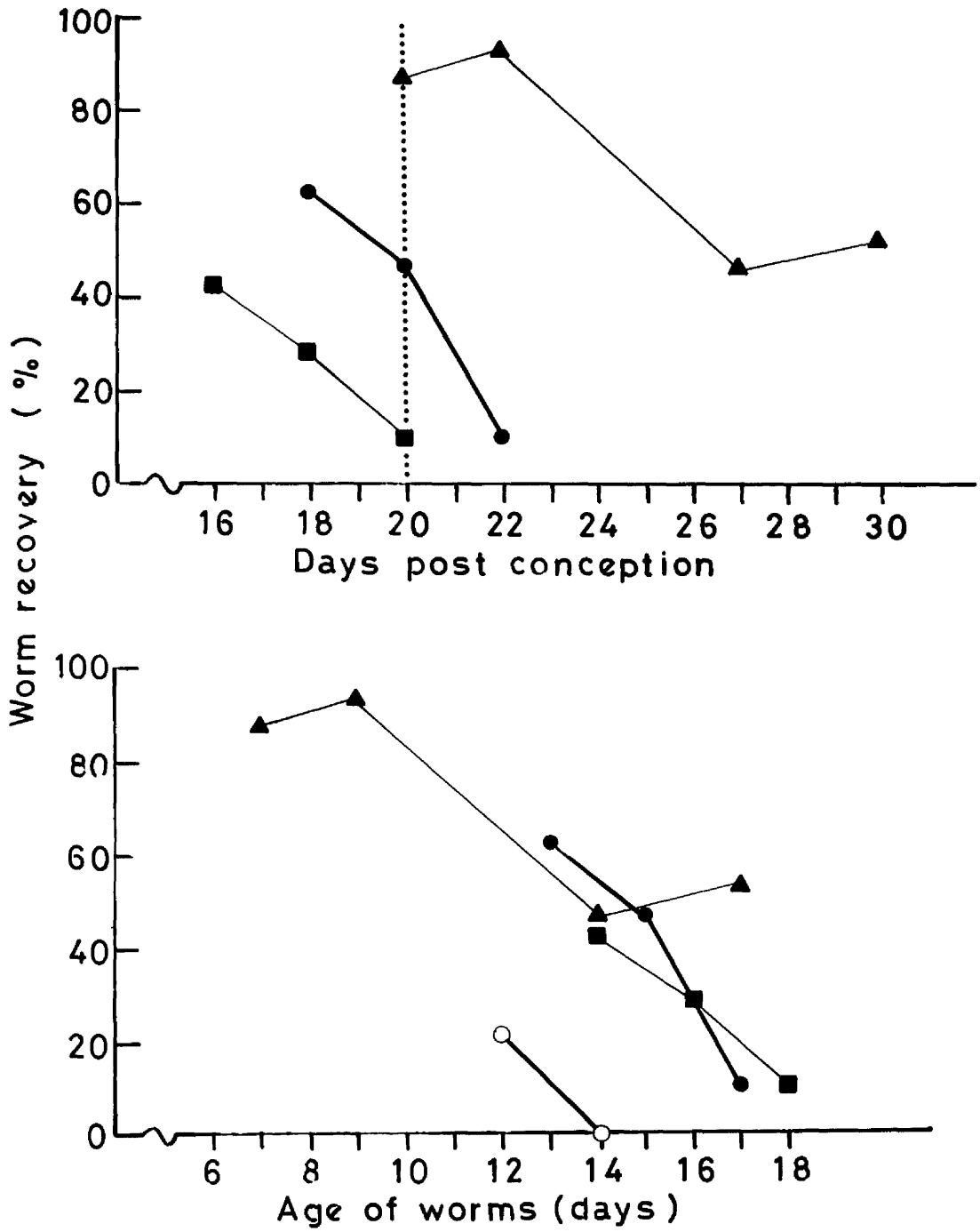
100
80
60
40
20
0
Worm recovery (%)
16
18
20
22
24
26
28
30
Days post conception
100
80
60
40
20
0
6
8
10
12
14
16
18
Age of worms (days)

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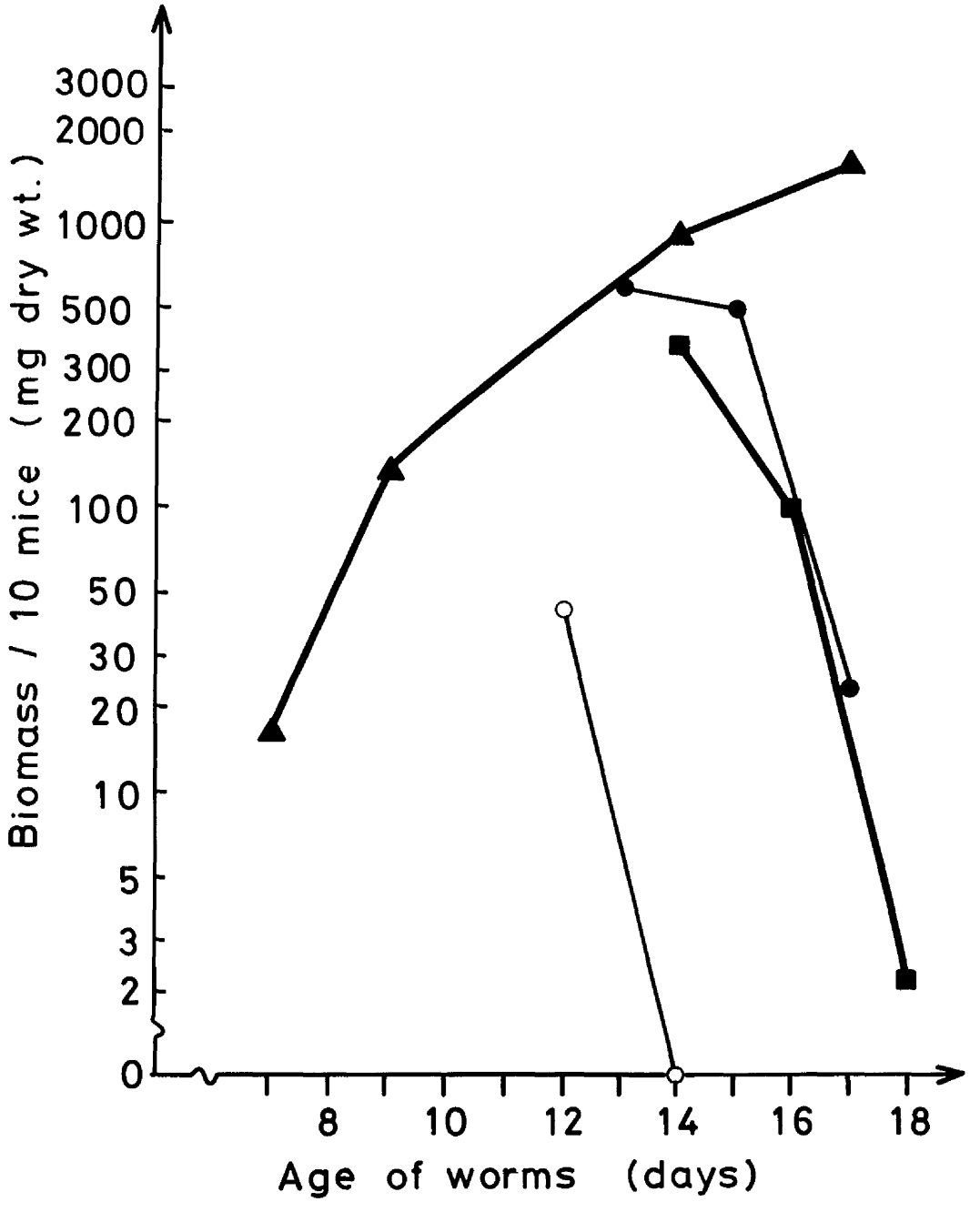
Figure I-8

Total biomass of H. diminuta from groups of 10 mice infected with eight cysticercoids. o, control; pregnant mice infected on days 2 (▩), 5 (●) or 13 (▲) of pregnancy.


Biomass / 10 mice (mg dry wt.)
3000
2000
1000
500
300
200
100
50
30
20
10
5
3
2
0
8
10
12
14
16
18
Age of worms (days)

similar to the other pregnant groups (Fig. I-7), but the recovery on day 17 p.i. was similar to that on day 14 p.i. rather than lower; this may indicate that lactation is suppressing the response to H. diminuta more strongly than pregnancy. The mice in this third group also supported heavier worms than the other groups on days 14 & 17 p.i. (Fig. I-9), indicating that the worms were growing better during lactation. Comparison of growth and rejection of worms from pregnant and lactating mice is covered more fully later in this Chapter.

(ii) Effect of early pregnancy

From the previous experiment it is clear that pregnancy will depress rejection of H. diminuta from mice infected as early as day 2 of pregnancy. In the next experiment, this observation was extended by infecting CFLP mice with five cysticercoids four days before mating; the mice were removed three days after mating so that the pregnant mice had been infected on days -4 to -6 of pregnancy. Pregnant and nulliparous control mice were autopsied on days 10, 12 & 14 p.i. The percentage recoveries, weights of worms from individual mice and means of worm weight per mouse and weight per surviving worm are displayed in Fig I-10.

Clearly, there is no evidence from these results that early pregnancy has any depressive effect on rejection; indeed, the recovery on day 10 p.i. was substantially lower in the pregnant group than in the controls (56% & 84% respectively), and on day 12 p.i. there was no difference in recovery between the two groups. In both groups, rejection was essentially complete by day 14, 11% & 16% of the worms from control and pregnant groups remaining.

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Figure I-9

Mean dry weight per surviving H. diminuta worm from nulliparous mice (o), or mice infected on days 2 (▩), 5 (●) or 13 (▲) of pregnancy.

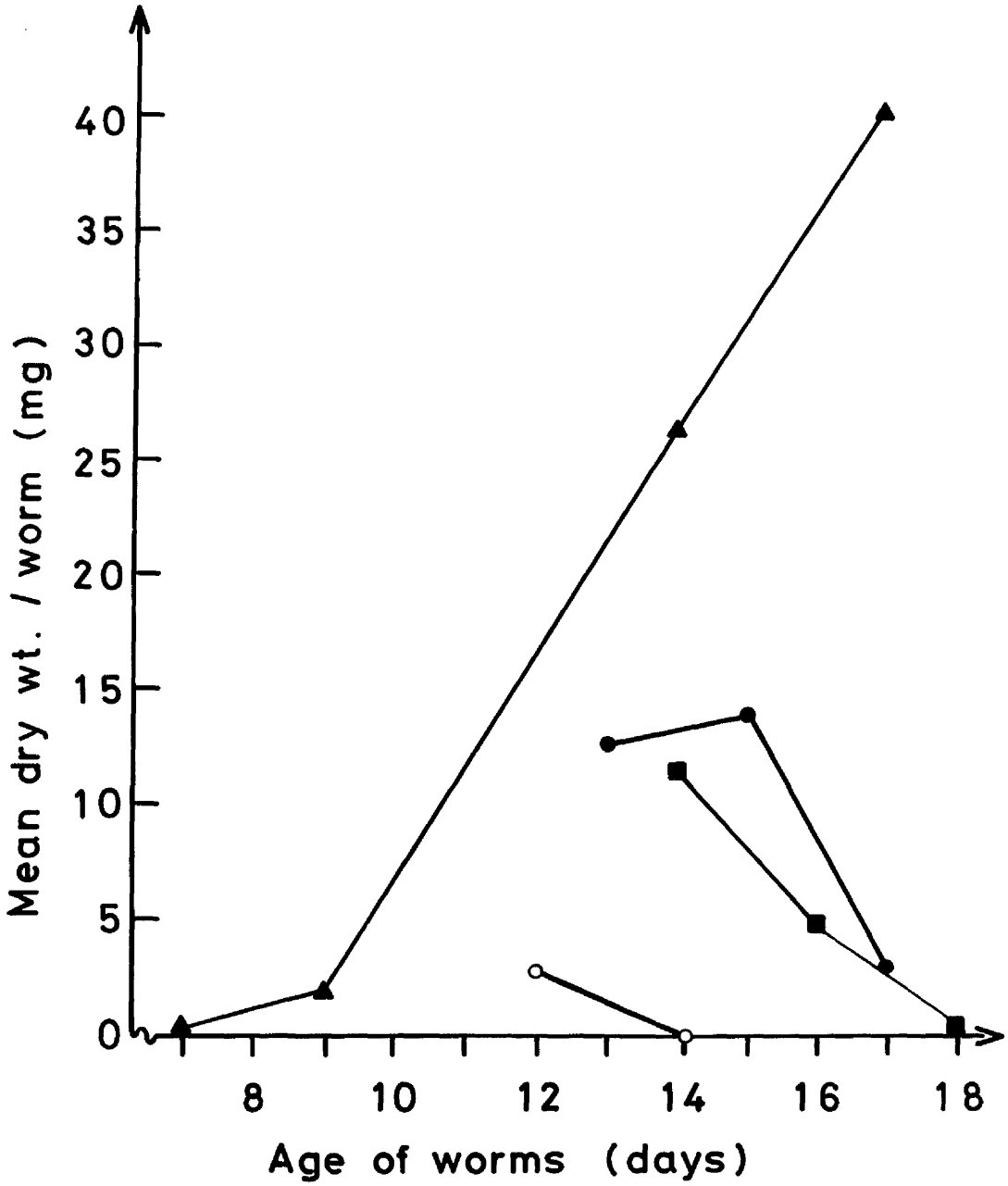
Mean dry wt. / worm (mg)
40
35
30
25
20
15
10
5
0
8
10
12
14
16
18
Age of worms (days)

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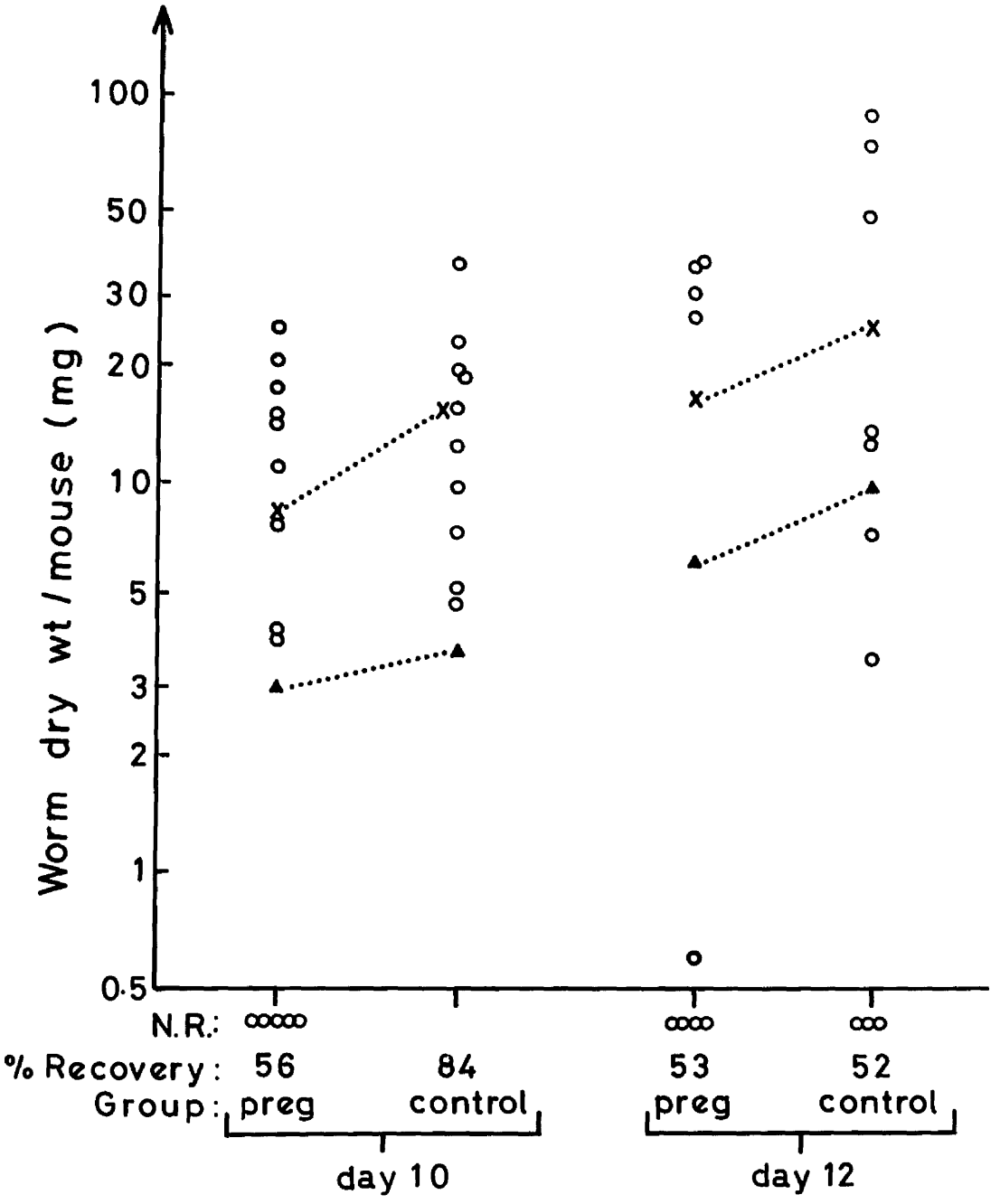
Figure I-10

Dry weight and recovery (%) of H. diminuta from pregnant and nulliparous mice infected with five cysticercoids; pregnant mice infected on day -6 to -4 of pregnancy.
o, total weight of worm tissue from a single mouse; x, mean weight of worm tissue per mouse; ▲, mean weight per surviving worm; N.R., mice from which no weighable worms were recovered.


Worm dry wt / mouse (mg)
100
50
30
20
10
5
3
2
1
0·5
N.R.:
% Recovery: 56 84 53 52
Group: preg control preg control
day 10
day 12

Worm weights did not appear to be affected by early pregnancy, at least in the manner shown in the previous experiments. The biomass per mouse and the mean weight of surviving worms were higher (though probably not significantly so) in the controls than in the pregnant mice.

d) Sensitisation to H. diminuta during pregnancy.

To test whether the immunodepressive effectof pregnancy was due to impaired induction of the specific immune ersponse, mice were infected on day 5 of pregnancy and the worms were removed with anthelmintic on day 10 p.i. (the previous experiments indicated that rejection from pregnant mice did not begin until after day 10 post infection). Litters were removed immediately post partum, and mice were challenged (in the first experiment) with eight cysticercoids 12 days post partum, autopsied on day 7 p.i., or (in the second experiment) challenged surgically eight days post partum with one worm, autopsied on day 5 p.i. Results from these mice were compared with results from similarly immunised nulliparous mice and naive nulliparous controls.

In both experiments it was clear that mice immunised during pregnancy were at least as immune to challenge as their immunised nulliparous counterparts. In the cysticercoid challenge experiment (Fig. I-II) the challenge worms were weighed en masse for the whole group because of the small amount of worm tissue involved. On day 7 p.i. the weight of worms from the nulliparous sensitised mice was substantially lower than from naive controls, as was the total recovery of worms and proportion of worms over 0.1 mg. The values obtained for the pregnant sensitised group were lower

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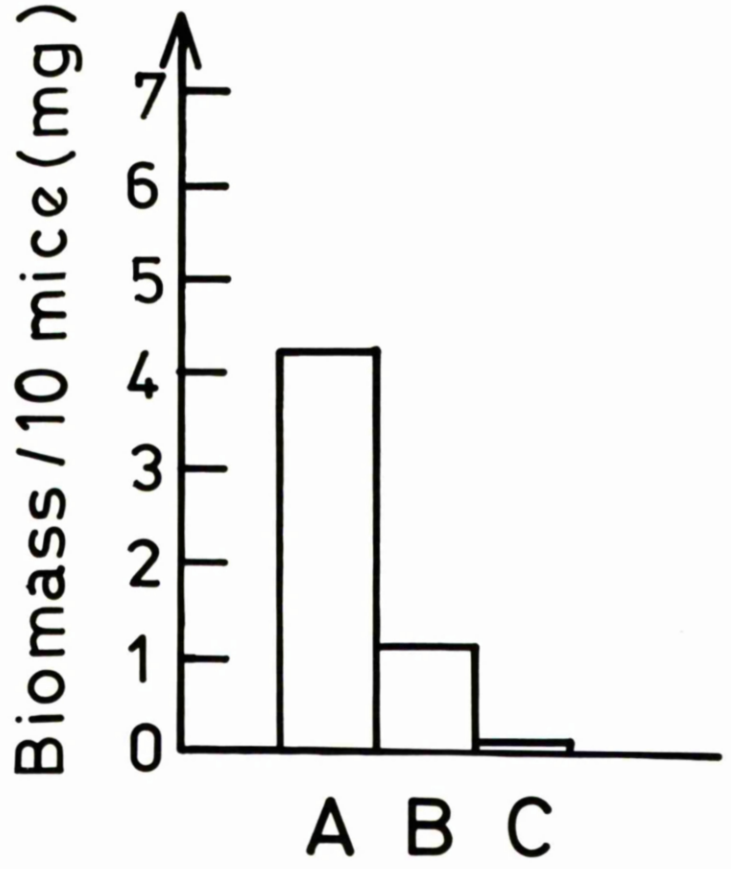
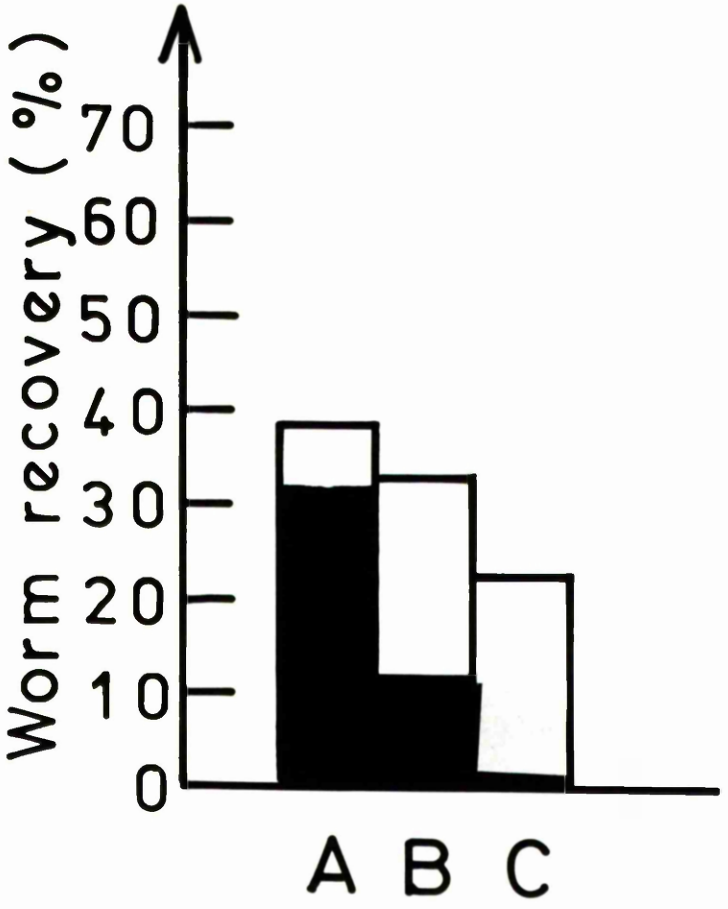
Figure I-II

Total biomass of worm tissue per group of 10 mice and recovery (%) of H. diminuta from challenge infections with eight cysticercoids in nulliparous naive mice (A), nulliparous immunised mice (B), and mice immunised during pregnancy, challenged post partum (C).

n = 10 in all three groups; shaded areas represent percentage of worms recovered $\geq$ 0.1 mg.


Biomass / 10 mice (mg)
0
1
2
3
4
5
6
7
A B C


Worm recovery (%)
0
10
20
30
40
50
60
70
A B C

still, indicating that the mice infected with H. diminuta during pregnancy were highly sensitised against a challenge infection.

This result was confirmed in the second experiment, using surgical challenge to assess the immune status of the mice. Worms recovered from the nulliparous immunised group (Fig I-12) were lighter than those from the naive control group ($p<0.05$), and worms from the pregnant-sensitised mice were lighter still ($p<0.01$, compared with naive controls). Recovery of worms from the nulliparous immunised group was higher than might have been expected (e.g. see Fig. 4-2); one possible reason for this is the truncation of the immunising infection at day 10 p.i. (see Befus, 1975b)

e) Effect of pregnancy on a secondary response

It can be concluded from the foregoing experiments that the induction of memory to H. diminuta was not depressed by pregnancy; I therefore decided to test the effector arm of the immune response by immunising nulliparous mice and challenging them with H. diminuta during subsequent pregnancy.

In a preliminary experiment, mice were infected with eight cysticercoids for ten days, after which the infection was terminated with anthelmintic. Thirteen days after this, the mice were mated, and were infected with eight cysticercoids on day 5 of pregnancy. Pregnant immune mice, nulliparous immune mice and naive controls were killed on day 7 p.i. Worms were weighed en masse for each group.

The results (Fig I-13) show that the immunised nulliparous mice carried a lighter weight of worms than the naive controls, but

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Figure I-12

Dry weight of *H. diminuta* recovered from surgical infection of nulliparous naive mice (A), nulliparous immunised mice (B), and mice immunised during pregnancy, challenged *post partum* (C). Each point represents the total weight of worm tissue from a single mouse.

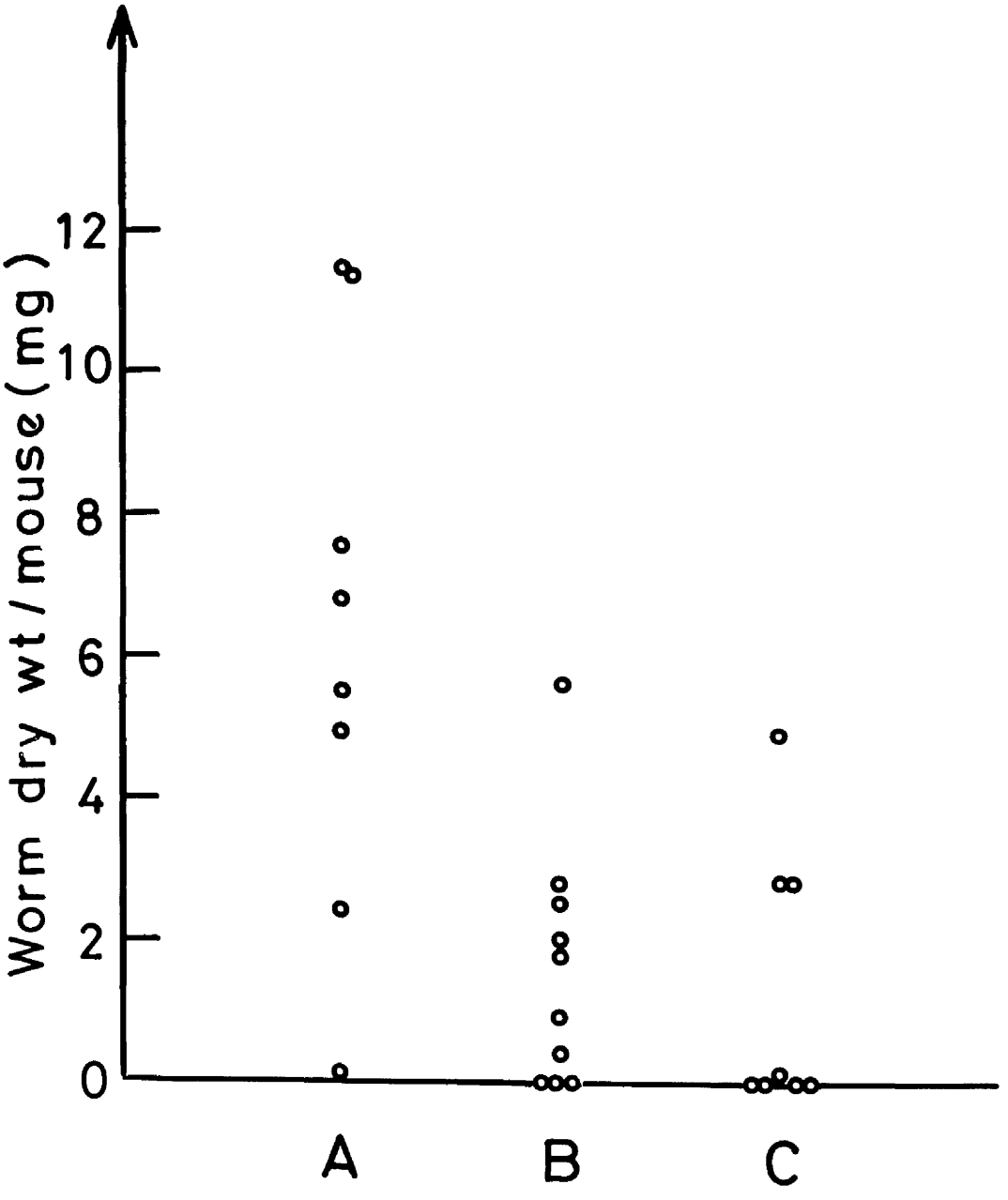
Worm dry wt / mouse (mg)
12
10
8
6
4
2
0
A
B
C

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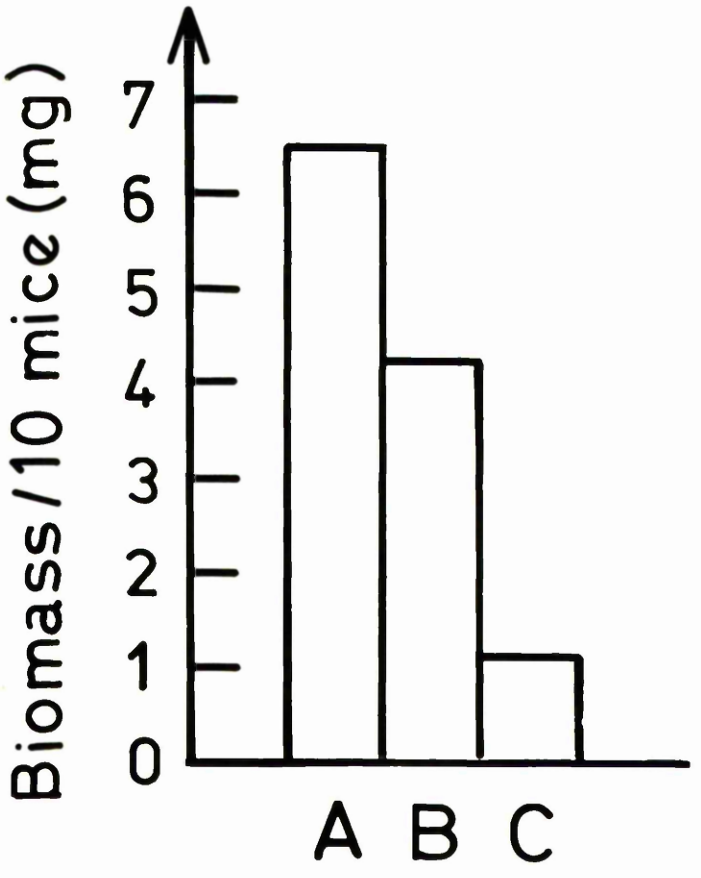
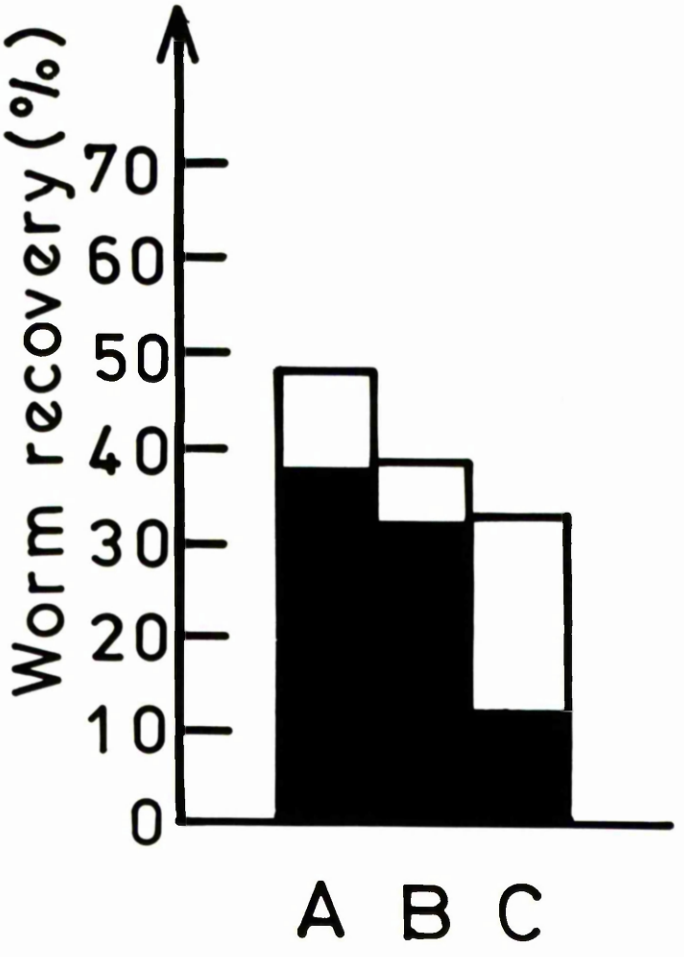
Figure I-13

Total biomass of worm tissue per group of 10 mice, and recovery (%) of H. diminuta from challenge infections with eight cysticercoids in immunised pregnant mice (A), nulliparous naive mice (B), and nulliparous immunised mice (C).

n = 7 in group A; n = 10 in groups B & C

Shaded areas represent recovery (%) of worms $\geq$ 0.1 mg.


Biomass/10 mice (mg)
0
1
2
3
4
5
6
7
A B C


Worm recovery (%)
0
10
20
30
40
50
60
70
A B C

the immunised mice challenged during pregnancy had heavier worms than the controls, indicating that pregnancy could depress a secondary response.

This experiment was repeated, and enlarged by the inclusion of a group of previously uninfected pregnant mice. CFLP mice were infected with five cysticercoids, given anthelmintic 11 days later, and mated the following day. Mice were challenged with five cysticercoids on day 7 of pregnancy, and autopsied on day 7 p.i. Control groups of naive and immunised nulliparous mice and, as stated above, naive pregnant mice, were similarly treated as appropriate. The experimental protocol is outlined in Table 1-3: results are shown in Fig. 1-14.

Table 1-3

Group	Days				
	-19	-8	-1	0	+7
A)	I	Z	Mated	I	K
B)	-	Z	Mated	I	K
C)	I	Z	-	I	K
D)	-	Z	-	I	K

I, infected with five H. diminuta
Z, anthelmintic
K, killed

Worms recovered from group C were lighter than group D worms, indicating that the mice of group C were sensitised against H. diminuta . Pregnant mice (group B) supported heavier worms than the nulliparous controls (group D), and although the previously immunised mice of group A had lighter worms than group B ($p<0.05$), the biomass per 10 mice was slightly greater in group A (12.6 mg)

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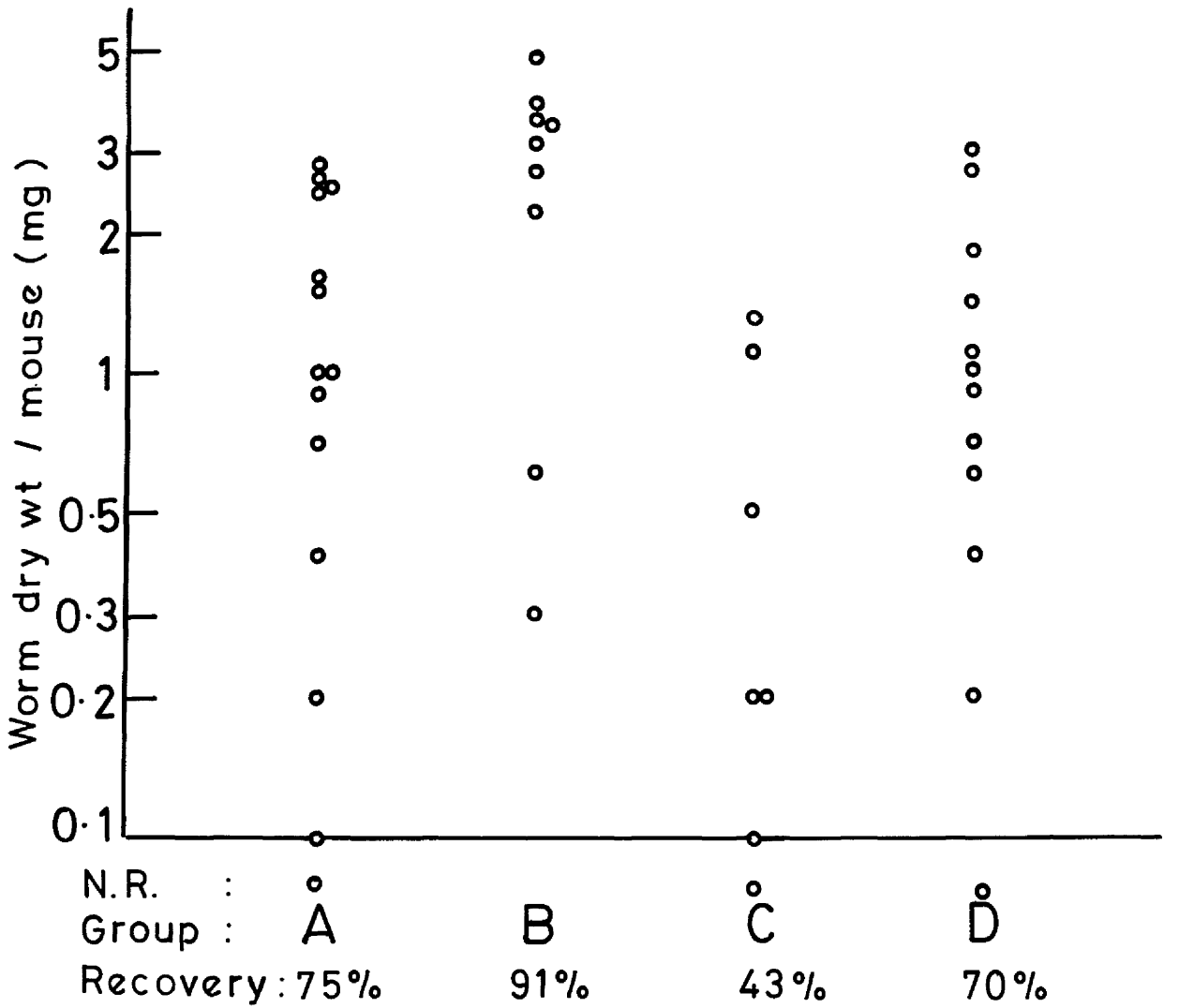
Figure I-14

Dry weight and recovery (%) of H. diminuta from five-cysticercoid infections of immunised pregnant mice (A), naive pregnant mice (B), immunised nulliparous mice (C), and naive nulliparous mice (D). Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.


Worm dry wt / mouse (mg)
5
3
2
1
0·5
0·3
0·2
0·1
N.R. :
Group : A B C D
Recovery : 75% 91% 43% 70%

than in group D (11.5 mg), indicating that although there is some degree of enhanced responsiveness in the pregnant mice of Group A, the degree of responsiveness is much less than in nulliparous immunised mice. The percentage recoveries support this view; the recoveries in groups A and D differ by only 5% (75% & 70% respectively, whereas 91% and 43% of the worms were recovered from groups B and C respectively.

In the third and final experiment of this series, various modifications were made to the protocol; firstly, the immunising infection of five cysticercoids was left until day 18 p.i. before giving anthelmintic. The mice would probably have rejected all their worms by this time, and the immunisation would probably be stronger than with an infection terminated after 10 or 11 days. Secondly, the challenge infection was with three cysticercoids; in lowering the challenge, the naive mice would probably allow worms to grow more rapidly and thus perhaps accentuate the difference between immune mice and controls. Thirdly, the day of pregnancy at challenge was day 14, also to accentuate differences between groups; killing on days 8 & 10 p.i., the mice would thus have littered 1 & 2 days previously. To avoid interference by the effects of lactation, litters were removed as soon as possible post partum. The experimental protocol thus appears as shown in Table 1-4.

The results (Fig. 1-15) show that there was a large difference between the nulliparous primary and secondary infected mice (groups C & D); on both days 8 and 10 p.i. the only recoveries from group C were one worm from one mouse on each day. On day 8 p.i. the immunised pregnant group (group A) had significantly lighter worms than the naive pregnant group B ($p<0.05$), but group A was not significantly different from group D, although group A had

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Figure I-15

Dry weight and recovery (%) of H. diminuta from three-cysticercoid infections of immunised pregnant mice (A), naive pregnant mice (B), immunised nulliparous mice (C), and naive nulliparous mice (D). Each point (o) represents the total weight of worm tissue from a single mouse. x, mean dry weight per mouse; N.R., mice from which no weighable worms were recovered; d.p.i., days post infection.

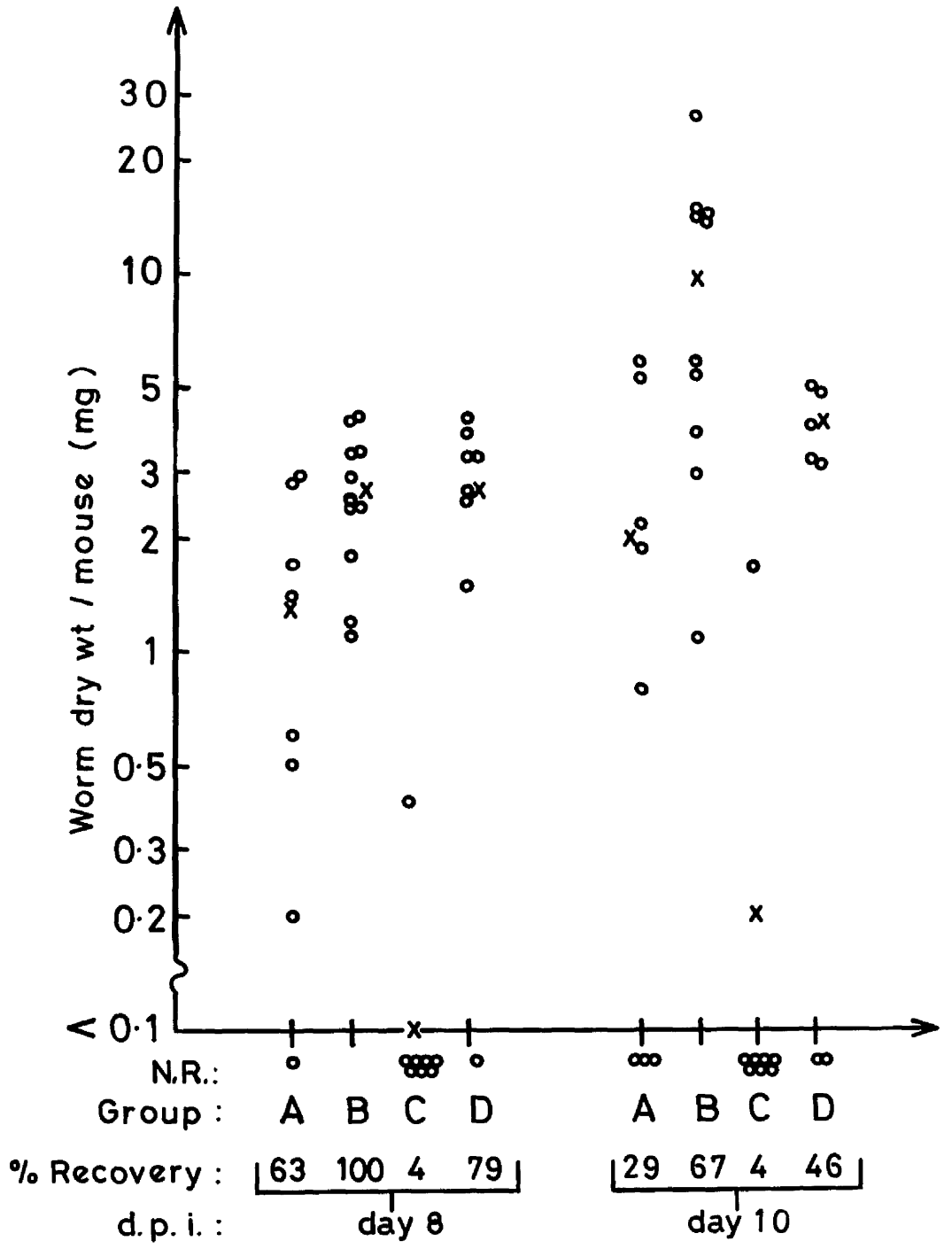
Worm dry wt / mouse (mg)
30
20
10
5
3
2
1
0·5
0·3
0·2
< 0·1
N.R.:
Group :
A B C D
A B C D
% Recovery :
63 100 4 79
29 67 4 46
d. p. i. :
day 8
day 10

Table I-4

Group	Days					
	-35	-17	-14	0	+8	+10
A)	I*	Z	Mated	I**	K	K
B)	-	Z	Mated	I	K	K
C)	I	Z	-	I	K	K
D)	-	Z	-	I	K	K

I*, infected with five cysticercoids
I**, infected with three cysticercoids

significantly heavier worms than group C ($p<0.01$), indicating, as in the previous experiment, that a previously immunised pregnant mouse gives a similar response to that of a naive nulliparous animal. This interpretation is confirmed by the day 10 result, in which group A worms were again significantly lighter than those of group B ($p<0.01$), although not significantly different from group D.

f) Effect of pregnancy on rejection of H. diminuta by inbred mice

As it is known that pregnancy has a much less immunodepressive effect in syngeneic matings compared with allogeneic matings (see Introduction), an experiment was carried out in which inbred NIH mice were mated with syngeneic NIH males, and infected with five H. diminuta cysticercoids on day 4 of pregnancy. Infected pregnant mice were killed on days 10, 12, 14 & 16 p.i., and nulliparous female control mice were killed on days 10 and 12 p.i.

The recovery of worms from the controls on day 10 p.i. was unexpectedly low (Fig. I-16), but as 100% of the worms were

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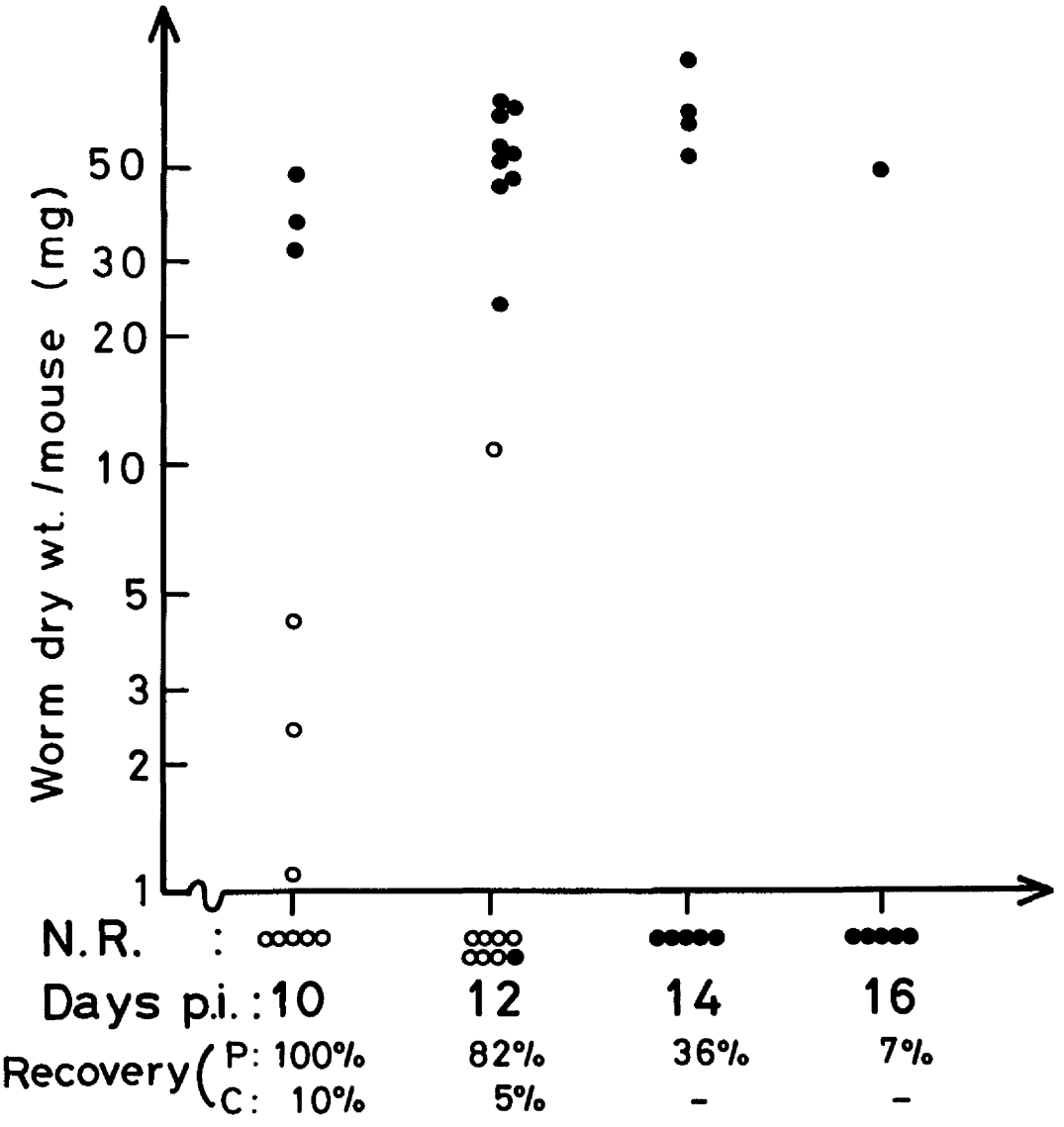
Figure I-16

Dry weight and recovery (%) of H. diminuta from five-cysticercoid infections of pregnant (●) and nulliparous (o) NIH mice. Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection; P, pregnant; C, control.


Worm dry wt./mouse (mg)
50
30
20
10
5
3
2
1
N.R. :
Days p.i. : 10 12 14 16
Recovery (P: 100% 82% 36% 7%
C: 10% 5% - -

recovered from the pregnant mice on this day, there is no reason to suspect the viability of the cysticercoids; it is much more likely that the controls were rejecting the worms very quickly, a result that was unexpected at that time. The pregnant mice, however, retained 82% of their worms on day 12 p.i., and over one third of the worms still remained on day 14 p.i., indicating that rejection was indeed slower in the pregnant mice. Although the maximun worm weight (on day 14 p.i.) was under 100 mg, this is within the limits of the same result in CFLP mice (Fig. 1-2), and it was subsequently found that cysticercoid infections with H. diminuta grow less well in NIH than in CFLP mice.

2) Lactation

a) Effect of lactation on the primary course of infection with H. diminuta

In the initial experiment to investigate the effects of lactation on growth and rejection of H. diminuta, CFLP mice were infected with three cysticercoids on days 2-6 of lactation (day of parturition = day 0 of lactation). Nulliparous control mice were killed on days 8, 10, 12 & 14 p.i., and lactating mice were killed on days 8, 10, 14, 15, 19 & 21 p.i.

The weight of worm tissue recovered per mouse (Fig. 1-17) was significantly higher in the lactating groups than in the controls on all days studied ($p < 0.01$). The degree of enhanced growth is apparently greater than for pregnant mice, in that as early as day 8 p.i. there was no overlap in the results for lactating and control mice. The day 8 worms in lactating mice reached up to about 10 mg each, a weight that would be unusually high for this age of worm even in a rat. The total biomass per group of eight mice from the lactating mice was 8 and 11 times the control values on days 8 and 10 p.i. respectively; it is also worth noting that some lactating mice on days 14 and 15 p.i. were carrying worm burdens in excess of 500 mg dry weight.

The percentage worm recoveries (Fig. 1-18) confirm that the control mice were rejecting strongly, rejection being virtually complete on day 12 p.i. Rejection from lactating mice proceeds only after day 10 p.i.; rejection was substantially slower than in control mice, not being complete until after day 21 p.i. The delay in rejection appears to be in the order

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Figure I-17

Dry weight of H. diminuta from three-cysticercoid infections of lactating (●) and nulliparous (o) CFLP mice. Each point represents the total weight of worm tissue from a single mouse.
days p.i., days post infection; N.R., mice from which no weighable worms were recovered.

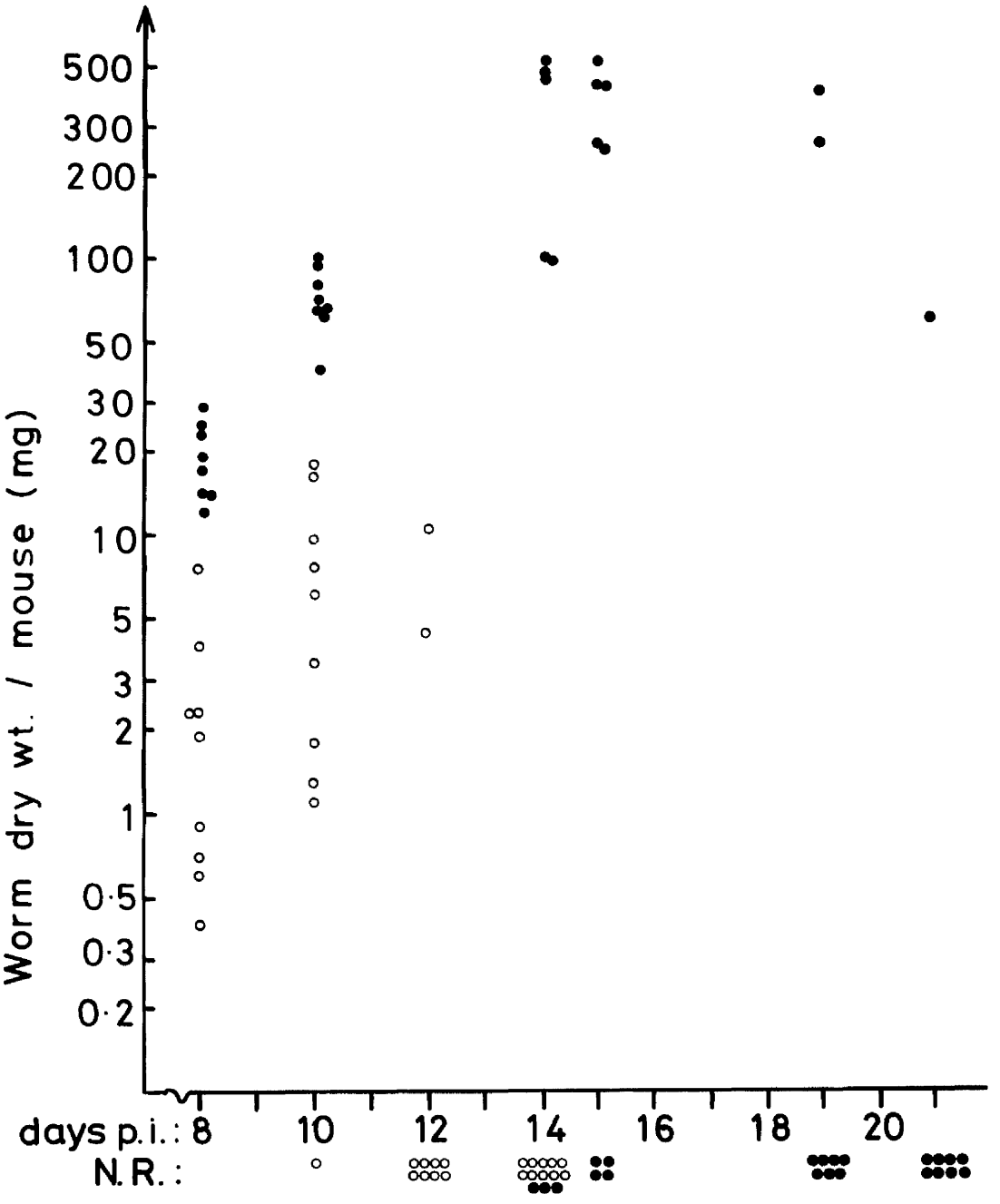
Worm dry wt. / mouse (mg)
500
300
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
days p.i.: 8
10
12
14
16
18
20
N.R.:

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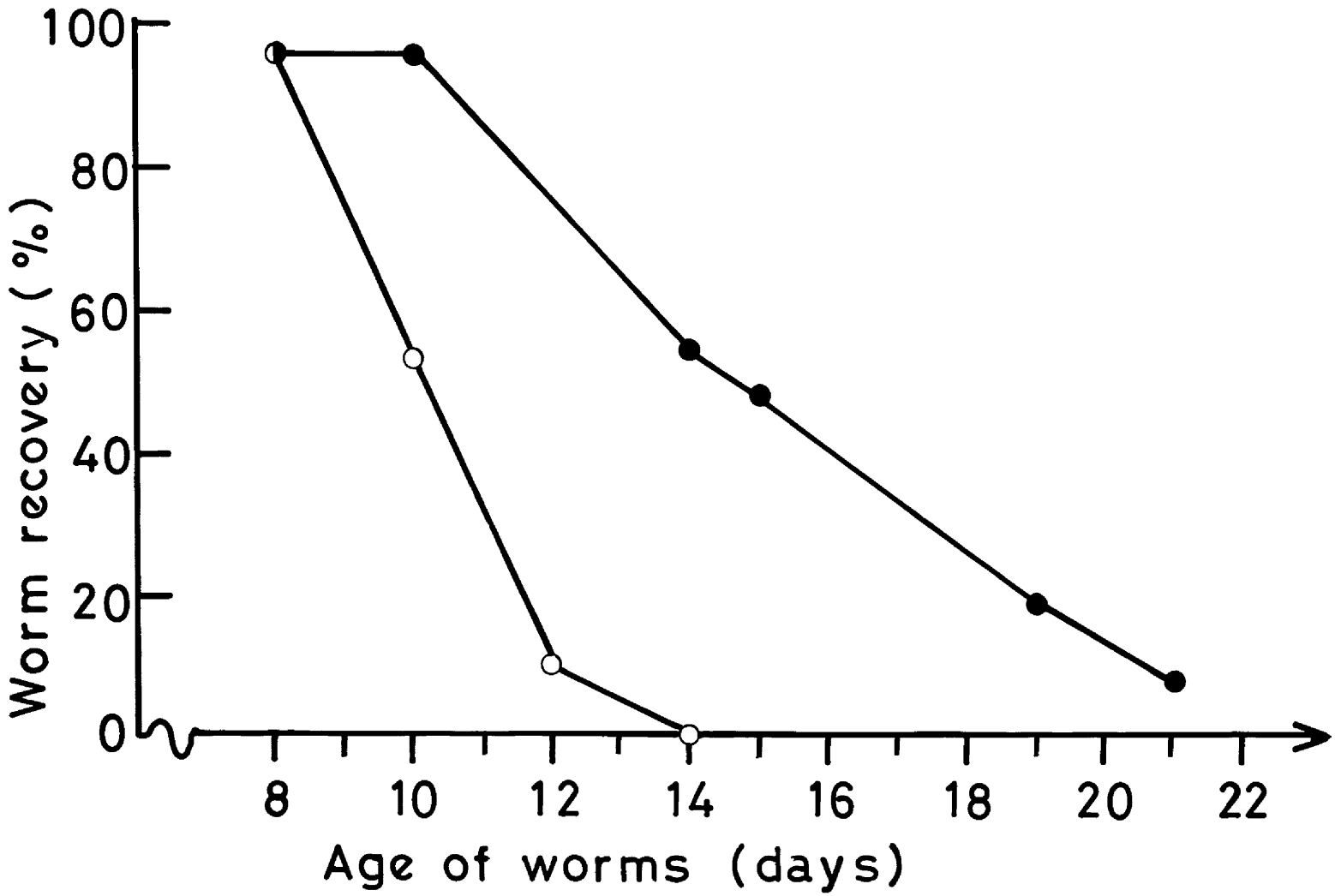
Figure I-18

Recovery (%) of H. diminuta from three-cysticercoid infections of lactating (●) and control (o) CFLP mice.


100
80
60
40
20
0
Worm recovery (%)
8
10
12
14
16
18
20
22
Age of worms (days)

of 4-7 days, i.e. 2-5 days longer than that observed in the experiments on pregnant mice.

b) Effect of varying the time of infection

In order to investigate, as for pregnancy, whether the loss of worms from lactating mice was due to a lifting of the immunodepression at the end of lactation (day 18-21 after parturition in the previous experiment), or to a slow loss mediated by a depressed but not completely suppressed mechanism, CD-1 mice were infected with eight cysticercoids on day 16 of pregnancy, day 2 of lactation, or day 10 of lactation. Lactating and control mice were autopsied as shown in table 1-5.

Table 1-5

Group	Day of lactation at infection	Day of autopsy p.i.
Control	-	8, 10, 12, 14
A	-4 (day 16 pregnancy)	14, 16, 18, 20, 22
B	+2	12, 14, 16, 18, 20
C	+10	10, 12, 14, 16

Litters were removed on day 18 of lactation to standardise the end of lactation.

The weights of worm recovered per mouse (Fig. 1-9) for the various days of autopsy show that control mice were reacting very strongly against the infection, rejection being virtually complete by day 12 p.i. It is clear from this graph that group C (in which day 8 p.i. coincides with the end of lactation) is

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Figure I-19

Dry weight of H. diminuta from eight-cysticercoid infections of control (o) and lactating mice.

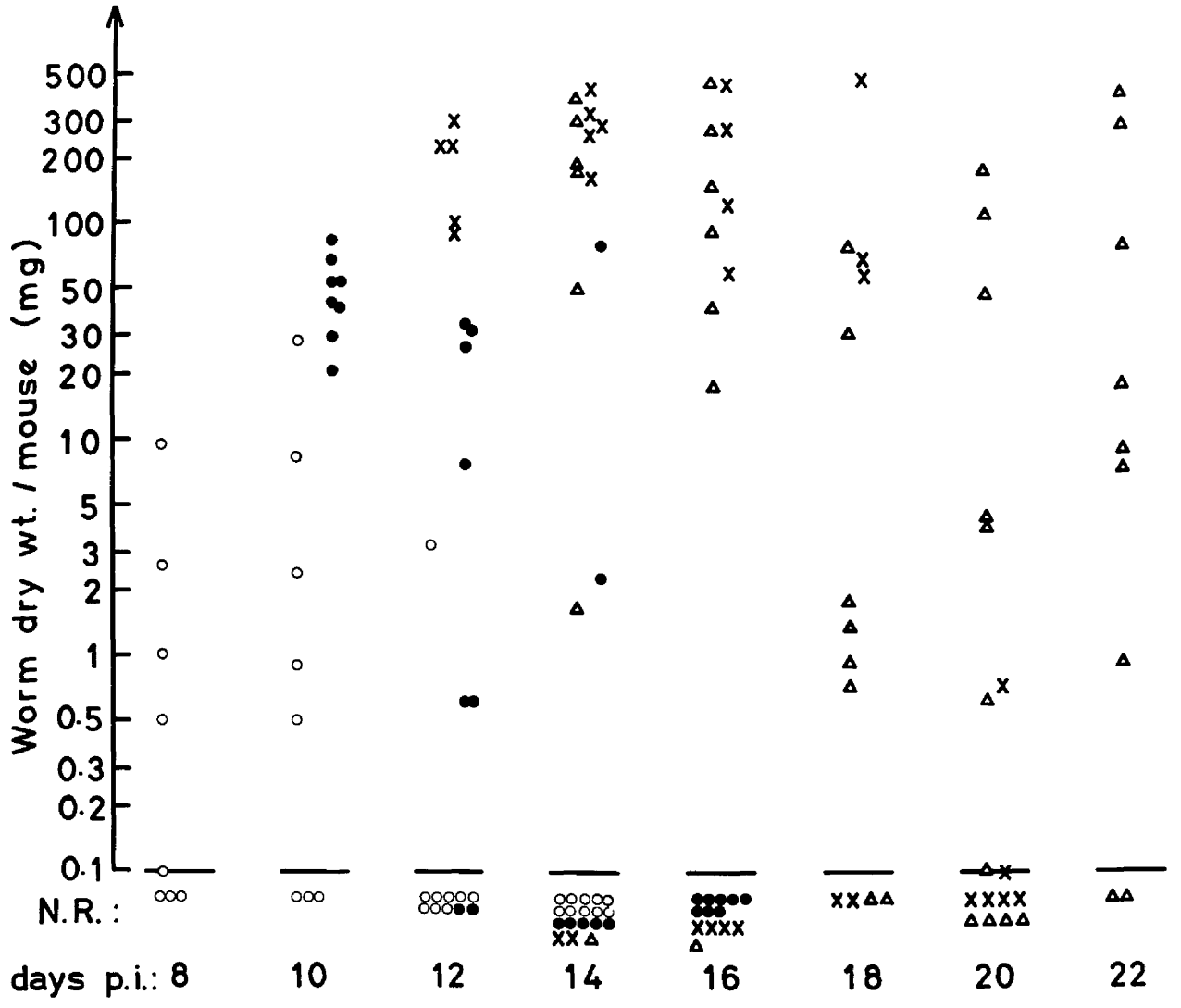
Group A (Δ), infected on day 16 of pregnancy

Group B (x) , " " 2 of lactation

Group C (●) , " " 10 of lactation

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; days p.i., days post infection.


Worm dry wt. / mouse (mg)
500
300
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
N.R. :
days p.i.: 8
10
12
14
16
18
20
22

responding more slowly than the controls, and significantly heavier worms are carried by group C than controls on days 8 and 10 p.i. ($p<0.01$); however, it is also clear that rejection, complete by day 16 p.i. is very much faster in group C than in groups A and B, indicating that an earlier finish to lactation hastens rejection. Groups A and B were similar as regards biomass of worms until day 20 p.i., when a substantial number (7/11) of mice in group A (in which the end of lactation does not occur until day 22 p.i.) retained a varied population of H. diminuta; however, all but one of the group B mice retained only destrobilated worms. As the end of lactation occurs in group B on day 16 p.i., it confirms the results from group A suggesting that rejection takes place rapidly after the end of lactation. The graph of percentage recovery (Fig. 1-20) confirms this interpretation; group C mice were slower to reject than controls, but were faster than groups A or B. Group A mice retained a substantial proportion of worms on days 20 and 22 p.i., at which point rejection was virtually complete in group B. It can also be seen from this graph that rejection was in progress in groups A and B long before the end of lactation; in both groups, only about 50% of the worms remained at the end of lactation. Thus it appears that slow loss does occur throughout lactation, as indicated in the previous experiment, but complete rejection follows quickly after the end of lactation.

This experiment was repeated using CFLP mice infected with five cysticercoids; the experimental protocol is shown in Table 1-6. On this occasion, lactation was of natural duration; litters were not removed from any of the groups.

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Figure I-20

Recovery (%) of H. diminuta from eight-cysticercoid infections of control (o) and lactating mice.

Group A (△), infected on day 16 of pregnancy

Group B (x), " " 2 of lactation

Group C (●), " " 10 of lactation

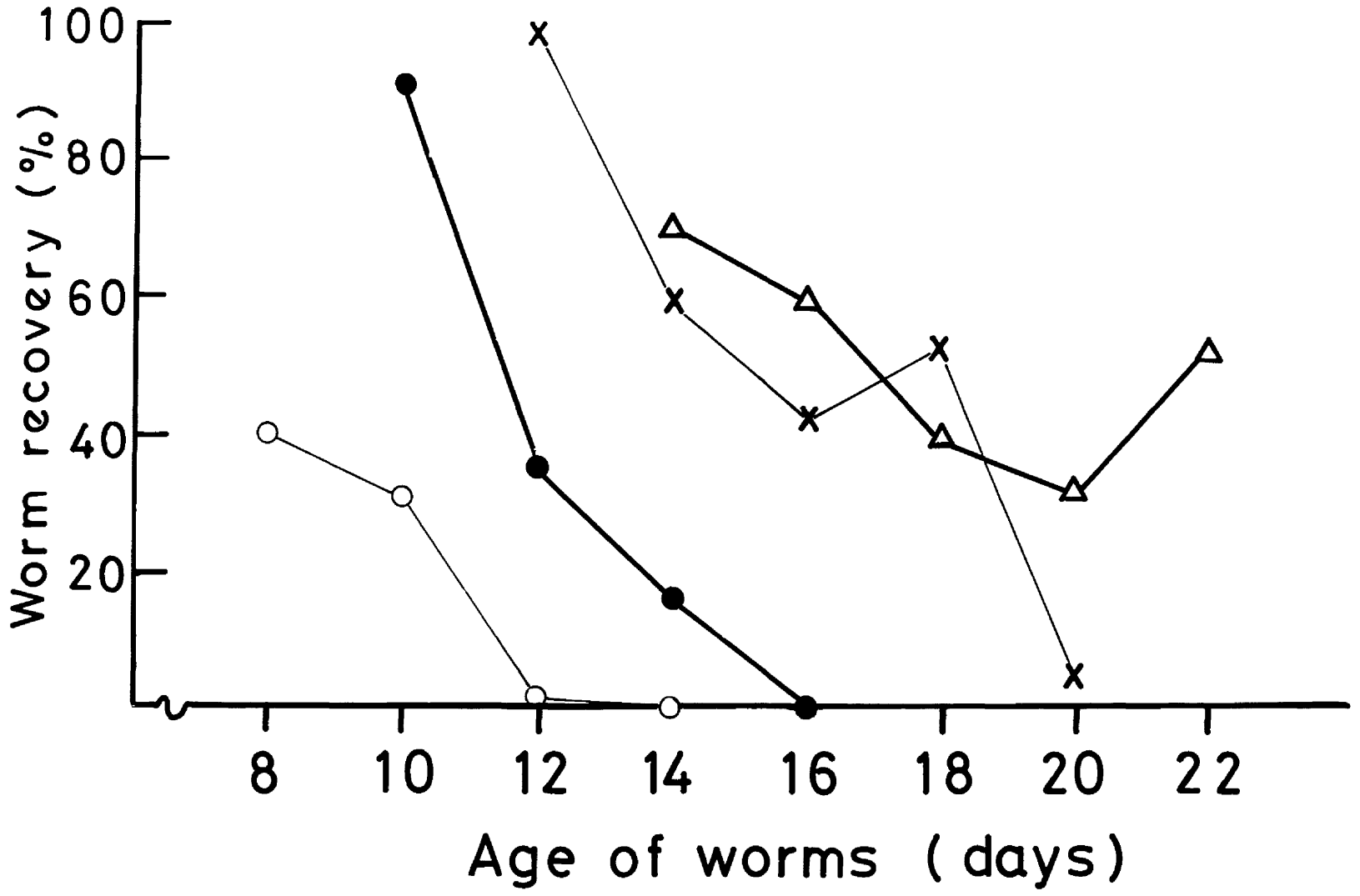
100
80
60
40
20
Worm recovery (%)
8
10
12
14
16
18
20
22
Age of worms (days)

Table I-6

Group	Day of lactation at infection	Day of autopsy p.i.
Control	-	8, 10, 12, 14
A	-4	14, 16, 18, 26
B	+2	12, 14, 16, 18
C	+10	8, 10, 12, 14, 16

The picture presented by the percentage recovery results (Fig. I-21) is similar to that of the previous experiment, but the differences between the groups were less marked. Group C was again responding more slowly than the controls, but only marginally faster than groups A and B; again there was no difference between groups A and B on days 14, 16 & 18 p.i.

The graph of worm weights per mouse (Fig. I-22) confirms the difference seen in the previous experiment between control worm weights and those from group C on days 8, 10 & 12 p.i. ($p < 0.01$), but again the differences between groups A, B and C were less marked than in the previous experiment.

Rejection of H. diminuta from control mice was even faster in this experiment than in the previous one, being almost complete on day 10 p.i., and rejection from groups A and B was also slightly faster than in the previous experiment. However, rejection from group C was, if anything, slower in this experiment, probably as a result of not terminating lactation on day 18 post partum. It is probable that lactation continued past this point in at least some of the mice, leading to slower and less clear-cut rejection.

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Figure I-21

Recovery (%) of H. diminuta from five-cysticercoid infections of control (o) and lactating mice.

Group A (Δ), infected on day 16 of pregnancy

Group B (x), " " 2 of lactation

Group C (⊙), " " 10 of lactation

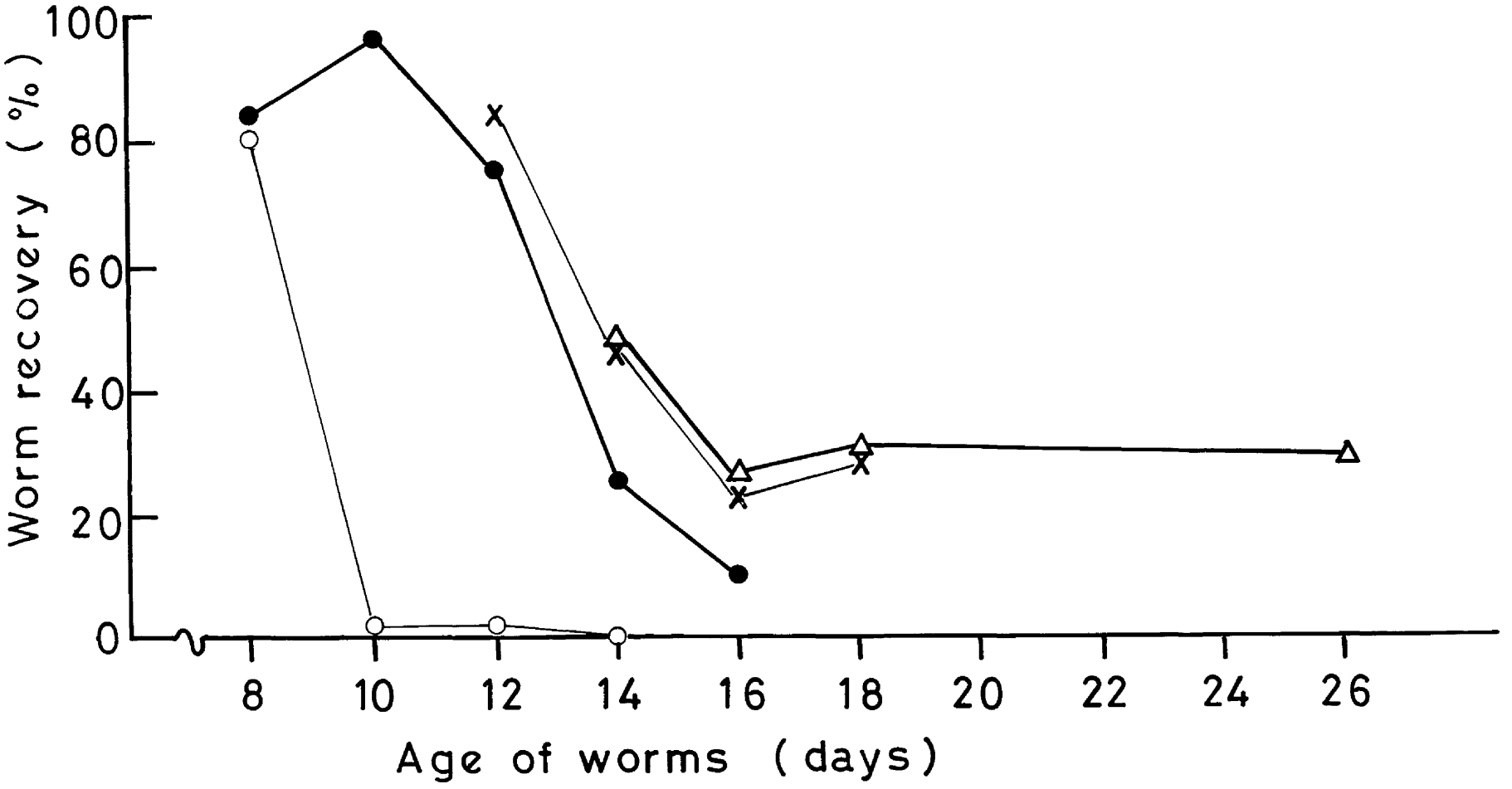
Worm recovery (%)
100
80
60
40
20
0
8
10
12
14
16
18
20
22
24
26
Age of worms (days)

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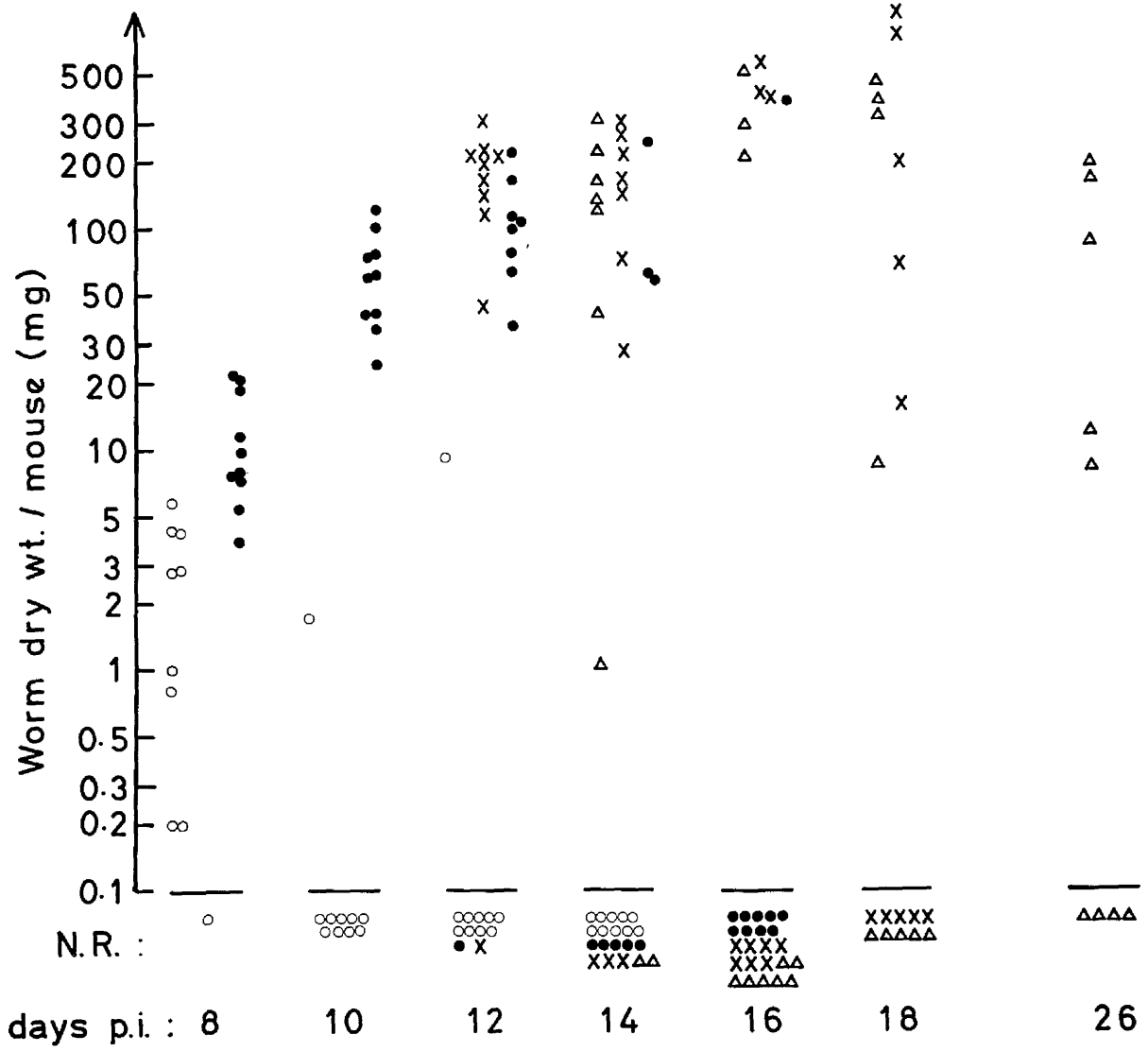
Figure I-22

Dry weight of H. diminuta from five-cysticercoid infections of control (o) and lactating mice. Group A (Δ), infected on day 16 of pregnancy Group B (x), " " 2 of lactation Group C (●), " " 10 of lactation Each point represents the total weight of worm tissue from a single mouse. N.R., mice from which no weighable worms were recovered; days p.i., days post infection.


Worm dry wt. / mouse (mg)
500
300
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
N.R. :
days p.i. : 8
10
12
14
16
18
26

c) Sensitisation to H. diminuta in lactating mice

To test whether induction of memory was affected by lactation, CD-1 mice were infected with eight cysticercoids on day 4 of lactation, and this infection was terminated with anthelmintic 10 days later. Six days after this, mice were challenged surgically with one worm and were autopsied on day 5 of the challenge infection. Nulliparous immunised mice and nulliparous naive controls received appropriate treatment at the same times, as outlined in Table 1-7. All litters were removed

Table 1-7

Group	Days				
	-20	-16	-6	0	+5
A	Parturition	I	Z	Op	K
B	-	I	Z	Op	K
C	-	-	Z	Op	K

I, infected with eight cysticercoids
Op, surgical infection

on the day of administration of anthelmintic.

It can be seen from the results (Fig. 1-23) that groups A and B gave very similar recoveries and weights of worms, and both are substantially lower than from the naive mice of group C ($p < 0.01$), indicating that, as is the case in pregnancy, the inductive part of the response is not depressed in lactating mice. The total biomass per group of seven mice in group A was slightly lower than in group B (15.5 mg and 18.2 mg respectively), but the biomass for group C was approximately eight times higher (133.6 mg).

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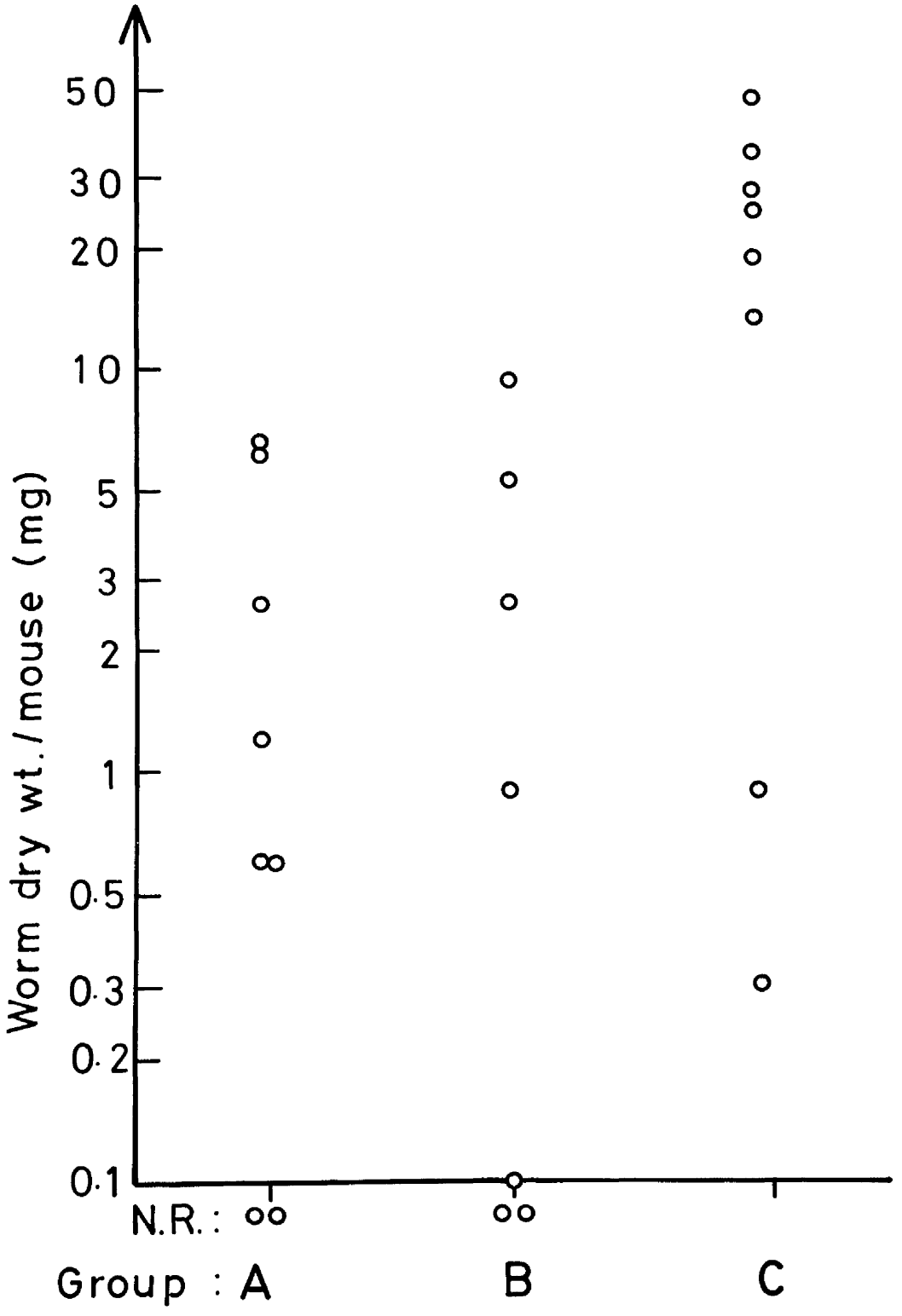
Figure I-23

Dry weight of H. diminuta from surgical infections of mice immunised during lactation (A), nulliparous immunised mice (B), and nulliparous naive mice (C). Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.


Worm dry wt./mouse (mg)
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
N.R.:
Group : A
B
C

d) Effect of lactation on a secondary response

To determine the effect of lactation on the response of previously sensitised mice, CD-1 mice were infected with five cysticercoids for 10 days, then treated with anthelmintic. One day later they were mated, and were challenged with three cysticercoids on day 7 of lactation; animals were autopsied on day 7 of challenge infection. Relevant controls were included as described in Table 1-8.

Table 1-8

Group	Days					
	-38	-28	-27	-7	0	+7
A	1*	Z	Mated	P	1**	K
B	-	Z	Mated	P	1	K
C	1	Z	-	-	1	K
D	-	Z	-	-	1	K

1*, infected with five cysticercoids
1**, infected with three cysticercoids
P, parturition

The worm weights recovered per mouse (Fig. 1-24) show that the worm burdens from the immunised mice of group C were significantly lighter than those from the naive mice of group D ($p < 0.01$). The lactating immunised mice of group A, however, supported a heavier weight of worms than either groups C or D ($p < 0.01$), indicating that not only does lactation suppress a secondary response, but also allows worms to grow faster than in an immunologically naive mouse. There was no significant difference between groups A and B in this experiment, indicating that lactation completely annuls the effect of previous sensitisation.

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Figure I-24

Dry weight of H. diminuta from three-cysticercoid infections of immunised or naive lactating and nulliparous CD-1 mice.

A, immunised lactating mice

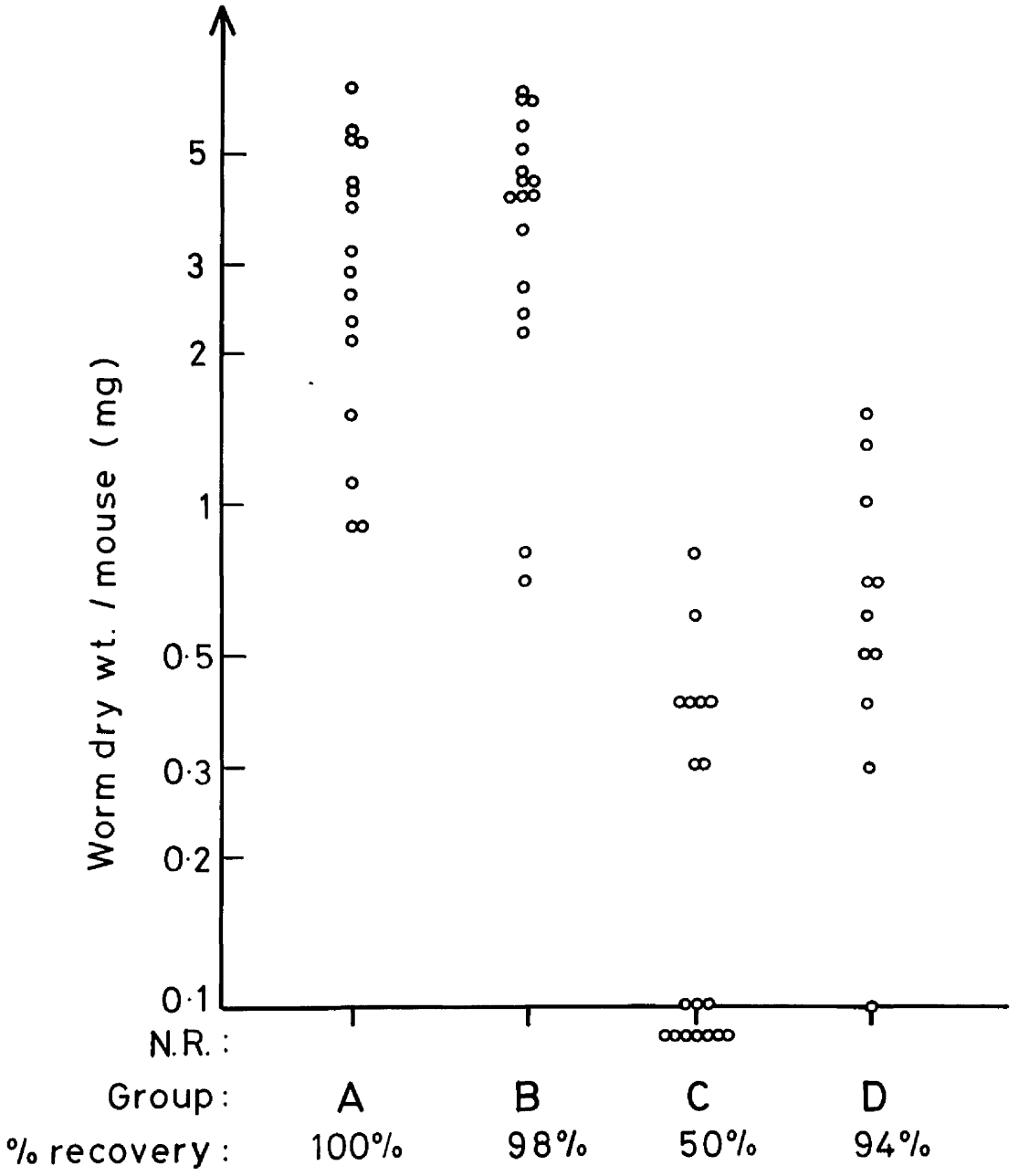
B, naive lactating mice

C, immunised nulliparous mice

D, naive nulliparous mice

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered


Worm dry wt. / mouse (mg)
5
3
2
1
0·5
0·3
0·2
0·1
N.R. :
Group :
A
B
C
D
% recovery :
100%
98%
50%
94%

To test this, a further experiment was performed in which CFLP mice were immunised for a longer period, and autopsy was on days 10 and 12 after challenge; these changes were intended to maximise any difference between immunised and naive lactating mice. The experimental design was as described in Table I-9.

Table I-9

Group	Days						
	-48	-32	-28	-8	0	+8	+10
A	I*	Z	Mated	P	I**	K	K
B	-	Z	Mated	P	I	K	K
C	I	Z	-	-	I	K	K
D	-	Z	-	-	I	K	K

I*, infected with five cysticercoids
I**, infected with three cysticercoids

The results of this experiment (Fig. I-25) show that on day 8 p.i. the immunised mice of group C had fewer and lighter worms than group D ($p < 0.05$). The immunised lactating mice (group A) again carried more worms than the immunised mice of group C (96% and 25% respectively), but the group A burdens were lighter than from the naive lactating mice of group B ($p < 0.01$) and not significantly different from the nulliparous controls (group D), indicating that immunological memory is able to penetrate the suppressive effects of lactation, if only to a small extent. The situation on day 10 p.i. was rather different; although worms were again lighter in group A than in group B (a difference not wholly accounted for by the lower recovery from group A mice), there was no statistically significant difference between the two groups. Also on this day, group A was very different from group D (88%

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Figure 1-25

Dry weight of H. diminuta from three-cysticercoid infections of immunised or naive lactating and nulliparous mice.

A, immunised lactating mice

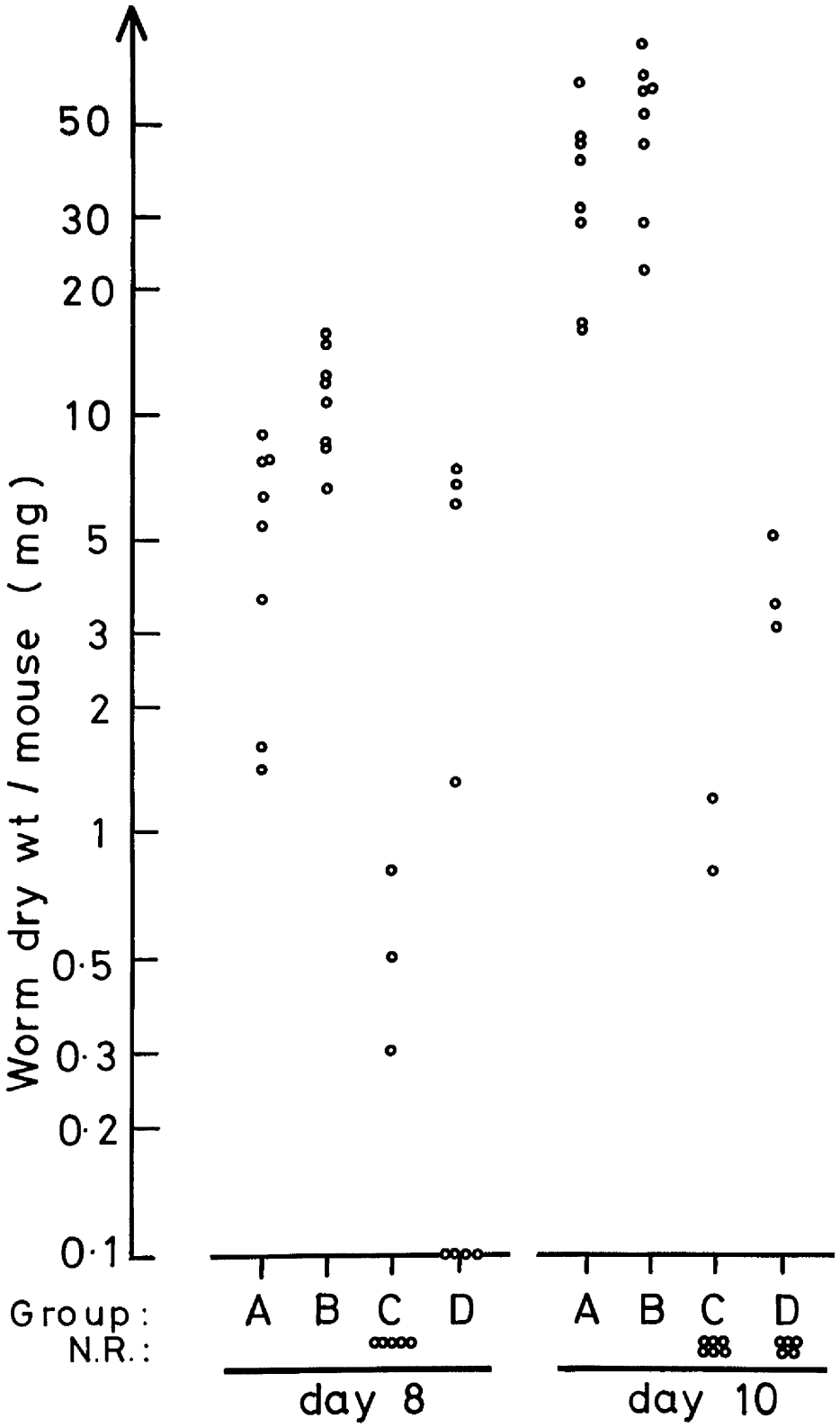
B, naive lactating mice

C, immunised nulliparous mice

D, naive nulliparous mice

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.


Worm dry wt / mouse (mg)
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Group :
N.R. :
A B C D
A B C D
day 8
day 10

and 21% worm recoveries respectively).

The combined results of these two experiments thus suggest that the response in previously immunised lactating mice to a challenge infection with H. diminuta may not differ materially from that of naive lactating mice or, at best, may be expressed at the level of the response of a naive nulliparous animal.

e) The relative effects of various stages of pregnancy and lactation on growth and rejection of H. diminuta

Results from various experiments on the effects of pregnancy and lactation were compiled, and the percentage worm recoveries plotted against the day of pregnancy or lactation on which the mice were autopsied. The points for the mice killed on day 12 p.i. were then connected, and points for days 14, 16 & 17 were similarly connected up. From this exercise (Fig. 1-26) it was apparent that there was a peak in worm recovery (i.e. possibly a maximum point of immunodepression) for each of the autopsy days in the second week of lactation. Consequently, an experiment was designed in which CFLP mice were infected with five cysticercoids at various times during pregnancy and lactation, namely days 0, 4, 10 & 16 of pregnancy, and days 2 and 8 of lactation. All mice were killed on day 14 p.i. Unmated control mice were infected at the same times and autopsied on days 8 and 14 p.i.; establishment in day 8 controls was over 90%, and no weighable worms were recovered from any of these mice on day 14 p.i. (all control groups contained eight mice).

The results from the mated groups (Fig.1-27) show that of the mice infected on day 0 of pregnancy, 4/14 carried a

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Figure 1-26

Recovery (%) of H. diminuta from five- or eight-cysticercoid infections of pregnant and lactating mice.

~~~~~~~~, infected 12 days previously

————, infected 14 " "

▬▬▬▬, infected 16 " "

—·—·—, infected 17 " "

Day 0 of lactation = day 20 of pregnancy.
~~~~~~~~

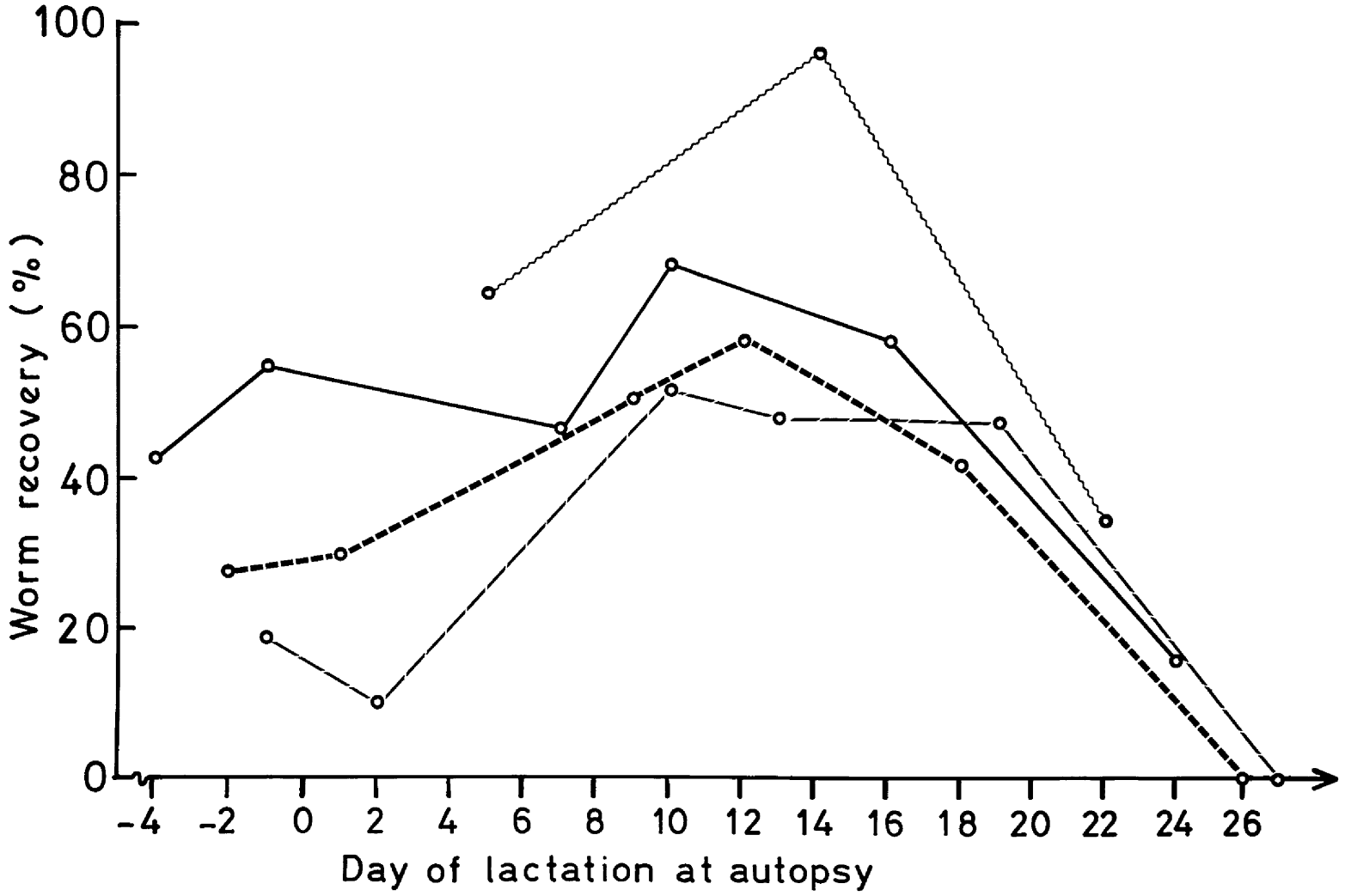
100
80
60
40
20
0
Worm recovery (%)
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Day of lactation at autopsy

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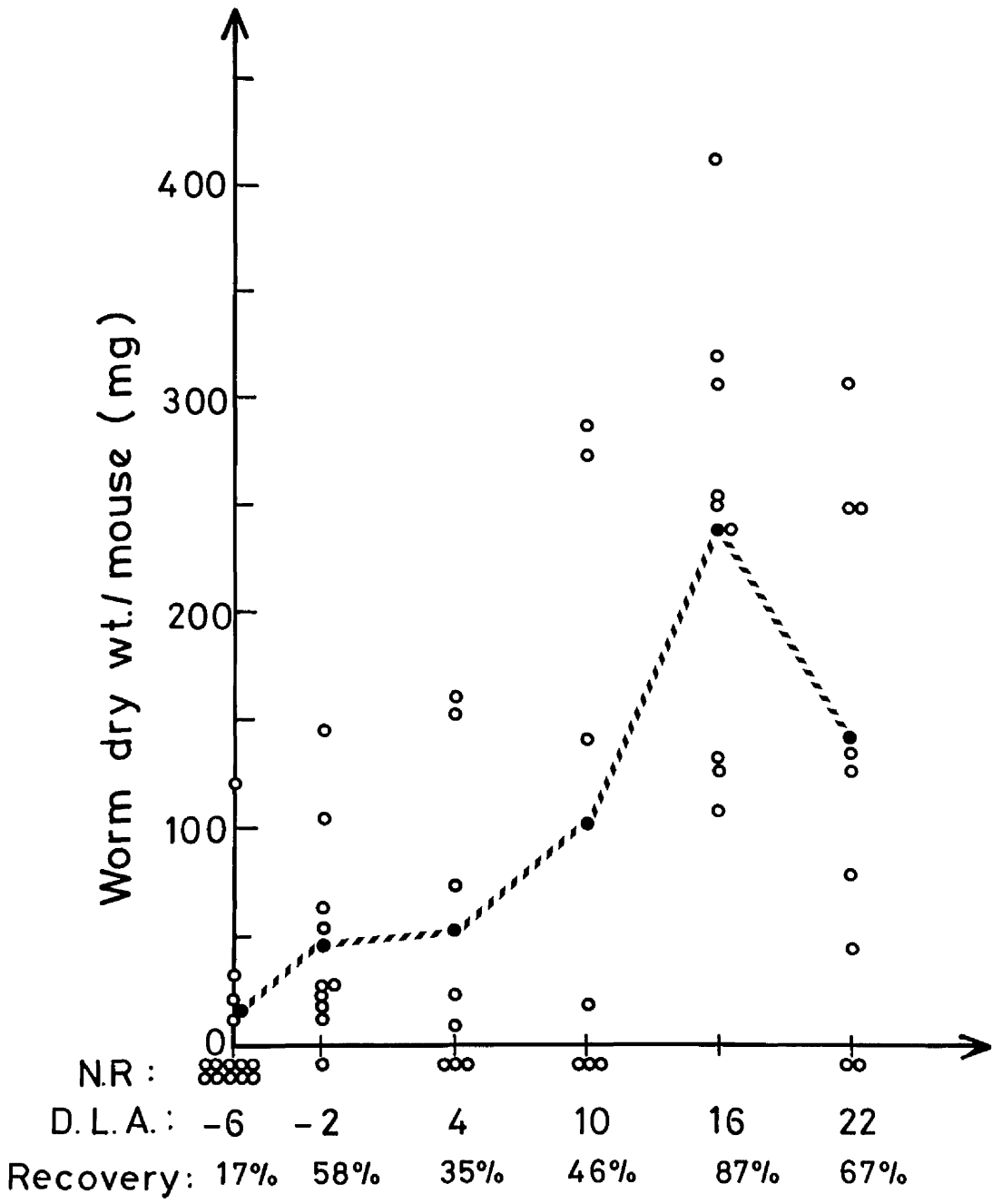
Figure 1-27

Dry weight and recovery (%) of H. diminuta from five-cysticercoid infections of pregnant and lactating CFLP mice. All mice were autopsied on day 14 p.i. on the day of pregnancy or lactation shown. Each point (o) represents the total weight of worm tissue from a single mouse. ●▰▰●, mean dry weight of H. diminuta per mouse; N.R., mice from which no weighable worms were recovered; D.L.A., day of lactation at autopsy. Day 0 of lactation = day 20 of pregnancy.


Worm dry wt./ mouse (mg)
400
300
200
100
0
N.R :
D. L. A. : -6 -2 4 10 16 22
Recovery: 17% 58% 35% 46% 87% 67%

substantial weight of worm tissue, and infection on day 4 of pregnancy increased this proportion to 9/10 mice; percentage recovery and mean weight of worms per mouse were also increased on this day. The increase in biomass continued to a peak at autopsy on day 16 of lactation (i.e. infected on day 2 of lactation) at which point the maximum percentage recovery (87%) was also obtained. The worms recovered on day 22 of lactation (litters were removed on day 18 of lactation, hence four days after the end of lactation) were lighter than those recovered on day 16 of lactation ($p < 0.01$). The growth of the individual worms recovered (expressed as mean weight per surviving worm - Fig. 1-28) also shows a progressive increase in the size of the worms during pregnancy, with a peak again in mice killed on day 16 of lactation, and a lower value for the mice killed on day 22 of lactation.

This experiment confirms the impression from experiments presented thus far that lactation is more immunosuppressive than pregnancy; the maximum recovery and weight of H. diminuta was from the group infected on day 2 of lactation and killed on day 16 of lactation. The nature of the host-parasite system does not allow a point estimate of immune status during lactation, but as days 8-14 p.i. are the days on which the worms would normally be rejected from nulliparous mice, it seems reasonable to conclude that the period of maximal immunodepression during pregnancy and lactation in the mouse with respect to H. diminuta infection is roughly days 10 to 16 of lactation.

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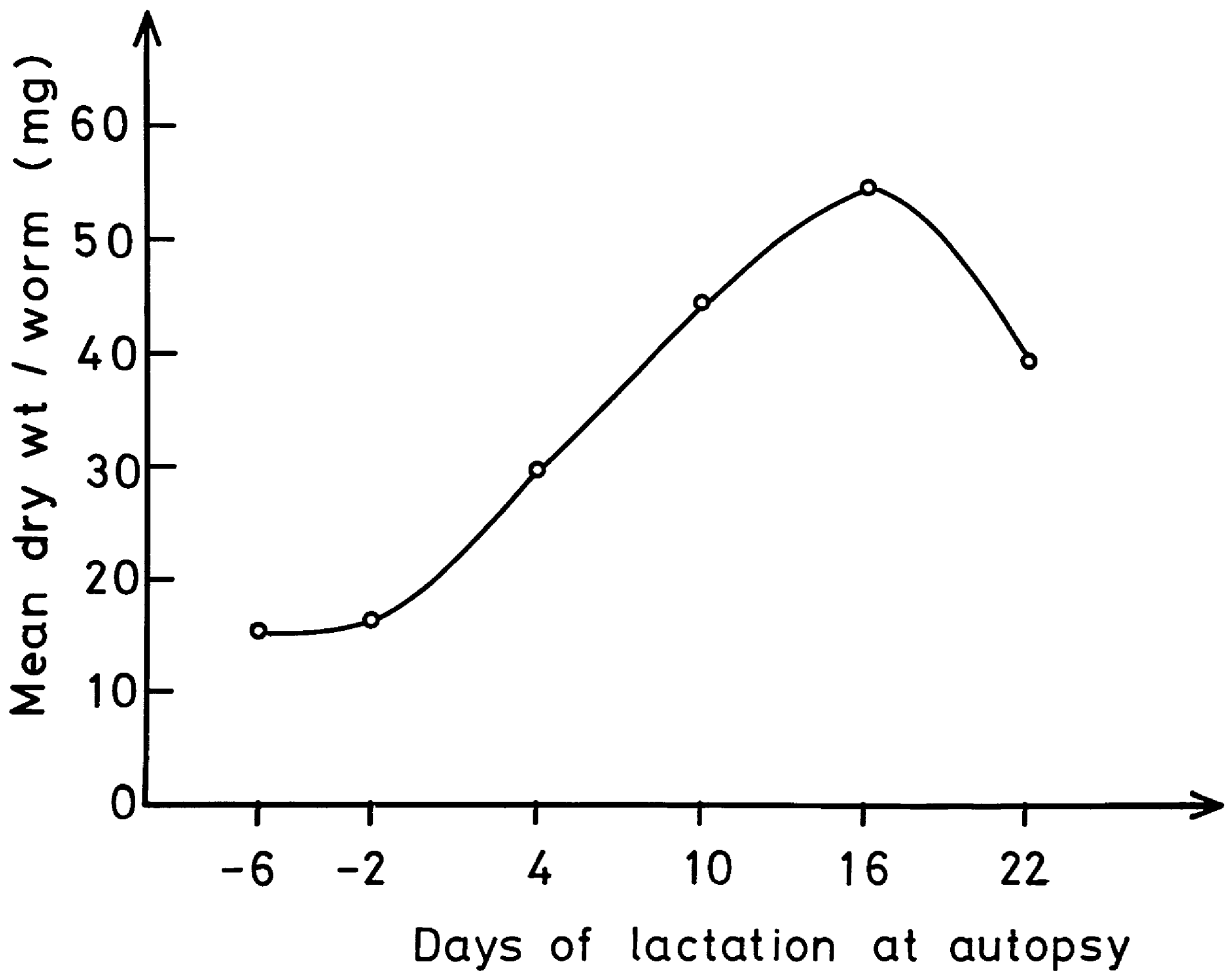
Figure I-28

Mean dry weight per surviving H. diminuta worm from pregnant and lactating mice. All mice were autopsied on day 14 p.i. on the day of lactation shown.

Day 0 of lactation = day 20 of pregnancy.


Mean dry wt / worm (mg)
60
50
40
30
20
10
0
-6
-2
4
10
16
22
Days of lactation at autopsy

f) The relative importance of food intake and immunodepression in increasing growth of H. diminuta during pregnancy and lactation

The higher percentage recoveries of worms and the longer persistance of strobilate worms in pregnant and lactating mice is ample proof that immunological reactivity to the parasite is depressed. However, as pregnant and lactating mice eat more food to support their offspring (in utero or post partum), the dry weights of the worms recovered may well be substantially altered by the increased dietary intake. Separation of the components of food intake and immunodepression in this system is a well-nigh impossible task, but some information may be gained by studying the growth of worms in pregnant and lactating mice, and comparing the growth of worms in pregnant and lactating mice treated with an immunosuppressive drug. These results in turn could be compared with those from nulliparous immunosuppressed and untreated groups of mice. One could then possibly estimate the degree of immunodepression involved, and thus estimate the role of food intake in increasing worm growth.

(i) Pregnancy

CFLP mice were infected with five cysticercoids on days 6-7 of pregnancy, treated with cortisone as described in General Materials & Methods, and killed on day 9 p.i. A second group was treated identically, except that no cortisone was given; nulliparous controls (cortisone treated and untreated) were also infected, and were killed on day 9 p.i. This experiment was repeated, and the pooled results are shown in Fig. 1-29.

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Figure I-29

Dry weight of H. diminuta from five-cysticercoid infections of CFLP mice.

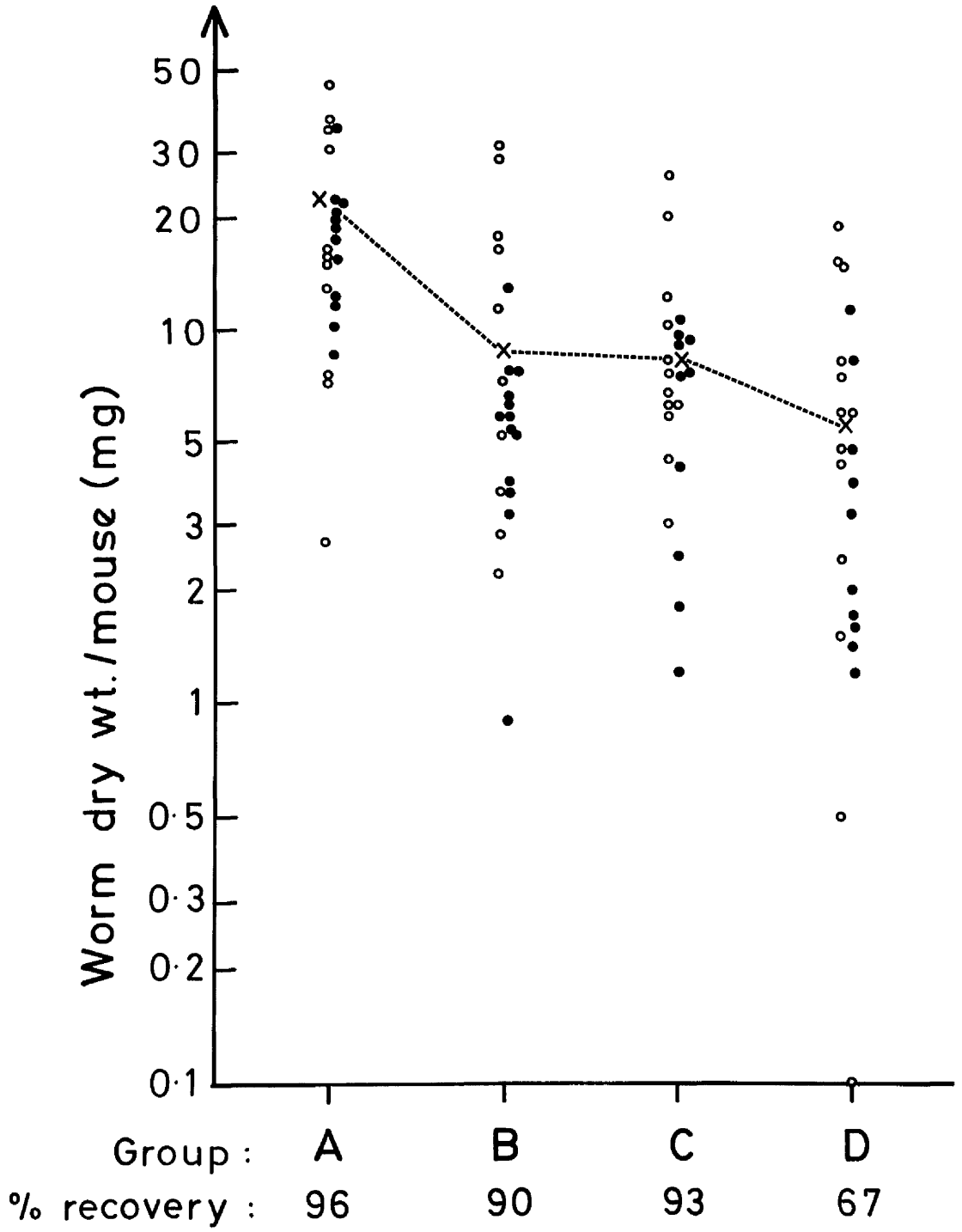
Group A, pregnant + cortisone

Group B, pregnant

Group C, nulliparous + cortisone

Group D, nulliparous

Each point represents the total weight of worm tissue from a single mouse; o & ● represent results from separate experiments; x----x, mean dry weight of worms per mouse.


Worm dry wt./mouse (mg)
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Group : A B C D
% recovery : 96 90 93 67

Treatment of nulliparous mice with cortisone raised the mean worm weight per mouse from 5.7 mg to 8.3 mg, an increase of 46%. The mean weight of worms per mouse from pregnant and cortisone-treated nulliparous mice was almost identical (8.3 mg and 8.8 mg respectively), but the mean weight per mouse for the cortisoned pregnant group was 22.3 mg, an increase of 152% over the untreated pregnant group. This latter relatively large increase in weight suggests that pregnant mice are not fully immunosuppressed, and growth of worms in pregnant mice may be markedly enhanced by full immunosuppression. This in turn suggests that the increase in weight of worms in pregnant mice over control values may be largely due to altered food intake.

(ii) Lactation

CFLP mice were mated, infected with five cysticercoids on days 4-5 of lactation, and either treated with cortisone or left untreated. Nulliparous treated and untreated mice were also infected, and all mice were killed on day 9 p.i.

The results of this experiment (Fig. 1-30) show that while cortisone treatment increased the mean weight of worms per mouse in nulliparous mice by 203% (an increase of 102% in mean weight per surviving worm), cortisone treatment of lactating mice resulted in an increase of only 36% over untreated lactating values, indicating that full immunosuppression of lactating mice has little effect on worm growth. This implies in turn that lactating mice are already highly immunodepressed. The fact that the worms from untreated lactating mice were heavier than those from nulliparous cortisone-treated mice indicates that there is also a large

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Figure I-30

Dry weight and recovery (%) of H. diminuta from five-cysticercoid infections of CFLP mice.

Group A, lactating + cortisone

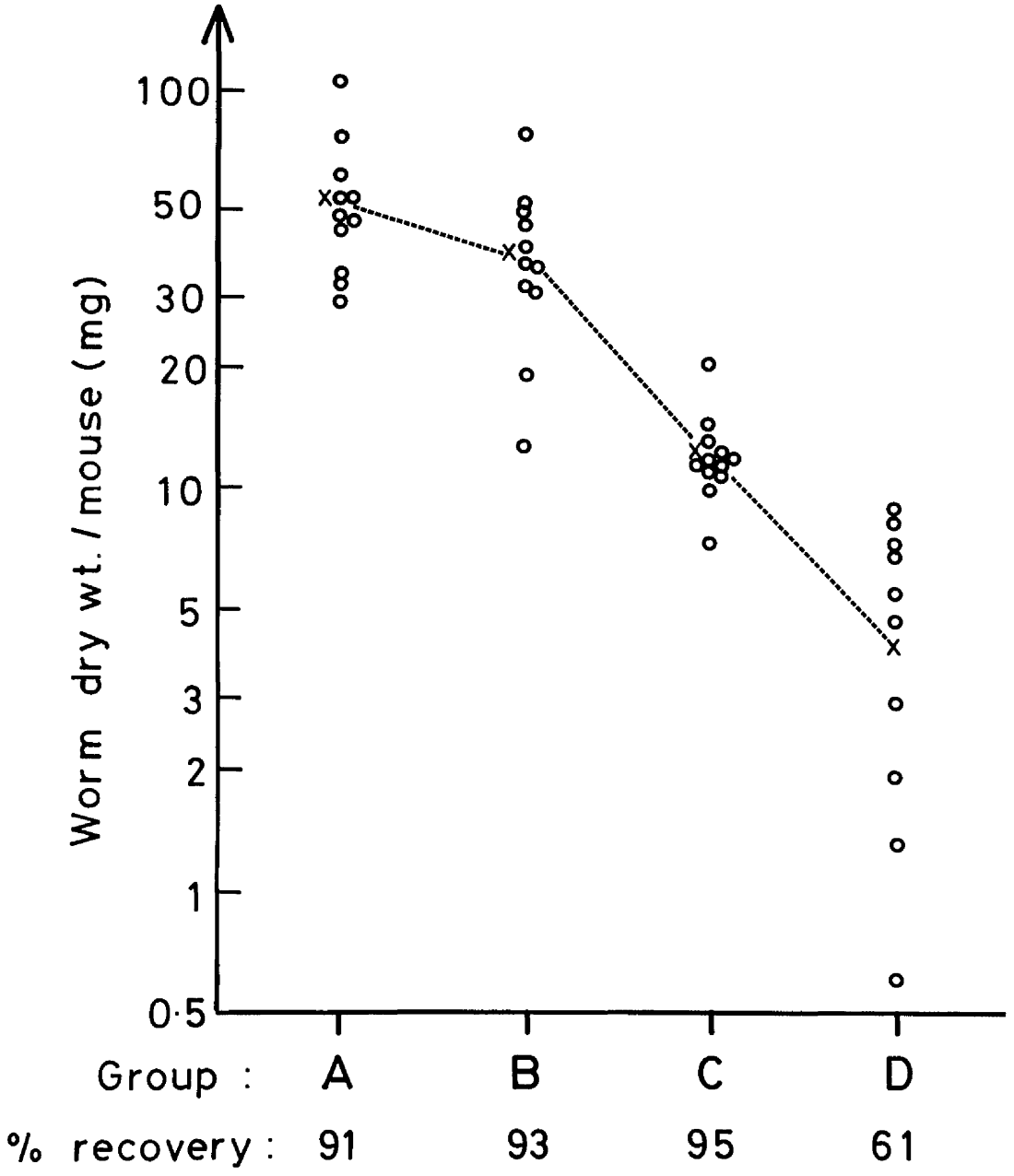
Group B, lactating

Group C, nulliparous + cortisone

Group D, nulliparous

Each point represents the total weight of worm tissue from a single mouse.

x----x, mean dry weight of worms per mouse.


Worm dry wt. / mouse (mg)
100
50
30
20
10
5
3
2
1
0·5
Group :
A
B
C
D
% recovery :
91
93
95
61

component of the increased worm growth that is due to increased food intake.

The results from this group of experiments thus confirm also that immunodepression is more marked during lactation than during pregnancy.

3) Transfer of immunity to offspring

As it has been demonstrated in other host-parasite systems that immunity transferred from mother to offspring fades by six weeks after birth (see Introduction), three experiments were designed, to test for the existence of maternal transfer of immunity to H. diminuta; offspring from immunised or naive mothers were infected at 2, 3, or 6 weeks after birth.

a) Challenge at six weeks old

40 CFLP mice were divided into two groups of 20; the 'immune' group was infected with five cysticercoids and the infection was terminated with anthelmintic 10 days later; control mice received anthelmintic at this time also. Both groups of mice were mated 1-4 days later and the immunised group was infected with one cysticercoid at six intervals between day 5 of pregnancy and parturition; during lactation a further four infections with one cysticercoid were administered. The offspring for each group were pooled, and infected at six weeks of age with three cysticercoids. The offspring were autopsied on days 8, 9, 10, 11, 12, 13, 14 & 16 p.i. Both male and female mice were used; groups autopsied contained equal numbers of both sexes.

The graph of weight of worms recovered (Fig. 1-31) shows that while small differences between the two groups did exist on any one day, these differences were not consistent, the offspring from immunised mothers carrying a heavier mass of worms than the control group on two of the days studied. This lack of difference was further emphasised by the percentage recovery results

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Figure I-31

Dry weight of H. diminuta from three-cysticercoid infections of CFLP mice at six weeks old.

●, mice with immunised mothers

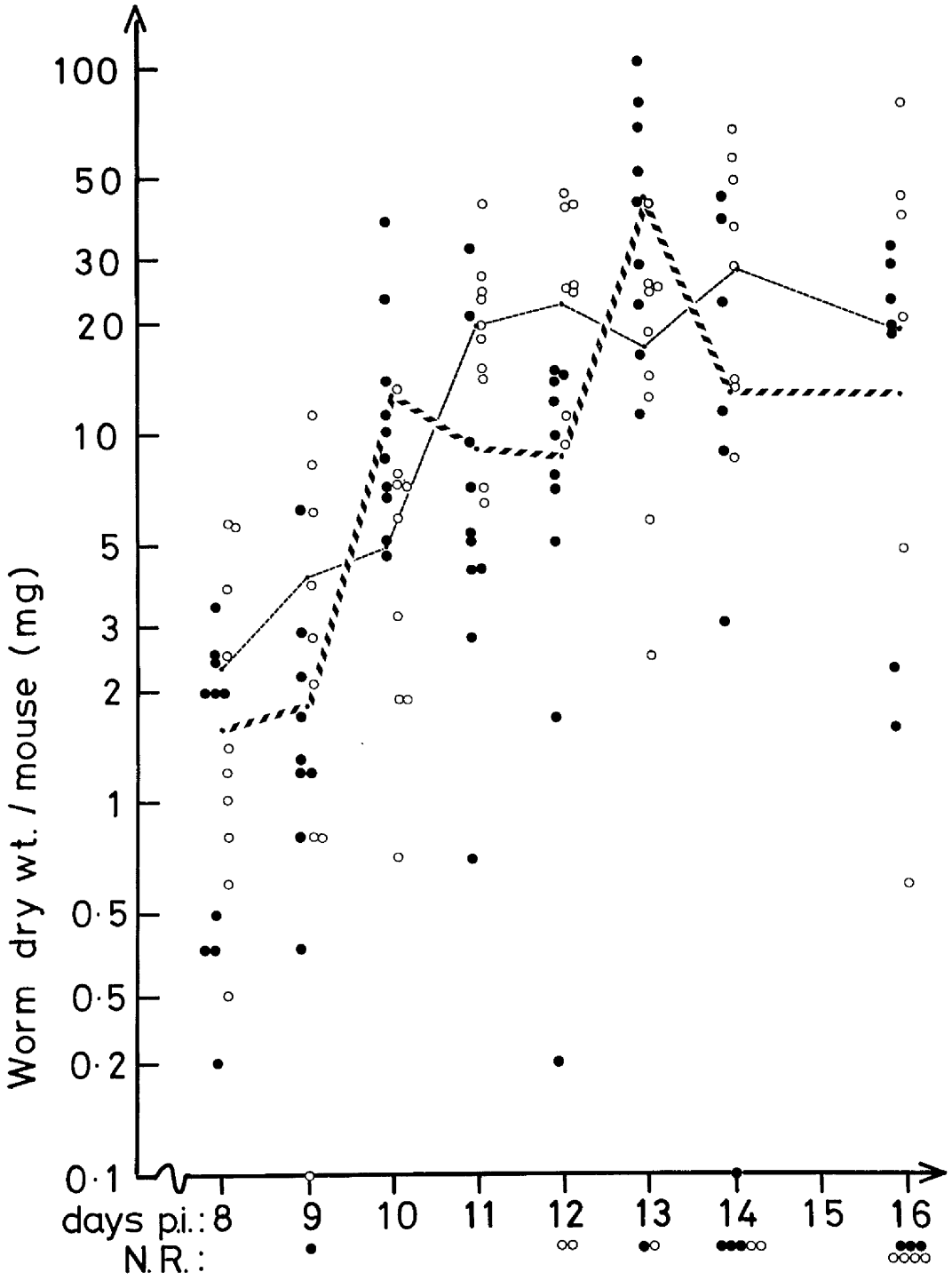
o, mice with naive mothers

Each point represents the total weight of worm tissue from a single mouse.

▰▰▰▰▰, mean weight of worms per mouse (immunised mothers)

----------, mean weight of worms per mouse (uninfected mothers)

Days p.i., days post infection; N.R., mice from which no weighable worms were recovered.


Worm dry wt. / mouse (mg)
100
50
30
20
10
5
3
2
1
0·5
0·5
0·2
0·1
days p.i.: 8 9 10 11 12 13 14 15 16
N.R.:

(Fig. I-32) in which the rate of loss of worms from the two groups was very similar. There is no evidence, therefore, that any immunity transferred from mother to progeny is evident at six weeks post-natally; this is not a totally unexpected result if one considers the time limits of effectiveness defined for other host-parasite systems.

b) Challenge at three weeks old

Two groups of 15 CFLP mice were used. One group was given five cysticercoids; both groups received anthelmintic 10 days later, and were mated 1-4 days after this. The immunised group received four infections with two cysticercoids during pregnancy and a further three infections during lactation. On day 21 post partum the offspring were pooled and infected with five cysticercoids; groups of mice containing equal numbers of both sexes were autopsied on days 8, 10, 13, 15, 17 & 19 p.i.

Once again, there was no consistent difference between the two sets of mice in either percentage recovery or worm weight (Fig. I-33). The only large difference between the two groups recorded in this experiment was on day 19 p.i.; however, as the percentage recovery declines very sharply from day 15 to day 19 p.i. this difference could easily be due to chance, particularly in the absence of a result after day 19 p.i.

So, in summary, there was again no difference evident between the mice with immunised or uninfected mothers at three weeks after birth.

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Figure 1-32

Recovery (%) of H. diminuta from three-cysticercoid infections of six-week-old mice with immunised mothers (●▰▰▰●) or uninfected mothers (o------o).

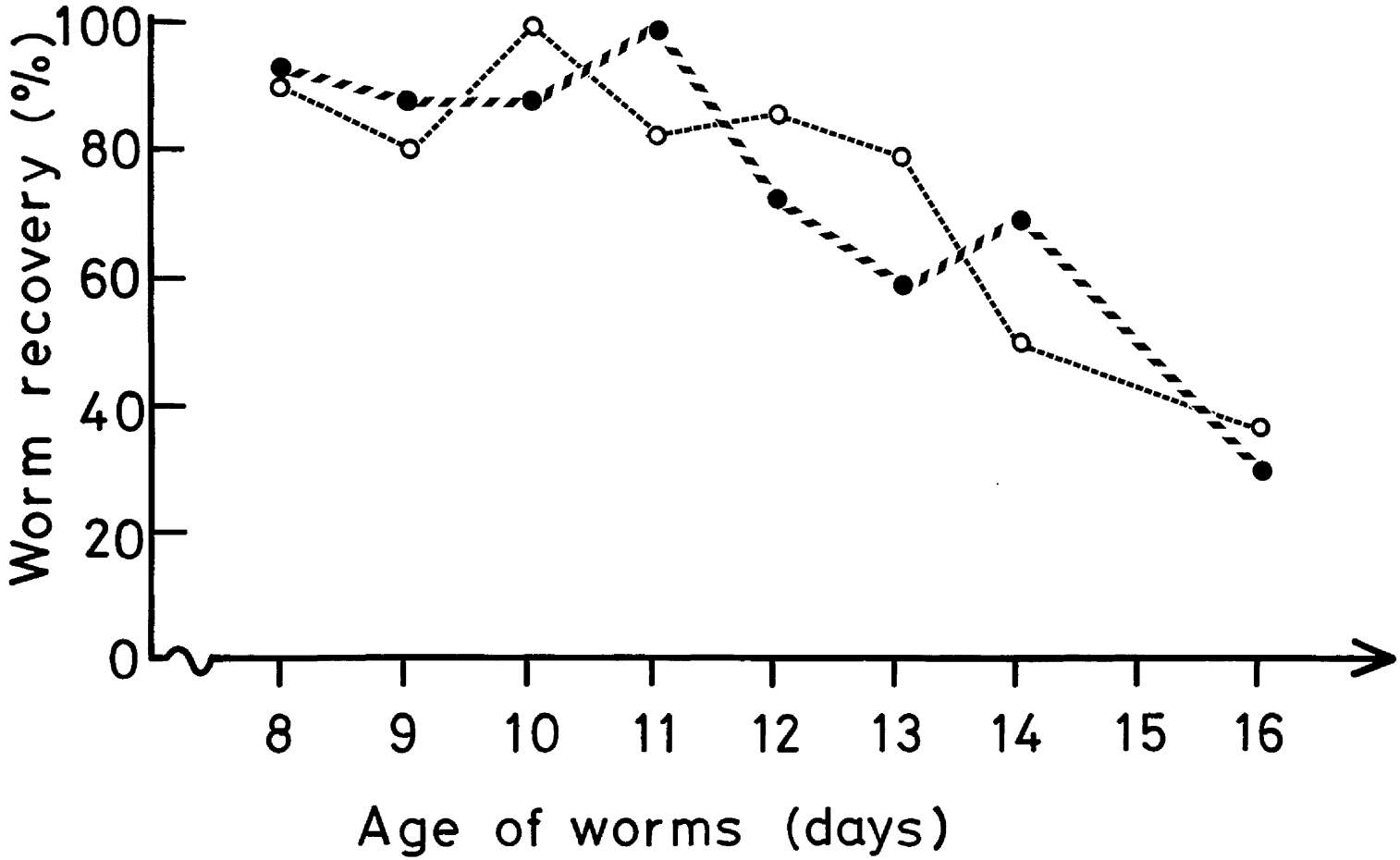
Worm recovery (%)
100
80
60
40
20
0
8
9
10
11
12
13
14
15
16
Age of worms (days)

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Figure I-33

Dry weight and recovery (%) of H. diminuta from five-cysticercoid infections of CFLP mice at three weeks old.

●, mice with immunised mothers

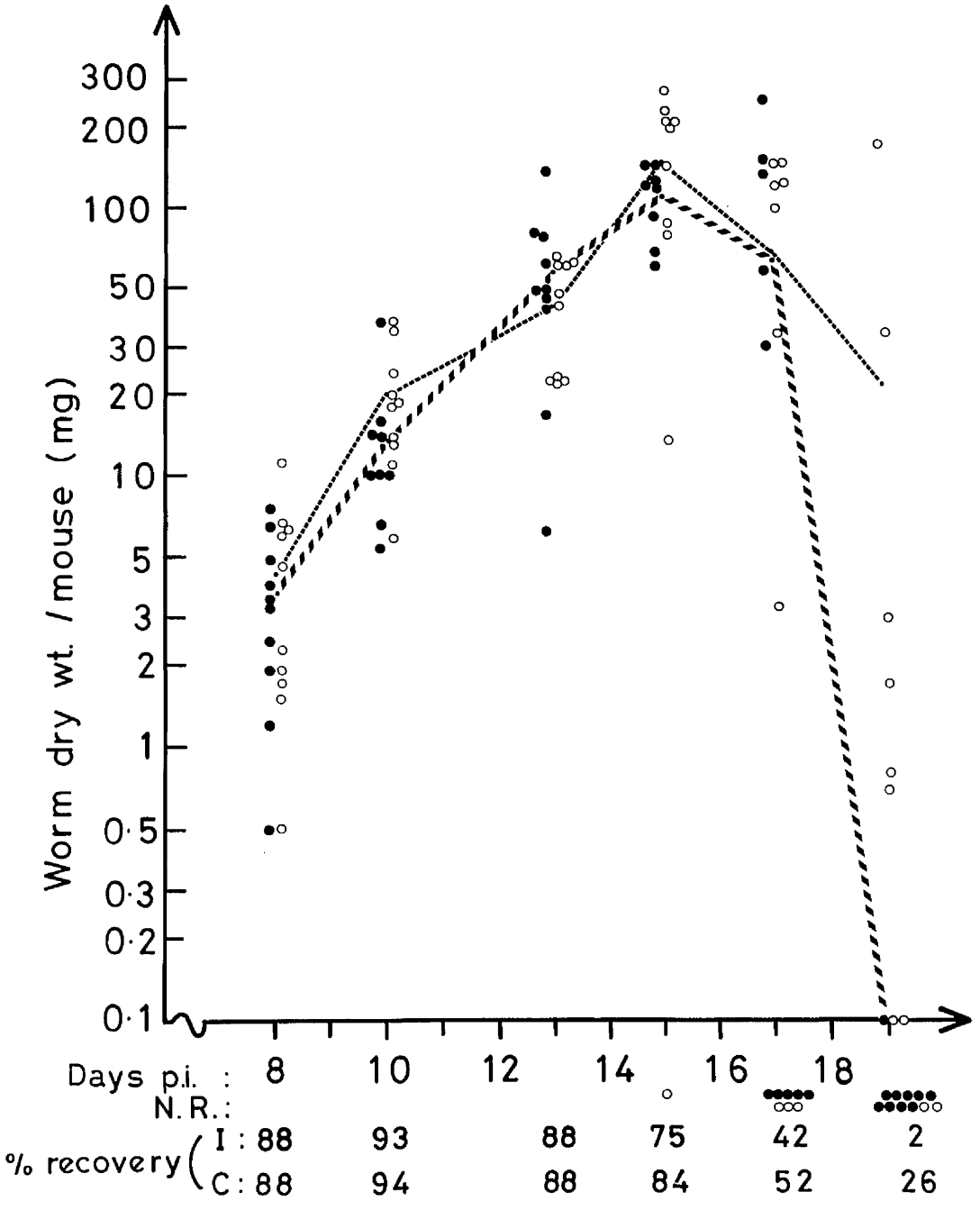
o, mice with uninfected mothers

Each point represents the total weight of worm tissue from a single mouse.

▰▰▰▰▰, mean worm weight per mouse (immunised mothers)

---------, mean worm weight per mouse (uninfected mothers)

Days p.i., days post infection; N.R., mice from which no weighable worms were recovered; I, immunised mothers; C, uninfected (control) mothers.


Worm dry wt. / mouse (mg)
300
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Days p.i. : 8 10 12 14 16 18
N.R.:
% recovery (I : 88 93 88 75 42 2
C : 88 94 88 84 52 26

c) Challenge at two weeks old

Two groups of 16 CFLP mice were used as mothers in this experiment; one group was infected 15 days and one day before mating with six cysticercoids. The offspring from both groups were infected with three cysticercoids at 14 days old, this being about the earliest age at which it is practicable to infect mice with the diameter of tubing necessary to accommodate the cysticercoids. The mice with immune or naive mothers were autopsied on days 8, 11, 13 & 18 p.i., and a group of mice with uninfected mothers was killed on day 26 p.i. Approximately equal numbers of both sexes were used, and litters remained with their natural mothers for the duration of the experiment.

The low recovery of worms from the control group on day 8 p.i. (Fig. 1-34) was probably due to the small size of the worms involved; this in turn may have been due to the fact that the mice with immunised mothers that were autopsied on this day weighed, on average, 3 g more than the offspring of the naive mothers. On day 11 p.i., however, recovery of worms from the uninfected mothers' offspring was 92%, and both groups of mice retained over 90% of the worms at day 13 p.i. without any significant difference in weights between the two groups. On day 18 p.i. rejection had commenced in both groups, but again worm weights and recoveries were very similar.

So, even infecting at two weeks of age, there is no evidence of any difference in worm growth or rejection in mice with immunised or naive mothers.

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Figure I-34

Dry weight and recovery (%) of H. diminuta from five-cysticercoid infections of CFLP mice at two weeks old.

●, mice with immunised mothers

o, mice with uninfected mothers

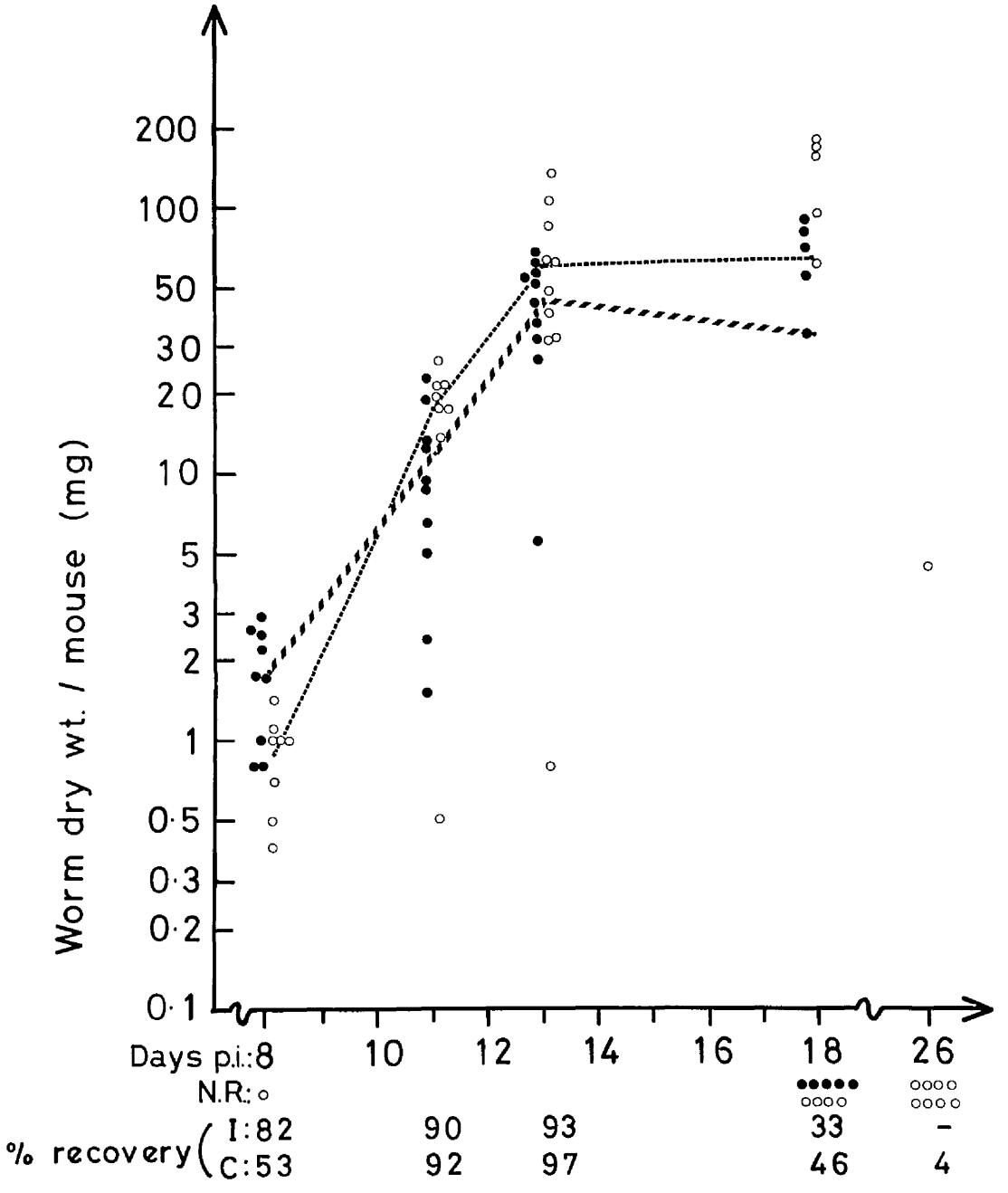
Each point represents the total weight of worm tissue from a single mouse.

▰▰▰▰▰, mean worm weight per mouse (immunised mothers)

----------, mean worm weight per mouse (uninfected mothers)

Days p.i., days post infection; N.R., mice from which no weighable worms were recovered;

I, immunised mothers; C, uninfected (control) mothers.


Worm dry wt. / mouse (mg)
200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Days p.i.: 8 10 12 14 16 18 26
N.R.: ○
% recovery (I: 82 90 93 33 –
C: 53 92 97 46 4

DISCUSSION

The above results demonstrate clearly that pregnancy and lactation have a profound effect on growth and rejection of *H. diminuta* in the mouse. In both phases of the reproductive cycle, the worms grow at a faster rate and rejection is considerably slower than in nulliparous mice.

In pregnant mice, the delay in rejection was found to be in the order of two days; this delay was certainly evident in mice infected on day 4 of pregnancy (Fig. 1-1), and a delay in rejection was noted when mice were infected on the day of mating (Fig. 1-28). However, infecting mice 4-6 days before mating produced no demonstrable difference in either growth or rejection from controls (Fig. 1-10). The mice in this latter experiment were killed on days 10 and 12 p.i., and were thus autopsied on days 4-8 of pregnancy. Clarke *et al.* (1978) detected immunodepression in early pregnancy in mice by studying rosette inhibition titres (RIT), and correlated this with the appearance of a high molecular weight immunosuppressive factor which appears in the maternal serum as early as six hours after fertilisation; they named this substance Early Pregnancy Factor (EPF). As immunosuppressants can halt rejection of *H. diminuta* at virtually any point in the course of infection with an attendant increase in growth rate (Hopkins & Stallard, 1976), it would appear that EPF plays little part in the impairment of the response to *H. diminuta*. Clarke *et al.* (1978) also found that EPF was replaced after day 5 of pregnancy by a second factor of low molecular weight which cross-reacts with anti-HCG antiserum. This rise in concentration of the second factor also coincides with the rise in steroid hormones such as progesterone

and corticosteroids (McCormack & Greenwald, 1974; Barlow et al., 1974), day 5 of pregnancy being the time of implantation of the embryo (Rugh, 1968). It therefore appears that the mechanism which depresses the response against H. diminuta in pregnant mice is similar to that detected by the many workers who have described a higher degree of immunosuppression in the later stages of pregnancy (e.g. Skowron-Cendrzak et al., 1975; Stimson et al., 1977; Bauminger & Peleg, 1978).

At parturition, there is a considerable flux of steroid hormones (McCormack & Greenwald, 1974; Barlow et al., 1974) and this has been reported as a time when immune responsiveness returns to normal levels. Morton et al. (1974) found that the RIT decreased shortly before parturition to control levels, and Hunninen (1935) reported that pregnant mice infected with H. nana ceased egg production within 2-3 days of parturition. However, it has also been found that human serum taken from mothers at the time of delivery is still markedly immunodepressive (Schiff et al., 1975), and that lymphocytes from newly-calved cows respond less well to mitogen stimulation than nulliparous control lymphocytes (Wells, Burrels & Martin, 1977). The present study does not indicate that parturition has any effect on the survival or growth of H. diminuta (Figs I-7, I-8), and growth of individual worms whose period of infection crossed the time of parturition was greater than worms of the same age in pregnant mice autopsied before parturition (Fig. I-29).

Lactation produced an effect similar to pregnancy in that rejection of H. diminuta was slower than in nulliparous controls, but the delay appears to be in the order of 4-7 days (Fig. I-18). Growth was also markedly enhanced, highly significant differences

between lactating and control groups being evident as early as day 8 p.i. (Fig. I-7), whereas in pregnant mice the difference is normally less marked on this day (e.g. Figs I-2, I-6). Worm burdens weighing around 500 mg were often recovered from lactating mice, a weight that is substantially heavier than the pregnancy 'upper limit' of around 200 mg. Lactation was shown to be the time of greater immunosuppression (Fig I-28), days 10 to 16 of lactation being the probable period of maximal immunosuppression.

In contrast to the end of pregnancy, the end of lactation is probably associated with a rapid return to immunological competence. Termination of lactation in mice results in rapid expulsion of T. muris (Selby & Wakelin, 1975) and T. spiralis (Ngwenya, 1977); in the present study, infecting with H. diminuta closer to the end of lactation caused earlier rejection of worms (Fig. I-19), an effect that was apparently reduced to some extent by allowing lactation to continue after day 18 post partum (Fig. I-22). Mice infected 6-8 days after parturition and removal of litters showed responses comparable to those of their nulliparous counterparts (Figs I-12, I-23).

It is an important feature that both pregnant and lactating mice show some degree of loss of H. diminuta, i,e, the rejection mechanism is not totally suppressed. This is in contrast to the situation with N. brasiliensis in the lactating rat, in which it was established by Connan (1972a) that no loss of worms occurs, even if lactation is extended to 36-37 days by substitution of younger litters.

One difficulty in interpretation of the results presented here is that pregnant and lactating mice eat more than their nulliparous counterparts, and associated with this is a marked

hyperplasia of the villi of the small intestine (Fell, Smith & Campbell, 1963) which, at least in the case of lactation, is caused by hormonal factors (Elias & Dowling, 1976). As hymenolepids are extremely sensitive to variations in host dietary intake, particularly carbohydrate (Read & Rothman, 1957b), pregnancy- or lactation-induced hyperphagia would be expected to increase the growth of H. diminuta. Growth was indeed very much increased, particularly during the second half of lactation (Fig. I-28), when the mother is feeding the heaviest weight of offspring. However, this is also the period of maximum worm recovery, and as it is unlikely that worms in a better nutritional environment are rejected more slowly, it is apparent that this is also the time of maximum immunosuppression. As the immune response of the mouse includes a restricting effect on growth of H. diminuta (Hopkins et al., 1972a), it is probable that growth of worms in pregnant and lactating mice is enhanced at least partly because of the depressed responsiveness of these mice. It is also known that, although increased food intake is a feature of lactation, the rate of absorption (particularly of glucose) by the small intestine is markedly enhanced (Elias & Dowling, 1976), thus making less nutrient available to the worms than would at first appear. Growth of H. diminuta in pregnant mice was substantially increased by treatment with cortisone, suggesting that a large proportion of the increased growth of worms in pregnant mice is due to increased food intake (Fig. I-30). Worms from lactating mice are heavier than worms of the same age from pregnant mice (Fig. I-29), and cortisone treatment of lactating mice had little effect on worm growth, indicating that lactating mice are already substantially immunodepressed (Fig. I-31). However, lactating mice carried heavier worms than cortisone-treated

nulliparous mice, indicating that increased food intake also made a substantial contribution to worm growth in lactating mice. Growth of H. diminuta in lactating mice may well be proceeding at the maximum rate physiologically possible for the worm, and cortisone treatment, even assuming it to be completely effective in ablating the immune response, would therefore have little effect. Thus, although worm growth is at least partially influenced by the status of the immune system in pregnant and lactating mice, it is virtually impossible to separate this from the effects of hyperphagia; percentage recovery of worms is therefore probably the better measure of the degree of immunological reactivity in this case, although the increase in worm growth may be a very important factor in the population dynamics of H. diminuta in wild mouse populations.

Having established that pregnancy and lactation cause reduced immunological responsiveness to H. diminuta, the problem is to determine which of the immunological events involved is/are depressed. Impaired induction of the immune response to T. spiralis in mice immunised with irradiated larvae while lactating was described by Ngwenya (1977); however, lactating mice infected with T. muris showed a near-normal secondary response to a subsequent challenge infection (Selby & Wakelin, 1975). Mice immunised with H. diminuta for a short period during pregnancy or lactation and subsequently challenged showed a response at least as strong as that of similarly immunised nulliparous mice, although worm rejection in pregnant or lactating mice did not normally commence until after the day on which anthelmintic was administered. These experiments imply that the inductive processes of the immune response (i.e. antigen uptake followed by specific sensitisation of lymphocytes with generation of immunological memory) occur without inhibition

in pregnant or lactating mice.

Following on from this, it is logical to assume that the effector arm of the response is depressed, probably either by inhibition of division of stimulated lymphocytes or by suppression of the expression of the immune response by effector lymphocytes or accessory cells. In support of the former argument, Mendelsohn *et al.* (1977) reported that inhibition of mitosis was the major effect on lymphocytes of steroid hormones such as progesterone and cortisol; Dineen & Kelly (1972) found that mesenteric lymph node cells (MLNC) from infected lactating rats could be used to transfer immunity to nulliparous recipients and *vice versa*, although immune MLNC from lactating donors were less efficient in lactating recipients, and these authors concluded that the differentiation of induced cells to effector cells was inhibited. However, Ogilvie & Parrott (1977) reported apparently unpublished experiments (citing Ogilvie & Love, 1974) showing that normal immune MLNC transferred to lactating rats harbouring adult (rather than larval) *N. brasiliensis* had no effect on the worms, indicating that some other specific cell function was depressed by a lactating serum factor, this cell function being essential for rejection of adult (lumen-dwelling) infections.

The strain of animal used may also be an important factor in determining the degree of immunodepression; Skowron-Cendrzak *et al.* (1975) reported that syngeneic mating in mice produced significantly less marked immunodepression during pregnancy than allogeneic mating. Maroni & deSousa (1973) found differences in weight of the lymph nodes draining the pregnant uterus in syngeneic and allogeneic matings, although the other lymphoid tissues were not consistently different in the two groups; this lack of difference

in the peripheral nodes was recently confirmed by Hetherington & Humber (1977). Many of the experiments in lactating animals infected with intestinal nematodes have been carried out with syngeneic animals (e.g. Dineen & Kelly, 1972; Ngwenya, 1976a), and immunity to H. diminuta was apparently markedly depressed in syngeneic (NIH x NIH) pregnancy (Fig. 1-16). However, the effect of pregnancy may also be related to the specific strain involved. Clarke (1979) reported interesting experiments showing that C57 Bl female mice immunised against and then mated with CBA male mice did not show the classic pregnancy-associated involution of the thymus; however, in the reciprocal case (CBA female immunised against and mated with C57 Bl males) thymic involution occurred normally. This therefore is an indication that the strain of mouse used in experiments on pregnancy and lactation may profoundly affect the results obtained.

One very important point that arises from the experiments presented here is that of anti-worm antibody production. As already summarised in the Introduction, mice are capable of producing antibodies against antigens during pregnancy and lactation, and pregnant mice may even have enhanced antibody potential (Fabris, 1973; Kenny & Diamond, 1977). H. diminuta in the mouse is normally coated with a variety of immunoglobulin classes that appear, from the sequential manner of their appearance on the tegument, to be specific antibody against the worm (Befus 1975b, 1977). Assuming that production of these antibodies may also be increased during pregnancy, the slower rejection of H. diminuta in pregnant mice does not support an independent role for antibody in rejection of H. diminuta, nor is the possibility that growth of the worms is affected adversely by antibody confirmed by the

experiments presented here.

The presence of the third component of complement (C_3) has also been observed on the surface of H. diminuta in mice (Befus, 1977); it is therefore possible that complement components in the presence of antibody may be involved in rejection of H. diminuta. However, the available evidence suggests that both the serum concentration of C_3 and complement activity increase during pregnancy (reviewed by Gusdon, 1976), suggesting that slower rejection of H. diminuta during pregnancy is not due to complement deficiency.

In the case of lactation there is direct evidence from other host-parasite systems that specific anti-worm antibody is manufactured, and antibody-damaged worms can be found in the intestinal lumen of lactating animals (Kelly & Ogilvie, 1972; Connan, 1973a; Selby & Wakelin, 1975). O'Sullivan (1974) found that lactation in the guinea-pig inhibited rejection of T. colubriformis, and attributed this to a functional deficiency of the myeloid component involved in rejection; however, with other models such as T. muris in the mouse (Selby & Wakelin, 1975) and N. brasiliensis in the rat (Dineen & Kelly, 1972) the deficient component is thought to be a lymphoid one, presumably T cells, indicating that the immune deficiency of lactation is functionally similar to that of pregnancy. The physiological mechanisms causing immunosuppression during lactation may therefore be similar to those during pregnancy. Thus, as antibody function and production is largely unaffected by lactation, this further supports the theory that antibodies do not adversely affect the growth or survival of H. diminuta.

Transfer of immunity to offspring by colostral and milk-borne antibody is a well-documented phenomenon, and this is a

particularly important mechanism with antigens presented from the lumen of the maternal small intestine. IgA is the major immunoglobulin of intestinal and mammary secretions (Brandtzaeg et al., 1970), and the relationship between intestinal immune responses and mammary IgA is so close that it has been suggested that IgA in the milk may be used as an accurate reflection of the antigenic exposure of the gut (Ahlstedt, Carlsson, Fallstrom, Hanson, Holmgren, Lidin-Janson, Lindblad, Jodal, Kaijser, Sohl-Akerlund & Wadsworth, 1977). Enteric bacteria such as Escherichia coli, Salmonella typhimurium and Vibrio cholerae will elicit specific antibody responses in the gut, and specific IgA antibodies can be subsequently detected in the colostrum and milk without substantial amounts of this antibody appearing in the serum (Goldblum, Ahlstedt, Carlsson, Hanson, Jodal, Lidin-Janson & Sohl-Akerlund, 1975; Allardyce, Shearman, McLelland, Marwick, Simpson & Laidlaw, 1974; Holmgren, Hanson, Carlsson, Lindblad & Rahimtoola, 1976).

IgA is secreted in the mammary gland by B cells (Ahlstedt et al., 1977). Love & Ogilvie (1977) have shown that thoracic duct lymphoblasts apparently migrate to mammary tissues of lactating rats if injected intravenously, indicating that cells stimulated by gut antigens (in this case N. brasiliensis) may migrate to the mammary tissues. This has recently been confirmed by McDermott & Bienenstock (1979), who demonstrated that IgA-producing MLNC will preferentially localise in mammary tissue. In the pig, intramammary or intramuscular injection of live transmissible gastroenteritis virus gives a high antibody titre in serum and colostrum, mainly IgG; colostral levels drop rapidly after the first 24 hours of lactation and levels of specific antibody in the milk are low

thereafter. In contrast, enteric infection of the mother gives low serum and colostral antibody, but much higher, persistent levels in the milk, and this antibody (which protects the progeny against infection) is mainly IgA (Bohl, Gupta, Olquin & Saif, 1972).

Cestodes such as H. nana and Taenia ovis have previously been shown to be adversely affected in offspring with immunised mothers (Larsh, 1942; Rickard, Boddington & McQuade, 1977), and in the case of T. taeniaeformis in the mouse, it has been shown that colostral IgA will protect young mice against challenge infection (Lloyd & Soulsby, 1978). In the experiments presented in this Chapter, there was no evidence for milk-borne antibody providing any protection against infection with H. diminuta. Lactation persists for about 21 days in the mouse, and challenge at 6, 3 or 2 weeks post partum showed no difference in growth or survival of the worms between groups with immunised or uninfected mothers. Larsh (1942) reported that the activity of milk-borne antibody against H. nana persisted for 37-41 days after birth, and similar figures for T. spiralis were recorded by Duckett et al. (1972), so any antibody directed against H. diminuta should have displayed some activity in the groups infected at 2 or 3 weeks old, even if not in the groups infected at six weeks of age.

Although no attempt was made to detect specific antibodies in the milk of mice infected with H. diminuta in the present study, it seems very likely that they are produced; no damage to the intestine is necessary for appearance in the milk of antibodies to the antigen, as was shown in (unpublished) work by Andre (1975), in which antibodies to sheep red blood cells were detected in the milk of guinea-pigs 10 days after oral administration of a suspension of sheep erythrocytes. Although the milk from mice

infected with H. diminuta may prove to be a useful source of anti-worm antibodies for future experiments, the experiments presented here indicate that IgA has no deleterious effect on the establishment, growth or survival of H. diminuta.

Taking the results of all the experiments presented in this Chapter, it appears unlikely that antibodies or complement are involved in rejection of H. diminuta; this further confirms the work of Isaak (1976), who reported that mice without antibody potential are able to expel H. diminuta. However, there are other possible interpretations. It has been suggested that the immunoglobulin on the surface of H. diminuta may protect the worm, masking its presence from the host (Befus & Podesta, 1976), and increased antibody production in pregnant and lactating mice may therefore enhance this function, causing slower rejection. There is as yet no evidence that the antibody coating on H. diminuta protects the worm in any way during a normal infection, rejection from the mouse being such a rapid process; it is much more probable that the effects of pregnancy and lactation arise from defects in the functions of effector T cells. Antibody, therefore, appears to have no independant action on the worm, although it may have some minor functions in the presence of effective T cells and/or myeloid components.

SUMMARY

1. The effects of pregnancy and lactation on immune responses and the possible mechanisms involved are briefly reviewed. In general, T effector lymphocyte responses are inhibited, but T helper and B lymphocyte responses are unaltered or enhanced.

2. It is shown that pregnant and lactating mice reject H. diminuta more slowly than normal mice; growth is also enhanced in these mice, partly because of increased food intake, partly because of the immunodepressive effects of pregnancy and lactation.

3. Lactation is shown to have a markedly greater immunodepressive effect than pregnancy.

4. The immunological defect is shown to lie in the effector arm of the response, as pregnant and lactating mice are slow to expel a secondary infection, and immunological sensitisation is uninhibited by pregnancy and lactation.

5. It is demonstrated that mice with immunised mothers gain no protection against H. diminuta infection, either by transplacental or milk-borne antibody transfer.

6. It is concluded that the immunological defect in pregnant and lactating mice is at the level of the T effector cell and/or associated accessory non-lymphoid cells, and that anti-worm antibody has no major independent effect on

the survival or growth of H. diminuta. It is suggested that future work should include careful monitoring of the food intake of pregnant and lactating mice to determine the extent to which the growth-limiting response of the mouse to the worm is depressed.

CHAPTER 2

THE LOCATION OF THE PROTECTIVE ANTIGENS OF *HYMENOLEPIS DIMINUTA*

INTRODUCTION

1. Investigation by transplantation of worms

In the investigation of the immune responses of the host to its parasites, it is a great advantage to be able to isolate the antigen (or antigens) which stimulates an effective immune response by the host, i.e. the 'protective antigen(s)'. This is valuable in experimental investigation for use both in *in vitro* immunological techniques and in *in vivo* development of vaccines or diagnostic reagents for use in helminth infections of medical or veterinary importance. Isolation of protective antigens from the multitude of different proteins contained in and produced by the parasite is a difficult process and has rarely been acheived; whole worm homogenates have frequently been used for immunisation, but with varying degrees of success (reviewed briefly by Wakelin & Selby, 1973; Cox, 1978). A more rewarding approach is the use of specific components of the worm's structure for homogenisation, in cases where the worm is divided into functional regions, such as is the case with trichuroid nematodes. The stichosome cells of these worms have proved to be a very valuable source of protective antigens in that the anterior part of worms such as *Trichinella spiralis* and *Trichuris muris* contains very little except the stichocytes.

Despommier and colleagues, working with *T. spiralis*, found that homogenates of larval worms could immunise against adult and larval challenge infection with *T. spiralis* (Despommier, Campbell & Blair, 1977a), and that the stichosome granules of *T. spiralis* larvae cross-react with immune serum and contain all the antigens

associated with excretory/secretory antigens (Despommier & Muller, 1976); these latter antigens are known to be protective (Campbell, 1955). Immunisation of mice with stichosome granules apparently protects the mice against a subsequent infection, causing a decrease in muscle larva recovery of 80-90% (Despommier & Muller, 1970), decrease in fecundity and acceleration of adult worm expulsion (see Jenkins & Wakelin, 1977).

Wakelin & Selby (1973) showed that vaccination of mice with soluble extracts of the anterior region of T. muris worms stimulated a high degree of resistance to infection, and attributed this to the presence of the stichocytes. This conclusion was confirmed by the work of Jenkins & Wakelin (1977), who showed that stichosome antigen and short term incubation fluid induced a high degree of protective immunity, and that both antigen preparations produced an identical single line in immunodiffusion against rabbit anti-whole worm antiserum.

In the case of intestinal cestodes, the situation is much more complex, owing largely to the functional organisation of the worm. The adult cestode has three general regions, namely the scolex, the undifferentiated neck region, and the strobila in various stages of maturation.

The source of the protective antigens of adult cestodes was previously thought to be the scolex, as this is the part of the worm most likely to penetrate the gut epithelium and thus be immunogenic (see Rees, 1967). Working with Echinococcus granulosus in dogs, Smyth (1963) described "secretion of small viscid droplets into the medium from the hook region", a secretion which was later shown to originate in the secretory cells of the rostellum (Smyth, 1964). Smyth (1969) postulated that the scolex of

E. granulosus often penetrated the epithelium of the dog small intestine and reported that, concerning the secretion droplets, "evidence has since been obtained that what is observed macroscopically is not, in fact, the secretion itself, but an antibody-antigen reaction taking place between it and antibody". Secretory gland cells in the scolex of the family Hymenolepididae have been reported by Baczynska (1914) and Fuhrmann (1918), so the scolex as the source of the protective antigen(s) appeared to be an attractive proposition.

Consideration of the strobila as a source of protective antigens previously appeared less attractive; it is only recently that this possibility has been considered, with general acceptance of the fact that uptake of antigenically intact proteins across the gut epithelium is possible (reviewed by Hemmings, 1978), and elucidation of the probable function of the dome of Peyer's patches in antigen uptake (Bockman & Cooper, 1973). The reluctance to accept that entirely lumenal parasites can be antigenic stems from a variety of causes. Many nutritionists made categoric statements to the effect that proteins were completely degraded to amino acids before absorption, and there was some argument over whether dipeptides or even tripeptides could be absorbed intact (see Wiseman, 1964, pp 51-54). This idea arose partly because of the insensitive techniques employed, and also perhaps because of the particular proteins used; e.g. albumen, which as Hemmings & Williams (1978) point out, "is an unfortunate choice of antigen fo this purpose" because it is largely broken down by peptic action in the stomach. As early as 1900 it was known that orally administered egg protein gave rise to specific serum precipitins (Uhlenhuth, 1900) , and Hecht, Mosko, Lubin,Sulzberger & Baer (1944)

showed that ragweed pollen proteins could be absorbed intact across the gut epithelium. This type of information did not find its way into the more general literature, and the principles exhibited remained in obscurity until recently. It is now generally accepted that antigen uptake by the gut takes place on a large scale; figures of 0.1-2.0% are given in the Ciba Symposium discussion (1977, pp 356-360), and Hemmings & Williams (1978) found that as much as 40% of bovine IgG could be absorbed by the rat gut as large breaksown products, of which at least some retain antigenicity.

The tegument of the adult cestode thus appears to be a potential source of protective antigens. The tegument is a metabolically active site: Oaks & Lumsden (1971) reported "appreciable replacement" of a carbohydrate-containing macromolecule component occurring 6-8 hours after its synthesis and incorporation into the brush border. It is also known that enzymes are produced by the worm which appear on the outer surface of the tegument; these may assist in digestion, or protect the worm from the action of host enzymes (see Pappas & Read, 1972a, b; Arme, 1976).

The present study arose primarily from the observation often made that single H. diminuta worms in mice survive longer and grow faster than worms in multiple-cysticercoid infections. The technique of transplanting directly into the duodenum (Hopkins & Zajac, 1976) was used in the present study to assess the relative importance of the amount of strobila in stimulating an immune response.

2. The effect of irradiation on growth, development and immunogenicity of Hymenolepis diminuta cysticercoids

The use of attenuated organisms in vaccination has been studied since the time of Pasteur, who produced the first medically important live attenuated vaccine (for anthrax) in 1881. The method of choice for attenuation of metazoan parasites is irradiation, usually of larval stages, ideally such that infection of the host with irradiated parasites will render it immune to challenge with virulent (i.e. non-attenuated) organisms of the same species. It is important that irradiated helminth vaccines should give rise to sterile adults, or not develop as far as the adult stage if the stimulation of immunity is a property of the larval stage (see Bickle, Taylor, James, Nelson, Hussein, Andrews, Dobinson & Marshall, 1979c), and also that tissue damage inflicted on the host is not severe.

Irradiation of helminths as a means of producing vaccines looked to be a promising prospect in the 1950's, with the appearance of a commercially available vaccine against the cattle lungworm Dictyocaulus viviparus (Jarrett, Jennings, McIntyre, Mulligan & Urquhart, 1960). An irradiated vaccine against the canine hookworm Ancylostoma caninum was developed by Miller (1970) and was also commercially available for a short period before its withdrawal for various reasons not associated with the vaccine itself (see Miller, 1978). The only other notably successful vaccine to date is that against D. filaria in sheep (Mulligan, 1976). Experimental work with a variety of other irradiated nematodes has produced promising results (briefly reviewed by Cox, 1978)

The possibilities of producing an irradiated vaccine for

human schistosomiasis have long been appreciated, as have the problems of storage of such a vaccine (Smithers & Terry, 1969), although storage by cryopreservation has been shown to be possible (James & Farrant, 1977). Immnuisation of animals against schistosomes is currently more promising than the prospects of human vaccination; successful results have been reported using irradiated vaccines against Schistosoma mattheei (Taylor, James, Nelson, Bickle, Dunne & Webbe, 1976) and S. bovis (Taylor, James, Bickle, Hussein, Andrews, Dobinson & Nelson, 1979) in the sheep, and S. incognitum in the dog (Tewari & Singh, 1977). The use of irradiated metacercariae of Fasciola hepatica has been successful in immunising rats against challenge infection (Armour & Dargie, 1974), but has met with little success in sheep and cattle (Nansen, 1975).

Irradiation of Hymenolepis spp. has been studied in the past, but with the exception of Tan & Jones (1967), the technique has been used for morphological studies. Probably the first attempt at irradiation of H. diminuta was by Palais (1933), who observed no effect of X-irradiation, possibly due to using too low a dosage (Tan & Jones, 1966). Schiller (1959b) studied irradiation of H. nana eggs; he found that 30 000 R* reduced the infectivity to mice, and stated that 40 kR was "completely lethal". Schiller also studied irradiation of H. nana cysticercoids in the range 5-20 kR and described morphological abnormalities in the resulting worms. More recently, Onyango-Abuje & Weinmann (1974) repeated some of this work and confirmed that adult worms were not obtained if eggs were exposed to more than 30 krad of

* 1.0 Roentgen (R) = 0.96 rad

X-irradiation.

Vilella, Gould & Gomberg (1960) studied the effect of ^{60}Co and X-ray irradiation of H. diminuta cysticercoids. They recorded that 12 kR of either type of radiation "prevented most cysticercoids from developing to tapeworms, and in some cases the few tapeworms that developed were stunted or sexually sterile". Doses of over 8 kR caused stunting of the worms, and a dose of 15 kR "rendered the cysts non-infective".

The present study was initiated partly to characterise the effects of high levels of irradiation on H. diminuta cysticercoids; up to 200 krad does not abolish invasiveness of H. nana oncospheres (Onyango-Abuje & Weinmann, 1974), and thus cysticercoids exposed to more than 15 krad may retain the ability to excyst and establish. The effect of this level of irradiation may be to cause severe stunting of the worms; H. diminuta of small size would probably not have been recovered by Vilella et al.(1960) due to the limitations of the technique used. If irradiation of H. diminuta could stunt growth of the worm without causing such damage that the worms had a short lifespan (see Tan & Jones, 1967), such stunted worms would be useful in assessing the role of the scolex in the stimulation of immunity to H. diminuta in the mouse.

MATERIALS & METHODS

1. Irradiation of cysticercoids

Cysticercoids were removed from beetles and placed in glass bijou bottles containing HBSS. The bottles were exposed to a 200 Ci ^{60}Co source for the appropriate length of time (dose rate 1.06 krad/minute), and the cysticercoids were administered to the mice within 90 minutes of isolation from the intermediate host.

2. Excystation of cysticercoids in vitro

Solution 1: 0.8 g pepsin (BDH, 1:2500)
100 ml HBSS
0.2 N HCl added to give pH 1.7-2.0

Solution 2: 0.2 g Na tauroglycocholate
0.3 g trypsin (BDH, 1:83)
0.2 N NaOH added to give pH 7.0-7.2

Cysticercoids were incubated in Solution 1 at 37°C for 11 minutes, in Solution 2 for 13 minutes at 37°C, and were then transferred to clean HBSS for inspection.

All other techniques are described in General Materials & Methods.

RESULTS

1. Transplantation of worms

a) Comparison of speed of rejection of large and small worms

In the first experiment, 12-week-old female CFLP mice were infected surgically with one worm each from cortisone-treated mouse donors. Half of the recipients were infected with worms that were eight days old (dry weight 1.0-1.5 mg), and the other half received worms that were four days old (less than 0.1 mg dry weight). Recipient mice from both groups were killed on days 7, 9 & 12 p.i.

On day 7 p.i., 7/8 of the small worms and 7/9 of the large worms were recovered, indicating successful establishment of the majority of the transplanted worms in both groups (Fig. 2-1). On day 9 p.i. there was a substantial reduction in the recovery of the large worms (2/7), but only a small drop in the number of small worms recovered (6/8). The difference between the two groups was still evident on day 12 p.i.; 2/10 and 5/11 worms were recovered from the mice infected with large and small worms respectively. The results of this experiment therefore indicate that rejection of the large worms was faster than the rejection of the small worms.

This result was confirmed in a similar experiment in which worm recipients (10 weeks old) were infected with 4 or 8 day old worms from cortisoned mouse donors; recipients were autopsied on days 7, 9 & 12 p.i., and an extra group of eight-day and four-day worm recipients was killed on days 5 and 14 p.i. respectively.

This experiment gave very similar results to the previous

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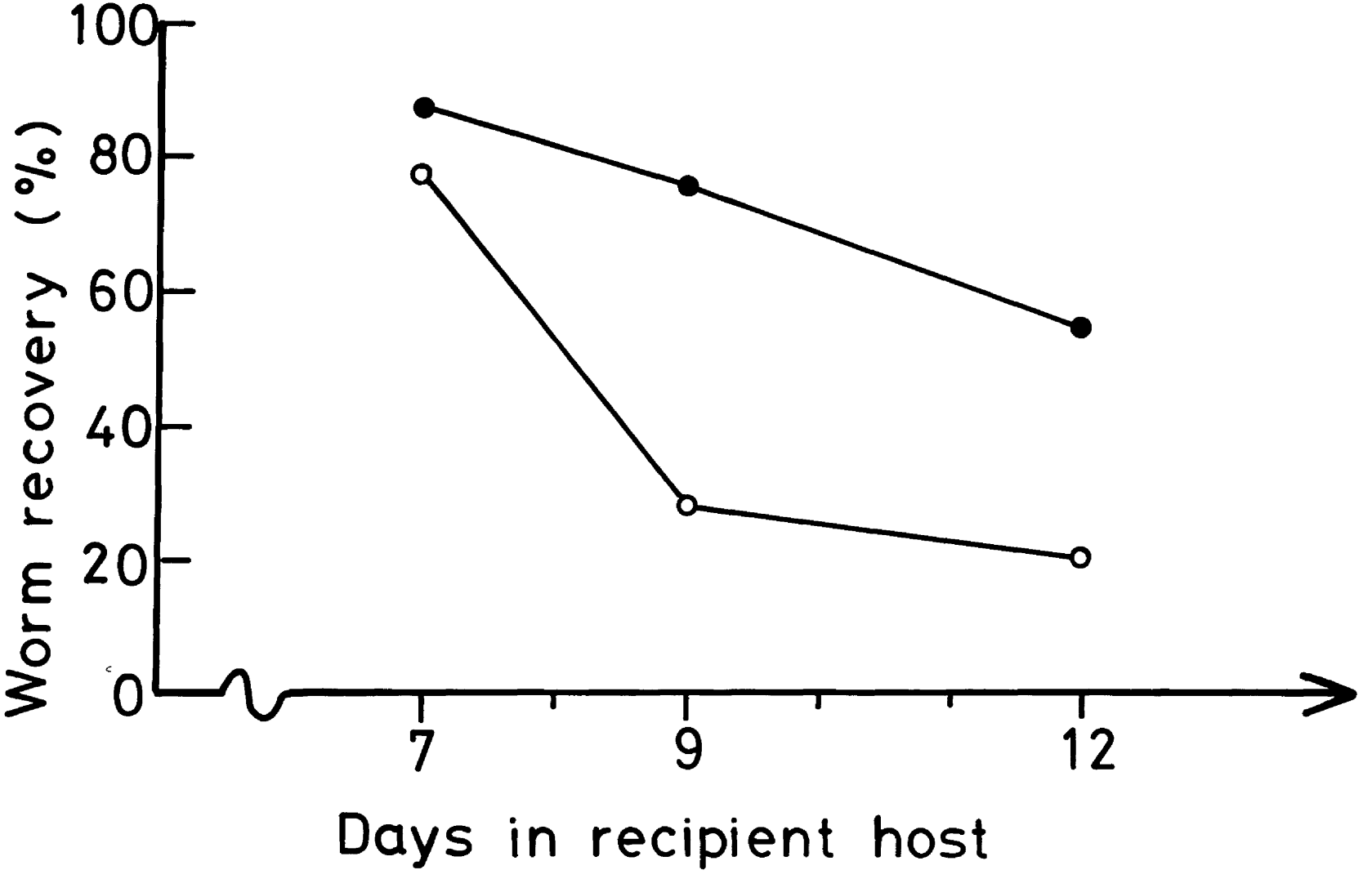
Figure 2-1

Recovery (%) of H. diminuta from CFLP mice infected surgically with four-day-old (●) or eight-day-old (o) worms from cortisoned donor mice.

n = 7-11 mice per group.


100
80
60
40
20
0
Worm recovery (%)
7
9
12
Days in recipient host

one; again, the majority of the recipients of the larger worms had rejected between days 7 and 9 p.i. (Fig. 2-2). Rejection of the small worms was considerably slower, 100% of the worms being recovered on day 9 p.i. and declining slowly to 71% on day 12 and 25% on day 14.

These experiments therefore indicate that, although both large and small worms posess a scolex, the worms with the greater amount of strobila are rejected more quickly. However, it is also possible that the observed results were due to the differing physiologies of the two ages of worm; the susceptibility to the immune response may differ, or production of the protective antigens may begin only when the worm is a certain age. The following experiments were designed to clarify this point by using worms of the same age, but of different sizes.

b) Rejection of worms of the same age but of differing size
(i) Cut worms

H. diminuta recovered from rat donors on day 7 p.i. were transplanted into mice either intact (about 2 mg dry weight) or cut to 2-3 mm (under 0.1 mg dry weight). Mice of both groups were autopsied on days 7 and 9 p.i.

On day 7 p.i., 7/8 of the uncut worms and 3/4 of the cut worms were recovered (Fig. 2-3). On day 9 p.i., 25% of the uncut worms were recovered, compared with 50% of the cut worms.

A subsequent experiment, however, demonstrated that the establishment of worms cut to this size was low (30-50%), probably due to the damage inflicted on the worms. The difference observed on day 9 p.i. between the two groups may therefore be more significant

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Figure 2-2

Recovery (%) of H. diminuta from CFLP mice infected surgically with four-day-old (●) or eight-day-old (o) worms from cortisoned donor mice

n = 8 mice per group, except groups marked *, where n = 7.

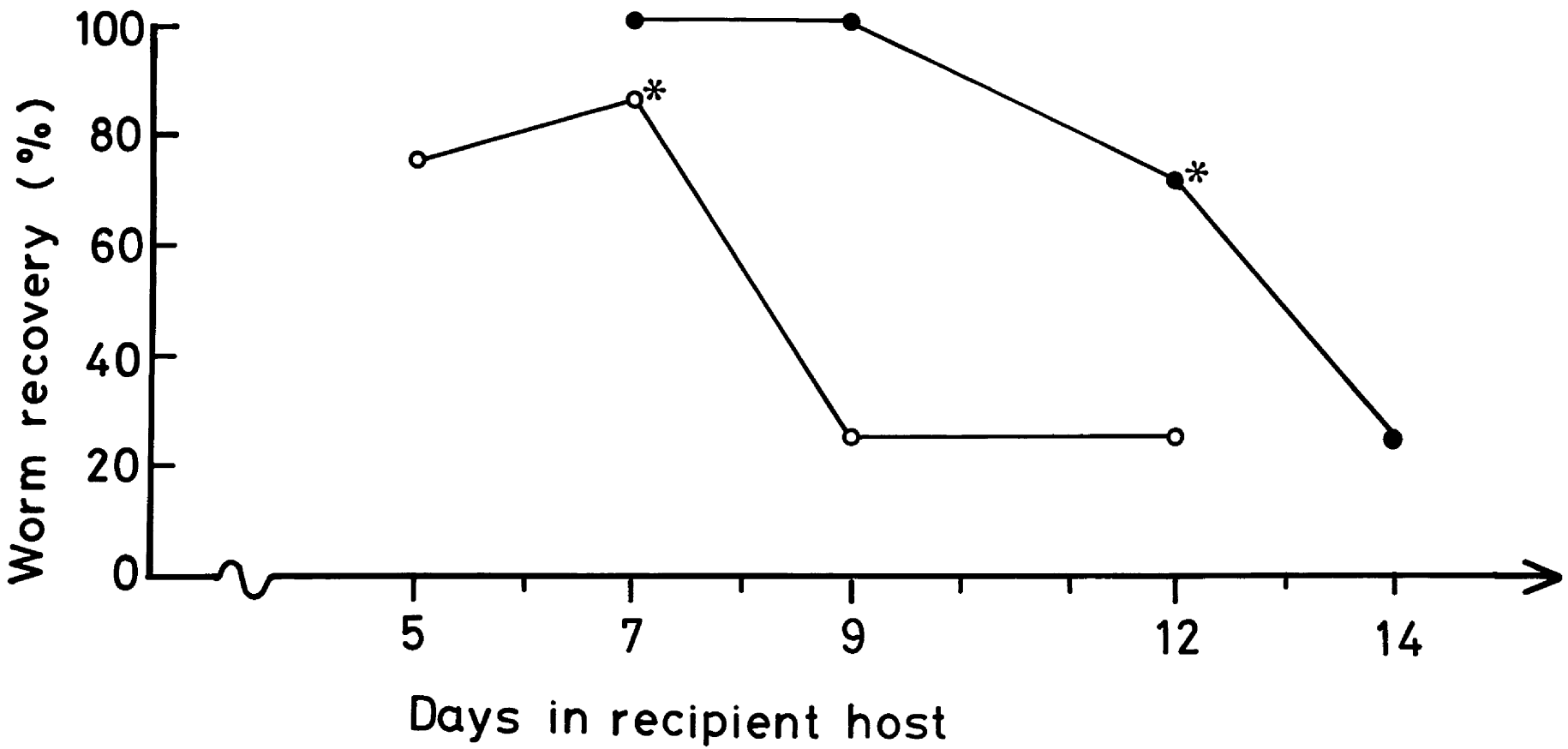
100
80
60
40
20
0
Worm recovery (%)
*
*
5
7
9
12
14
Days in recipient host

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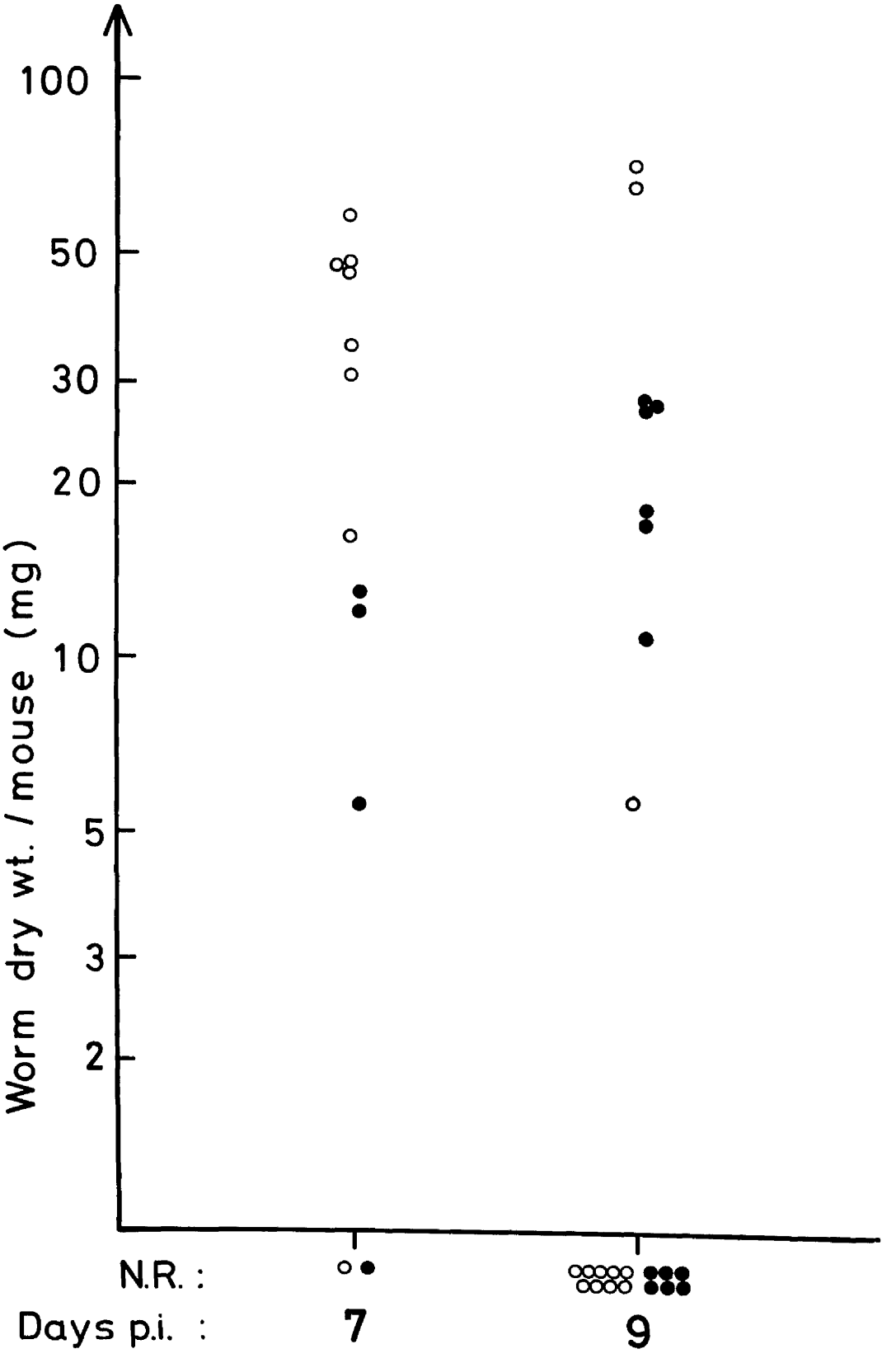
Figure 2-3

Dry weight of H. diminuta from CFLP mice infected surgically with intact (o) or cut (●) 7-day-old worms. Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


100
50
30
20
10
5
3
2
Worm dry wt. / mouse (mg)
N.R. :
Days p.i. :
7
9

than at first appears, as 50% recovery may represent 100% recovery of worms that survived the cutting proceedure.

(ii) Stunted worms

An experiment was performed in which the intention was to produce worms for transplantation of the same age but of differing sizes, by taking worms from mouse donors given large or small numbers of cysticercoids;the principle was that the worms of the heavy infection should be smaller than those of the lighter infection because of inter-worm competition.

Accordingly, cortisone-treated donor mice were infected with either 3 or 25 cysticercoids, and the worms recovered on day 8 p.i. weighed on average 2.8 mg and 0.4 mg respectively. These worms were then transplanted into naive recipient mice, and the course of infection followed in the two groups.

On day 7 p.i. (Fig. 2-4) the crowded worms were significantly lighter than the non-crowded worms ($p < 0.05$); the mean worm weight per mouse was 16.7 mg and 71.3 mg respectively. On day 9 p.i. 5/8 of the mice harbouring the normal worms had expelled their infection, but only 3/8 of the mice infected with stunted worms had rejected. To test whether or not this was a real difference, the remaining mice were killed the following day (day 10 p.i.), when it was found that both groups had rejected 75% of their worms. It is therefore unlikely that there was any difference in the speed of rejection between the two groups.

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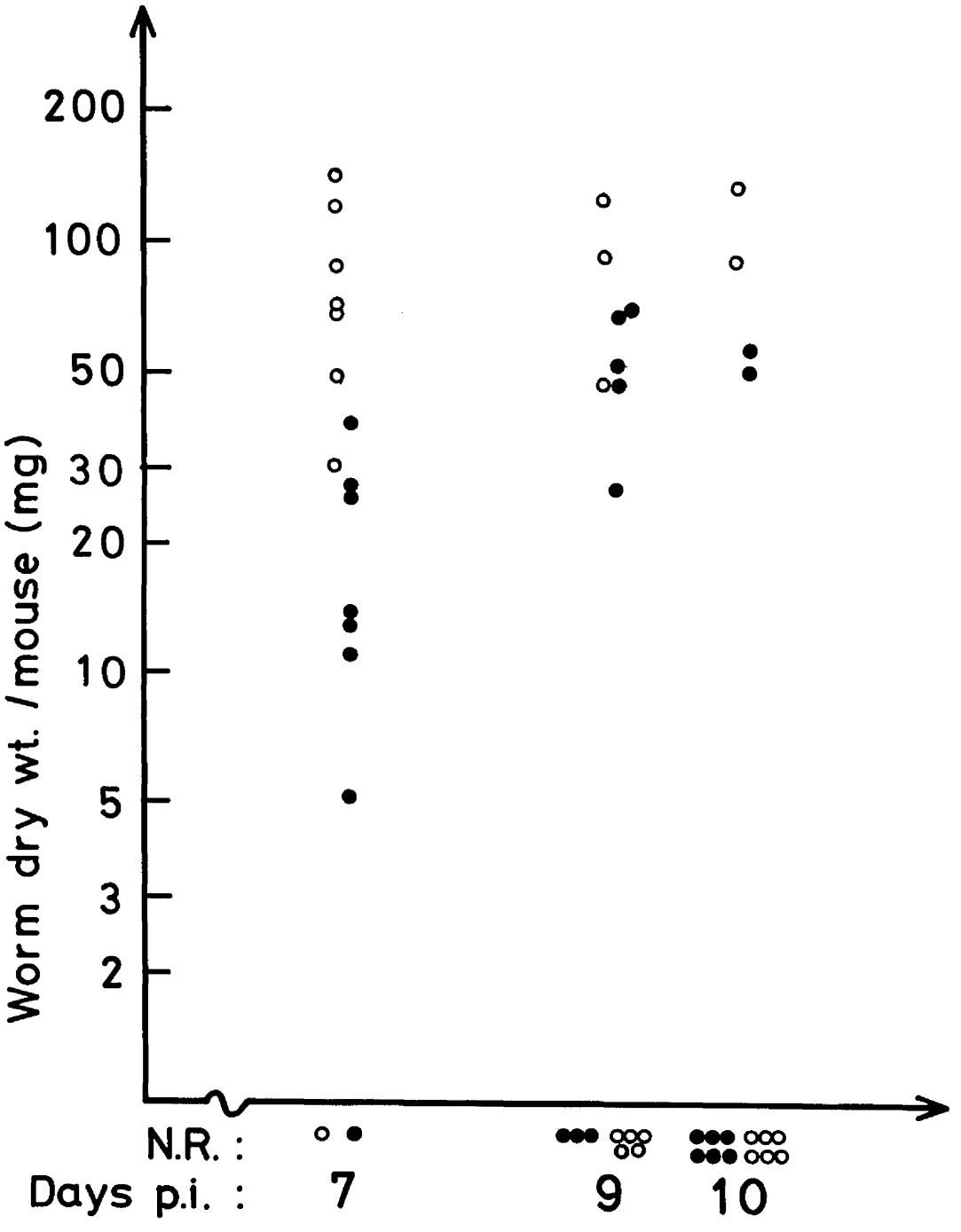
Figure 2-4

Dry weight of H. diminuta from CFLP mice infected surgically with normal worms (o) or worms stunted by crowding (●) from cortisoned mouse donors. Each point represents the total weight of worm tissue from a single mouse.
N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


200
100
50
30
20
10
5
3
2
Worm dry wt. /mouse (mg)
N.R. :
Days p.i. :
7
9
10

c) The effect of increasing the number of scolices

An experiment was carried out in which two groups of mice received one H. diminuta worm intra-duodenally (an eight-day worm from cortisoned mouse donors); one group also received four cysticercoids orally, after recovery from the anaesthetic. Killing these mice on days 5, 7 & 9 p.i. the cysticercoid worms should contribute very little biomass compared with the large tranplanted worm, while providing five times the scolex material.

Establishment of transplanted worms was 100% in both groups on day 5 p.i. (Fig. 2-5), but in contrast to previous experiments the worms had not been rejected by day 9 p.i. Up to day 9, the presence of four extra scolices had no deleterious effect on the growth or survival of a large worm, either by competition or by stimulating a stronger immune response. It is noticeable that the cysticercoid-derived worms were substantially lighter than one would normally expect; this may have been due to crowding effects because of the much larger transplanted worm, and/or interference with the host's eating habits following surgery.

In a second experiment, mice received one five day old worm from rat donors, and half of the mice also received nine cysticercoids orally after recovery from anaesthesia. The intention of using the smaller worm for transplanting (about 0.5 mg) was that this might lead to slower rejection and thus magnify differences between the groups. The recipient mice were killed on days 9, 12 & 15 p.i.

100% of the transplanted worms established and were present on day 9 p.i. (Fig. 2-6); the contribution of the cysticercoid worms to the total biomass was 11%, and the total biomass for the double-infected group was marginally lower (95.3 mg) than for the group

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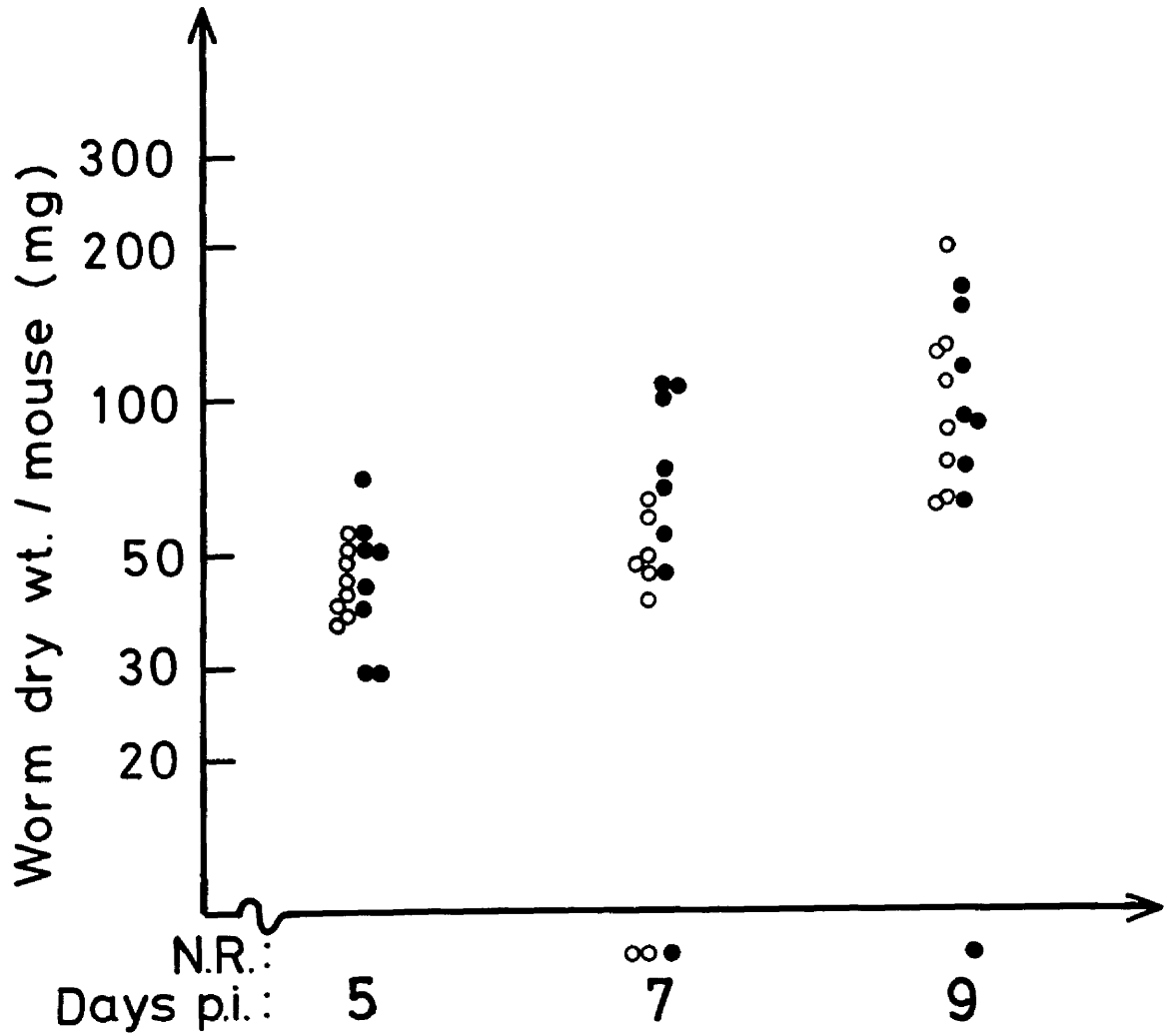
Figure 2-5

Dry weight of H. diminuta from CFLP mice infected surgically with one worm (o), or infected surgically with one worm and orally with four cysticercoids (●). Transplanted worms from cortisoned mouse donors infected eight days previously. Each point represents the total weight of worm tissue from a single mouse. N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.

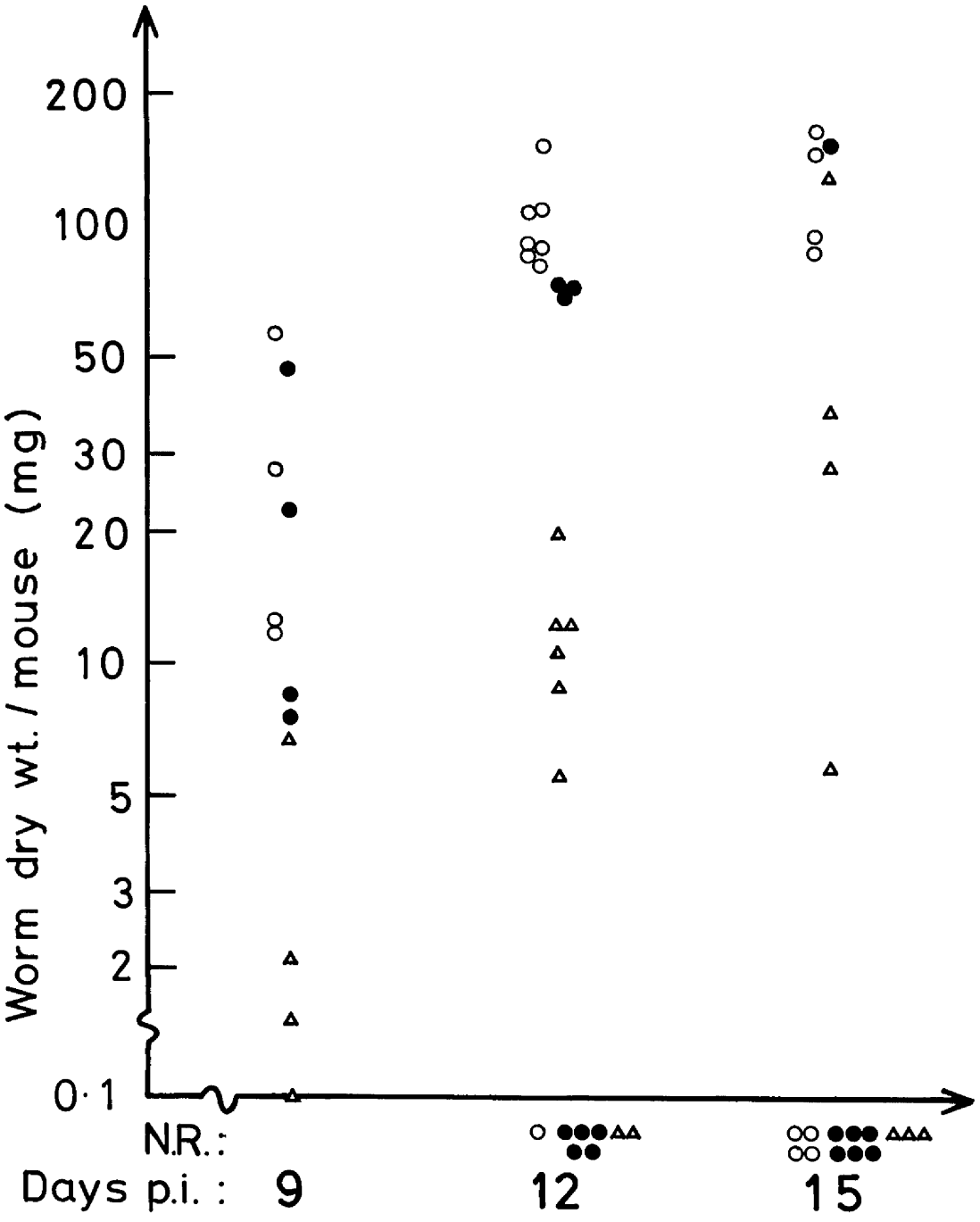

Worm dry wt. / mouse (mg)
300
200
100
50
30
20
N.R.:
Days p.i.:
5
7
9

Facing page 126

Figure 2-6

Dry weight of H. diminuta from CFLP mice infected surgically with one worm (o), or infected surgically with one worm and orally with nine cysticercoids; in the latter group, ● represents the transplanted worms, and Δ the total weight of cysticercoid-derived worm tissue. Transplanted worms from cortisoned mouse donors infected five days previously.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


200
100
50
30
20
10
5
3
2
0·1
Worm dry wt. / mouse (mg)
N.R.:
Days p.i. :
9
12
15

infected with the single worm (107.9 mg). On day 12 p.i., however, only 38% of the surgically introduced worms remained in the double-infected group, whereas 88% of the worms were recovered from the group infected with a single worm, a difference that was still evident on day 15 p.i. (14% and 50% respectively); the mean dry weight of worm tissue per infected mouse was again almost identical in the two groups on day 12 p.i. (80.0 mg and 99.6 mg respectively), although the surgically introduced worms were apparently rejected more quickly in the double-infected group. Thus the presence of the physiologically younger cysticercoid-derived worms substantially hastened rejection of the transplanted worms. However, the cysticercoid-derived worms contributed a substantial weight of strobila on days 12 and 15 p.i. (22% and 57% respectively of the total biomass), and this may have contributed to the earlier rejection by this group.

d) Immunisation with differing sizes of worms

If the presence of large amounts of strobila stimulates a faster immune response than that invoked by a smaller worm, it is possible that infection with a larger worm might stimulate a greater degree of resistance to reinfection. To test this, mice were infected orally with a single cysticercoid, or surgically with a worm recovered from cortisone-treated mouse donors infected eight days previously: a third group remained uninfected, but all three groups were subjected to surgery to allow for any effects of this procedure. All three groups received anthelmintic on day 16 p.i.; all mice were challenged surgically five days later and were killed six days after surgery.

100% of the worms were recovered from the naive control mice (Fig. 2-7), although the two lightest worms may have been damaged at insertion and subsequently regrown. The recovery from mice immunised with transplanted worms or with cysticercoids was 50% and 44% respectively, indicating that both sensitisation protocols had sensitised the intestine to a similar degree. There was neither any significant difference in the weights of the worms recovered from the two immunised groups.

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Figure 2-7

Dry weight of H. diminuta from CFLP mice on day 6 of a surgical challenge.

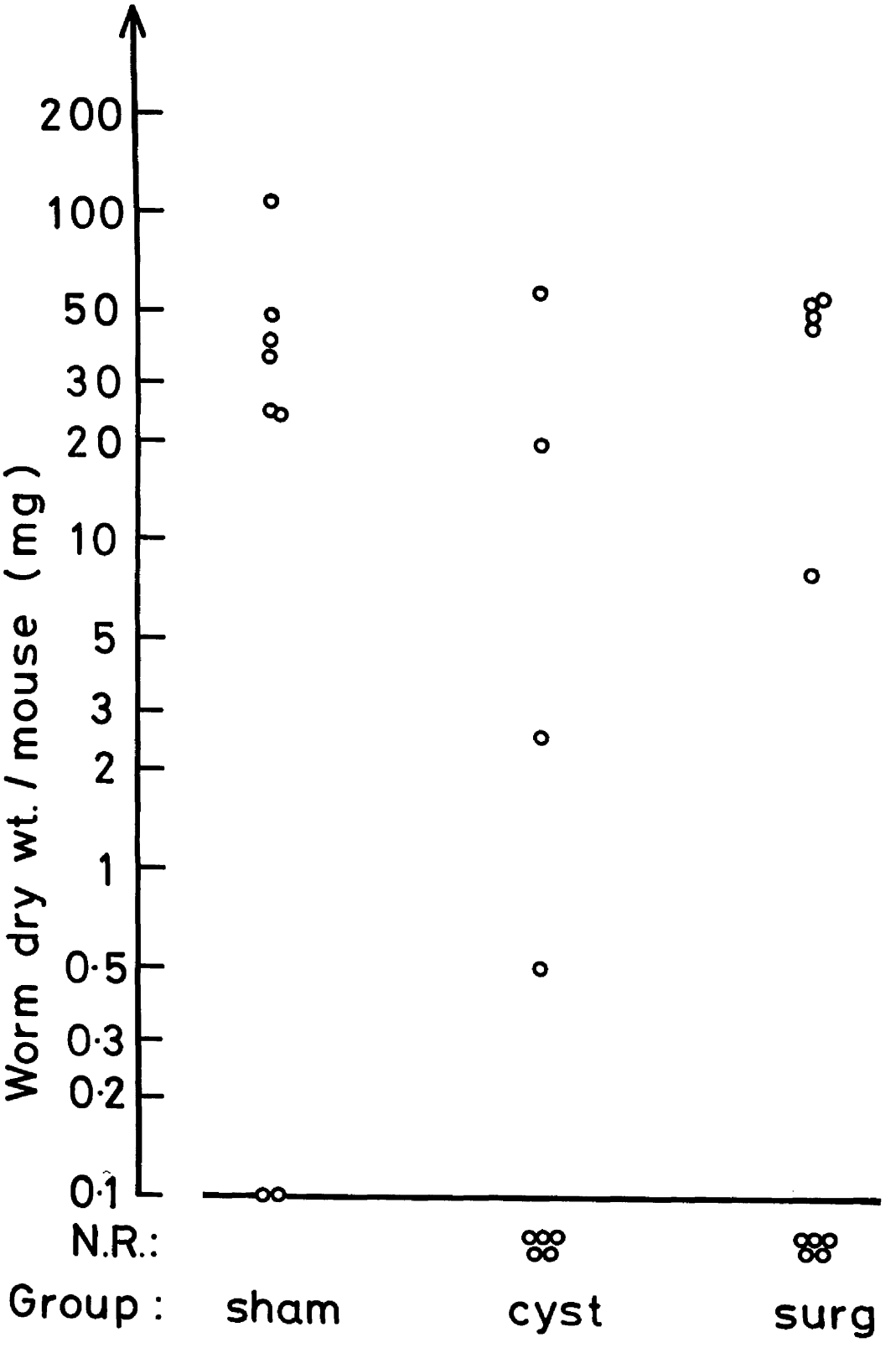
sham, mice exposed to surgery 21 days before challenge

cyst, mice exposed to surgery and infected orally with one cysticercoid 21 days before challenge

surg, mice infected surgically with one worm 21 days before challenge

All mice received anthelmintic five days before challenge. Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.


200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Worm dry wt. / mouse (mg)
N.R.:
Group :
sham
cyst
surg

2) Irradiation of cysticercoids

a) Calibration of the radiation dose

In a preliminary experiment, mice were infected with five cysticercoids which had been exposed to 0, 25 or 45 krad of gamma radiation from a ^{60}Co source. The mice were treated with cortisone, and were autopsied on day 8 p.i. No worms were visible in the washings from the small intestines of mice infected with irradiated cysticercoids, and the intestines were slit open longitudinally and incubated in HBSS at 37°C for 1-2 hours. Subsequent examination revealed the presence of tiny live worms consisting of a scolex with a stump of neck tissue (Plate 2-1). The scolex appeared normal, and the worm grossly resembled a destrobilated worm.

In a subsequent experiment, cysticercoids were exposed to 0, 10, 15 and 25 krad in an attempt to discover the lower limit of irradiation that would halt growth of the worm. Mice were infected with five cysticercoids, treated with cortisone, and were killed on day 10 p.i.: the results are shown in Table 2-1.

Over 90% of the worms were recovered in all the groups, indicating that up to 25 krad had no effect on the survival of the worms, at least up to day 10 p.i. 10 krad had no inhibitory effect on growth, but 15 krad lowered the total weight attained by the worms by about half. It was noted that the stunting effect of 15 krad was not uniform, some worms being more severely affected than others. All but one of the worms given 25 krad were of the severely stunted type described in the first experiment, but one worm grew to about 1 cm long (0.1 mg). Because of this and

Table 2-I

The effect of ^{60}Co irradiation of cysticercoids on growth and survival of *H. diminuta* in mice.

krad	Recovery (%)*	Biomass / four mice	n
0	100	67.0 mg	4
10	100	99.4 mg	4
15	93	35.6 mg	3
25	95	0.1 mg	4

*, includes worms of 0.1 mg.

subsequent results, 35 krad was taken as being the minimum dose that would effectively cause complete suppression of growth.

Measurements of 35 krad worms aged 14 days (from cortisone-treated mice) and of 20-hour old worms were taken after fixation. These two types of worm are shown in Plates 2-1 and 2-2 respectively. The total length of each worm, the scolex width and the median neck width were recorded using a X50 dissecting microscope fitted with camera lucida. 10 of each type of worm were measured, and the results are shown in Table 2-2.

It is notable that significant enlargement of irradiated worms did occur, but at this level of irradiation it is likely that this is due to cellular enlargement rather than cell multiplication (see General Discussion).

To test the effect of heavier doses of radiation on the ability of the cysticercoids to excyst, cysticercoids exposed to 0, 50, 60 & 70 krad were excysted *in vitro* as described in the Materials and Methods section of this Chapter.

The percentage excystation of cysticercoids in all four groups was very similar (Table 2-3), all figures falling between 67% and 85% excystation; the variation in the results that did exist was almost certainly due to inexpert use of the technique. Irradiation of cysticercoids with up to 70 krad thus appears to have no effect on the ability to excyst.

b) longevity of *H. diminuta* exposed to differing levels of irradiation

In a small preliminary experiment, cysticercoids exposed to 35 or 45 krad established well, but no worms were recovered on

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Plate 2-1

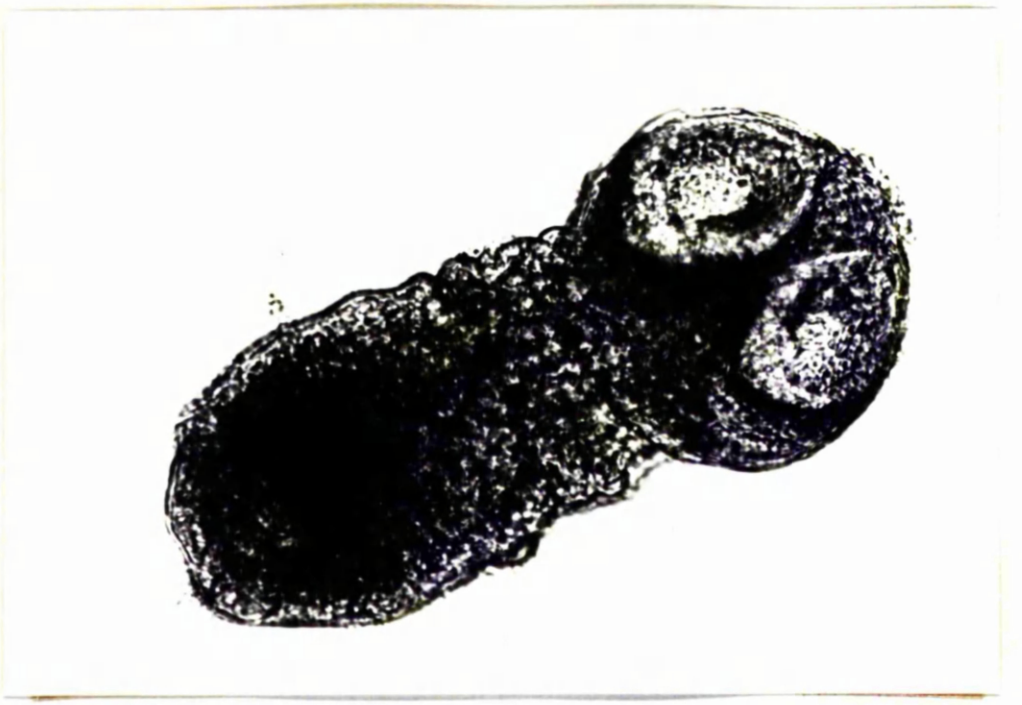
14-day-old worm arising from cysticercoids of H. diminuta exposed to 35 krad γ-radiation from a ^{60}Co source; worm from cortisone-treated mouse.

(For dimensions, see Table 2-2)

Plate 2-2

20-hour-old H. diminuta from a mouse.

Same scale as Plate 2-1; for dimensions, see Table 2-2.



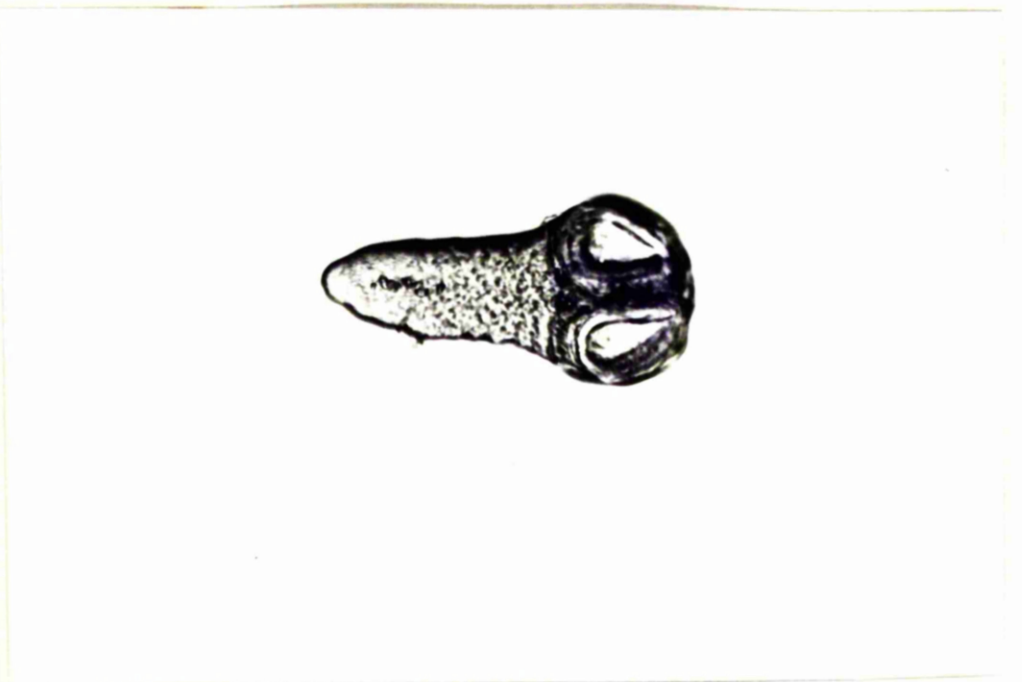


Table 2-2

The dimensions of young and irradiated H. diminuta

Parameter	20-hour worms	irradiated worms
Length (mm)	0.29 ± 0.03	0.37 ± 0.07*
Scolex width (mm)	0.14 ± 0.01	0.20 ± 0.02**
Neck width (mm)	0.08 ± 0.01	0.15 ± 0.04**

*, $p<0.01$; **, $p<0.001$, significant difference (Student's t test)

Irradiated worms 14 days old at time of measurement.

Table 2-3

The effect of high doses of ^{60}Co irradiation on the ability of H. diminuta to excyst in vitro.

krad	% excystation	n
0	67	9
50	60	20
60	85	20
70	70	20

day 14 p.i. from the mice, and therefore a more extensive experiment was carried out to investigate the longevity of the irradiated worms more fully. NIH mice were infected with 10 cysticercoids exposed to 0, 25, 35 & 45 krad, treated with cortisone, and killed on days 8, 13 & 16 p.i.

The non-irradiated worms were always recovered in greater numbers than the irradiated worms, but this probably reflects the technical difficulties involved in the recovery of small worms. As can be seen from Table 2-4, there was no evidence for loss of the irradiated worms in the course of the experiment; 60-80% of the irradiated worms were recovered, compared with 83-98% of the non-irradiated worms.

c) Immunological responses to irradiated H. diminuta

(i) Primary course of infection with irradiated cysticercoids

To determine whether the stunted worms arising from irradiated cysticercoids were rejected immunologically by mice, groups of six CFLP mice were infected with five normal or irradiated (35 krad) cysticercoids. The mice were treated with cortisone or left untreated, as outlined in Table 2-5.

Table 2-5

Group	Treatment	Days of autopsy p.i.
A	5 cysts	6, 9, 12
B	5 cysts + cortisone	9 21
C	5 irradiated cysts	6, 9, 11, 15, 21, 24
D	5 irr. cysts + cortisone	9 21

Table 2-4

Recovery (%) of H. diminuta after ^{60}Co irradiation of cysticercoids; infection in cortisone-treated mice.

krad	Days		
	8	13	16
0	94%	98%	83%**
25	72%	80%*	60%*
35	64%	76%	66%
45	70%	ND	80%+

All results based on 50 cysticercoids, except *, 40 cysticercoids
**, 30 " "
+, 10 " "
ND, not determined

Rejection of the worms from non-irradiated cysticercoids from the mice of group A was very rapid (Fig. 2-8); only 10% of these worms were recovered on day 9, and rejection was complete by day 12. Administration of cortisone (group B) effectively prevented the immune expulsion of these worms, 88% remaining on days 9 and 21 p.i. The irradiated worms in the untreated mice (group C) were also lost from the mice, but more slowly than from group A; worm recovery on day 11 was probably lower than it should have been, probably for technical reasons, but 40% of the worms were recovered on day 15. No irradiated worms were recovered on day 21, and only one worm was recovered on day 24 p.i. Administration of cortisone (group D) prevented loss of the irradiated worms

Loss of irradiated worms from the intestine of mice and abrogation of this loss by administration of an immunosuppressant strongly suggests that these irradiated worms are expelled from mice by an immunological mechanism. The subsequent experiments were designed to test

a) whether irradiated cysticercoids were capable of inducing immunological memory that would give an effective secondary response to a challenge with normal worms, and

b) if immunogenic, whether infections with irradiated cysticercoids were more, or less, immunogenic than normal cysticercoids.

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Figure 2-8

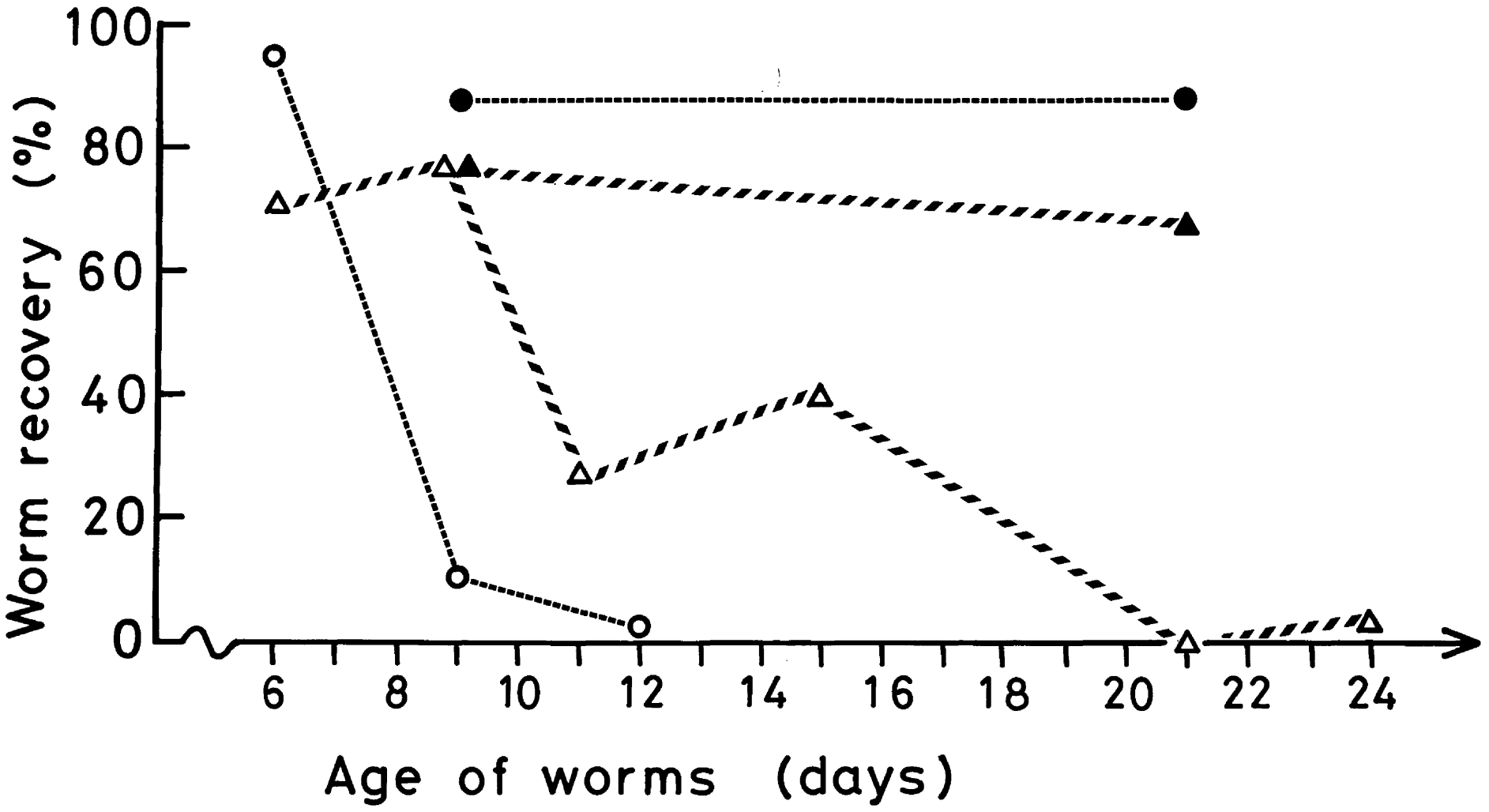
Recovery (%) of H. diminuta from CFLP mice.

o---------o, five normal cysticercoids day 0

●---------●, five normal cysts + cortisone

△▰▰▰▰△, five irradiated cysts (35 krad) day 0

▲▰▰▰▰▲, five irradiated cysts + cortisone


100
80
60
40
20
0
Worm recovery (%)
6
8
10
12
14
16
18
20
22
24
Age of worms (days)

(ii) Immunisation of mice with normal or irradiated cysticercoids

In the first experiment, CFLP mice were infected with five normal or irradiated cysticercoids, or remained uninfected. 25 days later, all mice were challenged with three normal cysticercoids, and were autopsied on day 8 of the challenge infection.

100% of the challenge infection was recovered from the naive control mice (Fig. 2-9), but only 6% of the worms were recovered as being over 0.1 mg in the mice immunised with the normal cysticercoids. Prior infection with irradiated cysticercoids reduced recovery of the challenge infection to 67%. The weight of worms recovered from the latter group was also intermediate between the values for the other two groups, although the total biomass from six mice (21.3 mg) was closer to the control value (36.7 mg) than to that of the normally-immunised mice (0.3 mg).

In the second experiment, NIH mice were again infected with five irradiated or normal cysticercoids, or left uninfected. All three groups were treated with anthelmintic 14 days later, and were challenged surgically 21 days after the immunising infection. Mice were killed 5 and 7 days p.i.

On day 5 p.i., 7/8 of the control mice carried large worms (Fig. 2-10); only one mouse of the group immunised with normal cysticercoids carried a worm heavier than at the time of transplantation, and apart from one 0.1 mg worm, all the other mice had rejected the challenge infection. As in the previous experiment, mice immunised with irradiated cysticercoids showed an intermediate level of immunity; 63% of the worms were recovered, and the total biomass for eight mice was 38.6 mg, compared with

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Figure 2-9

Dry weight and recovery (%) of H. diminuta from CFLP mice challenged with three cysticercoids and autopsied on day 8 p.i.

A, previously uninfected mice

B, mice infected with five irradiated cysticercoids 25 days before challenge

C, mice infected with five normal cysticercoids 25 days before challenge.

x, mean dry weight of worms per mouse.

Each point (o) represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.

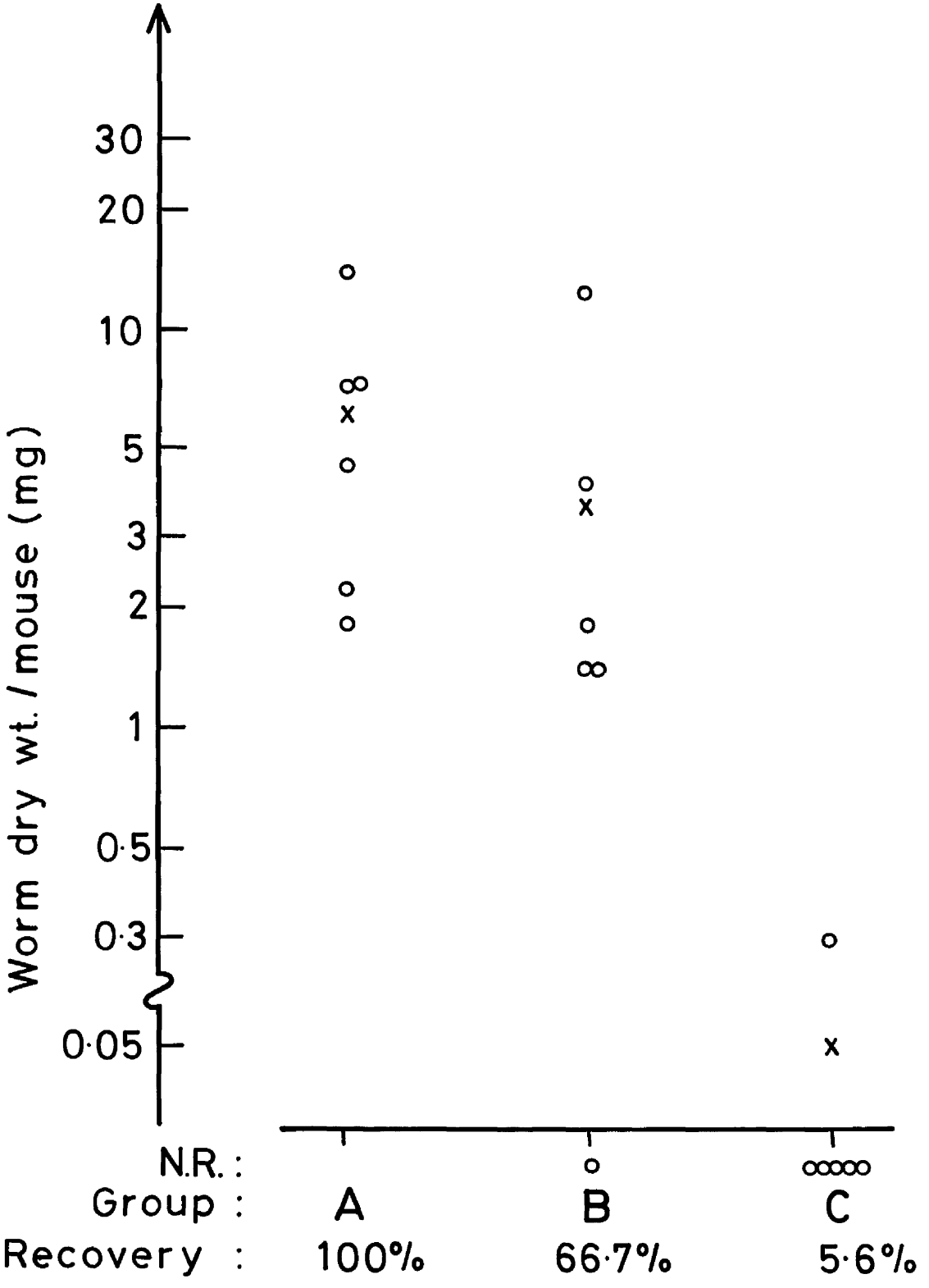
Worm dry wt. / mouse (mg)
30
20
10
5
3
2
1
0·5
0·3
0·05
N.R. :
Group :
A
B
C
Recovery :
100%
66·7%
5·6%

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Figure 2-10

Dry weight of H. diminuta recovered from NIH mice challenged surgically and autopsied on days 7 and 9 p.i.

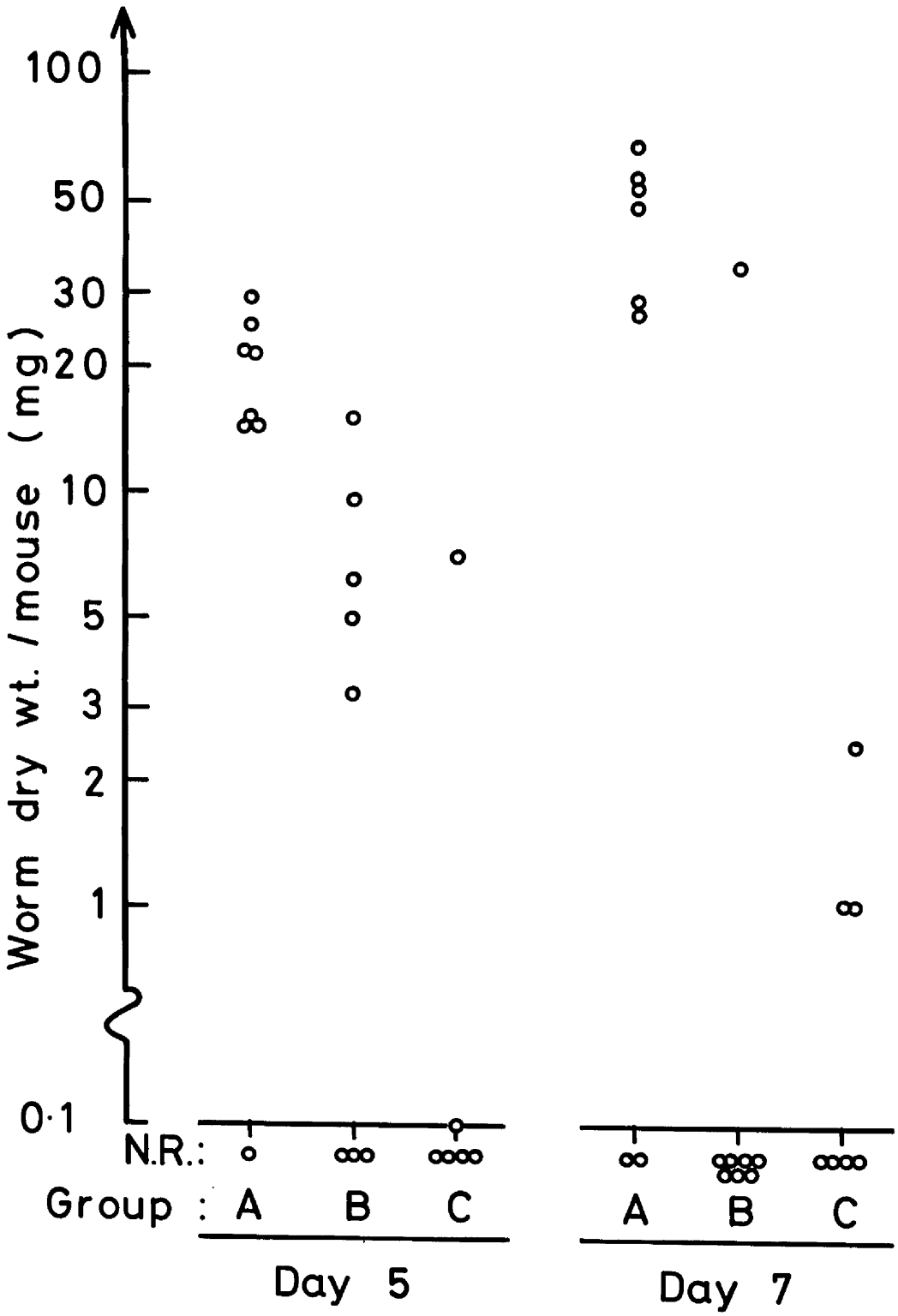
A, previously uninfected mice

B, mice infected with five irradiated cysticercoids 21 days before challenge

C, mice infected with five normal cysticercoids 21 days before challenge.

All mice given anthelmintic seven days before challenge. Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.


100
50
30
20
10
5
3
2
1
0·1
Worm dry wt. / mouse (mg)
N.R.:
Group : A B C
A B C
Day 5
Day 7

138.9 mg and 9.3 mg in the control and normally immunised groups respectively. On day 7 p.i., 6/8 worms remained in the control mice, but only one large worm and three small worms were recovered from the irradiated and normal immunised groups respectively; the total biomass for eight mice was 263.9 mg, 33.6 mg and 3.9 mg respectively.

DISCUSSION

The evidence presented in this chapter strongly suggests that it is some part of the strobila that plays the major role in stimulating protective immunity, and that the scolex plays no special part in induction on the immune response to H. diminuta.

The major part of the evidence for this comes from the first two experiments (Figs 2-1, 2-2), in which it was demonstrated that a large transplanted worm would be rejected more quickly (within about nine days) than a small worm (rejection essentially complete by day 14 p.i.). Both large and small worms posess a scolex, so the possibility exists that the difference in speed of rejection is due to the differing weights of strobila carried by the worms. While it is impossible to infect a mouse with a worm lacking a scolex in order to eliminate the scolex as a vital source of the antigen(s) which stimulate an effective immune response, the remainder of the experiments presented in this chapter support (albeit less directly) the contention that the strobila and neck of the worm supply the larger part of the protective antigen(s).

Assuming the whole worm to be responsible for stimulation of imunity, it follows that a mouse immunised with a large worm would be more resistant to a challenge infection than a mouse immunised with a cysticercoid. In the experiment designed to test this (Fig. 2-7) there was no apparent difference between the two immunised groups; however, as no anthelmintic was administered to terminate the infections, the cysticercoid worms probably persisted in the mice for longer than the transplanted worms. If we take the point of 50% rejection as being day 15 p.i. for the single cysticercoid infection (Hopkins et al., 1972a; Befus, 1975a),

and day 8 p.i. for a transplanted worm (Figs 2-1, 2-2) , and make the assumption that the worms weighed 1.0 mg on day 8 for the cysticercoid infection and day 0 for the surgical infection, assuming for simplicity that a worm of this age doubles in weight every 24 hours, we can see that the total value of 'mg days' for the two groups is almost identical, viz. 255 mg days. Thus, although the the immunising worms were probably present in the mice for differing lengths of time, the total output of antigen, as assessed by the response to a challenge infection, was largely identical, again suggesting a strobila-associated source of protective antigen.

One major criticism of the experiments cited thus far is that the difference in speed of rejection of large and small worms may be due to an age-related difference in the worms themselves, either because of sequential appearance of antigens (the antigen stimulating an effective immune response only appearing when the worm reaches a certain age), or because of a difference in the susceptibility to immune attack of the two ages of worm, older worms being more sensitive to the effects of the immune response.

These arguments appear unsatisfactory for a variety of reasons. Six-cysticercoid infections with H. diminuta are rejected more rapidly than one-cysticercoid infections (Befus, 1975a), so in this case the younger worms are expelled earlier than the older single worms. Immunity to H. diminuta can also apparently be stimulated by short-term (three days) infection with cysticercoids (Befus, 1975b), and thus the protective antigens must be present in immunologically significant amounts by this time. Serum antibodies arising during H. diminuta infection in the mouse have also been shown to cross-react with antigens present in cysticercoids (Andreassen et al., 1978a), suggesting that the antigens likely to

be involved in the stimulation of the immune response are present from the earliest stage of development in the mouse.

Determining the sensitivity of worms of differing ages to the immune response of the mouse is a more difficult problem. If the strobila is the major source of protective antigen, rejection of older worms is faster because of the larger amount of strobila present. Christie, Wakelin & Wilson (1979) found that H. diminuta in the rat was more susceptible to the effects of inflammation when younger, old worms being relatively resistant; this was also the conclusion reached by Howard, Christie, Wakelin, Wilson & Behnke (1978) and Behnke, Bland & Wakelin (1977) for H. microstoma and H. diminuta in the mouse.

The experiments in this Chapter that were designed to investigate the age-related sensitivity of H. diminuta met with little success. The experiment with cut worms gave promising results, which may be more significant than at first appears; as explained in the Results section, worms cut to a small size (about 2 mm) may have substantially reduced viability, so it may be that cut worms were in fact rejected more slowly than uncut worms of the same age.

An important contribution to the study of the roles of scolex and strobila in the stimulation of the immune response was made with the discovery of severely stunted worms produced by heavy (over 25 krad) irradiation of cysticercoids. One must assume that these tiny worms went unnoticed by previous workers such as Vilella et al. (1960). Irradiated worms always appeared to be healthy and vigourously moving when recovered, even at day 21 p.i.; while it is likely that the ability to undergo mitosis has been ablated by the radiation damage, some enlargement of the animal does take place (Table 2-2). It therefore appears likely that the

irradiated worms are functioning normally as regards feeding and detecting location signals (over 90% of these worms were found in the anterior half of the small intestine), and thus may be producing antigenic substances in amounts comparable to the scolex and early neck region of a normal worm.

Using 35 krad as the standard dose (the lowest dose that caused severe stunting of all of the worms), five-cysticercoid infections with irradiated worms were not completely rejected until day 21 p.i., very much slower than in the control (non-irradiated) infection. Further evidence that irradiated cysticercoids give rise to weakly immunogenic worms was obtained from experiments in which mice were immunised with normal or irradiated worms. Challenge with cysticercoids or adult (surgically introduced) worms revealed that specific sensitisation of the mice infected with irradiated cysticercoids had occurred, but was to an intermediate extent between naive mice and normally immunised mice. The weak response to irradiated H. diminuta by the mouse contrasts sharply with the work on immunisation of mice with Schistosoma mansoni. Increasing the dose of irradiation given to schistosomula up to 20 krad substantially increases the antigenicity as assessed by resistance to a normal challenge infection (Bickle, Taylor, Doenhoff & Nelson, 1979a). A similar effect is observable with the nematode Nematospiroides dubius (Hagan, personal communication), but in both cases the increase in immunogenicity may reflect impaired migration in the tissue phases (Bickle, Dobinson & James, 1979b), making the parasites more accessible to local immunocytes, and thus stimulating an effective resistance to reinfection, operating on the early tissue stages of development.

Although secretion of protective antigen is probably

largely a function of the strobila, there is probably a spectrum of activity in antigen production along the length of the worm. The externally measurable metabolic activity (uptake of metabolites per mg worm tissue) decreases along the length of the worm, the most anterior 15-20 mg dry weight being most active in the uptake of methionine (Read, Rothman & Simmons, 1963) and glucose (Henderson, 1977).

That an immune response is mounted against irradiated worms, and that memory against a normal challenge is stimulated, indicates that protective antigens are produced by the scolex and neck, but the weak nature of those responses further confirms that the scolex is not the major source of protective antigen. The course of rejection of the five irradiated worms was very similar to that observed for infections with a single cysticercoid (Hopkins et al., 1972a; Befus, 1975a) and it would thus appear that irradiated worms are as much as one fifth as immunogenic as normal worms, and this again supports the idea that the anterior part of the worm is most immunogenic. However, to assign an arithmetic estimate of antigenicity in this fashion is probably naive; the kinetics of antigenicity could be tested by comparing rejection of a single worm plus five irradiated worms with that of a two-worm infection.

Higher metabolic activity in the anterior part of the worm could also explain the observations of Andreassen et al. (1978a), who noted that infections with five worms could be rejected earlier than infections with two worms at a point when the weight of the worms in the former group is less than that of the latter. These authors attributed this result to the scolex being the source of the protective antigen(s); however, the result may reflect the higher antigenic activity of the anterior part of the worm. It is

also possible that induction of the immune response depends on the stimulation provided by the worm(s) on the first few days of infection, and as crowding factors do not operate on the first few days of infection with H. diminuta (Goodchild & Harrison, 1961), five worms would weigh proportionally more than two worms at this stage of infection.

In support of this latter point, I found no difference in the rate of rejection of stunted and non-crowded worms (Fig. 2-4), and although there was a large difference between the dry weights of the transplanted worms (0.4 mg and 2.8 mg) the stunted worms may not have been light enough to cause slower rejection. A worm of 0.4 mg may be capable of stimulating the mouse to respond at almost full efficiency, so a difference in the speed of rejection may be apparent between worms of 0.4 and 0.04 mg, but not between worms of 4.0 and 0.4 mg.

What then of the nature of the protective antigen? It is highly unlikely that the protective antigen(s) is/are to be found in the interior of the worm, as this is normally inaccessible to the immune system. It seems much more likely that the tegument is the source, either by exocytosis of proteins or by exposure of transport locus proteins or structural glycoproteins in the plasma membrane. Medium in which H. diminuta has been incubated contains potent inactivators of trypsin and chymotrypsin (Pappas & Read, 1972a, b). An immune response directed against this molecule causing its removal or inactivation would presumably open the surface of the worm to enzymic attack, and thereby cause rejection by forcing the worm to leave its preferred location, and it has been reported that dead or dying tapeworms are digested by the host

(Lumsden, 1975). Alternatively, fragments of plasma membrane carrying transport or structural glycoproteins may constitute protective antigen. Specific loci exist for facilitated transport of a variety of metabolites such as amino acids, some sugars, acetate, purines, and pyrimidines (reviewed by Arme, 1976). Immune attack on the worms could, therefore, be mediated by loss of transport function; Bland (1976b) found that uptake of methionine and acetate (but not glucose) was lower in worms subjected to immunological attack. Membrane glycoproteins are continuously synthesised, incorporated into the membrane and replaced. This is a very rapid process (Oaks & Lumsden, 1971), and it is possible that some reutilisation of the membrane occurs (see Oaks, Knowles & Cain, 1977); these molecules must be thrown off continuously, and would certainly be detected as foreign by the mouse.

Production of antibodies directed against antienzymes or membrane glycoproteins seems the most likely method of effecting worm discomfort,but as discussed above (General Introduction; Chapter 1) it is unlikely that antibody is directly involved in causing stunting or expulsion of H. diminuta. It is also unlikely that lymphokines would inhibit these molecules on a specific basis, so the most likely mechanism would be rejection mediated by non-specific factors produced by lymphoid or non-lymphoid cells, under the control of T cells which are specifically sensitised to membrane glycoproteins.

SUMMARY

1. The relative roles of the scolex and strobila of H. diminuta in stimulating the immune response of the mouse were investigated by transplantation of worms with large or small weights of strobila. It was found that large worms are rejected more quickly than small worms; it is argued that this is a size-related difference, and not age-related sensitivity of the worms or a timed appearance of protective antigen.

2. ^{60}Co-irradiated cysticercoids were found to give rise to severely stunted worms in mice. The calibration of the radiation dose is described; 35 krad was found to cause almost complete stunting of all of the worms.

3. Although rejected by an immunological mechanism, irradiated cysticercoid worms are shown to be poorly immunogenic; they are rejected very slowly by mice, and stimulate poorer memory to reinfection than normal cysticercoid worms.

4. It is concluded that the protective antigen(s) arise from the tegument, and that these are produced in greater quantity per unit weight by the anterior end of the worm. This theory is shown to be a possible explanation of several established observations on H. diminuta in mice, and routes of investigation for vaccination studies are suggested.

5. The nature of the protective antigen(s) and the immunological mechanisms that may be involved is briefly discussed.

CHAPTER 3

INTERACTION OF *HYMENOLEPIS DIMINUTA* AND *H. MICROSTOMA* WITH *TRICHINELLA SPIRALIS* IN RATS AND MICE

PREFACE

Hymenolepis diminuta and *H. microstoma* cross-react immunologically in the mouse such that a mouse immunised against *H. microstoma* is resistant to challenge with *H. diminuta*, and *vice versa* to a certain extent (Hopkins *et al.*, 1977). *H. microstoma*, at least at low levels of infection, will persist in the mouse for long periods of time (Hopkins *et al.*, 1977). Although *H. diminuta* is rejected immunologically by the mouse, even in single-worm infections, this worm will survive almost indefinitely in the rat (e.g. Chappell & Pike, 1976*a*, *b*) at low levels of infection. *H. microstoma* may remain unaffected by the immune response of the mouse because the scolex and neck are inside the bile duct (Howard, 1977); *H. diminuta* in the rat and mouse dwells exclusively in the intestinal lumen, and therefore has no such 'protected site'.

One way of investigating the factors which determine whether or not rejection takes place is to induce a reaction that is non-specific in effect by infection with a completely unrelated parasite, and then to study the effects of this reaction on hymenolepids that either are or are not normally rejected by the host animal.

Study of host-parasite relationships of *Hymenolepis* using this method of non-specific (i.e. not involving immunological cross-reaction) interaction was initiated by Behnke, Bland & Wakelin (1977), who monitored the effect of the expulsion phase of the nematode *Trichinella spiralis* on the growth and survival of *H. diminuta* in mice. They found that the inflammatory rejection phase of *T. spiralis* infection (i.e. days 4-19 of the infection)

adversely affected *H. diminuta* concurrently present in the small intestine, such that the cestode either failed to establish or to grow, and was prematurely expelled; the recovery of *H. diminuta* was lowest when the cysticercoids were administered on day 7 of a primary infection with *T. spiralis*. Using the more precise timing of the onset of inflammation in a secondary infection with *T. spiralis*, these authors confirmed that the response against the nematode caused destrobilation of the cestode. Infection with one of the parasites did not immunise the mouse against infection with the other, nor was there evidence for direct inter-worm competition or interaction. While *H. diminuta* does not stimulate a grossly visible inflammatory response in the small intestine of the mouse, a factor produced by the mouse in response to *T. spiralis* caused damage to and expulsion of the cestode in a manner very similar to that normally observed in rejection of *H. diminuta* by mice.

It was thus of great interest to study the effect of the inflammatory response to *T. spiralis* on cestodes that are not normally rejected by their hosts, namely *H. microstoma* in the mouse and *H. diminuta* in the rat.

The work presented here falls into three parts; parts a) and b) are contained in the following two papers reproduced from *Parasitology*.

Parasitology (1978), 77, 273–279

a) The effect of concurrent infection with *Trichinella spiralis* on *Hymenolepis microstoma* in mice

R. J. HOWARD, P. R. CHRISTIE,* D. WAKELIN, M. M. WILSON *and* J. M. BEHNKE†

Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow, G61 1QH

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SUMMARY

The intestinal changes brought about by rejection of *Trichinella spiralis* from mice were studied in relation to their effects on a concurrent infection with *Hymenolepis microstoma*, a cestode not normally rejected from mice. The rejection phase of *T. spiralis* was associated with a marked stunting of growth of *H. microstoma* given just before, during, or just after rejection of the nematode. The survival of *H. microstoma* was affected only when rejection of *T. spiralis* coincided with the intestinal phase of the cestode: if *T. spiralis* rejection was timed to occur after the scolex of the cestode had entered the bile duct there was no loss of *H. microstoma*. It is suggested that the adverse effects on growth and establishment of *H. microstoma* were due to the non-specific inflammatory component of the host's response to infection with *T. spiralis*.

INTRODUCTION

The expulsion of *Nippostrongylus brasiliensis* from rats has been reported to require not only an antibody response and a specific cellular element but also a non-specific element derived from bone marrow (Dineen & Kelly, 1973). After reviewing the immune mechanisms of various nematode infections, Ogilvie (1974) suggested that 'whatever the exact sequence of immunological events which trigger expulsion (of nematodes) it is probably caused by the release of some non-specific effector'.

Bruce & Wakelin (1977) showed that the expulsion of *Trichinella spiralis* from mice resulted in the non-specific expulsion of a concurrent *Trichuris muris* infection and that this effect was reduced by the administration of the anti-inflammatory drug indomethacin. A similar non-specific rejection of *Hymenolepis diminuta* from mice is associated with the rejection of a concurrent *T. spiralis* infection and it was suggested that this was attributable to the inflammation in the intestine initiated by the latter parasite (Behnke, Bland & Wakelin, 1977). These observations on interspecific interactions were extended in the present

* Reprint requests to P. R. Christie.

† Present address: Department of Zoology, University of Nottingham, University Park Nottingham, NG7 2RD.

report by determining the effect of a *T. spiralis* infection upon a concurrent *H. microstoma* infection. In contrast to all the above parasites, *H. microstoma* is not normally rejected from mice (Moss, 1971; Howard, 1977), although a secondary infection grows more slowly than a primary infection (Tan & Jones, 1967, 1968; Howard, 1976). The work presented in this paper was initiated to establish whether or not *H. microstoma* is susceptible to rejection processes, utilizing the severe host response to *T. spiralis* infection.

MATERIALS AND METHODS

Helminth-free male and female NIH (Anglia Laboratory Animals) mice were used in Experiments 1 and 2 respectively, male C3H (Bantin and Kingman Limited, Yorkshire) mice were used in Experiment 3, and outbred male CFLP (bred at the Wellcome Laboratories) mice were used in Experiment 4. All mice received food and water *ad libitum*.

The infection and autopsy procedures have been described previously for *H. microstoma* (Howard, 1976, 1977) and *T. spiralis* (Wakelin & Lloyd, 1976*a*). Briefly, mice received cysticercoids of the cestode by stomach tube whilst under light ether anaesthesia. The cestodes were recovered by slitting open the bile duct and small intestine and incubating these tissues at 37 °C for 1–2 h. Infective larvae of *T. spiralis* were recovered from rats or mice by digestion of the animals in acid pepsin for 2 h at 37 °C. These larvae were suspended in 0·2 % agar and the mice were infected with 500 (Exps 1 and 3) or 350 (Exps 2 and 4) larvae by oral inoculation into the stomach with a syringe and blunted cannula. The adult nematodes were recovered by a modified Baermann technique.

The dry weights and numbers of cestodes recorded from different groups of mice were compared using the Wilcoxon rank sum test (Remington & Schork, 1970).

NIH mice reject *T. spiralis* starting on day 8 of infection and rejection is complete by day 11·5 (Wakelin & Lloyd, 1976*a*); C3H and CFLP mice are slower in rejecting *T. spiralis*, but major worm loss generally occurs between days 10 and 12, rejection being complete by day 18 (Wakelin, unpublished observations).

RESULTS

The effect of a T. spiralis *infection on the growth and survival of a pre-existing* H. microstoma *infection*

Investigation of the effect of *T. spiralis* infection on a pre-existing *H. microstoma* infection began with experiments (Exps 1 and 2) in which groups of mice were infected with either 5 cysticercoids of *H. microstoma*, or *H. microstoma* and *T. spiralis* 5 days later, or *T. spiralis* only. These groups were infected and killed as shown in Table 1, and the results are given in Table 2.

The establishment and rejection of the nematode were apparently not affected by concurrent infection with the cestode (Group B). Similarly, the survival of the cestode was not affected by the nematode (Groups B, E, H and K). However,

Table 1. *The effect of* Trichinella spiralis *infection on a pre-established* Hymenolepis microstoma *infection: experimental design*

(Letters in parentheses are group designations.)

Group*	Day			
Experiment 1	0	5	9	16
Hm only	Hm	—	K† (A)	K (D)
Hm + Tsp	Hm	Tsp	K (B)	K (E)
Tsp only	—	Tsp	K (C)	K (F)

	Day				
Experiment 2	0	5	13	17	35
Hm only	Hm	—	—	K (G)	K (J)
Hm + Tsp	Hm	Tsp	—	K (H)	K (K)
Tsp only	—	Tsp	K (L)	K (I)	—

* Hm, infected with 5 cysticercoids of *H. microstoma*; Tsp, infected with 500 (Exp. 1) or 350 (Exp. 2) larvae of *T. spiralis*.
† K, day of autopsy.

Table 2. *The effect of* Trichinella spiralis *infection on a pre-established* Hymenolepis microstoma *infection: results*

(Experimental mice received 5 cysticercoids of *H. microstoma* on day 0 followed by *T. spiralis* infection on day 5.)

Group*	Day of autopsy	Mean Tsp recovery	Mean Hm recovery	Mean dry weight of Hm/mouse (mg)	No. of mice
Experiment 1					
A. Hm only	9	—	5·0	6·95	10
B. Hm + Tsp	9	—	4·1	5·34	10
	9	196·8	—	—	5
C. Tsp only	9	170·8	—	—	5
D. Hm only	16	—	4·6	95·18	9
E. Hm + Tsp	16	—	4·7	59·75†	9
	16	0·4	—	—	5
F. Tsp only	16	2·2	—	—	5
Experiment 2					
G. Hm only	17	—	4·9	79·87	7
H. Hm + Tsp	17	—	4·9	55·81†	7
I. Tsp only	17	0·0	—	—	5
J. Hm only	35	—	5·0	94·64	7
K. Hm + Tsp	35	—	4·9	81·99	7
L. Tsp only	13	163·2	—	—	5

* Hm, *H. microstoma*; Tsp, *T. spiralis*.
† Significant difference ($P < 0·01$).

H. microstoma recovered from dual-infected mice were lighter than those from control mice, but the difference was significant only in mice killed immediately after rejection of the nematode had commenced (Groups E and H killed 11 and 12 days after infection with *T. spiralis* respectively). The weight of worms recovered from the dual-infected mice 30 days after *T. spiralis* infection (Group K) was lower than that from control mice but not significantly so, indicating that the stunting of the cestode was not permanent.

The establishment and growth of H. microstoma *given before, during, and after the rejection phase of* T. spiralis

The results of the previous experiments showed that the growth, but not the survival, of *H. microstoma* was affected during the expulsion of *T. spiralis*. The cestode may not have been lost during the rejection phase of *T. spiralis* because (1) the scolex of *H. microstoma* is completely resistant to the changes in the gut associated with rejection of the nematode, or (2) the scolex was protected from contact with these intestinal changes by being inside the bile duct.

In an attempt to clarify this situation, an experiment (Exp. 3) was designed in which 4 groups of *T. spiralis*-infected mice were challenged with *H. microstoma* at various times so that (1) the scolex of the cestode was inside the bile duct (3–4 days after infection) before expulsion of the nematode had begun (Group B), (2) the cestode was migrating in the intestine during the early part of the expulsion phase of the nematode (Group D), (3) as (2), but during the latter part of the expulsion phase (Group F) and (4) the cestode was establishing after the nematode had been expelled (Group H).

The scope of this experiment was extended in Exp. 4 by infecting a group of mice with *H. microstoma* before infecting with *T. spiralis*, so that the cestode had only experienced the pre-rejection phase of *T. spiralis* at autopsy (Group J); and by infecting another group as for (4) above, but later, after expulsion of the nematode. All mice were autopsied 10 days after infection with *H. microstoma* and the design and results of Exps 3 and 4 are summarized in Table 3.

In mice infected with *H. microstoma* 3 days before giving *T. spiralis* (Groups I and J) there was a similar number of cestodes recovered from dual-infected and control mice and there was no loss of weight in the cestodes recovered from the dual-infected group: in fact, the average weight was slightly higher than that from the control group.

In mice infected with *H. microstoma* 1 day after infection with *T. spiralis* (Groups A and B) there was a similar number of cestodes recovered from both groups, but the weight of the cestodes from the dual-infected group was significantly lower than that from control mice.

In mice infected with *H. microstoma* 8 or 12 days after *T. spiralis* (Groups C–D, E–F and K–L) significantly fewer cestodes were recovered from the dual-infected mice than from control mice and these worms were significantly lighter than those from control mice (the differences between Groups F and L may be attributable to the use of different strains of mice and/or different numbers of *T. spiralis* administered).

Table 3. *The effect of varying the time of* Hymenolepis microstoma *infection with respect to* Trichinella spiralis *infection*

(*T. spiralis* (Tsp) given on day 0; mice killed 10 days after *H. microstoma* (Hm) infection).

Group	Day infected with Hm	Mean Hm recovery	Mean dry weight of Hm/mouse (mg)	No. of mice
Experiment 3				
A. Hm	1	4·0	6·61	7
B. Hm+Tsp	1	3·9	1·63*	7
C. Hm	8	4·1	5·38	8
D. Hm+Tsp	8	0·6*	0·01*	8
E. Hm	12	4·5	6·78	8
F. Hm+Tsp	12	0·8*	0·11*	8
G. Hm	21	4·4	5·93	7
H. Hm+Tsp	21	3·4	1·56†	7
Experiment 4				
I. Hm	−3	4·1	6·57	9
J. Hm+Tsp	−3	4·5	7·57	8
K. Hm	+12	4·7	6·39	9
L. Hm+Tsp	+12	2·6*	1·20*	8
M. Hm	+29	4·7	4·03	9
N. Hm+Tsp	+29	4·4	3·82	9

* Significant difference ($P < 0{\cdot}01$).
† Significant difference ($0{\cdot}02 > P > 0{\cdot}01$).

In mice infected with *H. microstoma* 21 or 29 days after giving *T. spiralis* (Groups G–H and M–N), the recoveries from the dual-infected groups were not significantly lower than from controls. Cestodes recovered from the group given *T. spiralis* 21 days before infection with *H. microstoma* were significantly lighter than controls, but no significant difference in weight was evident in the group given *H. microstoma* 29 days after infection with *T. spiralis*.

DISCUSSION

The results of the experiments presented in this paper suggest that the effect of concurrent infection with *T. spiralis* on survival and growth of *H. microstoma* depends greatly on the relative timing of the infections. In mice infected with *H. microstoma* before *T. spiralis* and autopsied before rejection of the nematode had commenced, there was no significant effect on the weight of the cestode, indicating that the pre-rejection phase of *T. spiralis* had no harmful effects on the cestode.

However, *H. microstoma* which had been exposed to the rejection phase of *T. spiralis* were always lighter than those from control mice, and this deleterious effect on the cestode appeared to persist until at least 21 days after infection with the nematode. There was no significant difference between the weights of *H. microstoma* recovered from dual-infected mice and those from control mice when

infection with the cestode took place 29 days after infection with *T. spiralis*, indicating that this effect is unlikely to be due to an immunologically specific cross-reaction. Stunting of *H. microstoma* exposed to the rejection phase of *T. spiralis* was severe, but apparently not permanent: there was no significant difference between the weights of worms recovered from mice infected with *H. microstoma* only and those infected with *H. microstoma* 30 days after infection with *T. spiralis* (Table 2, Exp. 2, Groups J and K).

Rejection of *T. spiralis* from the mouse appears to involve both specific humoral and cell-mediated responses (Love, Ogilvie & McLaren, 1976; Wakelin & Lloyd, 1976*b*), but the final effector mechanism may well be a non-specifically acting inflammation of the intestine (Larsh, 1967; Larsh & Race, 1975). As there appears to be no direct cross-immunity between *H. microstoma* and *T. spiralis*, it is apparently the non-specific inflammatory component that is responsible for the observed effects on survival and growth of *H. microstoma*. However, mice reduce their food consumption during rejection of *T. spiralis* (Larsh, Goulson & Van Zandt, 1962; Goulson & Larsh, 1964) and it is well known that reduced intake of carbohydrates may retard the growth of hymenolepids (Read & Rothman, 1957*a*). The reduction in growth of *H. microstoma* may, therefore, have been at least partially due to the altered dietary intake of the mouse, although it is also conceivable that a reduced intake might be offset by the malabsorption of nutrients associated with *T. spiralis* infection (Castro, Olson & Baker, 1967). However, it is unlikely that altered intake would markedly affect the initial establishment of the worms (Read & Rothman, 1957*b*): the significant reduction in numbers of *H. microstoma* recovered that did occur in some groups was probably, therefore, caused by the inflammation associated with the rejection of *T. spiralis*. The role of inflammation in retarding growth of the cestode is less clear.

In secondary infections of *H. microstoma* in mice, worm growth is retarded by the host response during the first 4 days of infection: after entering the bile duct, growth resumes at a similar rate to that in primary infections (Howard, 1977). This resembles the situation described in the present paper in which, following establishment of the scolex of *H. microstoma* in the bile duct, the worm was not sufficiently affected by inflammation for loss to occur: it may be that changes in the intestine during inflammation do not extend into the bile duct. It is also possible that the scolex itself changes after entry into the bile duct and becomes resistant to inflammatory products; the inflammation of the peribiliary tissues which is initiated by the cestode itself does not appear to affect its survival (Lumsden & Karin, 1970), but the components in this inflammation may differ considerably from *T. spiralis*-induced intestinal inflammatory products.

REFERENCES

Behnke, J. M., Bland, P. W. & Wakelin, D. (1977). Effect of the expulsion phase of *Trichinella spiralis* on *Hymenolepis diminuta* infections in mice. *Parasitology* **75**, 79–88.

Bruce, R. G. & Wakelin, D. (1977). Immunological interactions between *Trichinella spiralis* and *Trichuris muris* in the intestine of the mouse. *Parasitology* **74**, 163–73.

CASTRO, G. A., OLSON, L. T. & BAKER, R. D. (1967). Glucose malabsorption and intestinal histopathology in *Trichinella spiralis*-infected guinea-pigs. *Journal of Parasitology* **53**, 595–612.

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**, 504–12.

GOULSON, H. T. & LARSH, J. E. (1964). The effect against *Trichinella spiralis* of a 72-hour treatment schedule with cadmium oxide in mice given one–three infections. *Journal of the Elisha Mitchell Scientific Society* **80**, 84–90.

HOWARD, R. J. (1976). The growth of secondary infections of *Hymenolepis microstoma* in mice: the effect of various primary infection regimes. *Parasitology* **72**, 317–23.

HOWARD, R. J. (1977). *Hymenolepis microstoma*: a change in susceptibility to resistance with increasing age of the parasite. *Parasitology* **75**, 241–9.

LARSH, J. E. (1967). The present understanding of the mechanism of immunity to *Trichinella spiralis*. *The American Journal of Tropical Medicine and Hygiene* **16**, 123–32.

LARSH, J. E. & RACE, G. J. (1975). Allergic inflammation as a hypothesis for the expulsion of worms from tissues: a review. *Experimental Parasitology* **32**, 251–66.

LARSH, J. E., GOULSON, H. T. & VAN ZANDT, P. D. (1962). The control of *Trichinella spiralis* in mice by cadmium oxide in feed given on an intermittent schedule. *Journal of the Elisha Mitchell Scientific Society* **94**, 262–72.

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.

LUMSDEN, R. D. & KARIN, D. S. (1970). Electron microscopy of peribiliary connective tissues in mice infected with the tapeworm *Hymenolepis microstoma*. *Journal of Parasitology* **56**, 1171–83.

MOSS, G. D. (1971). The nature of the immune response of the mouse to the bile duct cestode, *Hymenolepis microstoma*. *Parasitology* **62**, 285–94.

OGILVIE, B. M. (1974). Immunity to parasites (Helminths and Arthropods). *Progress in Immunology* II, **4**, 127–35.

READ, C. P. & ROTHMAN, A. H. (1957*a*). The role of carbohydrate in the biology of cestodes. II. The effect of starvation on glycogenesis and glucose consumption in *Hymenolepis*. *Experimental Parasitology* **6**, 280–7.

READ, C. P. & ROTHMAN, A. H. (1957*b*). The role of carbohydrates in the biology of cestodes. IV. Some effects of host dietary carbohydrate on growth and reproduction of *Hymenolepis*. *Experimental Parasitology* **6**, 294–305.

REMINGTON, R. D. & SCHORK, M. A. (1970). *Statistics with Applications to the Biological and Health Sciences*, pp. 313–15. Englewood Cliffs, N.J.: Prentice-Hall, Incorporated.

TAN, B. D. & JONES, A. W. (1967). Autoelimination by means of X-rays: distinguishing the crowding factor from others in premunition caused by the mouse bile duct cestode, *Hymenolepis microstoma*. *Experimental Parasitology* **20**, 147–55.

TAN, B. D. & JONES, A. W. (1968). Resistance of mice to reinfection with the bile duct cestode *Hymenolepis microstoma*. *Experimental Parasitology* **22**, 250–5.

WAKELIN, D. & LLOYD, M. (1976*a*). Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology* **72**, 173–82.

WAKELIN, D. & LLOYD, M. (1976*b*). Accelerated expulsion of adult *Trichinella spiralis* in mice given lymphoid cells and serum from infected donors. *Parasitology* **72**, 307–15.

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b) The effect of the expulsion phase of *Trichinella spiralis* on *Hymenolepis diminuta* infection in rats

P. R. CHRISTIE, D. WAKELIN *and* M. M. WILSON
Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow, G61 1QH

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SUMMARY

The effect of the intestinal changes brought about by the expulsion of *Trichinella spiralis* in rats was studied in relation to the growth and survival of a concurrent infection with *Hymenolepis diminuta*, a cestode not normally rejected by the rat in low-level infections. Growth of *H. diminuta* was stunted in rats given *T. spiralis* just before, or after, infection with *H. diminuta*, the stunting being more pronounced when the cestode was given closer to the period of inflammation. There was no loss of the cestode from dual-infected rats and no evidence for destrobilation was found. Lower *T. spiralis* burdens had a correspondingly weaker effect on growth of *H. diminuta*, and stunting was abolished by administration of the anti-inflammatory drug cortisone acetate. It is concluded that the stunting of *H. diminuta* is probably due to the non-specific inflammatory component of the rat's response to *T. spiralis* infection.

INTRODUCTION

Hymenolepis diminuta in the rat was regarded as being non-immunogenic (Heyneman, 1962): recent studies have shown that there is a specific serum antibody response to worm antigens (Harris & Turton, 1973), but there is no evidence that the antigens responded to are in any way involved in a protective response. In low-level infections (< 15 worms, depending on the strain of rat) *H. diminuta* will apparently survive indefinitely (Read, 1967), but there is a gradual and incomplete loss of worms from heavier infections (Roberts & Mong, 1968; Andreassen, Hindsbo & Hesselberg, 1974; Hesselberg & Andreassen, 1975; Chappell & Pike, 1976*a*, *b*). There is some controversy over the cause of this loss of worms: some workers consider it to be immunologically mediated (Andreassen *et al.* 1974) whereas others consider that it may be due to some form of inter-worm competition (Roberts & Mong, 1968).

If it be assumed that the rejection of *H. diminuta* from the rat is immunologically mediated, then the nature of the effector mechanism remains obscure. It is becoming clear that, in many cases, rejection of helminths from the mammalian intestine is the result of inflammatory reactions that are non-specific in effect, albeit mediated through specific immunological mechanisms (Larsh & Race, 1975; Dineen, Gregg, Windon, Donald & Kelly, 1977). *Trichinella spiralis* elicits

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a severe inflammatory response in mice and rats (Larsh, 1963); in the mouse, intestinal inflammation initiated by *T. spiralis* has been shown to be effective in causing concomitant (i.e. early) rejection of *Trichuris muris* (Bruce & Wakelin, 1977), *H. diminuta* (Behnke, Bland & Wakelin, 1977), and *H. microstoma* (Howard, Christie, Wakelin, Wilson & Behnke, 1978). It was, therefore, of interest to investigate the effect of inflammation induced by *T. spiralis* in the rat intestine on the growth and survival of *H. diminuta*.

MATERIALS AND METHODS

Rats

Male and female CFHB rats (Anglia Laboratory Animals) were infected at 8–10 weeks of age. Rats were housed under conventional conditions with food (Rat and Mouse Breeding Diet – Grain Harvesters Ltd) and water available *ad libitum*. A minimum of 6 rats was used/group.

Parasites

The Rice strain of *H. diminuta* was used throughout. Maintenance of the parasite and recovery of cysticercoids has been described elsewhere (Hopkins, Subramanian & Stallard, 1972). Rats were infected with 3 cysticercoids each and worms were recovered by flushing out the intestine with balanced salt solution. Using this technique, worms larger than 1 cm long (> 0·1 mg dry weight) were easily recovered and the dry weights of worm burdens from individual rats were recorded. In the first experiments described in this paper (Fig. 1), a category ('not recovered') is included for worms weighing less than 0·1 mg: this implies that although the worms may have been present, the technique was not adequate to recover them.

The strain of *T. spiralis* and the methods used for infection and recovery have been described elsewhere (Wakelin & Lloyd, 1976).

Cortisone

Cortisone acetate ('Cortistab', Boots) was administered by subcutaneous injection at a dose of 15 mg every 2nd day. Cortisone-treated and relevant control rats were given oxytetracycline HCl (Terramycin, Pfizer Ltd) in their drinking water at a concentration of 165 mg/l.

EXPERIMENTAL DESIGN AND RESULTS

Course of T. spiralis *infections*

The following parameters of *T. spiralis* infections in CFHB rats were established during the course of the work. About 50% of the larvae administered became established as mature worms. In a primary infection worm burdens are stable for approximately 8 days and then there is a rapid loss of worms. Very few, or no, worms can be recovered from the small intestine after the 12th day. The ability to expel worms, and the time course of expulsion are independent of the number

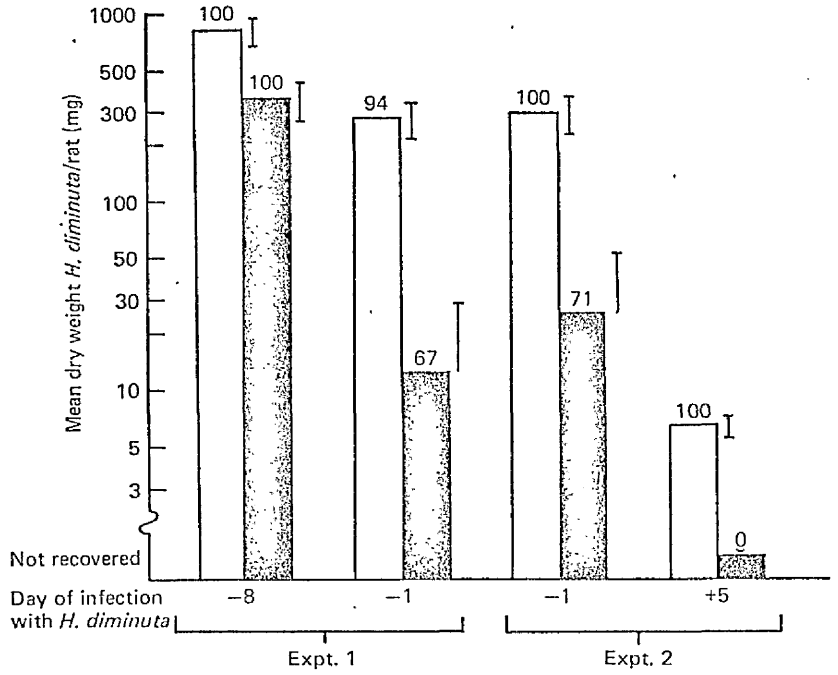

Fig. 1. Effect on growth of *Hymenolepis diminuta* of a concurrent infection with *Trichinella spiralis*. Mean dry weight/rat (± S.D.) of *H. diminuta* recovered from groups of 6 rats infected with 2000 larvae of *T. spiralis* on day 0, and 3 cysticercoids of *H. diminuta* on day −8 or −1 (Exp. 1), day −1 or +5 (Exp. 2). Shaded bars, dual-infected rats; open bars, *H. diminuta* only. All rats autopsied on day 12: superscripts are percentage recovery of *H. diminuta*.

of larvae used for infection; rats infected with 200 or 2000 larvae showed a similar pattern of worm expulsion. Worm expulsion is accompanied, and probably caused, by marked inflammatory changes in the intestine. These changes are apparent 5–6 days after infection and are most intense during expulsion, but the intestine then recovers rapidly. In the majority of experiments described in this paper infections of 2000 larvae were given, and the course of infection monitored in rats killed after 7 and 12 days. As the results were consistent throughout, details of the *T. spiralis* worm recoveries are excluded from the experimental results.

The effect on H. diminuta *of challenging with* T. spiralis *at different times*

Two experiments were carried out in which infections with *H. diminuta* were established 8 days and 1 day before (Exp. 1) or 1 day before and 5 days after (Exp. 2) infection with 2000 *T. spiralis* larvae. Rats were killed 12 and 22 days after infection with *T. spiralis*: the results of the day 12 autopsy are shown in Fig. 1.

There was a severe depression of growth of the cestode in all the dual-infected groups; the reduction in the dry weight of worms recovered compared with controls was 60, 90 and > 99% in groups given *H. diminuta* on days −8, −1 and

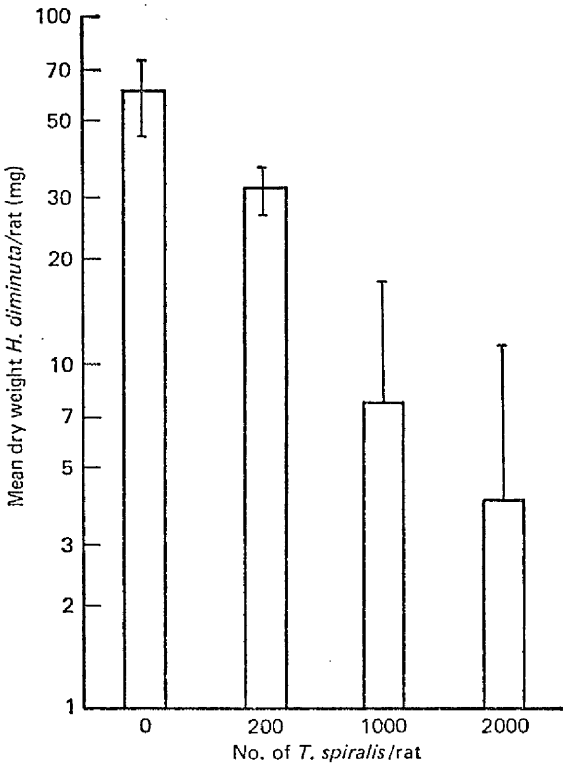

Fig. 2. Effect on growth of *Hymenolepis diminuta* of a concurrent infection with varying numbers of *Trichinella spiralis*. Mean dry weight/rat (± S.D.) of *H. diminuta* recovered from groups of 6 or 7 rats given 0, 200, 1000 or 2000 *T. spiralis* larvae + 3 cysticercoids of *H. diminuta* on day 0, and autopsied on day 10.

+5 respectively. This progressive effect on weight appeared to be paralleled by an increasing loss of worms. However, the recovery of cestodes from rats killed on day +22 was 100% from all 4 control groups and 100, 100 and 87·5% from dual-infected groups given *H. diminuta* on days −8, −1 and +5 respectively. This indicates that although there was a pronounced stunting effect, there was no significant loss of cestodes once established. (The low recovery on day 12 may reflect a failure to recover small worms – see Materials and Methods.)

In a third experiment, rats were infected with *H. diminuta* on day 8 of *T. spiralis* infection and autopsied 21 days later: there was 100% recovery of *H. diminuta* from control rats, and 83% recovery from dual-infected rats: the weight of cestodes recovered from the latter group was 45% of that from the controls.

The effect of varying the number of T. spiralis *administered on the growth of* H. diminuta

Four groups of rats were infected as follows: *H. diminuta* only, *H. diminuta* + 200 *T. spiralis*, *H. diminuta* + 1000 *T. spiralis* and *H. diminuta* + 2000 *T. spiralis*. Both parasites were administered on day 0 and rats were autopsied on day 10 (Fig. 2). The extent to which growth of *H. diminuta* was reduced was linked to

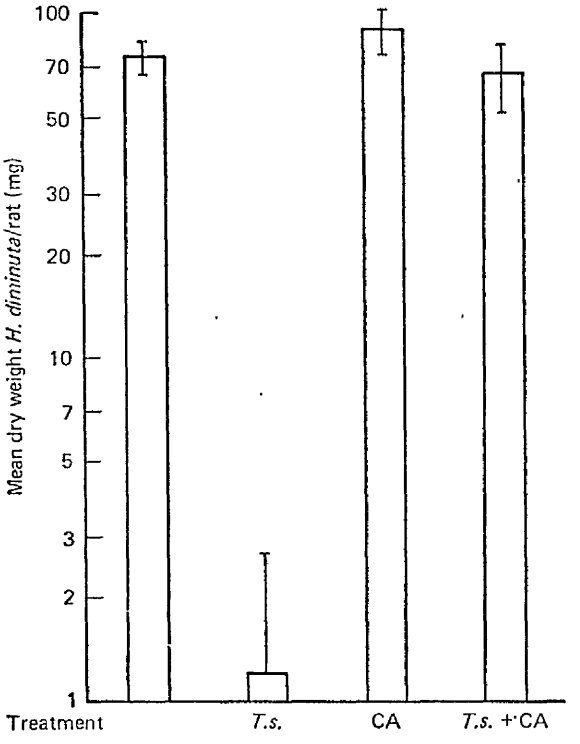

Fig. 3. Effect of cortisone acetate (CA) treatment on growth of *Hymenolepis diminuta* in rats concurrently infected with *Trichinella spiralis*. Mean dry weight/rat (± S.D.) of *H. diminuta* recovered from groups of 6 rats. All rats were given 3 cysticercoids of *H. diminuta* on day 0 and autopsied on day 10. *T.s.*, 1000 *T. spiralis* larvae on day 0.

the number of *T. spiralis* given: 2000 and 1000 *T. spiralis* caused 94% and 88% reduction in weight compared with controls and even 200 *T. spiralis* caused a 50% reduction in weight (equivalent to 24 h growth lost).

The effect of administration of cortisone on the stunting of growth of H. diminuta *during concurrent infection with* T. spiralis

Four groups of rats were infected and treated as follows: *H. diminuta* only, *H. diminuta* + *T. spiralis*, *H. diminuta* + cortisone acetate, and *H. diminuta* + *T. spiralis* + cortisone acetate. Both parasites were administered on day 0 and rats were autopsied on day 10 (Fig. 3). There was a 98% reduction in dry weight of *H. diminuta* attained in the dual-infected group compared with controls (7 mg and 450 mg respectively), but in the dual-infected group given cortisone growth was restored to control levels. Giving cortisone did not *per se* cause a large increase in the growth of the worms, the dry weight of the group receiving *H. diminuta* + cortisone exceeding the control weight by only 19%.

The effect of T. spiralis *infection on established* H. diminuta: *to investigate whether inflammation can cause destrobilation of* H. diminuta

The above results show that growth is reduced but that there is no loss of established *H. diminuta*. In the mouse (Behnke *et al.* 1977) the response to *T. spiralis* causes destrobilation of *H. diminuta* (a loss of strobila behind the neck) and it was of interest to study the effect of *T. spiralis* infection on the integrity of established cestodes in the rat.

Rats were infected with *H. diminuta* 15 days before administration of *T. spiralis* on day 0: the cestode would, therefore, have an extensive strobila at the onset of inflammation (approximately 6 days after giving *T. spiralis*) and destrobilation would be obvious at autopsy on day 10. When the rats were killed, 94% of the cestodes were recovered in both control and dual-infected rats, and all were strobilate. However, growth of the cestode in dual-infected rats had been retarded, the mean dry weight recovered/rat being 321 mg from dual-infected compared with 571 mg from control rats.

DISCUSSION

The major observable effect of concurrent infection with *T. spiralis* on *H. diminuta* was a marked stunting of the cestode, i.e. a slowing or stopping of growth. The extent of this effect appeared to be dependent on the relative timing of the 2 infections and the number of *T. spiralis* administered.

When *H. diminuta* was given several days before *T. spiralis* there was relatively little effect on growth compared with the severe stunting seen when the cestode was administered closer to, or during, the time of expulsion when the gut is markedly inflamed. This could be attributed to the more anterior position of the scolex of the older worms in the small intestine (Chandler, 1939; Bråten & Hopkins, 1969) causing the proliferative neck region to be in a less inflamed habitat and hence better able to grow, whereas smaller worms would be further down the intestine in a more inflamed environment. Unfortunately, we have no information from the present experiments about the effect of *T. spiralis* infection on the distribution and location of the cestodes. One other possibility is that there is a physiological change in the cestode, older worms being more resistant to the effects of inflammation. It is of interest to note that there was a slightly lower recovery of *H. diminuta* when given during the period of expulsion of *T. spiralis* (5 and 8 days after infection with *T. spiralis*): this, however, probably reflects a poorer establishment in the inflamed intestine due to disruption of location signals.

The stunting effect was more marked when heavy *T. spiralis* infections (2000 larvae) were given than when lighter *T. spiralis* infections were given (1000 and 200 larvae), implying that as the *T. spiralis* burden (and hence the degree of inflammation) increases, the growth of the cestode decreases. These observations, taken in consideration with the abolition of the stunting effect with cortisone, suggest that the non-specific inflammatory component of the rat's response to *T. spiralis* infection is having a deleterious effect on the cestode. Intestinal tape-

worms are extremely sensitive to changes in host dietary intake (Read & Rothman, 1957) and as heavy *T. spiralis* infections cause a reduction in dietary intake (Saowakontha, 1975) this may be at least partly responsible for the stunting effect. However, as even low level infections of *T. spiralis* (200 larvae) caused a marked reduction of worm growth, it appears likely that the inflammation does play a part in the stunting. Indeed, the intestinal malabsorption observed in *T. spiralis* infections (Castro, 1976) may offset the stunting effect of reduced dietary intake by making more nutrients, particularly carbohydrates, available to the cestode.

Perhaps the most interesting point raised by the results is that neither destrobilation nor loss of *H. diminuta* occurred in the face of subsequent severe intestinal inflammation. In the mouse, *H. diminuta* is rejected immunologically (Hopkins *et al.* 1972), and Behnke *et al.* (1977) showed that *T. spiralis*-induced inflammation caused premature, i.e. non-specific, destrobilation and loss of the cestode. The bile duct cestode *H. microstoma*, not normally rejected by mice, is also removed if its intestinal phase coincides with *T. spiralis*-induced inflammation (Howard *et al.* 1978). Once the scolex is inside the bile duct, no rejection of *H. microstoma* can occur, suggesting that the bile duct is an immunologically privileged site. In the present study *H. diminuta*, although not rejected immunologically from the rat at low level infections, has no such 'protected site' and is, therefore, presumably as vulnerable to attack by the rat's response to *T. spiralis* as it is in the mouse. However, no rejection or even destrobilation (a 'marker' of stress on the cestode, such as immunological attack) takes place, and this ability to survive is difficult to explain. It is possible that the rat's response to *T. spiralis* differs qualitatively from that of the mouse, the factor effective in removal of *H. diminuta* from the mouse being absent in the rat. Alternatively, it may be that *H. diminuta* in the rat is physiologically insusceptible to inflammatory responses. The resolution of this problem awaits further evidence of immunity to *H. diminuta* in the rat and studies on the immune mechanisms involved.

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REFERENCES

Andreassen, J., Hindsbo, O. & Hesselberg, C. A. (1974). Immunity to *Hymenolepis diminuta* in rats: destrobilation and expulsion in primary infections, its suppression by cortisone treatment and increased resistance to secondary infections. *Proceedings of the 3rd International Congress of Parasitology, Munich*, 2, 1056–7.

Behnke, J. M., Bland, P. W. & Wakelin, D. (1977). Effect of the expulsion phase of *Trichinella spiralis* on *Hymenolepis diminuta* in mice. *Parasitology* **75**, 79–88.

Bråten, T. & Hopkins, C. A. (1969). The migration of *Hymenolepis diminuta* in the rat's intestine during normal development and following surgical transplantation. *Parasitology* **59**, 891–905.

Bruce, R. G. & Wakelin, D. (1977). Immunological interaction between *Trichinella spiralis* and *Trichuris muris* in the intestine of the mouse. *Parasitology* **74**, 163–73.

Castro, G. A. (1976). Spatial and temporal integration of host responses to intestinal stages of *Trichinella spiralis*. In *Biochemistry of Parasites and Host-Parasite Relationships* (ed. H. van den Bossche), pp. 343–58. Amsterdam: Elsevier/North-Holland Biomedical Press.

CHANDLER, A. C. (1939). The effects of number and age of worms on development of primary and secondary infections of *Hymenolepis diminuta* in rats, and an investigation into the true nature of 'premunition' in tapeworm infections. *American Journal of Hygiene* **29**, 105–14.

CHAPPELL, L. H. & PIKE, A. W. (1976*a*). Loss of *Hymenolepis diminuta* from the rat. *International Journal for Parasitology* **6**, 333–9.

CHAPPELL, L. H. & PIKE, A. W. (1976*b*). Interactions between *Hymenolepis diminuta* and the rat. In *Biochemistry of Parasites and Host-Parasite Relationships* (ed. H. van den Bossche), pp. 379–84. Amsterdam: Elsevier/North-Holland Biomedical Press.

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**, 211–15.

HARRIS, W. G. & TURTON, J. A. (1973). Antibody response to tapeworm (*Hymenolepis diminuta*) in the rat. *Nature, London* **246**, 521–2.

HESSELBERG, C. A. & ANDREASSEN, J. (1975). Some influences of population density on *Hymenolepis diminuta* in rats. *Parasitology* **71**, 517–24.

HEYNEMAN, D. (1962). Studies on helminth immunity. II. Influence of *Hymenolepis nana* (Cestoda: Hymenolepididae) in dual infections with *H. diminuta* in white mice and rats. *Experimental Parasitology* **12**, 7–18.

HOPKINS, C. A., SUBRAMANIAN, G. & STALLARD, H. (1972). The development of *Hymenolepis diminuta* in primary and secondary infections in mice. *Parasitology* **64**, 401–12.

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**, 273–9.

LARSH, J. E. JR. (1963). Experimental trichiniasis. *Advances in Parasitology* **1**, 213–86.

LARSH, J. E. JR. & RACE, G. J. (1975). Allergic inflammation as a hypothesis for the expulsion of worms from tissues: a review. *Experimental Parasitology* **37**, 251–66.

READ, C. P. (1967). Longevity of the tapeworm *Hymenolepis diminuta*. *Journal of Parasitology* **53**, 1055–6.

READ, C. P. & ROTHMAN, A. H. (1957). The role of carbohydrates in the biology of cestodes. IV. Some effects of host dietary carbohydrate on growth and reproduction of *Hymenolepis*. *Experimental Parasitology* **6**, 294–305.

ROBERTS, L. S. & MONG, F. N. (1968). Developmental physiology of cestodes. III. Development of *Hymenolepis diminuta* in superinfections. *Journal of Parasitology* **54**, 55–62.

SAOWAKONTHA, S. (1975). The effect of trichinosis on weight gain and food intake of rats fed low and high protein diet. *South East Asian Journal of Tropical Medicine and Public Health* **6**, 247–50.

WAKELIN, D. & LLOYD, M. (1976). Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology* **72**, 173–82.

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c) Interaction of *Hymenolepis diminuta* and *Nippostrongylus brasiliensis* in the rat

An additional experiment was performed in which the nematode *Nippostrongylus brasiliensis* was used as a source of an intestinal inflammatory response (Taliaferro & Sarles, 1939), to investigate further the status of *H. diminuta* in the rat. The foregoing paper (Christie *et al.*, 1979) indicated that there may be some loss of *H. diminuta* in dual-infected rats, but only if the initial establishment of the worms coincided with the period of most intense inflammation (in this case, 5 and 8 days after infection with *T. spiralis*). The experiment using *N. brasiliensis* was therefore designed to establish two points: firstly, that administration of *H. diminuta* cysticercoids during the period of inflammation will reduce the establishment of the cestode; and secondly, that the effect on *H. diminuta* observed by Christie *et al.* (1979) was not solely a property of the response to *T. spiralis*.

Female CFHB rats aged 8-12 weeks were infected subcutaneously with approximately 3000 *N. brasiliensis* larvae on day -6; these and control rats were infected with three cysticercoids of H. diminuta on day 0. Rats from both groups were killed on days +8 and +25 (14 and 31 days after infection with *N. brasiliensis*); this latter group was included to recover regrown tapeworms from dual-infected rats that may not have been large enough to detect on day +8. Two rats infected with the nematode only were killed on day 7 p.i. to record establishment; a mean of 2769 worms were recovered.

On day 8 of infection with *H. diminuta* (Table 3-1), 100% of the control *H. diminuta* infection was recovered, but no worms of greater than 0.1 mg were recovered from the dual-infected group. Killing the second two groups 17 days later, 100% of the cestodes were again recovered from the control, but only 56% of the worms were recovered fron the dual-infected rats. The individual worms that were recovered from the latter group were 24% lighter than those from controls.

Table 3-1

Recovery (%) and weight of *H. diminuta* from rats infected with *N. brasiliensis* six days previously.

Group	day	Recovery (%)	Mean worm dry weight / rat	n
C	8	100	15.2 mg	6
Nb	8	0	-	6
C	25	100	618 mg*	6
Nb	25	56	260 mg*	6

C, three *H. diminuta* day 0; Nb, ca. 3000 *N. brasiliensis* day -6.

*, dry weight estimated as 1/5 x wet weight

DISCUSSION

1) Interactions between *H. diminuta* and *N. brasiliensis* in the rat

This experiment demonstrated the two points raised in the introductory paragraph; the stunting effect on *H. diminuta* in the rat is not limited to the response to *T. spiralis*, as a very marked stunting effect was observed in rats concurrently infected with *N. brasiliensis*; infection with the cestode during a period when the intestine is inflamed does apparently alter the establishment or survival of the worm. Excystation or location stimuli may be upset; alternatively, the young tapeworm may be susceptible to the effects of the inflammatory response, becoming resistant at a later stage.

In the light of this information, some reinspection of the *H. microstoma*/ *T. spiralis*/ mouse work (Howard *et al.*, 1978) may be necessary, as only about five days elapse until the scolex is inside the bile duct. As the establishment of the cysticercoids may have been affected, one should perhaps qualify the conclusion that *H. microstoma* is susceptible to rejection processes while in the lumen of the small intestine. Transplantation of mature *H. microstoma* into mice appropriately infected with *T. spiralis* using a similar protocol to that of Howard (1977) would perhaps clear this point up.

2) Interaction studies on *Hymenolepis*: general conclusions

The present study and that of Behnke *et al.* (1977) have raised some interesting points, both with respect to *T. spiralis* and *H. diminuta*. Although the number of intestinal mast cells and

globule leukocytes apparently increases during the course of infection with H. diminuta in the mouse (Andreassen et al., 1978a), treatment of mice with the antihistamine and anti-5-hydroxytryptamine drugs promethazine, methysergide or cyproheptadine HCl has no effect on growth or rejection of this parasite (Hopkins, personal communication), suggesting that these products of the inflammatory response are not involved in expulsion of H. diminuta. Treatment of mice with disodium chromoglycate (a potent inhibitor of mast cell degranulation) has very little effect on rejection of T. spiralis , expulsion in treated mice being delayed by only about one day (Michalska & Karmanska, 1976). The expulsion of both H. diminuta and T. spiralis in dual-infected mice (Behnke et al., 1977) may therefore have been due to other factors, acting singly or in combination, such as acidosis (Castro, Cotter, Ferguson & Gordon, 1973), increased phospholipase activity (Larsh, Ottolenghi & Weatherly, 1974), or release of prostaglandins (Dineen, Kelly, Goodrich & Smith, 1974).

The response of the rat and the mouse to T. spiralis possibly differs, in that in the mouse the factors produced cause early expulsion of H. diminuta, whereas in the rat no expulsion or destrobilation takes place. T. spiralis stimulates an inflammatory response in both hosts, and the apparent difference in the components involved may be due to different mechanisms for expulsion of T. spiralis. Rejection of T. spiralis by the mouse is a strongly thymus-dependent process, which may involve myeloid (bone marrow derived) accessory cell products acting directly on the worms, with T cells an essential component of this response (Wakelin & Wilson, 1979a, b), whereas B cells and antibody have been strongly implicated in expulsion of T. spiralis from rats

(Love *et al.*, 1976; Despommier *et al.*, 1977b). The inflammation of the rat gut may therefore be a mechanism to allow passage of antibody into the lumen of the intestine (see Barth, Jarrett & Urquhart, 1966). If the apparent qualitative difference in the responses of the rat and the mouse to *T. spiralis* could be further characterised, perhaps this would give a clue to the identity of factor(s) which expel(s) *H. diminuta* from the mouse.

SUMMARY

1. The effect of the inflammatory expulsion phase of *T. spiralis* on growth and survival of hymenolepids not normally rejected by their hosts was studied with *H. microstoma* in the mouse and *H. diminuta* in the rat.

2. Growth of the cestodes in dual-infected animals was markedly depressed, and the severity of the effect on growth increased if the cestode was administered closer to the time of expulsion of *T. spiralis*.

3. *H. microstoma* was found to be susceptible to the effects of inflammation while migrating in the small intestine, but once the scolex was inside the bile duct, no loss or destrobilation took place. *H. diminuta* in the rat was not expelled or caused to destrobilate by the inflammation, but establishment was probably affected if rats were infected with the cestode during the period of severest inflammation caused by *T. spiralis* or *N. brasiliensis*.

4. It is suggested that the bile duct is an immunologically privileged site for *H. microstoma*, and that the factors secreted by the inflamed intestines of rats and mice infected with *T. spiralis* differ qualitatively.

CHAPTER 4

LYMPHOCYTE TRANSFER IN MICE INFECTED WITH *HYMENOLEPIS DIMINUTA*

INTRODUCTION

1. Transfer of immunity with lymphocytes

Transfer of immunological memory and immune responsiveness from one animal to another with lymphocytes has proved to be one of the most useful techniques in immunological studies, in particular in investigation of the nature of the immune response to parasitic organisms. There are numerous models in which the immune response to helminths has been studied using lymphocyte transfer; the most extensively studied systems are Nippostrongylus brasiliensis in the rat (Kelly & Dineen, 1972; reviewed by Ogilvie & Love, 1974) and Trichinella spiralis in the mouse (Wakelin & Lloyd, 1976a). Other host-parasite systems in which immunity has been successfully transferred with lymphocytes include T. spiralis in the rat (Love et al., 1976), Trichuris muris in the mouse (Wakelin, 1975), Trichostrongylus colubriformis in the guinea-pig (Wagland & Dineen, 1965), Ascaris in the guinea-pig (Khoury, Stromberg & Soulsby, 1977), Strongyloides ratti in the rat (Moqbel, 1976), and Fasciola hepatica in the mouse (Lang, Larsh, Weatherly & Goulson, 1967) and in the rat (Corba, Armour, Roberts & Urquhart, 1971). This technique has also proved useful in investigation of immunity to protozoan parasites such as Plasmodium in rats (Phillips, 1970) and Trypanosoma in mice (Takayanagi, Kambara & Enriquez, 1973; Pouliot, Viens & Targett, 1977).

Use of lymphocyte transfer, whole-body irradiation and lymphocyte separation techniques has allowed considerable progress in identification of the specific components involved in immune expulsion of intestinal parasites, particularly nematodes. Thus, it

has been postulated that rejection of N. brasiliensis by the rat involves a specific sequence of action by lymphocytes, antibody and a bone marrow component (reviewed by Ogilvie & Love, 1974), although Ogilvie, Love, Jarra & Brown (1977) suggested that a bone marrow component may not be necessary, because heavily irradiated rats can rapidly expel antibody-damaged worms after transfer of Ig-negative thoracic duct lymphocytes.

Likewise in the mouse, the response to T. spiralis has been shown to be transferable to irradiated recipients with T lymphocytes alone, highly purified B cell populations having no effect on the worm survival; however, it appears that a non-lymphoid component is also necessary in bringing about the changes in the intestinal environment which cause worm expulsion (Wakelin & Wilson, 1979a, b). This is in contrast to the work of Despommier, McGregor, Crum & Carter (1977) with T. spiralis in rats; these authors postulate that T cells act principally as helper cells, and that B cells cause more efficient rejection than T cells, possibly by production of IgA. This may, as Wakelin (1978) pointed out, reflect fundamental differences in the composition of the response to T. spiralis in the mouse and rat, a conclusion supported by comparison of the results of Christie et al. (1979) and Behnke et al. (1977) (see Chapter 3).

The response of the mouse to H. diminuta is lymphocyte dependent, displaying sensitivity to immunosuppressants (Hopkins et al., 1972b) and thymic insufficiency (Bland, 1976a, b), and long-term memory (Hopkins, 1980). This system should thus be amenable to transfer of immunity with lymphocytes. Mesenteric lymph node cells (MLNC) have been used successfully in most cases of transfer of immunity against intestinal parasites, the mesenteric lymph node (MLN) being the regional draining node for the intestine.

In rats and mice, all lymphatic drainage from the small intestine passes through the MLN (Tilney, 1971; Carter & Collins, 1974).

Attempts by Bland (1976b) to transfer immunity against H. diminuta in mice using MLNC and spleen cells met with little success, and the present study was initiated to expand this work by varying various parameters in the search for effective adoptive transfer of immunity.

2. Lymphocyte migration and accumulation in the intestine

It is now well-known that isotopically labelled lymphoblasts from the thoracic duct or MLN injected into a recipient animal will preferentially localise in the lamina propria of the small intestine (Gowans & Knight, 1964; Griscelli, Vassalli & McCluskey, 1969). This capacity for intestinal localisation is a feature of both T and B lymphoblasts (Guy-Grand, Griscelli & Vassalli, 1974; McWilliams, Phillips-Quagliata & Lamm, 1975) and is not a property shared by small T lymphocytes (DeFreitas, Rose & Parrott, 1977) or peripheral lymph node lymphoblasts or Peyer's patch lymphoblasts (Griscelli et al., 1969; Guy-Grand et al., 1974; Parrott, Rose, Sless, DeFreitas & Bruce, 1975).

It has been shown with various nematode infections that the presence of the worms in the small intestine causes enhanced accumulation of blast cells in the mucosal layer of the small intestine, e.g. T. spiralis in mice (Rose, Parrott & Bruce, 1976a) and rats (Despommier et al., 1977b; Love & Ogilvie, 1977), N. brasiliensis in rats (Love & Ogilvie, 1977), and T. colubriformis in guinea-pigs (Dineen, Ronai & Wagland, 1968). Traffic of lymphoblasts to the infected small intestine has been studied

extensively with T. spiralis in the mouse, and this work has shown that the enhanced accumulation of lymphoblasts in the small intestine is a property of T blasts and not of B blasts (Rose et al., 1976b) and is, antigenically speaking, an entirely non-specific phenomenon, lymphocytes from non-infected animals displaying enhanced accumulation in the infected small intestine, but not vice versa (Rose et al., 1976a). Nonspecific intestinal inflammation induced by orally administered turpentine also enhances lymphoblast accumulation in the small intestine (Parrott et al., 1975). It has also been shown by Love & Ogilvie (1977) that thoracic duct lymphoblasts from rats infected with N. brasiliensis. show enhanced accumulation in the intestine of rats infected with T. spiralis, and vice versa, again suggesting a lack of antigenic specificity.

It is also notable that peripheral lymph node blasts will also display enhanced accumulation in the small intestine of mice infected with T. spiralis (in fact an increase of 118-205% over control values, compared with an increase of 57% with MLN blasts), although the absolute number of these peripheral lymphoblasts reaching the gut is relatively much smaller than is observed with MLN blasts, and peripheral lymphoblasts are easily diverted to peripheral sites of inflammation (Rose et al., 1976b).

The present study was initiated to determine if there was any correlation between the results of the experiments on transfer of immunity and the lymphocyte traffic to the small intestine of mice infected with H. diminuta.

MATERIALS & METHODS

1. Lymphocyte transfer

Lymphocytes for transfer into recipients were taken from the MLN of donor mice. The MLN was removed, cleaned of fat and connective tissue, and stored in Medium 199 (including L-glutamine and 25mM HEPES buffer: Gibco Europe Ltd.) + 5% heat-inactivated foetal calf serum (Flow Laboratories) + 10 i.u. heparin / ml, on melting ice. When all the nodes had been collected, any remaining fat that had become apparent because of the low temperature was cleaned off, and the nodes were pressed through a fine nylon sieve with a plastic syringe plunger. The large fragments were allowed to settle out over 1-2 minutes; the supernantant was decanted into round-bottomed centrifuge tubes and centrifuged at 200 g for 4 minutes at 4°C. The supernantant was discarded and the cell pellet was made up to a known volume with fresh medium. After agitation with a pasteur pipette, the suspension consisted almost entirely of single cells. A little of this suspension was mixed 1 in 20 with a 2% w/v solution of trypan blue in HBSS, and the cells were counted with a haemacytometer. Viability, assessed by exclusion of the dye, was always over 90%. The original cell suspension was diluted to give the correct number of cells/ml, and the cells were injected into a tail vein of recipient mice in 0.2-0.5 ml.

Surgical challenge with strobilate worms was chosen as the method of assessing the transfer of immunity, as this has been found to be more consistent and sensitive than cysticercoid challenge (Hopkins & Zajac, 1976; personal observations).

2. Lymphocyte traffic

Lymphocytes were prepared as above, except that the medium used was prepared by taking 100 ml of RPMI 1640 (without glutamine or bicarbonate: Flow Laboratories) to which was added 0.8 ml of 7.5% sodium bicarbonate (Flow Laboratories), 1.0 ml of 200mM glutamine (Gibco Europe Ltd.) and 10 ml of heat-inactivated foetal calf serum (Flow Laboratories). 1 ml of cell suspension was added to a sterile plastic tissue culture grade tube (Nunclon Delta) containing 4 ml of medium including L-(^{75}Se)selenomethionine (The Radiochemical Centre, Amersham). The specific activity of the isotope was in the range 3-20 mCi/mg selenomethionine; isotope and cell concentration per ml at incubation is given for each experiment in the Results section, but was usually 1 μCi/10^7 cells/ml. The cells were incubated at 37$\pm$0.5^{o}C, centrifuged at 200g for 4 minutes at room temperature, and the supernatant removed. The cells were washed three times in Medium 199 (without serum or heparin), counted and tested for viability, and 5×10^7 cells in 0.5 ml were injected into recipient mice via a tail vein. In accordance with the findings of Rose et al. (1976a) who found maximum localisation of cells 24 hours after injection, recipient mice were killed 24 hours after injection of labelled cells. The small intestine, MLN, spleen and liver were removed, rinsed externally in HBSS, and placed in separate plastic vials for measurement of radioactivity. The organs were counted for gamma-emission on a Packard Tricarb Liquid Scintillation Spectrometer. The counts for the various organs, one dose of each batch of labelled cells (to assess the total injected radioactivity), and blank tubes (for background radiation counts) were recorded on a printer and the

figures treated as follows.

The percentage of the injected dose recovered from each of the small intestine, spleen, MLN and liver (%ID) was calculated as:

$$\%ID = \frac{\text{organ count - background count}}{\text{injected dose count - background count}} \times \frac{100}{1}$$

The percentage in an organ of the total radioactivity recovered from all four organs (%TR) was calculated as:

$$\%TR = \frac{\text{organ count - background count}}{\text{total count from all organs - (4 x background)}} \times \frac{100}{1}$$

All experiments in this Chapter were carried out with inbred NIH strain mice. Sterile instruments, vessels and media were used throughout, and cells were prepared for injection under very clean (but not necessarily sterile) conditions.

RESULTS

1. Attempts to transfer immunity against H. diminuta with MLNC

a) Characterisation of the response of NIH mice to surgical infection with H. diminuta

To test the response of NIH strain mice to surgical infection with H. diminuta and to make a comparison with the well-characterised CFLP strain, groups of NIH and CFLP female mice were challenged surgically at 9 weeks old with a single worm recovered from rat donors infected six days previously. Mice from both groups were killed on days 7, 9, 11, 13 & 15 p.i., and the dry weights of the worms recovered are shown in Fig. 4-1.

The majority of worms established successfully in both groups (judged by the recoveries on day 7 p.i.), and it is clear that there was no substantial difference in growth or survival of H. diminuta on subsequent days between the two strains of mice. In both strains, rejection commenced at about day 9 p.i.; recovery on day 11 p.i was around 50%, and rejection was virtually complete by day 13 p.i.

To investigate whether the NIH mouse is capable of mounting a strong secondary response to H. diminuta, immunised and non-immunised animals of this strain were challenged surgically with six-day-old rat worms. The immunised mice had been previously infected with five cysticercoids, and both groups were given anthelmintic 16 days later. All mice were surgically challenged 4-6 days after this, and autopsied on days 5 and 7 p.i.

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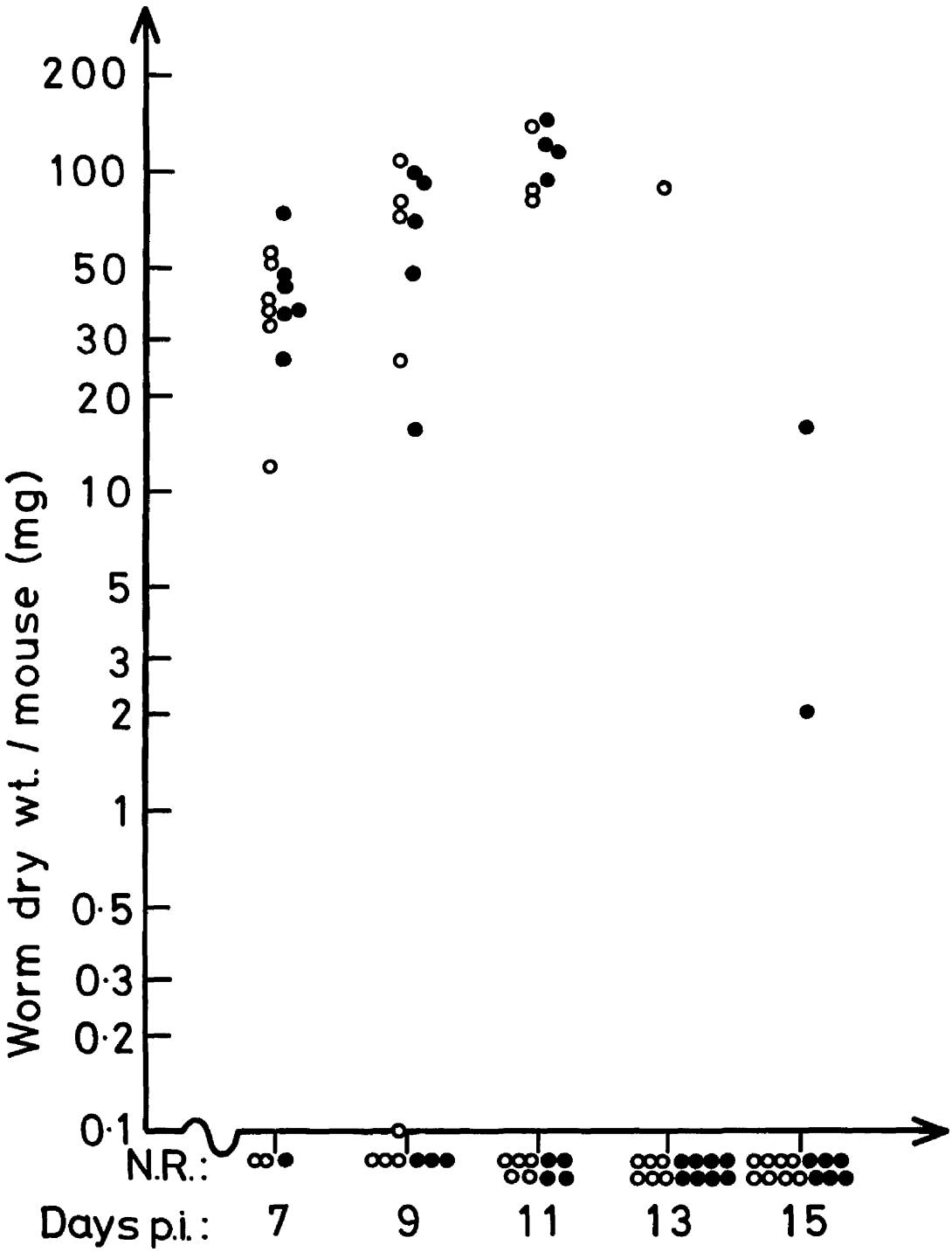
Figure 4-1

Dry weight of H. diminuta from surgically infected CFLP (o) or NIH (●) mice. Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


200
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Worm dry wt. / mouse (mg)
N.R.:
Days p.i.: 7 9 11 13 15

On day 5 p.i., 7/8 of the worms were recovered from the immunised mice (Fig. 4-2), but only 1/8 was recovered from the immunised mice; this latter worm weighed 0.8 mg (compared with a mean of 25.6 mg per surviving worm in the controls), and only destrobilated worms were recovered from the other seven mice. On day 7 p.i., 6/8 of the control mice retained worms, indicating that rejection of the worms had not commenced in this group; however, there were a large number of worms under 2 mg, which may indicate that the immune response was under way or that an unusually high proportion of the worms had been damaged at insertion and subsequently regrown. In any event, the immunised group again showed almost complete rejection, 1/9 of the worms remaining. This experiment therefore demonstrated that NIH mice are capable of mounting a fast, efficient secondary response, of a similar efficiency to that in CFLP mice (Hopkins & Zajac, 1976).

b) Test of cell transfer technique

Cell transfer technique was tested at the outset by transferring immunity against sheep erythrocytes. Cell donors were injected intraperitoneally with a suspension of sheep erythrocytes, and serum antibody response to challenge was found to be markedly enhanced in cell recipients. As stated in Materials & Methods for this Chapter, cell viability at transfer was always greater than 90%, but after a number of unsuccessful results with H. diminuta, the technique was re-tested as follows.

Five NIH mice were infected with 350 T. spiralis larvae, and the enlarged MLN's removed eight days later. 7×10^7 of the pooled cells were transferred to each of five recipients. These and five

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Figure 4-2

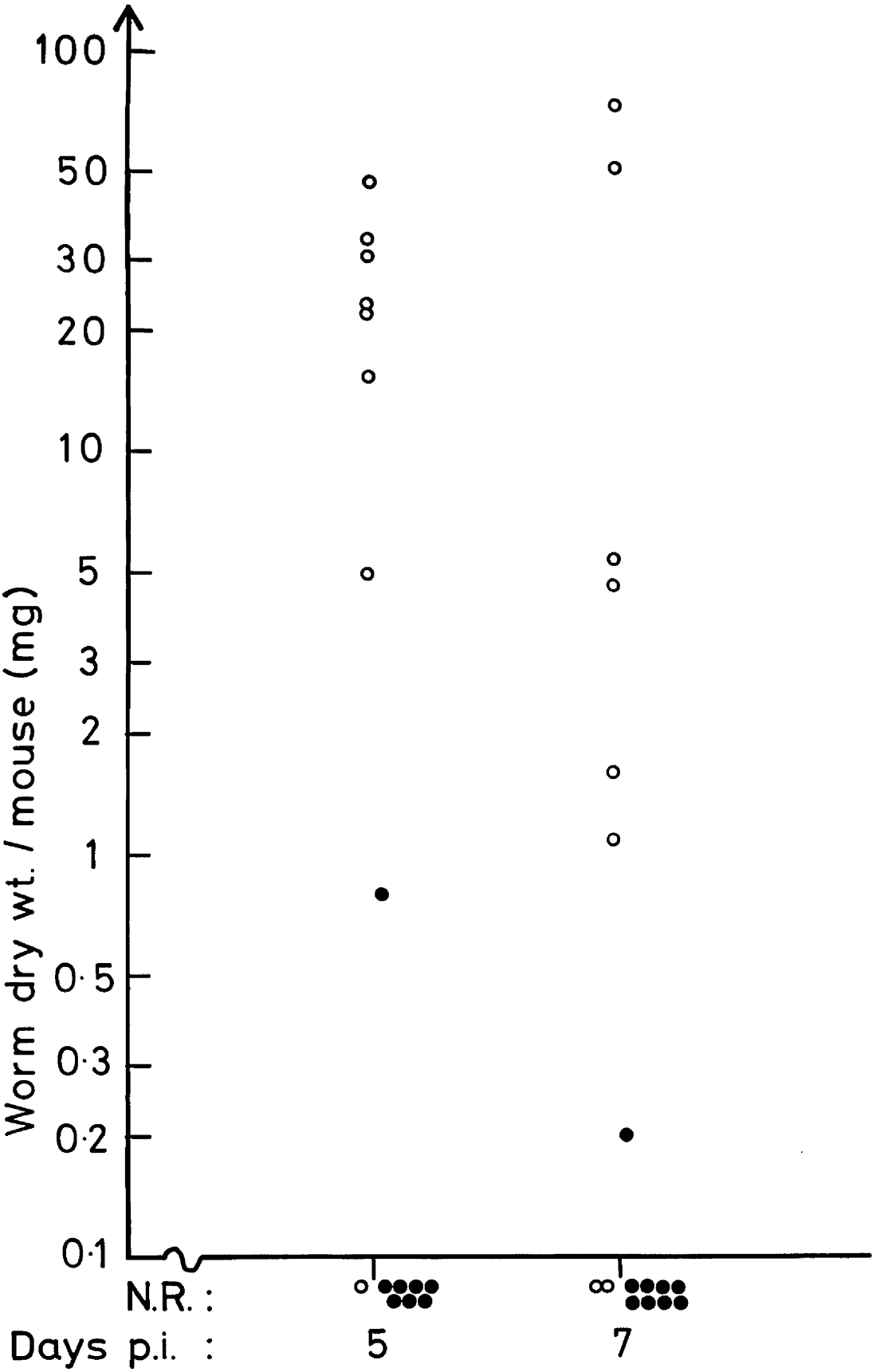
Dry weight of H. diminuta from surgical challenge of NIH mice.

o, previously uninfected mice

•, mice infected with five cysticercoids 20-22 days before challenge.

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Worm dry wt. / mouse (mg)
N.R. :
Days p.i. :
5
7

control mice were challenged with 350 T. spiralis larvae on the day of cell transfer, and autopsied eight days later for counting adult worms in the small intestine. A mean of 225±39 worms was recovered from the control mice, and a mean of 83±62 worms from the cell recipients. The worms recovered from tha latter group were also obviously smaller, but no measurements were taken. This experiment thus shows that the technique employed was indeed sufficient to transfer immunity against an intestinal parasite.

c) Transfer of immunity against H. diminuta: lymphocytes taken during a primary infection

Four experiments were carried out in which cell donors were infected with six cysticercoids of H. diminuta, and the MLNC were harvested on days 7, 14, 16 & 21 p.i. (one day for each experiment). $5x10^7$ MLNC were injected into recipient mice, which were challenged surgically one day later. Cell recipients and control (no cells) mice were autopsied on day 6 p.i.; the results for all four experiments are shown in Fig. 4-3.

Although the control results for the day 14 and 16 MLNC experiments were less than satisfactory (probably due to transplanting damaged worms), there was no indication from any of these experiments that transfer of $5x10^7$ MLNC taken from mice at various stages of infection had any effect on establishment, growth or survival of a challenge infection.

I had noticed in many experiments (mainly from the work for Chapter 2) that mice infected surgically with H. diminuta often had greatly enlarged MLN's with many germinal centres, particularly on

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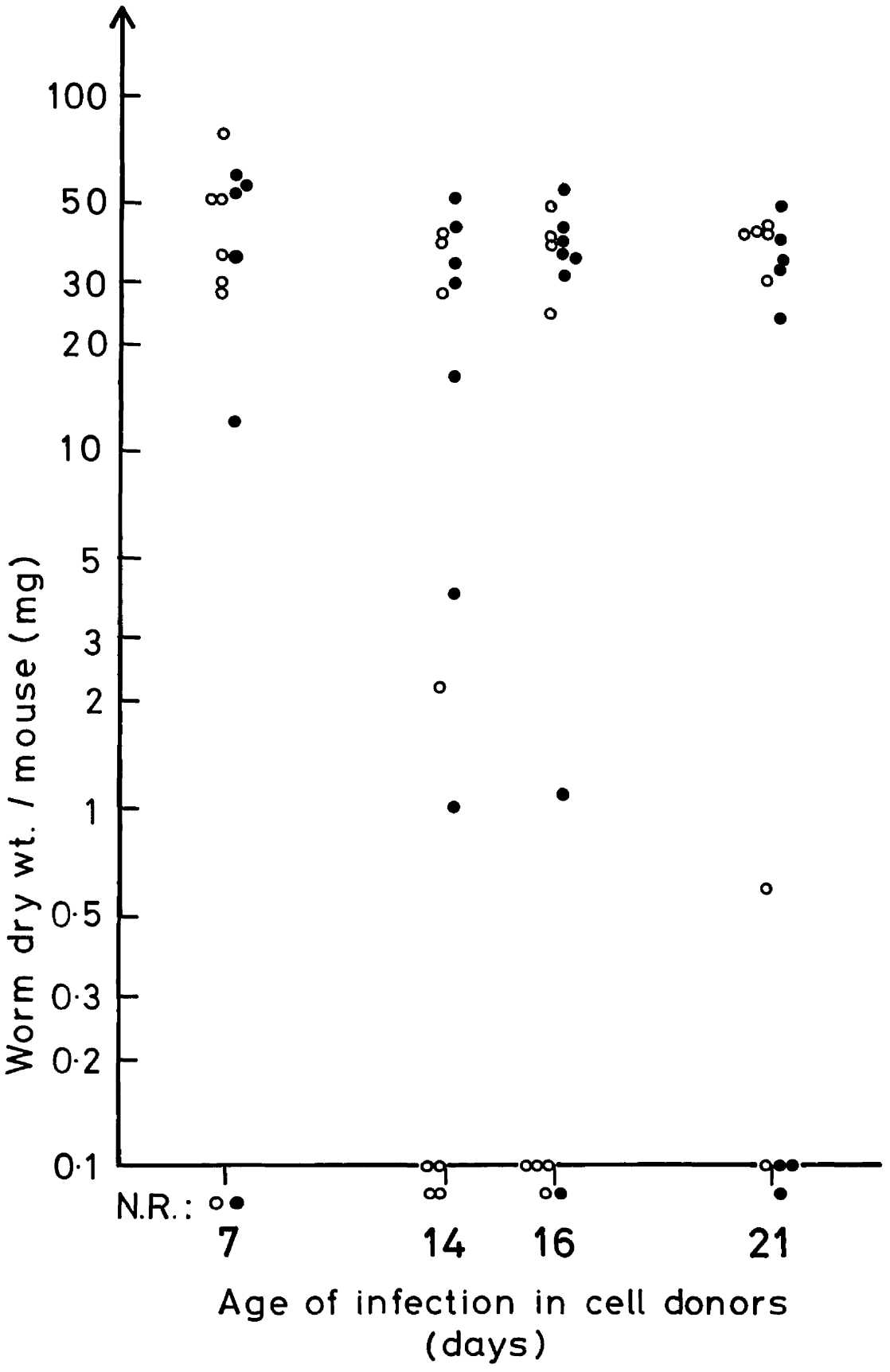
Figure 4-3

Dry weight of H. diminuta from NIH mice six days after surgical challenge.

o, control mice (no cells given)

●, mice injected i.v. with 5×10^7 MLNC from donors infected at the times shown.

N.R., mice from which no weighable worms were recovered.


100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
Worm dry wt. / mouse (mg)
N.R.:
7
14
16
21
Age of infection in cell donors (days)

day 9 p.i. This may have been due to microorganisms introduced into the gut lumen or wall while transplanting the worms, escape of microorganisms from the gut lumen, nonspecific stimulation of division of lymphocytes by surgical stress, or intense stimulation of the node by the large worm in the gut lumen.

To test if the node was in fact reacting to the presence of the worm, $5x10^7$ day 9 MLNC from surgically infected cell donors were transferred into recipient mice. Cell recipients and control mice were challenged surgically one day later and autopsied on day 7 p.i.

The results (Fig. 4-4) again showed that there was no difference in growth or survival of a challenge infection. 100% of the worms were recovered from both groups, and the mean dry weight per mouse was actually higher in the cell recipients (29.0 mg) than in the controls (26.9 mg)

Experiments were carried out to determine whether the number of cells transferred in the above experiments was too low to effectively transfer immunity against H. diminuta infection. $2x10^8$ MLNC is approaching the maximum tolerable intra-venous dose for a mouse, so this level of transfer was studied.

In the first two experiments, MLNC were taken from donors infected eight days previously with five cysticercoids. Recipient mice received $2x10^8$ MLNC, and in the first experiment (Expt. 1) recipient and control mice were challenged surgically two days later. A preliminary kill on day 7 p.i. indicated no difference in worm weights or recovery between the two groups, so the majority of the mice in this experiment were killed on day 9 p.i.

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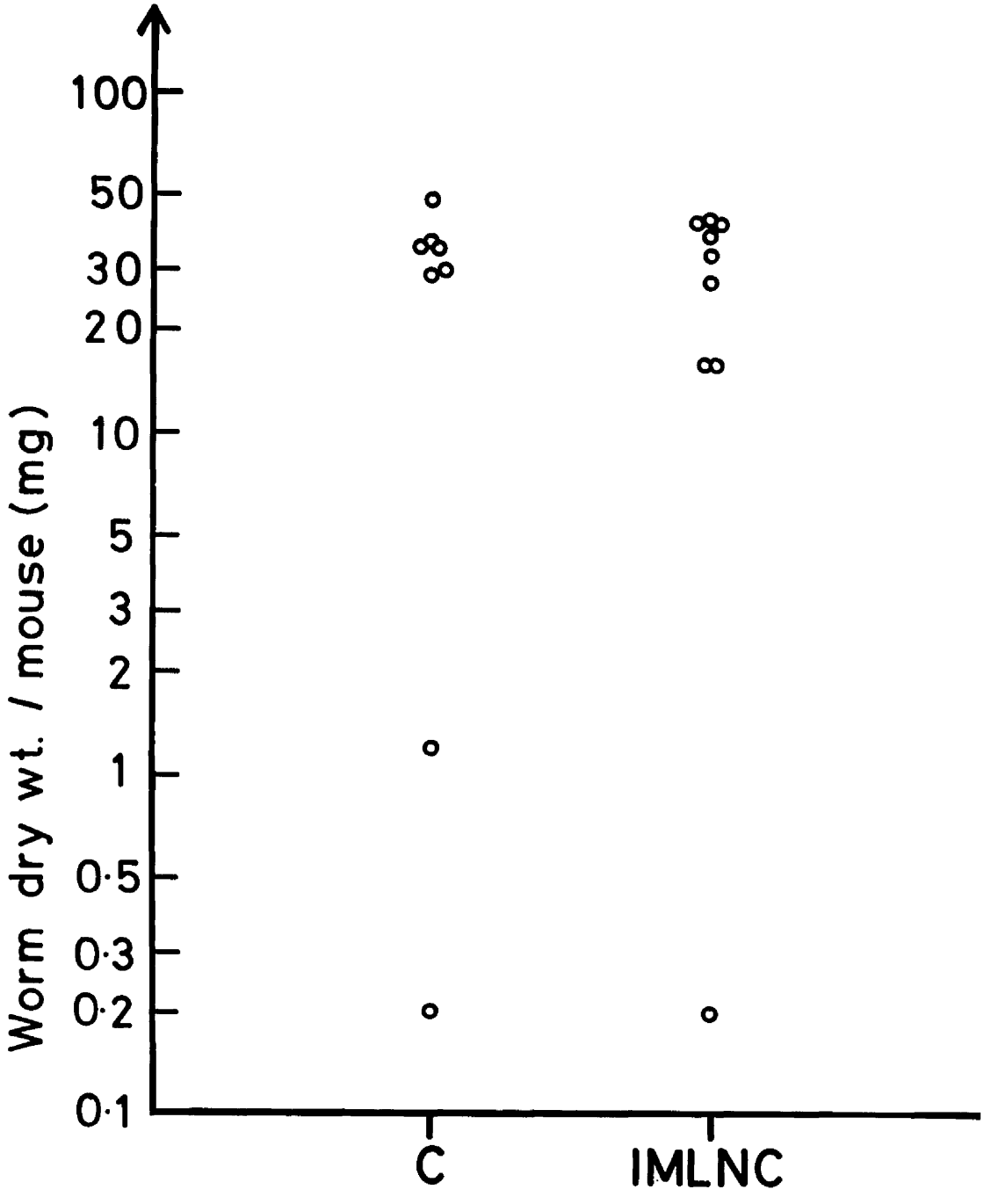
Figure 4-4

Dry weight of H. diminuta from NIH mice seven days after surgical challenge.

C, control mice (no cells given)

IMLNC, mice receiving 5×10^7 MLNC from donor mice infected surgically nine days before cell transfer.

Each point represents the total weight of worm tissue from a single mouse.


Worm dry wt. / mouse (mg)
100
50
30
20
10
5
3
2
1
0·5
0·3
0·2
0·1
C
IMLNC

9/12 of the control mice retained worms (Fig. 4-5, Expt. 1), but only 2/12 of the cell recipients were found to be infected, indicating that the cell recipients were rejecting their worms earlier than the control mice.

A repeat of this experiment was conducted, only this time the surgical challenge was seven days after cell transfer. In addition to the normal controls, a group of mice given 1.5×10^8 naive MLNC (i.e. from uninfected donors) was included. All mice were again killed on day 9 p.i.

The results (Fig. 4-5, Expt. 2) show that although the mean worm weight per mouse of the group receiving immune MLNC was lower (35.0 mg) than that of the group receiving naive MLNC (58.3 mg), it was similar to the mean for the controls (38.0 mg), and none of the groups was significantly different from any other group.

In the next experiment, MLNC were taken from mice infected with five H. diminuta 21 days previously. Cell recipients were injected with 2×10^8 MLNC; cell recipients and controls were challenged surgically seven days later, and were autopsied on days 7 and 9 p.i.

On day 7 p.i., 7/8 of the controlworms were recovered (Fig. 4-6), whereas only 3/8 of the worms were recovered from cell recipients. On day 9 p.i., all of the cell recipients had lost their worms, but 3/7 of the control worms remained. This experiment therefore suggests that 2×10^8 day 21 immune MLNC were effective in accelerating expulsion of H. diminuta.

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Figure 4-5

Dry weight of *H. diminuta* from NIH mice killed nine days after surgical challenge.

C, control (no cells given)

IMLNC, 2×10^8 MLNC from donor mice infected with five cysticercoids eight days before cell transfer

NMLNC, 1.5×10^8 MLNC from naive (uninfected) donor mice.

N.R., mice from which no weighable worms were recovered.

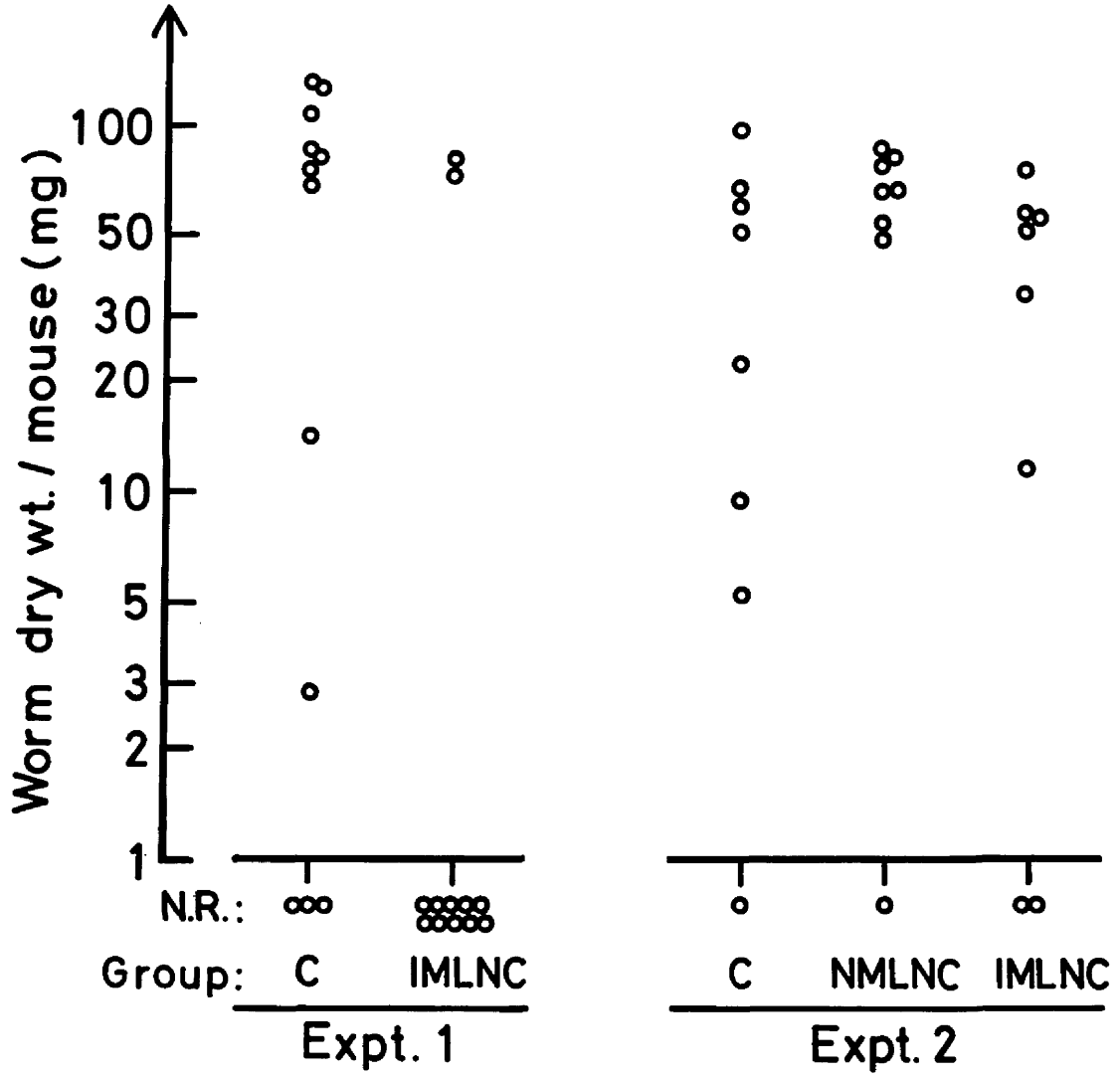
Worm dry wt. / mouse (mg)
100
50
30
20
10
5
3
2
1
N.R.:
Group:
C
IMLNC
C
NMLNC
IMLNC
Expt. 1
Expt. 2

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Figure 4-6

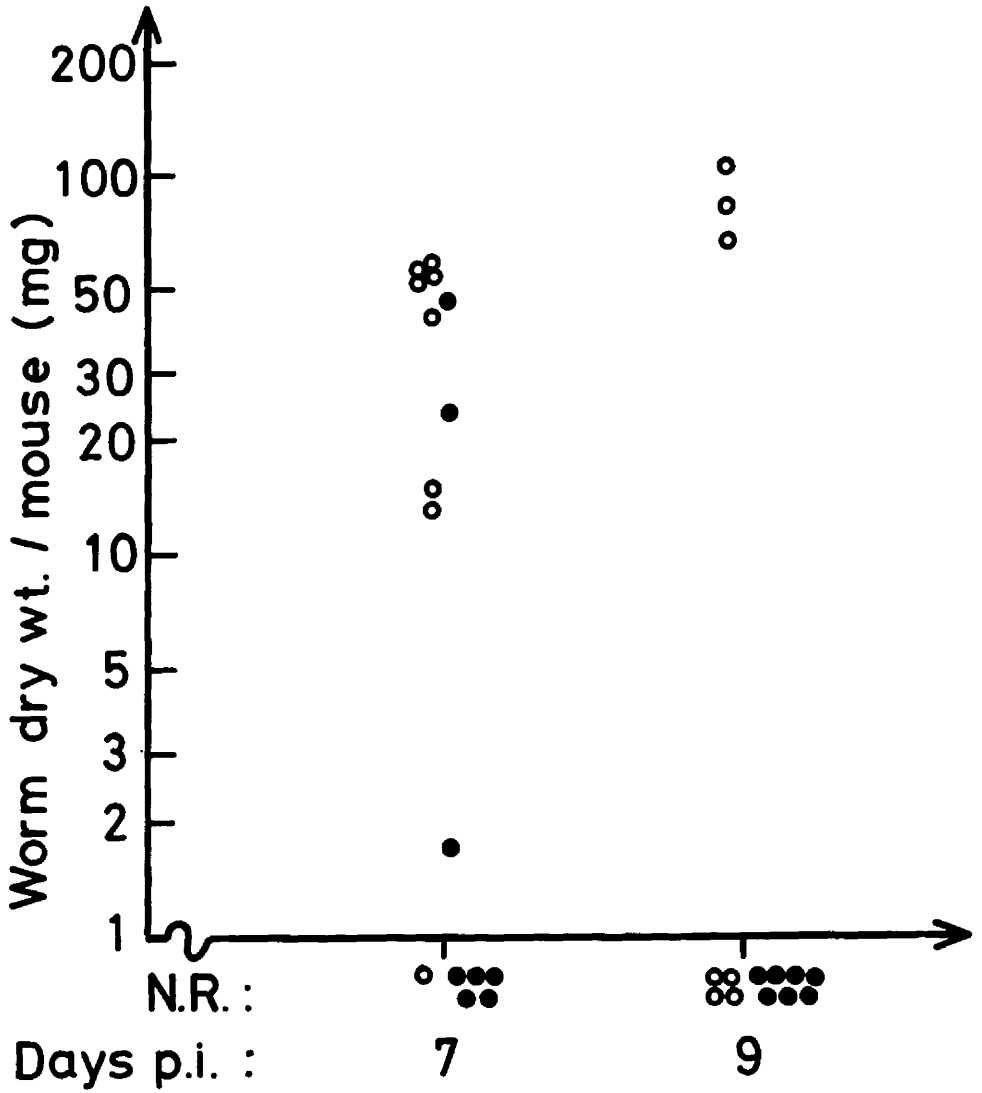
Dry weight of H. diminuta from NIH mice after surgical challenge.

o, control mice (no cells given)

●, mice injected i.v. with 2×10^8 MLNC from donors infected with five cysticercoids 21 days prior to cell transfer

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


200
100
50
30
20
10
5
3
2
1
Worm dry wt. / mouse (mg)
N.R. :
Days p.i. :
7
9

d) Transfer of immunity against H. diminuta: lymphocytes taken during a secondary infection

(i) Using low numbers of cells

A series of experiments was conducted to determine whether a second exposure to H. diminuta would stimulate an enhanced response in the MLN that would make transfer of immunity possible with fewer than $2x10^8$ MLNC.

In the first experiment, cell donor mice were infected with five H. diminuta and given a challenge infection with a further five worms 21 days later. MLNC from these mice were collected on day 7 of the challenge infection, and $7x10^7$ MLNC were transferred to each recipient. Cell recipients and control mice were challenged surgically two days later, and killed on days 7 and 9 p.i.

The results (Fig. 4-7, Expt. 1) show that 6/8 of the worms were present in the cell recipients, and these worms were, if anything, heavier than those recovered from the small number of control mice. On day 9 p.i., both groups retained similar numbers of worms (3/8 and 3/9 in cell recipient and control groups respectively), the surviving worms from the control mice being slightly heavier than those from the cell recipients.

In a repeat experiment, donor mice were infected with five cysticercoids, and challenged with a further five on day 20 p.i. Seven days after challenge, the MLNC were collected, and $6.5x10^7$ MInc transferred into each recipient. Cell transfer and control mice were challenged surgically seven days later, and as a preliminary kill on day 7 p.i. indicated no difference between the groups, the majority of the mice were killed on day 9 p.i.

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Figure 4-7

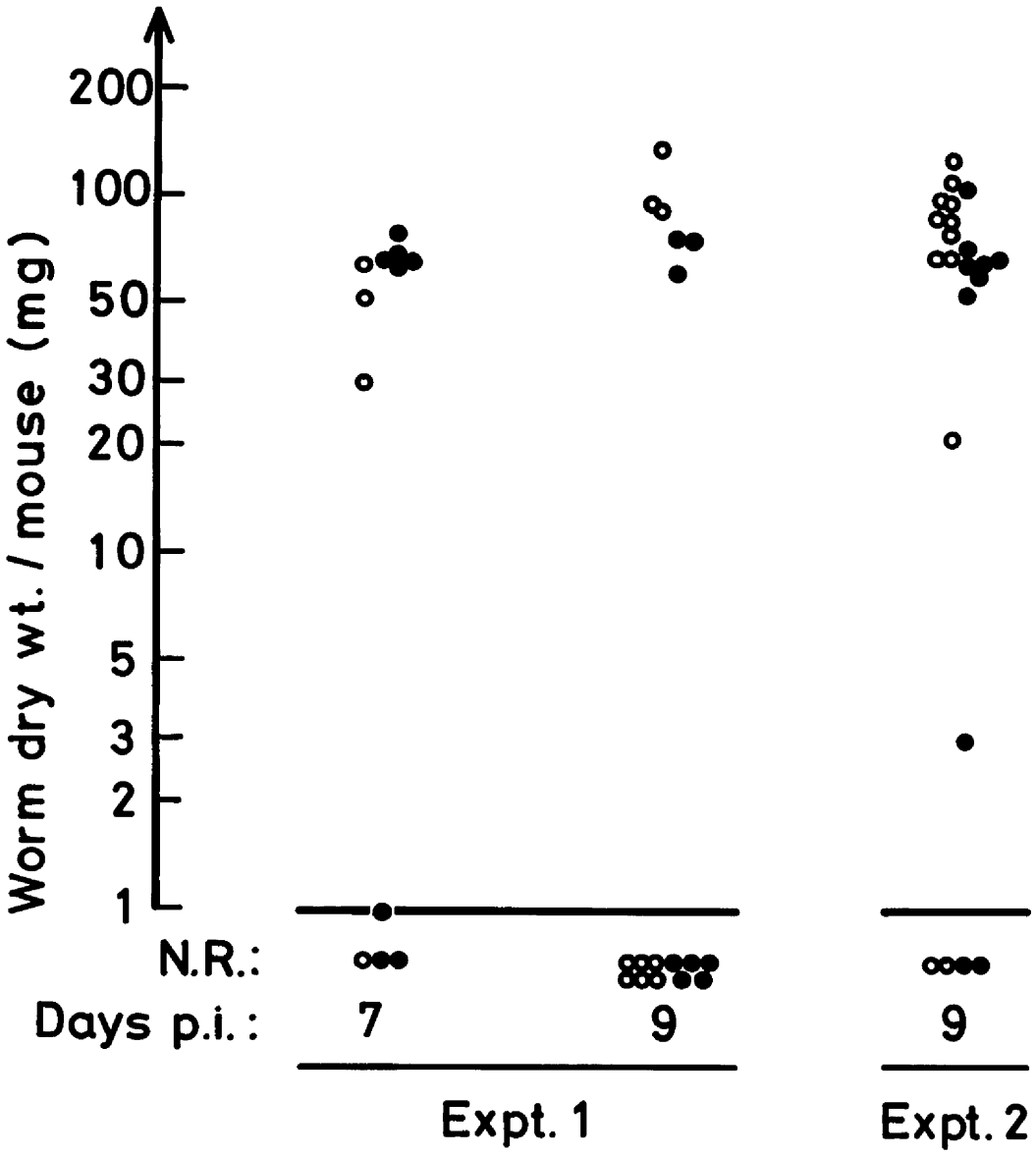
Dry weight of H. diminuta from NIH mice on days 7 and 9 (Expt. 1) or day 9 (Expt. 2) of a surgical infection.

o, control mice (no cells given)

●, mice injected i.v. with 7×10^7 MLNC from donor mice infected with five cysticercoids 28 or 27 days and 7 days prior to cell transfer.

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


Worm dry wt. / mouse (mg)
200
100
50
30
20
10
5
3
2
1
N.R.:
Days p.i.:
7
9
9
Expt. 1
Expt. 2

Although there was essentially no difference in recovery of worms between the two groups (Fig. 4-7, Expt. 2), the worms recovered from the cell recipients were significantly lighter than those from control mice ($p<0.01$); the reduction in mean dry weight was about 30%.

(ii) Using high numbers of cells

Donor mice were infected with five cysticercoids and challenged with a further five on day 19 p.i. MLN were collected seven days later and cell recipients were injected with $1.4-1.7\times10^8$ MLNC. These and control mice were challenged surgically seven days later and autopsied on days 7 and 8 p.i.

On day 7 p.i., 5/8 of the worms were recovered from the control group (Fig. 4-8, Expt. 1), whereas only 1/7 of the cell recipients was found to be infected. As the control result was not very satisfactory, the remainder of the mice were killed on day 8 p.i. to test the observed difference. On day 8 p.i. the situation was very similar, 6/8 and 2/7 worms remaining in the control and cell transfer mice respectively. Combining the results for the two days, 73% of the worms were recovered from control mice, but only 21% from the cell recipients. This therefore indicated that some degree of enhanced immunity had been transferred.

This experiment was repeated, using 2×10^8 MLNC taken from mice immunised with five worms for 21 days followed by seven days of an identical challenge infection. Cell recipients and control mice were challenged surgically seven days after cell transfer, and autopsied on day 7 p.i.

The results (Fig. 4-8, Expt. 2) show that this time there

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Figure 4-8

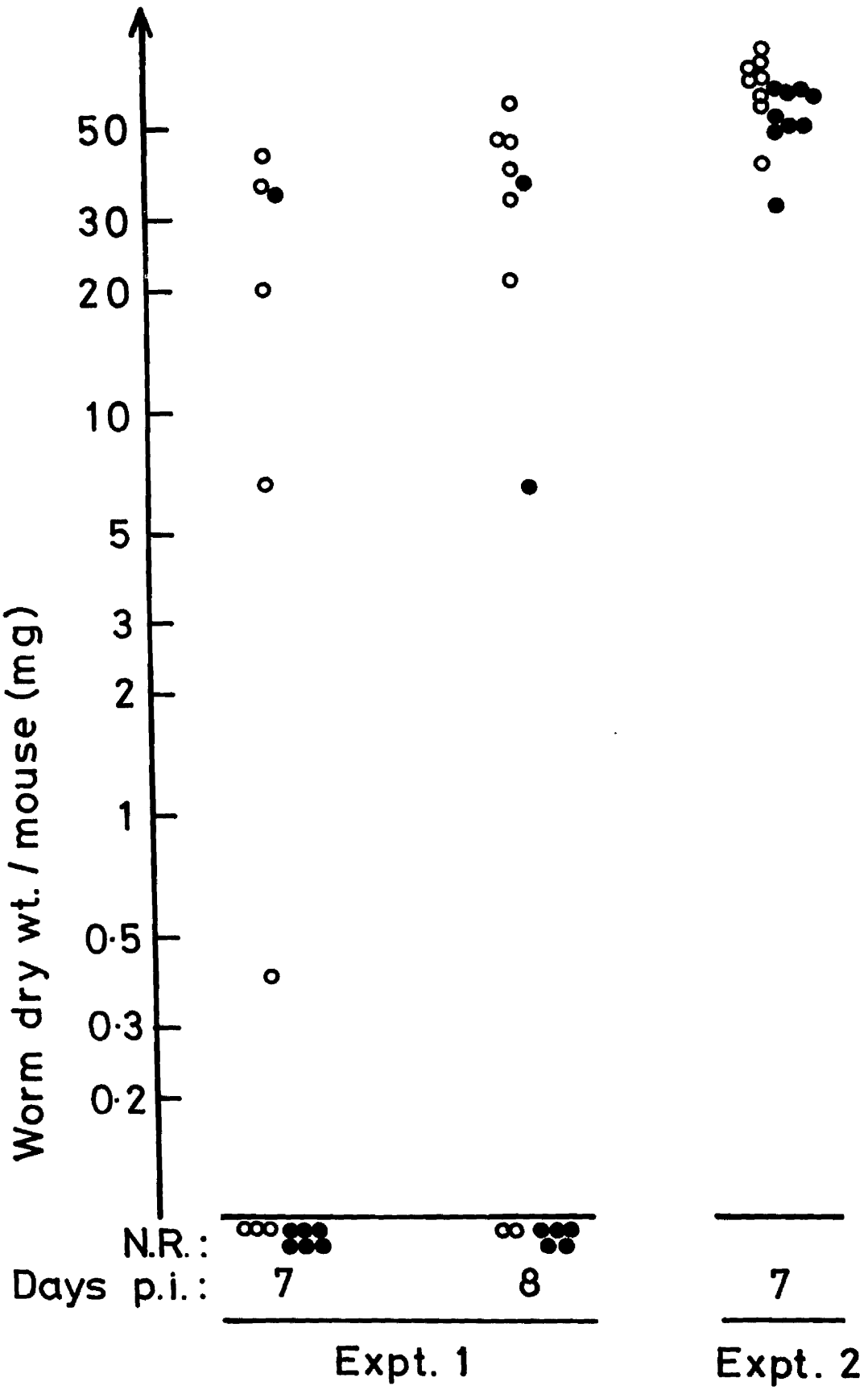
Dry weight of H. diminuta from NIH mice on days 7 and 8 (Expt. 1) or day 7 (Expt. 2) of a surgical infection.

o, control mice (no cells given)

•, mice injected i.v. with 2×10^8 MLNC from donor mice infected with five cysticercoids 26 and 7 (Expt. 1) or 28 and 7 (Expt. 2) days prior to cell transfer.

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered; Days p.i., days post infection.


Worm dry wt. / mouse (mg)
50
30
20
10
5
3
2
1
0·5
0·3
0·2
N.R.:
Days p.i.:
7
8
7
Expt. 1
Expt. 2

was no difference in recovery of worms between the two groups (100% from both groups), and although the worms from the cell recipients were lighter than the control worms ($p<0.05$), the mean worm weight was only 16% lower. This batch of mice was subsequently found to be heavily infected with an intestinal protozoan, probably Hexamita sp., and this may have adversely affected the outcome of this experiment.

In a third experiment, cell donors were infected with five cysticercoids and challenged with a further five on day 21 p.i. MLN were collected on day 4 of the challenge infection, and 1.3×10^8 MLNC were transferred into recipient mice. these and control mice were challenged surgically seven days later, and autopsied on day 7 p.i.

No evidence for rejection was found in either group (Fig. 4-9); 7/8 and 6/6 worms were recovered from controls and cell recipients respectively. the mean weights of the worms recovered were also very similar (45.6 mg and 44.6 mg respectively), indicating that transfer of 1.3×10^8 day 4 secondary infection MLNC had no effect on a challenge infection with H. diminuta.

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Figure 4-9

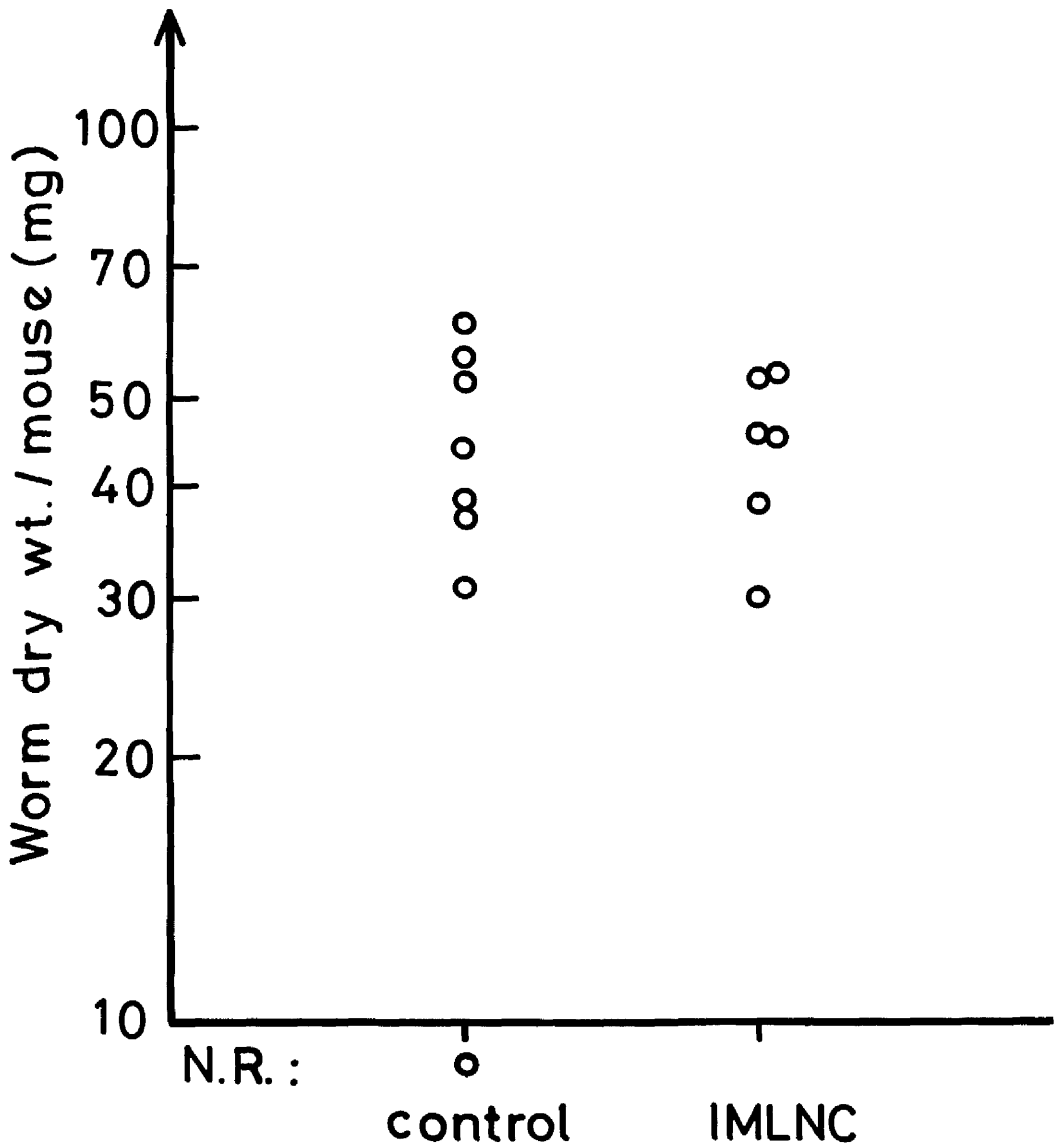
Dry weight of H. diminuta from NIH mice seven days after surgical challenge.

control, no cells given

IMLNC, mice injected i.v. with 1.3×10^8 MLNC from donor mice infected with five cysticercoids 4 and 25 days prior to cell transfer.

Each point represents the total weight of worm tissue from a single mouse.

N.R., mice from which no weighable worms were recovered.


Worm dry wt./mouse (mg)
100
70
50
40
30
20
10
N.R. :
control
IMLNC

2) Lymphocyte traffic in mice infected with H. diminuta

a) Test of method and technique using T. spiralis

A preliminary experiment was carried out to determine firstly, that the enhanced migration of cells to the small intestine of mice four days after infection with T. spiralis (Rose et al., 1976a) was detectable using L-(^{75}Se)-selenomethionine , and secondly that the techniques involved were sound. MLNC were taken from uninfected mice or mice infected with T. spiralis four days previously, and were incubated at 2 μCi/10^7 cells/ml. Uninfected recipients were injected with the cells from the uninfected donors, and the cells from the infected donors were injected into mice infected with T. spiralis four days previously. All cell recipients were killed 24 hours later, and the percentage of the injected dose (%ID) and the percentage of the total recovered radioactivity (%TR) calculated from the radioactive counts (Table 4-1).

The radioactivity recovered from the small intestine, and thus probably the number of lymphocytes accumulating in the lamina propria, was significantly greater both as %ID and %TR in the group infected with T. spiralis, confirming the findings of Rose (1976a), and establishing that the technique and isotope used were capable of detecting this difference.

b) Comparison of the accumulation of labelled lymphocytes in the small intestine of mice infected with T. spiralis or H. diminuta

In the first experiment studying distribution of lymphocytes in H. diminuta infection, a group of mice infected with T. spiralis

Table 4-1

Radioactivity in the small intestine of mice infected with *T. spiralis* following i.v. injection of (^{75}Se)selenomethionine labelled MLNC.

	Control (n = 4)	*T. spiralis* (n = 3)	increase in infected mice
%ID	9.9 ± 0.6	14.7 ± 3.6 *	48.5%
%TR	22.4 ± 2.4	29.9 ± 3.1 *	33.5%

%ID, percentage of the injected dose recovered

%TR, percentage of the total recovered radioactivity

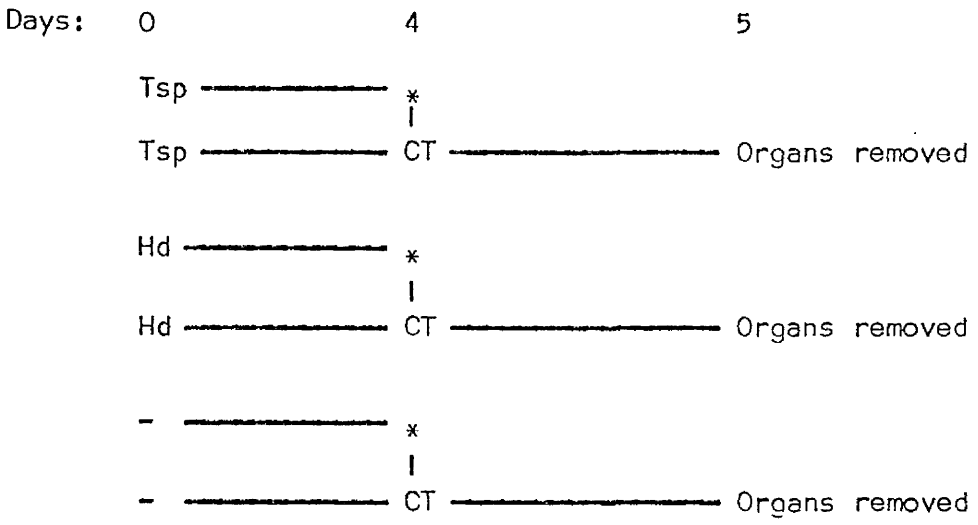
T. spiralis, 350 *T. spiralis* larvae four days previously

*, significantly different from controls ($p < 0.05$)

(Student's t test)

was included as an extra control to indicate that the technique was sound in the event of a negative result with H. diminuta. Three groups of four mice were injected with labelled MLNC from donors treated identically to themselves, i.e. uninfected, infected with 350 T. spiralis or five H. diminuta four days previously, as shown in Table 4-2.

Table 4-2



Tsp, 350 T. spiralis; Hd, 5 H. diminuta

*, MLNC removed and incubated with isotope; CT, MLNC injected i.v.

MLNC were incubated at 1 μCi/10^7 cells/ml. The values of %ID and %TR were calculated for the small intestine, MLN, spleen and liver for the three groups.

The value of %ID for the small intestine was higher than the control value in both infected groups (Table 4-3), though only significantly so in the group infected with T. spiralis, which showed an increase over the control value of 111%. However, an increase in %TR occurred only in the mice infected with T. spiralis,

Table 4-3

Recovery of the radioactivity from organs of mice infected with 350 *T. spiralis* (Tsp) or five *H. diminuta* (Hd) four days previously.

Group		Small Intestine	MLN	Spleen	Liver	Total	n
Control	%ID	9.1 ± 1.6	5.3 ± 2.0	6.5 ± 1.9	12.1 ± 1.0	33%	4
	%TR	29.3 ± 3.4	15.5 ± 5.3	19.3 ± 6.1	36.0 ± 4.6		
Tsp	%ID	19.2 ± 1.0**	8.0 ± 1.9	6.2 ± 0.7	15.1 ± 1.3	48.5%	4
	%TR	39.4 ± 0.5**	16.3 ± 3.4	12.8 ± 1.9	31.3 ± 3.2		
Hd	%ID	12.4 ± 1.4	6.7 ± 1.9	7.4 ± 0.2	15.4 ± 1.9	41.9%	4
	%TR	29.6 ± 2.8	15.9 ± 4.6	17.8 ± 0.5	36.8 ± 4.6		

**, $p<0.01$, significant difference from controls (Student's t test)

again a highly significant difference. MLN counts were identical in the three groups, but %TR for the spleen was lower in the mice infected with T. spiralis, indicating some redistribution of the labelled cells to offset the increase in localisation in the intestine.

It can also be seen from Table 4-3 that the total label recovered expressed as a percentage of the injected dose was higher in the two infected groups than in controls, with the value for the mice infected with T. spiralis highest of all. While this could be explained as a primary increase of MLNC at the small intestine (as described by Rose et al., 1976a), in this experiment the sequence of processing the cells was the same (T. spiralis, H. diminuta, controls) and the three groups of cells took up progressively less isotope. When this latter phenomenon occurred in subsequent experiments, the isotope-containing medium was held on ice in a single bottle until required, rather than dispensed into tubes at the outset of the experiment and waiting for 1, 2 or 3 hours before the cells were added. This procedure reduced the tendency somewhat, presumably by reducing adsorption of the isotope onto the culture tubes, but the differing uptake of isotope may have been at least partly due to the metabolic activity of the population of MLNC being incubated; actively dividing lymphoblasts would presumably take up more of the label than small lymphocytes, and as mice infected with T. spiralis have an increased proportion of blast cells in the MLN (Rose et al., 1976a), one might expect MLNC from these mice to take up more label. This topic is further pursued in the Discussion.

c) Lymphocyte traffic in mice infected with 50 H. diminuta

MLNC were taken from control and infected mice, the latter group having received 50 cysticercoids four days previously. The cells were incubated at 1 μCi/10^7 cells/ml, and the cells from the infected donors were injected into recipients also infected with 50 H. diminuta four days previously, and control cells into uninfected recipients.

In this experiment (Table 4-4) there was no increase in arrival of labelled cells at the small intestine of the infected animals, either as %ID or as %TR. However, MLN counts in the infected group were increased by 59% and 43% respectively (statistically significant difference from controls for both figures), indicating that the MLN of the mice heavily infected with H. diminuta was more attractive to MLNC than the resting (control) MLN.

d) Lymphocyte traffic in mice infected six or eight days previously with five H. diminuta

As increasing the level of challenge had no effect on lymphocyte accumulation in the small intestine (part c) above), it is possible that the migration of cells to the intestine occurs at a different time to that observed with T. spiralis. Accordingly, an experiment was designed in which one group of mice (donors and recipients) remained uninfected, and two other groups (donors and recipients) were infected with five cysticercoids six or eight days prior to cell incubation and transfer. MLNC were incubated at 2 μCi/10^7 cells/ml.

Again there was no significant difference between the amounts

Table 4-4

Recovery of radioactivity from the organs of mice infected with 50 *H. diminuta* four days before injection of labelled cells.

Group		Small intestine	MLN	Spleen	Liver	Total	n
Control	%ID	10.0 ± 0.2	6.0 ± 0.9	7.4 ± 0.7	13.4 ± 1.4	36.8%	4
	%TR	27.3 ± 1.9	16.4 ± 1.9	20.2 ± 1.6	36.3 ± 2.9		
Hd	%ID	10.1 ± 1.3	9.6 ± 1.5**	8.0 ± 1.4	13.2 ± 0.9	40.9%	4
	%TR	24.8 ± 1.8	23.4 ± 2.1**	19.5 ± 3.3	32.3 ± 1.2		

Hd, *H. diminuta*; **, $p < 0.01$, significant difference from controls (Student's *t* test)

of radioactivity recovered from the small intestine of control mice and either infected group (Table 4-5). The %ID values for MLN, spleen and liver were higher in the group infected eight days previously, but again this was the first group of cells to be labelled; the count per injected dose was higher in this group, and this may have accounted for the increase in total recovered radioactivity.

e) Lymphocyte traffic in mice with a secondary infection of H. diminuta

In an attempt to magnify any differences which exist in lymphocyte accumulation (but which were not detectable with the techniques employed) in the small intestine of mice infected with H. diminuta, mice were immunised with five cysticercoids and challenged with a further five on day 21 p.i. MLNC were collected on day 4 of the challenge infection, and naive and immune cells were incubated at 1 μCi/10^7 cells/ml. Labelled cells were injected into naive and immune recipients respectively (recipients treated identically to donors).

The %ID figures for small intestine, MLN, spleen and liver were all increased in the infected animals, but again a greater percentage of the radioactivity was recovered from the infected group (Table 4-6). Again, it was the infected group cells that were processed first, but the injected dose count was under 10% higher than that for the control cells, indicating that the increase in total recovered radioactivity (almost 50%) may have been due to other factors operating within the recipient mice.

Table 4-5

Recovery of radioactivity from the organs of mice infected with five *H. diminuta* six and eight days before injection of labelled cells.

Group		Small intestine	MLN	Spleen	Liver	Total	n
Control	%ID	9.3 ± 1.5	4.9 ± 0.5	6.6 ± 0.6	16.7 ± 2.8	37.5%	3
	%TR	24.7 ± 1.3	13.2 ± 2.1	17.7 ± 2.0	44.3 ± 3.0		
Day 6 p.i.	%ID	9.2 ± 1.6	4.6 ± 0.7	7.2 ± 2.0	15.0 ± 2.9	36.0%	5
	%TR	25.7 ± 3.0	12.9 ± 0.8	20.0 ± 3.8	41.4 ± 1.3		
Day 8 p.i.	%ID	10.1 ± 1.0	6.9 ± 0.7	8.4 ± 1.1	20.6 ± 1.8	46.0%	5
	%ID	22.0 ± 1.7	15.0 ± 1.4	18.2 ± 2.8	44.8 ± 2.8		

Table 4-6

Recovery of radioactivity from the organs of mice injected with labelled lymphocytes during a secondary infection with *H. diminuta*

Group		Small intestine	MLN	Spleen	Liver	Total	n
Control	%ID	10.1 ± 0.2	4.4 ± 0.3	4.4 ± 0.9	12.4 ± 0.8	31.3%	5
	%TR	32.3 ± 1.8	13.9 ± 1.2	14.0 ± 2.5	39.8 ± 1.7		
Hd	%ID	13.6 ± 2.2**	6.2 ± 1.1	6.4 ± 1.2	19.8 ± 1.7	46.0%	8
	%TR	29.3 ± 3.0	13.6 ± 2.1	13.8 ± 1.3	43.3 ± 1.3		

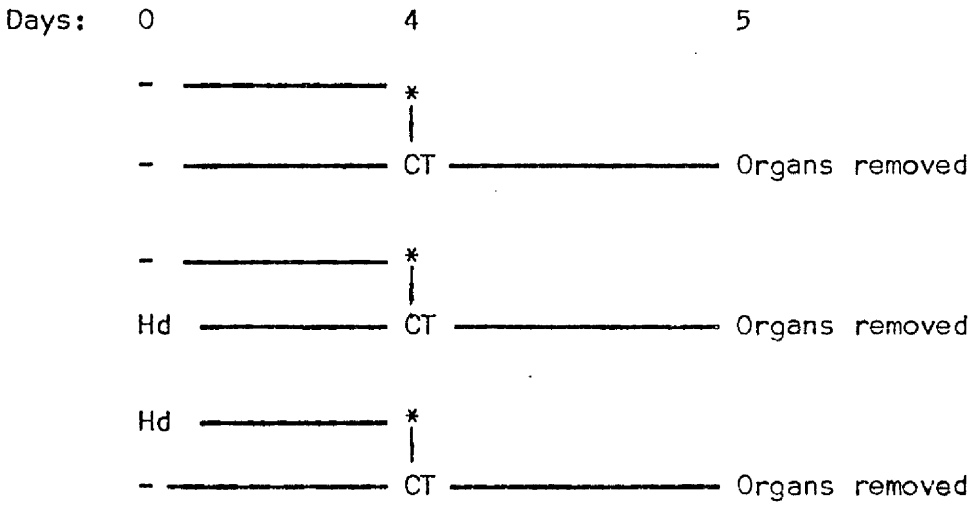
Hd, mice infected with five *H. diminuta* 25 and 4 days before cell transfer

**, $p < 0.01$, significant difference from controls. (Student's t test)

f) Transfer of 'immune' and naive lymphocytes into naive and 'immune' mice

To test if the few observed differences in cell accumulation noted thus far were due to the use of differing cell populations for the infected and uninfected recipients, an experiment was carried out in which naive cells were transferred after labelling into infected and uninfected recipients. Athird group was included in which cells from infected mice were injected into naive recipients; the design of the experiment is shown in Table 4-7.

Table 4-7



Hd, five H. diminuta

*, MLNC removed and incubated with isotope; CT, MLNC injected i.v.

The 'immune' mice were infected with five cysticercoids four days before cell transfer (donors and recipients), and the lymphocytes were incubated at 1 μCi/10^7 cells/ml.

There were no differences in either %ID or %TR figures between the immunised and naive recipients of the naive cells (Table 4-8), except that the spleen values were marginally higher

Table 4-8

Recovery of radioactivity from the organs of infected and uninfected mice injected with labelled lymphocytes from infected or uninfected donors.

Donor	Recipient		Small intestine	MLN	Spleen	Liver	Total	n
N	N	%ID	9.5 ± 0.8	5.4 ± 1.2	6.1 ± 0.5	17.0 ± 0.7	38.0%	5
		%TR	25.0 ± 1.6	14.2 ± 3.1	16.1 ± 1.4	44.8 ± 1.8		
N	I	%ID	9.5 ± 1.2	4.7 ± 1.2	7.8 ± 1.1	16.4 ± 0.4	38.4%	4
		%TR	24.7 ± 1.2	12.2 ± 2.5	20.3 ± 1.6	43.2 ± 2.6		
I	N	%ID	9.1 ± 0.5	5.5 ± 0.7	7.6 ± 1.2	14.6 ± 1.3	36.8%	5
		%TR	24.8 ± 0.8	15.1 ± 1.9	20.6 ± 3.4	39.5 ± 2.5		

N, uninfected (naive) mice; I, infected mice

in the infected recipients. The naive recipients of immune cells also showed a slight increase in the spleen counts, and the amount of isotope reaching the liver was slightly lower than in the controls. This experiment therefore demonstrated that use of differing cell populations did not disguise differences in migration to the small intestine of animals infected with H. diminuta four days previously.

3. The number of lymphocytes in the MLN during infection with H. diminuta and T. spiralis in mice

Data on the number of MLNC obtained per donor mouse were available from several of the cell transfer experiments reported in this Chapter, and this information was collected and plotted against the age of infection with H. diminuta or T. spiralis (Fig. 4-10). While this is unsatisfactory for many reasons, not least the lack of uninfected controls for the longer infections, it does give an idea of the difference in the response of the MLN to infection with these two parasites. In mice infected with T. spiralis, the number of cells in the node increases rapidly over the first few days of infection from a resting level of about 4×10^7 cells per node to a plateau (Wakelin, personal communication) at about 8×10^7 cells per node by day 8 p.i. In the case of H. diminuta infection, however, the cell number increases much more slowly, not reaching 7×10^7 cells until day 28 p.i. (this latter group received a challenge infection on day 21 p.i.).

Although the variation between the groups and the lack of controls for the effects on the node of maintenance under 'conventional conditions' make precise interpretation difficult, it is clear that the response of the MLN to H. diminuta infection is much slower and/or less profound than the response to the presence of T. spiralis.

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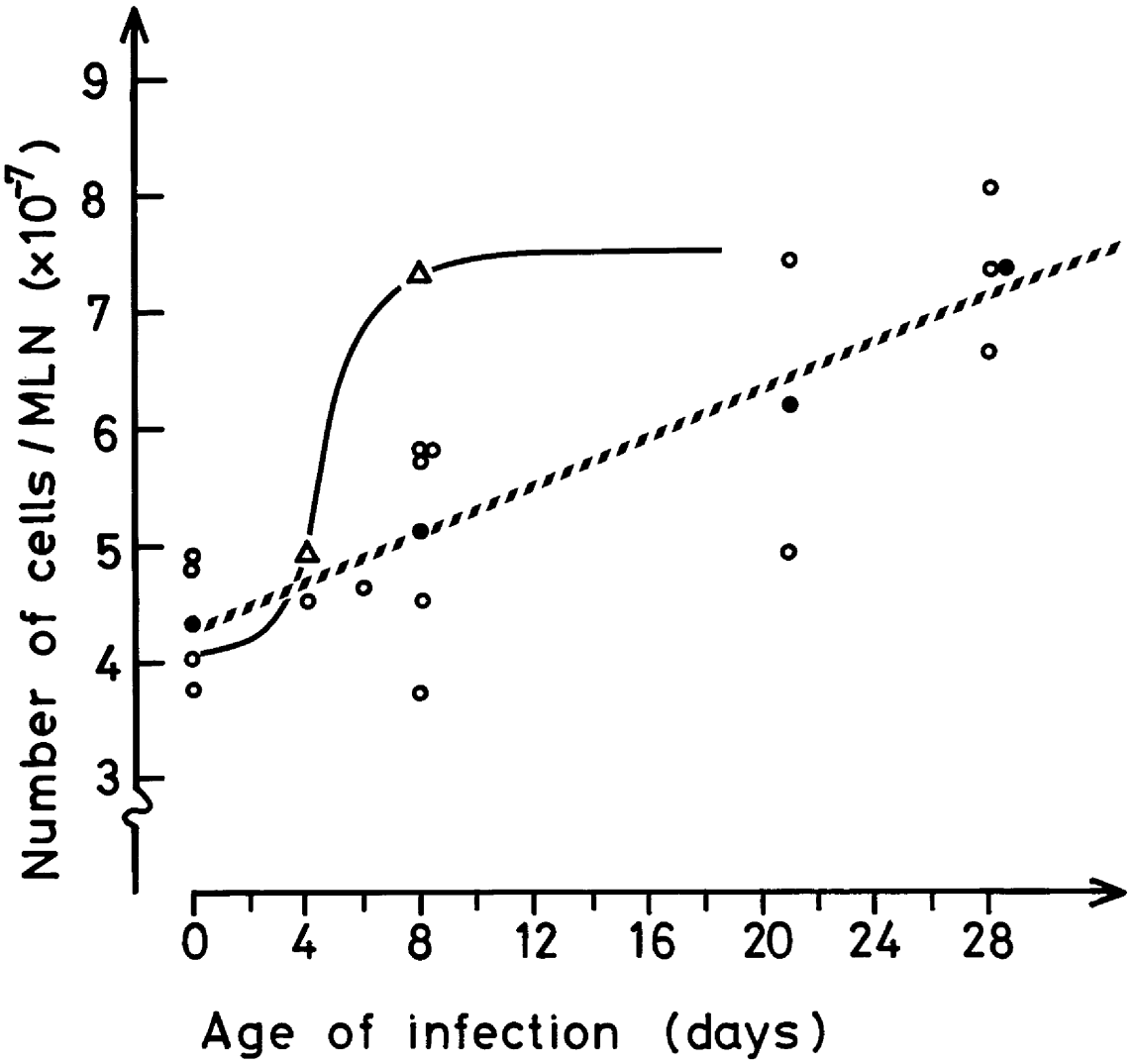
Figure 4-10

The mean number of cells in the MLN of NIH mice infected with *H. diminuta* (o) or *T. spiralis* ( ) .

Each point represents a single experiment, and is based on 5-10 mice (*T. spiralis*) or 15-30 mice (*H. diminuta*).

●, mean value for *H. diminuta* experiments.

T. spiralis (———) and *H. diminuta* (▰▰▰▰) curves fitted by inspection.


Number of cells / MLN (x10⁻⁷)
9
8
7
6
5
4
3
0
4
8
12
16
20
24
28
Age of infection (days)

DISCUSSION

Attempts to transfer immunity against H. diminuta using MLNC from donors infected with five or six worms were consistently unsuccessful with 5-7x10^7 MLNC taken at verious times during a primary and secondary infection, although this number of cells is higher than the total number normally found in the MLN of a single mouse. Using 2x10^8 MLNC per recipient (a donor:recipient ratio of about 4:1), successful reduction of the worm burden of the cell recipients was achieved with cells taken on day 8 of a primary infection, but more convincingly so with cells from day 21 p.i. and day 7 of a secondary infection (i.e. a difference in worm recovery was detected on day 7 of challenge, before rejection could have commenced in the controls). Although this latter finding was not consistent, the repeat experiments were carried out at a time when a protozoal infection of the intestine (probably Hexamita sp.) was widespread both in our own animals and in mice obtained from commercial suppliers, and this may have altered the behaviour of the cells in both donors and recipients. 1.3x10^8 MLNC taken on day 4 of a secondary infection caused no premature loss of H. diminuta in cell recipients, so presumably the minimum effective dose of MLNC in transferring immunity lies between 1.3 and 2.0x10^8 cells.

Transfer of high numbers of cells has apparently no profound effect on the normal kinetics of lymphocyte half-life or thymic output (Wallis, Leuchars, Chaudhuri & Davies, 1979), but 2x10^8 lymphocytes represents a very considerable addition to the lymphoid pool of the mouse; estimates of the total number of T cells per mouse vary from 1.5x10^8 (Sprent, 1973) to 4x10^8 (Wallis et al., 1979).

Rejection of H. diminuta from mice is a very effective and

rapid process, comparable to T. spiralis not only in that they are both intestinal parasites, but also in that both parasites are rejected immunologically by the mouse via a thymus-dependent process (Bland, 1976a; Wakelin & Wilson, 1979a) over a similar time-scale (the present study; Wakelin & Lloyd, 1976a). Response to a secondary infection is very rapid in both host-parasite systems (Hopkins & Zajac, 1976; Wakelin & Lloyd, 1976a), and a long-lasting immunological memory after infection is also common to both (Hopkins, 1980; Wakelin & Lloyd, 1976a).

However, the cellular kinetics of the response to T. spiralis differ profoundly from those of the response to H. diminuta in mice. Cells capable of transferring immunity against T. spiralis appear in the MLN of infected mice as early as day 4 p.i. (Wakelin & Wilson, 1977a). On day 8 p.i., which is considered by Wakelin & Lloyd (1976b) to be the day when most effective transfer of immunity is obtained, it is possible to transfer immunity against T. spiralis with as few as $1x10^7$ MLNC (Wakelin & Wilson, 1977a) or $3x10^6$ T cells (Wakelin & Wilson, 1979a). Not only was a large number of cells necessary for successful transfer of immunity, but also the most effective cells were obtained after day 8 p.i. with H. diminuta.

The differences between the two systems are further exemplified by the experiments investigating lymphocyte traffic to the small intestine. However, before discussing the results obtained, it is necessary to look critically at the techniques used. L-(^{75}Se)-selenomethionine is taken up by cells in a manner almost indistinguishable from the sulphur analogue methionine (Yousef & Luick, 1969), and has been recommended as a label for lymphocytes (Bainbridge, 1976; Rose & Micklem, 1976) with properties similar to ^{51}Cr as regards distribution after injection of labelled cells,

while being markedly less toxic than ^{51}Cr (Rolstad & Toogood, 1978). L-(^{75}Se)-selenomethionine was criticised by Rannie & Donald (1977) for the degree of utilisation of the label after elution from the lymphocytes, but as Bainbridge (1976) points out, elution tends to occur only in low-density accumulations of lymphocytes, so it is possible that reutilised label in non-lymphoid tissue is a reflection of the passage of lymphocytes through the organ as well as the radioactivity contained in the lymphocytes in the tissue at the time of measurement of radioactivity. Injected selenomethionine has a propensity for localisation in the liver and small intestine (Bainbridge, 1976; Rose & Micklem, 1976). Owing to lack of time the proper controls of measurement of free isotope transferred with the lymphocytes were not conducted in the present study, an omission that does not necessarily invalidate the results obtained; while the high liver counts recorded in the present study indicate some contamination of the cells with free isotope (see Bainbridge, 1976), this would tend to make the technique less sensitive rather than useless.

Bearing these caveats in mind, differences in the percentage of the injected label (%ID) and in the distribution of the recovered label in the major abdominal organs were found in mice infected with T. spiralis ; the differences in the small intestine counts were comparable to those described by Rose et al. (1976a) using the blast cell label (^{125}I)iodo-deoxyuridine (^{125}IUdR). In mice infected with H. diminuta there was, however, no appreciable change in the number of cells arriving at the small intestine at 4, 6 or 8 days p.i. compared with uninfected mice. The other organs (MLN, spleen and liver) sometimes showed differences from control results, but the only major difference was that observed in the MLN of mice

on day 4 of a 50-cysticercoid infection, indicating some degree of trapping of the labelled cells by the MLN.

In the experiment using mice with a secondary infection of H. diminuta, there were substantial increases in the %ID for all four organs, but the %TR values remained unaltered (i.e. there was no redistribution of label that would indicate, for example, that the small intestine was a more attractive site when infected). This highlights another problem in the interpretation of the results on cell traffic presented in this Chapter, namely the use of cells from uninfected donors for uninfected recipients, and infected donors for infected recipients. While this may give a more accurate impression of what is actually happening in vivo, the risk is that in transferring different populations of cells, possibly with differing percentages of blast cells and thus a differing pattern of isotope uptake, the differences observed may be a characteristic of the cells used and not of the attractiveness of the infected small intestine. Some support from this comes from the fact that the total recovered radioactivity was not the same for infected and uninfected groups, although there are possibly technical reasons for this phenomenon (see Results section 2b). However, there were no important differences between control and infected (day 4 p.i., five cysticercoids) mice when naive cells were used for uninfected and infected recipients. This further confirms the difference between H. diminuta and T. spiralis infections, as Rose et al. (1976a) were able to detect a difference between uninfected recipients and mice infected with T. spiralis four days previously, using cells from uninfected donors.

If there were an enhanced movement of cells from the MLN to the infected intestine, it is reasonable to expect this to occur

within the first eight days of infection with H. diminuta (i.e. before rejection commences), and indeed Rose et al. (1976a) found enhanced migration of lymphoblasts to the small intestine of mice after 2 and 4 days of infection with T. spiralis, but not thereafter. This coincides temporally with intense activity in the MLN (Fig. 4-10) ; the slow response of the MLN to the presence of H. diminuta is further evidence of the low-key nature of the reaction to this parasite.

What then do the experiments presented here tell us of the cellular events leading up to rejection of H. diminuta? The first possibility is that the MLN is the source of a small population of lymphocytes which are very efficient in causing expulsion of H. diminuta. In injecting MLNC to try to transfer immunity, the number of these cells actually reaching the intestine may be too small to be effective in causing expulsion. It could be argued that the inflammation in the body wall and the skin caused by surgery might divert lymphocytes to those sites, thereby reducing the number of cells reaching the lamina propria. However, as Rose et al. (1976b) found that MLNC were not easily diverted to peripheral sites of inflammation, this proposition seems unlikely. There is the possibility that the NIH mice used were not fully inbred, but the successful results with NIH mice given cells two weeks before challenge (Wakelin & Wilson, 1977a) argue against this.

An alternative possibility is that the MLN, although it apparently enlarges slowly in response to H. diminuta infection, is not the generating site of the immune response after 'seeding' with cells from Peyer's patches. It is extremely unlikely that the spleen is involved in view of the almost undetectable levels of anti-worm antibody present in the serum (see General Introduction),

and the most promising alternative is that the Peyer's patches are the direct source of cells active against H. diminuta. These cells could either pass through the MLN or migrate laterally into the lamina propria. Using T. colubriformis in the guinea-pig, Dineen et al. (1968) found that Peyer's patch lymphocytes could transfer immunity to naive recipients, and would also migrate in increased numbers to the small intestine of infected animals. In rats infected with T. spiralis, Peyer's patch cells which are reactive to worm antigen do not appear until 12-21 days after infection (Levin, Ottesen, Reynolds & Kirkpatrick, 1976), but both studies indicate that Peyer's patch cells may be involved in the response to intestinal parasites. Although lateral migration from Peyer's patches is probably not a major route of population of the lamina propria with lymphocytes (Cebra, Kamat, Gearhart, Robertson & Tseng, 1977), Muller-Schoop & Good (1975) noted a higher concentration of IgA-producing lymphocytes in the areas around Peyer's patches, and Parrott & Ferguson (1974) demonstrated that fine efferent lymphatics run from the Peyer's patches to the adjacent villi, and that labelled lymphocytes can be seen to pass along these lymphatics.

Although it is a subjective impression (and therefore not particularly reliable), I have noticed that the Peyer's patches of animals chronically infected with H. microstoma are large and prominent. As H. microstoma and H. diminuta cross-react immunologically, (Hopkins et al., 1977), this adds weight to the possibility that Peyer's patches are involved in the response to H. diminuta.

A third alternative is that the small intestine itself could be the source of the effector lymphocytes; there are numerous long-lived small lymphocytes to be found in the lamina propria and epithelium (Lemmel & Fichtelius, 1971; Ropke & Everett, 1976), and

it is possible that these cells are stimulated and divide *in situ*, the wall of the intestine being the site of generation and expression of the immune response to the presence of *H. diminuta*.

Further investigations of cell transfer and cell migration should therefore include investigation of Peyer's patch cells as well as MLNC. Future experiments should also be based on much heavier infections with *H. diminuta*; although five worms stimulate a fast response and good immunological memory, giving 50 or 100 *H. diminuta* would perhaps magnify the effects on the immune system to a measurable level. *T. spiralis* in the mouse stimulates gross inflammation of the small intestine, which may act as an amplifying system of the effects that could have expelled lower numbers of worms; indeed, small numbers of *T. spiralis* are rejected by mice without any gross inflammation (Wakelin & Lloyd, 1976a; Wakelin, personal communication), but whether cellular transfer of immunity against *T. spiralis* would be successful with low numbers of parasites remains to be determined.

SUMMARY

1. Syngeneic transfer of mesenteric lymph node cells (MLNC) was used to study transfer of immunity against H. diminuta between mice, and to investigate the attractiveness of the infected small intestine to isotopically-labelled lymphocytes.

2. Enhanced responsiveness to H. diminuta was not transferable with fewer than 1.7×10^8 MLNC, which represents a 4:1 donor:recipient ratio and restricts the future usefulness of the technique; immunity was more effectively transferred with MLNC taken at day 21 p.i. than day 8 p.i. The results obtained are discussed in relation to the conditions for transfer of immunity against T. spiralis in mice.

3. No difference was recorded in the amount of isotope recovered from the small intestine of control and H. diminuta-infected mice injected with isotopically labelled MLNC. The limitations on interpretation imposed by the use of (^{75}Se)selenomethionine as a label for lymphocytes and by the protocols used are discussed; it is concluded that study of lymphocyte traffic in mice infected with H. diminuta is a potentially rewarding field, but it is stressed that much more sensitive techniques are required, possibly in conjunction with heavier infections.

4. Alternatives to the MLN as generating sites of the immune response to H. diminuta are considered, and it is suggested that future work includes investigation of Peyer's patches.

APPENDIX

THE EFFECT OF AGE ON INFECTIVITY OF *HYMENOLEPIS DIMINUTA* CYSTICERCOIDS

INTRODUCTION

One group of parameters that must be established in experimental host-parasite research is the conditions under which eggs or larvae mature, become infective, and remain infective. For *H. diminuta*, the development of the cysticercoid in the intermediate host *Tribolium confusum* has been extensively studied. The influence of environmental temperature on the rate of development of the cysticercoid to maturity was described in detail by Voge & Turner (1956), who found that at 25°C cysticercoids were fully mature by 14 days after infection of the beetle with eggs. This was the condition under which cysticercoids were maintained in our laboratory, and in all the experiments reported in this thesis, cysticercoids were allowed to mature for at least 21 days to give a reasonable safety margin.

For what length of time the cysticercoids remain infective has, as far as I know, never been fully studied. Schiller (1959a), working with *H.nana* in *T. confusum*, reported that "most of the larvae encysted in the haemocoele are capable of remaining alive and infective throughout the life of the infective host" (beetles kept at 28°C). Other workers investigating the longevity of cysticercoids have extended their studies only over the first six months of infection. Evans (1973) found that cysticercoids of *Hymenolepis* spp. excyst normally at four months old, confirming the work of Gerris & De Rycke (1972), which showed that cysticercoids of *H. microstoma* aged two weeks to five months did not differ in their ability to excyst.

Befus (1975b, p. 29) tentatively attributed a poor recovery of *H. diminuta* to the age of the cysticercoids (7 months), and

pointed out the lack of knowledge of the infectivity of the cysticercoids older than 5-6 months. It was to fill this gap in our knowledge that the present study was carried out.

MATERIALS & METHODS

As described in General Materials & Methods, cysticercoids were maintained in T. confusum kept in dark incubators at 25°C. Cysticercoids were recovered by hand, and were administered to mice within one hour of isolation from the intermediate host.

RESULTS & DISCUSSION

Two experiments were carried out, both with CFLP mice but with differing experimental protocols. In the first experiment (Expt. 1) mice were infected with five cysticercoids. One group of mice received cysticercoids that were one month old, the second cysticercoids that were five months old, and the third cysticercoids that were 10 months old. Mice were autopsied on day 8 p.i. and the worms from each individual mouse were weighed together.

In the second experiment, mice were each infected with a single cysticercoid in order to eliminate crowding effects. Three groups of mice were again used; this time, the cysticercoids were 2, 7 and 12 months old. All mice in this experiment (Expt. 2) were treated with cortisone, and were autopsied on day 10 p.i. The combined results of both experiments are shown in Fig. A-1.

Up to five months of age, establishment of worms was essentially 100%; the recovery of one month old worms (84%) may

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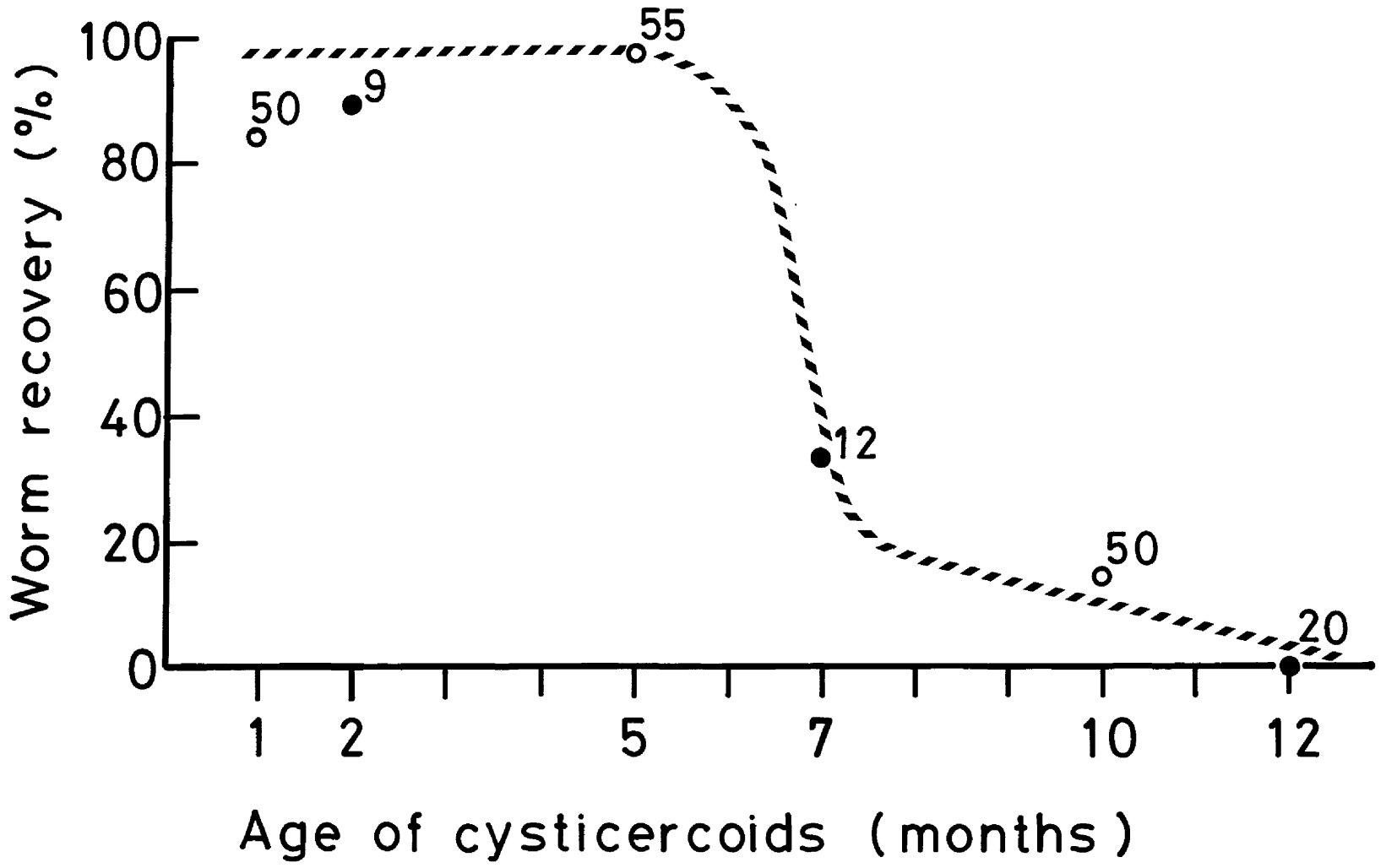
Figure A-1

Recovery (%) of H. diminuta from infections of CFLP mice with cysticercoids of differing ages.

o, Expt. 1 (five-cysticercoid infection)

●, Expt. 2 (one-cysticercoid infection)

Superscripts show the total number of cysticercoids administered to that group of mice; curve fitted by inspection.


Worm recovery (%)
100
80
60
40
20
0
50
9
55
12
50
20
1
2
5
7
10
12
Age of cysticercoids (months)

have been lower because of stunting or destrobilation of some of the worms, and the figure for the two month old cysticercoids (89%) represents a single worm not recovered. Viability at seven months was only 33%, and declined further to 14% at ten months and 0% at 12 months. The mean weight per surviving worm was always lower in the infections with older cysticercoids, but insufficient data were available to assign significance to this result.

The implication from these experiments is, therefore, that although cysticercoids are normally viable at five months old (see Introduction and Fig. A-1), the viability drops sharply thereafter, less than one third of the cysticercoids remaining infective at seven months old. There are probably several factors which may affect the rate of senescence in the cysticercoid, such as species or strain of either the parasite or the intermediate host, the number of cysticercoids carried by the intermediate host, or the temperature at which the infected beetles are maintained. Each of these factors may shorten or prolong the infective lifespan of the cysticercoid, but as higher temperatures greatly accelerate the speed of development of the cysticercoid from the oncosphere (Voge & Turner, 1956), it seems probable that higher temperatures would also hasten the senescence process.

GENERAL DISCUSSION

Hymenolepis diminuta in the mouse is a fascinating model. The swift, efficient rejection of this parasite contrasts sharply with the no less fascinating chronic nature of low-level infections with *H. diminuta* in the rat (e.g. Chappell & Pike, 1976a, b) and *H. microstoma* in the mouse (Hopkins *et al.*, 1977). The apparently complete lack of damage to the mucosa in infections with *H. diminuta* (Turton, 1968) puts this parasite in a separate class from many other parasitic infections; it is a truly enteral parasite, and the immune responses directed against the worm are intestinal responses in the purest sense.

Study of *H. diminuta* in the mouse has reached the awkward stage where the basic kinetics and characteristics of rejection are well established, but not enough completed work exists to allow the application of sophisticated immunological techniques; this problem is further compounded by the apparent dissimilarity in the mechanism of rejection between *H. diminuta* and the well-established nematode models. This thesis, I hope, will form a further part of the bridge between *H. diminuta* being a novel and a well-established, practical experimental host-parasite model.

Before discussing the implications of the work presented in this thesis, I feel it necessary (and, for future workers, perhaps useful) to say a little about the technical problems encountered during this study which affected either the results or the quantity of work that it was possible to carry out.

Firstly, *H. diminuta* in the mouse is a very variable system, as can be seen from many of the graphs presented in this thesis.

Although higher levels of infection (up to 6 or 8 cysticercoids) decrease the variability substantially (Befus, 1975), there was still a considerable variation between experiments conducted at different times, in terms of the timing of the onset and completion of rejection of the worms. Variability in the results appeared lower in inbred mice of the NIH strain, but was not eliminated by any means. Concurrent infections with protozoal and viral organisms encountered in 'conventional conditions' probably contribute enormously to this variability.

The second major problem was the extensive use of surgical infection in the experiments reported in this thesis. Surgical infection is a much more predictable and sensitive method of challenging mice, particularly older mice, than orally-administered cyticercoid infections. However, the trauma of surgery must inevitably affect the immune status of the mouse (Cooper, Irving & Turnbull, 1974; Kinnaert, Mahieu & Van Geertruyden, 1978), and also its eating habits, so important when the weight of worms is a major measured parameter. Hopkins & Zajac (1976) pointed out the desirability of being able to challenge mice with large worms given per os, possibly by use of inhibitors of acid secretion and/or drugs which decrease the transit time through the stomach, thereby circumventing surgery. Apart from the benefits to be gained in terms of assessing the response of the mouse, surgery is a difficult and tiring technique for the experimenter, and 16-18 operations per day represents a maximum number to perform while maintaining high standards of technique.

In the first Chapter it was demonstrated that both pregnancy and lactation cause changes which depress the response of the mouse

to H. diminuta. This was expressed as a reduced ability to either limit growth or to effect expulsion of the parasite. This is certainly of interest in an ecological sense, in that it has been recognised that pregnancy and lactation (particularly the latter) allow parasites to reach the egg-producing stage, whereas they would probably be rejected by normal hosts before the worms reach maturity; this is probably the case with Trichuris muris (Wakelin, 1970; Selby & Wakelin, 1975).

The thymus-dependency of the response of the mouse to H. diminuta was established by study of infections in congenitally athymic mice (Isaak et al., 1975; Bland, 1976a; Andreassen et al., 1978), but these experiments gave no information about the role of antibody in the host-parasite relationship, as antibody synthesis normally requires T helper cell cooperation (reviewed by Gisler, 1977). Pregnancy and lactation therefore offered a useful method for confirmation of the T cell dependency of the response, and also of the role of antibody in rejection of the parasite, as pregnancy and lactation cause depressed T effector cell responses and normal or enhanced antibody production (Fabris, 1973; Kelly & Ogilvie, 1972).

The findings that growth of the worms was enhanced and that rejection was inhibited in pregnant and (particularly) lactating mice provided strong evidence that anti-worm antibody has no independant action on the worm. This was convincingly supported by the results of the experiments on maternal transfer of immunity. Assuming that specific anti-worm antibody is manufactured in the small intestine, it appears unlikely that secretion of this antibody would not occur in the milk (Ahlstedt et al., 1977; McDermott & Bienenstock, 1979). As young mice have little or no

capacity to mount their own response to infection with H. diminuta (Befus & Featherston, 1974), study of maternal transfer of Ig offered an ideal system to test the activity of anti-worm antibody. In the event, neither growth nor survival of the worms in the offspring of immunised mothers were affected; these experiments therefore support the argument that antibodies have no direct effect on the worms, and confirm the as yet unpublished work of Isaak (1976), who found that ablation of the capacity to mount antibody responses (by treatment with anti-IgM antiserum) in mice did not remove the ability to reject H. diminuta.

Two major aspects of the studies on pregnancy and lactation remain to be characterised. Firstly, the contribution of the increase in food intake to the increased growth of the worms should be determined more accurately, in order to analyse separately the apparent depression of the facet of the immune response that normally stunts growth of the worms. It is difficult to see how this could be achieved, but close measurement of dietary intake during pregnancy and lactation would certainly be useful. Secondly, the nature of the agent(s) (hormonal or otherwise) that cause the immunodepression is still far from clear. This latter problem, however, is being studied by many immunologists and endocrinologists at the present time, and it is perhaps better to wait until a reasonable concensus of opinion has been reached before continuing research with H. diminuta in this line.

Chapter 2 established that the strobila, or more probably the tegument of H. diminuta is the major source of the immunogens that stimulate the protective immune response in the mouse. The use of worm transplantation techniques showed that small worms were rejected more slowly than large worms. Other experiments indicated that this

was not due to an age-related difference in the worms themselves, but represented a higher antigenic output by the worms with more strobila, i.e. a larger area of tegument. If this is so, the enormous worms supported by pregnant and lactating mice are more immunogenic than worms of the same age in normal hosts; when we add to this the increased retention of these large worms by the mice, it is apparent that the degree of immunodepression in pregnancy and lactation must be even greater than at first appears.

The conclusion that worms with larger surface area are more immunogenic was supported by experiments in which worms severely stunted by heavy irradiation (35 krad) stimulated a much lower level of immunity to normal challenge, and were themselves rejected more slowly than normal worms. The decrease in immunogenicity after irradiation contrasts markedly with the increase in immunogenicity of irradiated Schistosoma mansoni (Bickle et al., 1979a) and Nematospiroides dubius (Hagan, personal communication) in mice, but this is another reflection of the wholly lumenal nature of H. diminuta.

The tegument as a source of protective antigen fits the established observations that surgically implanted worms are rejected more quickly than oral cysticercoid worms in sham operated hosts (Hopkins & Zajac, 1976), and the increased metabolic activity per unit weight at the anterior end of the worm (Read et al., 1963; Henderson, 1977) is a valid explanation of why multiple-worm infections are expelled earlier than single- worm infections, even where the total dry weight of the latter exceeds the former.

This part of the study was initiated partly to help identify the source of the immunogens for future vaccination studies; it would be of no use to employ, for example, homogenates of strobila if the protective antigen turned out to be produced exclusively

by the scolex, or vice versa. In the event, the immunogens are probably released over the surface of the entire worm; stripped tegument (Oaks et al., 1977) or tegumental glycoproteins may prove to be valuable in vaccination with 'dead' material, and the use of young worms (or the anterior part of older worms) is indicated for studies on antigens secreted in culture or on immunisation by parenteral implantation of worms.

There is also much to be studied in the stunted worms arising from irradiated cysticercoids. One can only assume that these tiny worms were not detected by earlier workers such as Vilella et al. (1960), and there are, therefore, many possible avenues of research open. In particular, metabolic studies to determine the health of these worms in relation to normal worms would be useful in confirming that their low antigenicity is due to their small size and not to the damage inflicted on the metabolic processes of the worm by the radiation. Morphological and ultrastructural studies would prove very interesting not only per se, but also in assessing the nature of the damage to the cells and the extent to which the normal morphology is altered.

Chapter 3 was a study that is of considerable interest with respect to both Hymenolepis and Trichinella. The inflammatory response of the mouse to T. spiralis will cause non-specific expulsion of H. diminuta (Behnke et al., 1977) and of H. microstoma in the first few days of infection, i.e. before the scolex enters the bile duct. The response of the rat to T. spiralis has a much less marked effect on H. diminuta, neither destrobilation nor rejection of established cestodes occurring, even in the face of the response to heavy infections with T. spiralis. This may reflect a fundamental difference in the mechanisms of expulsion of T. spiralis by the

mouse and rat, rather than the lack of a certain active component in the inflammatory reaction of the rat. If rejection of T. spiralis by the rat depends on antibody, particularly IgA, to a much greater extent than in the mouse (Despommier et al., 1977b; Wakelin & Wilson, 1979 a), this in turn suggests that rejection of large numbers of H. diminuta by the rat (Andreassen et al., 1974a) associated with short term memory to challenge (Andreassen & Hopkins, 1980) may also be dependant on synthesis of specific anti-worm antibody. Immunoglobulin on the surface of H. diminuta in rats has been detected (Befus, 1977; Threadgold & Befus, 1977), but further information on the involvement of antibody in the response of rats to T. spiralis and H. diminuta is clearly necessary.

The lack of success in attempts to transfer immunity against H. diminuta in mice with MLNC (Chapter 4) was not totally unexpected in view of Bland's (1976b) preliminary results, but was disappointing nevertheless. So much can be deduced about the mechanisms of expulsion of a parasite by manipulation of lymphocyte transfer in conjunction with cell separation and isotope labelling techniques, and the establishment of a system for cellular transfer of immunity against H. diminuta would have opened many avenues of research, all the more interesting because expulsion of H. diminuta from the mouse appears to differ in many ways from the well-established nematode models such as T. spiralis, N. brasiliensis, T. muris and T. colubriformis. Although transfer of immunity against H. diminuta was intermittently achieved with over 10^8 MLNC, the large number of donors required and the amount of work involved in even the simplest experiment makes this a difficult success to exploit. I should also add that on more than one occasion $2x10^8$ MLNC given i.v. proved lethal to cell recipients for no apparent reason, so the

number of experiments presented here is smaller than it ideally would have been.

In the light of these results, it was hardly surprising that I was unable to detect any enhanced migration of lymphocytes to the small intestine infected with H. diminuta. The technical details that caused problems in interpretation of the results obtained are rehearsed in the Discussion of Chapter 4, and taking into account the limitations on sensitivity imposed by the techniques employed, I feel that this route of investigation (isotope uptake by lymphocytes in vitro and in vivo, and traffic of labelled lymphocytes) potentially still has something to offer. Firstly, use of a general lymphocyte label (L-(^{75}Se)-selenomethionine) was perhaps unwise; the reasoning at the time was that any differences in lymphocyte accumulation detected using this isotope could be differentiated by use of labels specific for lymphoblasts or small lymphocytes.

No experiments were conclusive in demonstrating enhanced accumulation of lymphocytes in the small intestine, but as noted above, the techniques employed may not have been sensitive enough. The difference in accumulation, if it exists, is obviously a very small one, and future experiments (which I believe would prove very valuable) should be designed with this in mind. T. spiralis infection increases the number of lymphoblasts in the MLN by about 50% (Rose et al., 1976a), and use of such cell populations in conjunction with a label specific for lymphoblasts (such as ^{125}IUdR) coupled with very high levels of infection with H. diminuta would detect far more sensitively any differences in the attractiveness of the small intestine to lymphoblasts.

This thesis has therefore presented results that further define, either positively or negatively, the factors involved in bringing about expulsion of H. diminuta from the mouse. The nature of the effector mechanism which the mouse uses to force this large metazoan to leave the intestine while still alive remains obscure; it must be said, though, that this is an unresolved problem for most, if not all, experimental intestinal helminth systems. The way forward in investigation of H. diminuta in the mouse must lie in study of the cellular changes in the intestine wall during the course of infection, either by histological and lymphocyte labelling studies or by characterisation of the effects of depleting specific cell populations on survival of the worms. The techniques for this type of study exist; whether sufficient enthusiasm and resources will be applied to this most difficult stage in the investigation of this unjustly neglected host-parasite model remains to be seen.

REFERENCES

ADCOCK, E.W., TEASDALE, F., AUGUST, C.S., COX, S., MESCHIA, G., BATTAGLIA, F.C., & NAUGHTON, M. (1973). Human chorionic gonadotropin: its possible role in maternal lymphocyte suppression. Science 181, 845-847.

AHLSTEDT, S., CARLSSON, B., FALLSTROM, S.P., HANSON, L.A., HOLMGREN, J., LIDIN-JANSON, G., LINDBLAD, B.S., JODAL, U., KAIJSER, B., SOHL-AKERLUND, A. & WADSWORTH, C. (1977). Antibodies in human serum and milk induced by enterobacteria and food proteins. In Ciba Foundation Symposium 46 (q.v.) 115-127.

ALLARDYCE, R.A., SHEARMAN, D.J.C., McCLELLAND, D.B.L., MARWICK, K., SIMPSON, A.J. & LAIDLAW, R.B. (1974). Appearance of specific colostrum antibodies after clinical infection with Salmonella typhimurium. British Medical Journal 3, 307-309.

ANDRE, C. (1975) Unpublished work. Cited p131 of the Ciba Foundation Symposium 46 (q.v.)

ANDREASSEN, J., HINDSBO, O. & RUITENBERG, E.J. (1978a). Hymenolepis diminuta infections in congenitally athymic (nude) mice: worm kinetics and intestinal histopathology. Immunology 34, 105-113.

ANDREASSEN, J., HINDSBO, O. & HESSELBERG, C.A. (1974). Immunity to Hymenolepis diminuta in rats: destrobilation and expulsion in primary infections, its suppression by cortisone treatment and increased resistance to secondary infections. Proceedings of the 3rd International Congress of Parasitology, Munich 2, 1056-1057.

ANDREASSEN, J., & HOPKINS, C.A. (1980). Immunologically mediated rejection of Hymenolepis diminuta by its normal host - the rat.

ANDREASSEN, J., JESPERSEN, S. & ROEPSTORFF, A. (1978b). Tapeworm (Hymenolepis diminuta) infections in rats and mice. Parasitology 77, xxv.

ANDRESEN, R.H. & MUNROE, C.W. (1962). Experimental study of the behaviour of adult skin homografts during pregnancy: a preliminary report. American Journal of Obstetrics and Gynecology 84, 1096-1101.

ANSELL, J.D., McDOUGALL, C.M., SPEEDY, G. & INCHLEY, C.J. (1978). Changes in lymphocyte accumulation and proliferation in the lymph nodes draining the pregnant uterus. Clinical and Experimental Immunology 31, 397-407.

ARME, C. (1976). Feeding. In Ecological Aspects of Parasitology (Ed. C.R. Kennedy) 75-97. North-Holland Publishing Co., Amsterdam.

ARMOUR, J. & DARGIE, J.D. (1974). Immunity to Fasciola hepatica in the rat. Successful transfer of immunity by lymphoid cells and by serum. Experimental Parasitology 35, 381-388.

BACZYNSKA, H. (1914). Etudes anatomiques et histologiques sur quelques nouvelles especes de cestodes d'oiseaux. Bulletin de la Societe Neuchateloise des Sciences Naturelles 40, 187-239.

BAINBRIDGE, D.R. (1976). Use of ^{75}Se-L-methionine as a label for lymphoid cells. Immunology 30, 135-144.

BARLOW, S.M., MORRISON, P.J. & SULLIVAN, F.M. (1974). Plasma corticosterone levels during pregnancy in the mouse: the relative contributions of the adrenal glands and foeto-placental units. Journal of Endocrinology 60, 473-483.

BARTH, E.E.E., JARRETT, 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.

BAUMINGER, S. & PELEG, S. (1978). Changes in immunological activity of rat lymphoid organs during pregnancy. Clinical and Experimental Immunology 32, 179-185.

BEER, A.E. & BILLINGHAM, R.E. (1974). Immunobiology of mammalian reproduction. Advances in Immunology 14, 2-76.

BEFUS, A.D. (1975a). Secondary infections of Hymenolepis diminuta in mice: effects of varying worm burdens in primary and secondary infections. Parasitology 71, 61-75

BEFUS, A.D. (1975b). Intestinal immune responses of mice to the tapeworms Hymenolepis diminuta and H. microstoma. Ph.D. Thesis, University of Glasgow.

BEFUS, A.D. (1977). Hymenolepis diminuta and H. microstoma: mouse immunoglobulins binding to the tegumental surface. Experimental Parasitology 41, 242-251.

BEFUS, A.D. & FEATHERSTON, D.W. (1974). Delayed rejection of single Hymenolepis diminuta in primary infections of young mice. Parasitology 69, 77-85.

BEFUS, A.D. & PODESTA, R.B. (1976). Intestine. In Ecological Aspects of Parasitology (Ed. C.R. Kennedy) 303-325. North-Holland Publishing Co., Amsterdam.

BEFUS, A.D. & THREADGOLD, L.T. (1975). Possible immunological damage to the tegument of Hymenolepis diminuta in mice and rats. Parasitology 71, 525-534.

BEHNKE, J.M., BLAND, P. & WAKELIN, D. (1977). Effect of the expulsion phase of Trichinella spiralis on Hymenolepis diminuta in mice, Parasitology 75, 79-88.

BICKLE, Q.D., DOBINSON, T. & JAMES, E.R. (1979b). The effects of gamma-irradiation on migration and survival of Schistosoma mansoni schistosomula in mice. Parasitology 79, 223-230.

BICKLE, Q.D., TAYLOR, M.G., DOENHOFF, M.J. & NELSON, G.S. (1979a). Immunisation of mice with gamma-irradiated intramuscularly injected schistosomula of Schistosoma mansoni. Parasitology 79, 209-222.

BICKLE, Q.D., TAYLOR, M.G., JAMES, E.R., NELSON, G.S., HUSSEIN, M.F., ANDREWS, B.J., DOBINSON, A.R. & MARSHALL, T.F.deC. (1979c). Further observations on immunisation of sheep against Schistosoma mansoni and S. bovis using irradiation-attenuated schistosomula of homologous and heterologous species. Parasitology 78, 185-193.

BJUNE, G., DUNCAN, E., BARNETSON, R.StC. & MELSOM, R. (1978). In vitro modulation of lymphocyte responses to PHA by plasma in mother and baby at the time of birth. Clinical and Experimental Immunology 32, 517-522.

BLAND, P. (1976a). Immunity to Hymenolepis diminuta: unresponsiveness of the athymic nude mouse to infection. Parasitology 72, 93-97.

BLAND, P. (1976b). The Immune Response of the Mouse to the Tapeworm Hymenolepis diminuta. Ph.D. thesis, University of Glasgow.

BOCKMAN, D.E. & COOPER, M.D. (1973). Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study. American Journal of Anatomy 136, 455-477.

BOHL, E.H., GUPTA, R.K.P., OLQUIN, M.V.F. & SAIF, L.J. (1972). Antibody responses in serum, colostrum and milk of swine after infection or vaccination with Transmissible Gastro-enteritis Virus. Infection and Immunology 6, 289-301.

BRANDTZAEG, P., FJELLANGER, I. & GJERULDSEN, S.T. (1970). Humansecretory immunoglobulins. I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. Scandinavian Journal of Haematology suppl. 12, 1-83.

BRATEN, T. & HOPKINS, C.A. (1969). The migration of Hymenolepis diminuta in the rat's intestine during normal development and following surgical transplantation. Parasitology 59, 891-905.

BRUCE, R.G. & WAKELIN, D. (1977). Immunological interaction between Trichinella spiralis and Trichuris muris in the intestine of the mouse. Parasitology 74, 163-173.

BULMER, R. & HANCOCK, K.W. (1977). Depletion of circulating T-lymphocytes in pregnancy. Clinical and Experimental Immunology 28, 302-305.

CAMPBELL, C.H. (1955). The antigenic role of the excretions and secretions of Trichinella spiralis in the production of immunity in mice. Journal of Parasitology 41, 483-491.

CARTER, P.B. & COLLINS, F.M. (1974). The route of enteric infection in normal mice. Journal of Experimental Medicine 139, 1189-1203.

CASTRO, G.A. (1976). Spatial and temporal integration of host responses to intestinal stages of Trichinella spiralis: retro- and prospective views. In Biochemistry of parasites and Host-parasite Relationships (Ed. H. van den Bossche) 343-358. Elsevier / North-Holland Biomedical Press, Amsterdam.

CASTRO, G.A., COTTER, M.V., FERGUSON, J.D. & GORDON, C.W. (1973). Trichinosis - physiologic factors possibly altering the course of infection. Journal of Parasitology 59, 268-276.

CASTRO, G.A., OLSON, L.T. & BAKER, R.D. (1967). Glucose malabsorption and intestinal histopathology in Trichinella spiralis-infected guinea-pigs. Journal of Parasitology 53, 595-612.

CEBRA, J.J., KAMAT, R., GEARHART, P., ROBERTSON, S.M. & TSENG, J. (1977). The secretory IgA system of the gut. In Ciba Foundation Symposium 46 (q.v.), 5-22.

CHAICUMPA, V., JENKINS, C.R. & ROWLEY, D. (1976). Passive transfer of immunity to infection with Nematospiroides dubius from immunised mice to their offspring. Australian Journal of Experimental Biology and Medical Science 54, 245-252.

CHANDLER, A.C. (1939). The effects of number and age of worms on development of primary and secondary infections with Hymenolepis diminuta in rats , and an investigation into the true nature of 'premunition' in tapeworm infections. American Journal of Hygeine 29, 105-114.

CEPPELLINI, R. (1971). In Progress in Immunology (Ed. Amos, B.), 973-1025. Academic Press, New York.

CHAPPELL, L.H. & PIKE, A.W. (1976a). Loss of Hymenolepis diminuta from the rat. International Journal for Parasitology 6, 333-339.

CHAPPELL, L.H. & PIKE, A.W. (1976b). Interactions between Hymenolepis diminuta and the rat. In Biochemistry of Parasites and Host-parasite Relationships (Ed. H. van den Bossche) 379-384. Elsevier /North-Holland Press, Amsterdam.

CHOROMANSKI, L. (1978). The influence of cyclophosphamide on the development of Hymenolepis diminuta in mice. Short Communications of the 4th International Congress of Parasitology E, 32-33.

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.

CIBA FOUNDATION SYMPOSIUM 46 (New Series) 1977. Immunology of the Gut. Elsevier / Excerpta Medica / North-Holland Press, Amsterdam.

CLAMAN, H.N. (1975). How corticosteroids work. Journal of Allergy and Clinical Immunology 55, 145-151.

CLARKE, A.G. (1979). Pregnancy-induced involution of the thymus can be prevented by immunising with paternal skin grafts: a strain-dependent effect. Clinical and Experimental Immunology 35, 421-424.

CLARKE, F.M., MORTON, H. & CLUNIE, G.J.A. (1978). Detection and separation of two serum factors responsible for depression of lymphocyte activity in pregnancy. Clinical and Experimental Immunology 32, 318-323.

COLEMAN, R.M., CARTY, J.M. & GRAZIODEI, W.D. (1968). Immunogenicity and phylogenetic relationships of tapeworm antigens produced by Hymenolepis nana and H. diminuta. Immunology 15, 297-304.

CONNAN, R.M. (1970). The effect of host lactation on the self cure of Nippostrongylus brasiliensis in rats. Parasitology 61, 27-33.

CONNAN, R.M. (1971). Hyostrongylus rubidis; the size and structure of worm populations in adult pigs. Veterinary Record 89, 186-191.

CONNAN, R.M. (1972a). The effect of host lactation on a second infection of Nippostrongylus brasiliensis in rats. Parasitology 64, 229-233.

CONNAN, R.M. (1972b). Passive protection with homologous antiserum against Trichostrongylus colubriformis in the guinea-pig. Immunology 23, 647-651.

CONNAN, R.M. (1973a). The immune response of the lactating rat to Nippostrongylus brasiliensis. Immunology 25, 261-268.

CONNAN, R.M. (1973b). Ovine parasitic gastro-enteritis: the Spring rise in the faecal egg count. In Helminth Diseases of Cattle, Sheep and Horses in Europe (G.M. Urquhart & Armour J., Eds), 36-41. Maclehose , Glasgow.

CONTRACTOR, S.F. & DAVIES, H. (1973). Effect of human chorionic somatommatropin and HCG on PHA induced lymphocyte transformation. Nature (New Biology) 243, 284

COOPER, A.J., IRVINE, J.M. & TURNBULL, A.R. (1974). Depression of immunological responses due to surgery. A model in the guinea-pig. Immunology 27, 393-399.

CORBA, A., ARMOUR, J., ROBERTS, R.J. & URQUHART, G.M. (1971). Transfer of immunity to Fasciola hepatica infection by lymphoid cells. Research in Veterinary Science 12, 292-295.

COX, F.E.G. (1978). Specific and nonspecific immunisation against parasitic infections. Nature 273, 623-626.

CRABBE, P.A., NASH, D.R., BAZIN, H., EYSSEN, H. & HEREMANS, J.F. (1970). Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Laboratory Investigation 22, 448-457.

CRAFT, I.L. (1970). The influence of pregnancy and lactation on the morphology and absorptive capacity of the rat small intestine. Clinical Science 38, 287-295.

CURRIE, G.A., DOORNINCK, W. van & BAGSHAWE, K.D. (1968). Effect of neuraminidase on the immunogenicity of early mouse trophoblast. Nature 219, 191-192.

DeFREITAS, A.A., ROSE, M.E. & PARROTT, D.M.V. (1977). Murine mesenteric and peripheral lymph nodes: a common pool of small T cells. Nature 270, 731-733.

DESPOMMIER, D.D., CAMPBELL, W.C. & BLAIR, L.S. (1977a). The in vivo and in vitro analysis of immunity to Trichinella spiralis in mice and rats. Parasitology 74, 109-119.

DESPOMMIER, D.D., McGREGOR, D.D., CRUM, E.D. & CARTER, P.B. (1977b). Immunity to Trichinella spiralis II. Expression of immunity against adult worms. Immunology 33, 797-805.

DESPOMMIER, D.D. & MULLER, M. (1970). Functional antigens of Trichinella spiralis. Journal of Parasitology 56 Section 2 Part I, 76.

DESPOMMIER, D.D. & MULLER, M. (1976). The stichosome and its secretion granules in the mature muscle larvae of Trichinella spiralis. Journal of Parasitology 62, 775-785.

DINEEN, J.K. & KELLY, J.D. (1972). The suppression of rejection of Nippostrongylus brasiliensis in lactating rats: the nature of the immunological defect. Immunology 22, 1-12.

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., 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 Immun Immunology 46, 360-374.

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., RONAI, P.M. & WAGLAND, B.M. (1968). The cellular transfer of immunity to Trichostrongylus colubriformis 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.

DUCKETT, M.G., DENHAM, D.A. & NELSON, G.S. (1972). Immunity to Trichinella spiralis V. Transfer of immunity against the intestinal phase from mother to baby mice. Journal of Parasitology 58, 550-554.

DUNSMORE, J.D. (1965). Ostertagia spp. in lambs and pregnant ewes. Journal of Helminthology 39, 159-184.

ELIAS, E. & DOWLING, R.H. (1976). The mechanism for small-bowel adaptation in lactating rats. Clinical Science and Molecular Medicine 51, 427-433.

EVANS, W.S. (1973). The in vitro cultivation of Hymenolepis nana and H. microstoma. Ph.D. Thesis, University of Glasgow.

FABRIS, N. (1973). Immunological reactivity during pregnancy in the mouse. Experientia 29, 610-612.

FELL, B.F., SMITH, R.A. & CAMPBELL, R.M. (1963). Hypertrophic and hyperplastic changes in the alimentary canal of the lactating rat. Journal of Pathology and Bacteriology 85, 179-188.

FERGUSON, A. & MACDONALD, T.T. (1977). Effects of local delayed hypersensitivity on the small intestine. In Ciba Foundation Symposium 46 (q.v.), 305-319.

FUHRMANN, O. (1918). Cestodes d'oiseaux de la Nouvelle-Caledonie et des iles Loyalty (Sarasin et Roux) Nova Caledonia. Archives de Zoologie Experimentale et Generale 2, 397-449.

FULCI, F. (1913). Die Restitutionsfahigkeit des Thymus der Saugetiere nach der Schwangerschaft. Zentralblatt fur allgemeine Pathologie und pathologische Anatomie 24, 968-974.

GEMMELL, M.A. (1976). Immunology and regulation of the cestode zoonoses. In Immunology of Parasitic Infections (S. Cohen & E.H. Sadun, Eds) 333-358. Blackwell Scientific Publications, Oxford.

GEMMELL, M.A. & MacNAMARA, F.N. (1972). Immune response to tissue parasites II. Cestodes. In Immunity to animal parasites (ed. E.J.L. Soulsby) 235-272. Academic Press, New York.

GERRIS, H.M. & DeRYCKE, P.H. (1972). Excystation of Hymenolepis microstoma I. Influence of age, population density and pretreatment of the cysticercoids. Zeitschrift fur Parasitenkunde 39, 277-283.

GILLES, H.M., LAWSON, J.B., SIBELAS, M., VOLLER, A. & ALLEN, N. (1969). Malaria, anaemia and pregnancy. Annals of Tropical Medicine and Parasitology 63, 245-263.

GISLER, R.H. (1977). Cellular regulation of immune responses. INSERM 72, 111-126.

GOLDBLUM, R.M., AHLSTEDT, S., CARLSSON, B., HANSON, L.A., JODAL, U., LIDIN-JANSON, G. & SOHL-AKERLUND, A. (1975). Antibody-forming cells in human colostrum after oral immunisation. Nature 257, 797-799.

GOODALL, R.I. (1972). The growth of Hymenolepis microstoma in the laboratory rat. Parasitology 65, 137-142.

GOODALL, R.I. (1973). Studies on the growth, location specificity and immunobiology of some hymenolepid tapeworms. Ph.D. Thesis, University of Glasgow.

GOODCHILD, C.G. & HARRISON, D.L. (1961). Growth of the rat tapeworm Hymenolepis diminuta , during the first five days in the final host. Journal of Parasitology 47, 819-829.

GOWANS, J.L. & KNIGHT, E.J. (1964). The route of recirculation of lymphocytes in the rat. Proceedings of the Royal Society B Biological Science 159, 257-282.

GRISCELLI, C., VASSALLI, P. & McCLUSKEY, R.T. (1969). The distribution of large dividing lymph node cells in syngeneic recipient rats after i.v. injection. Journal of Experimental Medicine 130, 1427-1451.

GUPTA, S. & GOOD, R.A. (1977). α-fetoprotein and human lymphocyte subpopulations. Journal of Immunology 118, 405-408.

GUSDON, J.P. (1976) Maternal immune responses in pregnancy. In Immunology of Human Reproduction (ed. J.S. Scott & W.R. Jones) 103-125. Academic Press, New York.

GUY-GRAND, D., GRISCELLI, C. & VASSALLI, P. (1974). The gut-associated lymphoid system: nature and properties of the large dividing cells. European Journal of Immunology 4, 435-443.

HARRIS, W.G. & TURTON, J.A. (1973). Antibody response to tapeworm (Hymenolepis diminuta) in the rat. Nature 246, 521-522.

HECHT, R., MOSKO, M.M., LUBIN, J., SULZBERGER, M.B. & BAER, R.L. (1944). The absorption of whole ragweed pollen from the gastrointestinal tract. Journal of Allergy 15, 9-13.

HELLSTROM, K.E., HELLSTROM, I. & BRAWN, J. (1969). Abrogation of cellular immunity to antigenically foreign mouse cells by a serum factor. Nature 224, 914-915.

HEMMINGS, W.A. (ed.) (1976). Maternofoetal Transmission of Immunoglobulins: Proceedings of a Symposium on Transmission of Immunoglobulins From Mother to Young. Cambridge University Press.

HEMMINGS, W.A. (1978). The transmission of high molecular weight breakdown products of protein across the gut of suckling and adult rats. In Antigen Absorption by the Gut (ed. W.A. Hemmings) 37-47. MTP Press Ltd.

HEMMINGS, W.A. & WILLIAMS, E.W. (1978). Transport of large breakdown product of dietary protein through the gut wall. Gut 19, 715-723.

HENDERSON, D. (1977). The effect of worm age, weight and number in the infection on the absorption of glucose by Hymenolepis diminuta. Parasitology 75, 277-284.

HERD, R.P. (1976). The cestocidal effect of complement in normal and immune sera in vitro. Parasitology 72, 325-334.

HESSELBERG, C.A. & ANDREASSEN, J. (1975). Some influences of population density on Hymenolepis diminuta in rats. Parasitology 71, 517-524.

HETHERINGTON, C.M. & HUMBER, D.P. (1977). The effect of pregnancy on lymph node weight in the mouse. Journal of Immunogenetics 4, 271-276.

HOLMGREN, J., HANSON, L.A., CARLSSON, B., LINDBLAD, B.S. & RAHIMTOOLA, J. (1976). Neutralising antibodies against Escherichia coli and Vibrio cholerae enterotoxins in human milk from a developing country. Scandinavian Journal of Immunology 5, 867-872.

HOPKINS, C.A. (1970). Diurnal movement of Hymenolepis diminuta in the rat. Parasitology 60, 255-271.

HOPKINS, C.A. (1980). Immunological memory to the adult tapeworm Hymenolepis diminuta in the mouse intestine.

HOPKINS, C.A., GOODALL, R.I. & ZAJAC, A. (1977). The longevity of Hymenolepis microstoma in mice, and its immunological cross-reaction with H. diminuta. Parasitology 74, 175-184.

HOPKINS, C.A. & STALLARD, H.E. (1974). Immunity to intestinal tapeworms: 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. (1977). Hymenolepis microstoma: a change in susceptibility to resistance with increasing age of the parasite. Parasitology 75, 241-249.

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.

HUNNINEN, A.V. (1935). Studies on the life history and host-parasite relations of Hymenolepis fraterna (H. nana var. fraterna, Stiles) in white mice. American Journal of Hygiene 22, 414-443.

HEYNEMANN, D. (1962). Studies on helminth immunity II. Influence of Hymenolepis nana (Cestoda: Hymenolepididae) in dual infections with H. diminuta in white mice and rats. Experimental Parasitology 12, 7-18.

INSLER, G.D. & ROBERTS, L.S. (1976). Hymenolepis diminuta: lack of pathogenicity in the healthy rat host. Experimental Parasitology 39, 351-357.

ISAAK, D.D. (1976). Analysis of the mechanisms of immune expulsion from mice of Hymenolepis diminuta and H. nana. Dissertation Abstracts International 37B, 2675.

ISAAK, D.D., JACOBSON, R.H. & REED, N.D. (1975). Thymus dependence of tapeworm (Hymenolepis diminuta) elimination from mice. Infection and Immunity 12, 1478-1479.

ITO, A. (1977). The mode of passive protection against Hymenolepis nana induced by serum transfer. International Journal for Parasitology 7, 67-72.

ITO, T. & HOSHINO, T. (1962). Studies on the influences of pregnancy and lactation on the thymus in the mouse. Zeitschrift fur Zellforschung und Mikroskopische Anatomie 57, 667-678.

JAMES, E.R. & FARRANT, J. (1977). Recovery of infective Schistosoma mansoni schistosomula from liquid nitrogen: a step towards storage of a live schistosomiasis vaccine. Transactions of the Royal Society for Tropical Medicine and Hygiene 71, 498-500.

JARRETT, E.E.E. & URQUHART, G.M. (1971). The immune response to nematode infections. International Review of Tropical Medicine 4, 53-96.

JARRETT, W.F.H., JENNINGS, F.W., McINTYRE, W.I.M., MULLIGAN, W. & URQUHART, G.M. 91960) Immunological studies on Dictyocaulus viviparus infection. Immunity produced by the administration of irradiated larvae. Immunology 3, 145-151.

JENKINS, S.N. & WAKELIN, D. (1977). The source and nature of some functional antigens of Trichuris muris. Parasitology 74, 153-161.

KASAKURA, S. (1971). A factor in maternal plasma during pregnancy that suppresses the reactivity of mixed leukocyte cultures. Journal of Immunology 107, 1296-1301.

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. (1973). The suppression of rejection of Nippostrongylus brasiliensis in Lewis strain rats treated with ovine prolactin. Immunology 24, 551-558.

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.

KENNY, J.F. & DIAMOND, M. (1977). Immunological responsiveness to Escherichia coli during pregnancy. Infection and Immunity 16, 174-180.

KHOURY, P.B., STROMBERG B.E. & SOULSBY, E.J.L. (1977). Immune mechanisms to Ascaris suum in inbred guinea-pigs. I. Passive transfer of immunity by cells and serum. Immunology 32, 405-412.

KINNAERT, P., MAHIEU, A. & VanGEERTRUYDEN (1978). Stimulation of antibody synthesis induced by surgical trauma in rats. Clinical and Experimental Immunology 32, 243-252.

KIRBY, D.R.S., BILLINGTON, W.D. & JAMES, D.A. (1966). Transplantation of eggs to the kidney and uterus of immunised mice. Transplantation 4, 713-718.

LANG, B.Z., LARSH, J.E., WEATHERLY, N.F. & GOULSON, H.T. (1967). Demonstration of immunity to Fasciola hepatica in recipient mice given peritoneal exudate cells. Journal of Parasitology 53, 208-209.

LARSH, J.E. (1942). Transmission from mother to offspring of immunity against the mouse cestode Hymenolepis nana (var. fraterna). American Journal of Hygiene 36, 187-194.

LARSH, J.E.(1949). The effect of pregnancy on the natural resistance of mice to Hymenolepis infection. Journal of Parasitology 35, suppl.37.

LARSH, J.E. (1963). Experimental trichiniasis. Advances in Parasitology 1, 213-286.

LARSH, J.E. (1967). The present understanding of the mechanism of immunity to Trichinella spiralis. The American Journal of Tropical Medicine and Hygiene 16, 123-132.

LARSH, J.E., GOULSON, H.T. & Van ZANDT, P.D. (1962). The control of Trichinella spiralis in mice by cadmium oxide in feed given on an intermittent schedule. Journal of the Elisha Mitchell Scientific Society 94, 262-272.

LARSH, J.E., OTTOLENGHI, A. & WEATHERLY, N.F. (1974). Trichinella spiralis: Phospholipase in challenged mice and rats. Experimental Parasitology 36, 299-306.

LEMMEL, E.M. & FICHTELIUS, K.E. (1971). Life span of lymphocytes within epithelium, Peyer's patch epithelium, epidermis and liver of mice. International Archives of Allergy and Applied Immunology 41, 716-728.

LEVIN, D.M., OTTESEN, E.A., REYNOLDS, H.Y. & KIRKPATRICK, C.H. (1976). Cellular immunity in Peyer's patches of rats infected with Trichinella spiralis. Infection and Immunity 13, 27-30.

LITCHFORD, R.G. (1963). Observations on Hymenolepis microstoma in three laboratory hosts: Mesocricetus auratus, Mus musculus and Rattus norvegius. Journal of parasitology 49, 403-410.

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. & OGILVIE, B.M. (1977). Nippostrongylus brasiliensis and Trichinella spiralis: Localisation of lymphoblasts in the small intestine of parasitised rats. Experimental Parasitology 41, 124-132.

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.

LUMSDEN, R.D. (1975). Surface ultrastructure and cytochemistry of parasitic helminths. Experimental Parasitology 37, 267-339.

LUMSDEN, R.D. & KARIN, D.S. (1970). Electron microscopy of peribiliary connective tissues in mice infected with the tapeworm Hymenolepis microstoma. Journal of parasitology 56, 1171-1183.

MARONI, E.L. & DeSOUSA, M.A.B. (1973). The lymphoid organs during pregnancy in the mouse. A comparison between a syngeneic and an allogeneic mating. Clinical and Experimental Immunology 13, 107-124.

MEDAWAR, P.B. (1954). Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symposia of the Society for Experimental Biology VII, 320-338.

MEITES, J. & NICHOLL, C.S. (1959). Hormonal prolongation of lactation for 75 days after litter withdrawal in post-partum rats. Endocrinology 65, 572-579.

MENDELSOHN, J., MULTER, M.M.,& BERNHEIM, J.L. (1977). Inhibition of human lymphocyte stimulation by steroid hormones: cytokinetic mechanisms. Clinical and Experimental Immunology 27, 127-134.

METTRICK, D.F. (1971). Hymenolepis diminuta: the microbial fauna, nutritional gradients, and physicochemical characteristics of the small intestine of uninfected and parasitised rats. Canadian Journal of Physiology and Pharmacology 49, 972-984.

METTRICK, D.F. (1972). Changes in the distribution and chemical composition of Hymenolepis diminuta and the intestinal nutritional gradients of uninfected and parasitised rats following a glucose meal. Journal of Helminthology 46, 407-429.

METTRICK, D.F. (1973). Competition for ingested nutrients between the tapeworm Hymenolepis diminuta and the rat host. Canadian Journal of Public Health 64, 70-82.

MICALSKA, Z. & KARMANSKA, K. (1976). Influence of inhibition of degranulation of mast cells by Intal (disodium cromoglycate) on the course of trichinellosis in mice. Acta Parasitologica Polonica 24, 69-80.

MILLAR, K.G., MILLS, P. & BAINES, M.G. (1973). A study of the influence of pregnancy on the thymus gland of the mouse. American Journal of Obstetrics and Gynecology 117, 913-918.

MILLER, T.A. (1970). Vaccination against canine hookworm. Journal of Parasitology 56, II:1, 239.

MILLER, T.A. (1978). Industrial development and field use of the canine hookworm vaccine. Advances in Parasitology 16, 333-342.

MIMS, C.A. (1977). The Pathogenesis of Infectious Disease p. 305. Academic Press (London) Ltd.

MOQBEL, R. (1976). Studies on the host-parasite relationship of Strongyloides ratti in rats. Ph.D. Thesis, University of London.

MORTON, H., HEGH, V. & CLUNIE, G.J.A. (1974). Immunosuppression detected in pregnant mice by rosette inhibition test. Nature 249, 459-462.

MOSS, G.D. (1971). The nature of the immune response of the mouse to the bile duct cestode Hymenolepis microstoma. Parasitology 62, 285-294.

MUCHMORE, A.V. & BLAESE, R.M. (1977). Immunoregulatory properties of fractions from human pregnancy urine: evidence that Human Chorionic Gonadotropin is not responsible. Journal of Immunology 118, 881-886.

MULLER-SCHOOP, J.W. & GOOD, R.A. (1975). Functional studies of Peyer's patches: evidence for their involvement in intestinal immune responses. Journal of Immunology 114, 1757

MULLIGAN, W. (1975). Preparation of radiation-attenuated vaccines against helminthic infections. In Nuclear techniques in Helminthology research, 1-11. IAEA, Vienna.

MULLIGAN, W. (1976) In Nuclear Techniques in Animal Production and Health, 413-419. IAEA, Vienna.

McCORMACK, J.T. & GREENWALD, G.S. (1974). Progesterone and Oestradiol -17β concentrations in the peripheral plasma during pregnancy in the mouse. Journal of Endocrinology 62, 101-107.

McDERMOTT, M.R. & BIENENSTOCK, J. (1979). Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory and genital tissues. Journal of Immunology 122, 1892-1898.

McLEAN, J.M., MOSLEY, J.G. & GIBBS, A.L.C. (1974). Changes in thymus, spleen and lymph nodes during pregnancy and lactation in the rat. Journal of Anatomy 118, 223-230.

McWILLIAMS, M., PHILLIPS-QUAGLIATA & LAMM, M.E. (1975). Characteristics of mesenteric lymph node cells homing to gut-associated lymphoid tissue in syngeneic mice. Journal of Immunology 115, 54-58.

NANSEN, P. (1975). Resistance in cattle to Fasciola hepatica induced by γ-ray attenuated larvae: results from a controlled field trial. Research in Veterinary Science 19, 278-283.

NGWENYA, B.Z. (1976a). Effect of lactation on CMI of Swiss mice to Trichinella spiralis. Cellular immunology 24, 116-122.

NGWENYA, B.Z. (1976b) Response of ovine prolactin-treated mice to initial infection with Trichinella spiralis. Journal of Parasitology 62, 871-873.

NGWENYA, B.Z. (1977). Response of nonsensitised and sensitised lactating mice to infection with Trichinella spiralis. International Journal for Parasitology 7, 41-46.

NICOLL, C.S. & BERN, H.A. (1972). On the action of prolactin among the vertebrates: is there a common denominator? In Ciba Foundation Symposium : Lactogenic Hormones (ed. Wolstenholme & Knight) 299.

OAKS, J.A. & LUMSDEN, R.D. (1971). Cytological studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate-containing macromolecules into tegument membranes. Journal of Parasitology 57, 1256-1268.

OAKS, J.A., KNOWLES, W. & CAIN, G.D. (1977). A simple method of obtaining an enriched fraction of tegumental brush border from Hymenolepis diminuta. Journal of Parasitology 63, 476-485.

OGILVIE, B.M. (1974). Immunity to parasites (Helminths and arthropods). Progress in Immunology II, Vol. 4 127-135.

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. & 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 relationship in the rat. Experimental Parasitology 29, 138-177.

OGILVIE, B.M. & JONES, V.E. (1973). Immunity in the parasitic relationship between helminths and hosts. Progress in Allergy 17, 93-144.

OGILVIE, B.M., LOVE, R.J., JARRA, W. & BROWN, K.N. (1977). Nippostrongulus brasiliensis infection in rats: the cellular requirement for worm expulsion. Immunology 32, 521-528.

OGILVIE ,B.M. & PARROTT, D.M.V. (1977). The immunological consequences of nematode infection. In Ciba Foundation Symposium 46 (q.v.), 183-194.

ONYANGO-ABUJE, J.A. & WEINMANN, C.J. (1974). Effects of gamma irradiation on the infectivity and development of Hymenolepis nana oncospheres. Zeitschrift fur Parasitenkunde 44, 111-116.

O'SULLIVAN, B.M. & DONALD, A.D. (1970). A field study of nematode parasitic populations in the lactating ewe. Parasitology 61, 301-315.

O'SULLIVAN, B.M. & DONALD, A.D. (1973). Responses to infection with Haemonchus contortus and Trichostrongylus colubriformis in ewes of different reproductive status. International Journal for Parasitology 3, 521-530.

O'SULLIVAN, B.M. (1974). Effects of lactation on Trichostrongylus colubriformis infections in the guinea-pig. International Journal for Parasitology 4, 177-181.

PALAIS, M. (1933). Les anomalies des cestodes; recherches experimentales sur Hymenolepis diminuta (Rud.). Annales Faculte Sciences de l'Universite Marseille 6, 109-163.

PALM, J., HEYNER, S.,& BRINSTLER, J. (1971). Differential immunofluorescence of fertilised mouse eggs with H-2 and non-H-2 antibody. Journal of Experimental Medicine 133, 1282-1293.

PANTELOURIS, E.M. (1971). Observations on the immunobiology of 'nude mice. Immunology 20, 247-252.

PAPPAS, P.W. & READ, C.P. (1972a). Trypsin inactivation by intact Hymenolepis diminuta. Journal of Parasitology 58, 864-871.

PAPPAS, P.W. & READ, C.P. (1972b). Inactivation of α- and β-chymotrypsin by intact Hymenolepis diminuta (Cestoda). Biological Bulletin 143, 605-616.

PARROTT, D.M.V. & FERGUSON, A. (1974). Selective migration of lymphocytes within the mouse small intestine. Immunology 26, 571-588.

PARROTT, D.M.V., ROSE, M.L., SLESS, F., DeFREITAS, A. & BRUCE, R.G. (1975). Factors which determine the accumulation of immunoblasts in gut and skin. In Future Trends in Inflammation II (ed. J.P. Giroud, D.A.Willoughby & G.P. Velo) 32-39. Birkhauser Verlag, Basel.

PERRY, R.H. (1974). Transfer of immunity to Trichinella spiralis from mother to offspring. Journal of Parasitology 60, 460-465.

PHILLIPS, R.S. (1970). Plasmodium berghei: passive transfer of immunity by antiserum and cells. Experimental Parasitology 27, 479-495.

PICKARD, R.E. (1968) Varicella pneumonia during pregnancy. American Journal of Obstetrics and Gynecology 101, 504

PLUM, J., THIERY, M. & SABBE, L. (1978). Distribution of mononuclear cells during pregnancy. Clinical and Experimental Immunology 31, 45-49

POULIOT, P., VIENS, P. & TARGETT, G.A.T. (1977). T lymphocytes in the transfer of immunity to Trypanosoma musculi in mice. Clinical and Experimental Immunology 27, 507-511.

PURTILO, D.T., HALLGREN, H.M. & YUNIS, E.J. (1972). Depressed maternal lymphocyte response to PHA in human pregnancy. Lancet i, 769-771.

RADERMECKER, M., BEKHTI, A., PONCELET, E.,& SALMON, J. (1974). Serum IgE levels in protozoal and helminthic infections. International Archives of Allergy and Applied Immunology 47, 285-295.

RANNIE, G.H. & DONALD, K.J. 91977). Estimation of migration of thoracic duct lymphocytes to non-lymphoid tissues. Cell and Tissue Kinetics 10, 523-541.

READ, C.P. (1955). Intestinal physiology and the host-parasite relationship. In Some Physiological Aspects and Consequences of Parasitism (ed. W.H. Cole), 27-43. Rutgers University Press.

READ, C.P. (1967). Longevity of the tapeworm Hymenolepis diminuta. Journal for Parasitology 53, 1055-1056.

READ, C.P. & KILEJIAN, A.Z. (1969).Circadian migratory behaviour of a cestode symbiote in the rat host. Journal of Parasitology 55, 574-578.

READ, C.P. & ROTHMAN, A.H. (1957a). The role of carbohydrate in the biology of cestodes II. The effect of starvation on glycogenesis and glucose consumption in Hymenolepis. Experimental Parasitology 6, 280-287.

READ, C.P. & ROTHMAN, A.H. (1957b). The role of carbohydrates in the biology of cestodes IV. Some effects of host dietary carbohydrate on growth and reproduction of Hymenolepis. Experimental Parasitology 6, 294-305.

READ, C.P., ROTHMAN, A.H. & SIMMONS, J.E. (1963). Studies on membrane transport with special reference to parasite-host integration. Annals of the New York Academy of Science 113, 154-205

READ, C.P. & VOGE, M. (1954). The size attained by Hymenolepis diminuta in different host species. Journal of Parasitology 40, 88-89.

REES, G. (1967). Pathogenesis of adult cestodes. Helminthological Abstracts 36(1), 1-23.

REMBIESA, R., PTAK, W. & BUBAK, M. (1974). The immunosuppressive effects of mouse placental steroids. Experientia 30, 82-83.

REMINGTON, R.D. & SCHORK, M.A. (1970). Statistics with applications to the Biological and Health Sciences, pp. 313-315. Englewood Cliffs, N.J.: Prentice-Hall Incorporated.

RICKARD, M.D., BODDINGTON, E.B. & McQUADE, N. (1977). Vaccination of lambs against Taenia ovis infection using antigens collected during in vitro cultivation of larvae: passive protection via colostrum from vaccinated ewes and the duration of immunity from a single vaccination. Research in Veterinary Science 23, 368-371.

ROBERTS, L.S. & MONG, F.N. (1968). Developmental physiology of cestodes III. Development of Hymenolepis diminuta in superinfections. Journal of Parasitology 54, 55-62.

ROLSTAD, B. & TOOGOOD, E. (1978). Toxicity of $Na_2{}^{51}CrO_4$ when used to label rat lymphocytes. Journal of Immunological Methods 21, 271-276.

ROPKE, C. & EVERETT, N.B. (1976). Proliferative kinetics of large and small intraepithelial lymphocytes in the small intestine of the mouse. American Journal of Anatomy 145, 395-408.

ROSE, M.L. & MICKLEM, H.S. (1976). (^{75}Se)-L-methionine: a new isotopic marker for lymphocyte localisation studies. Journal of Immunological Methods 9, 281

ROSE, M.L., PARROTT, D.M.V. & BRUCE, R.G. (1976a). Migration of lymphoblasts to the small intestine I. Effect of Trichinella spiralis infection on the migration of mesenteric lymphoblasts and mesenteric T lymphoblasts in syngeneic mice. Immunology 31, 723-730.

ROSE, M.E., PARROTT, D.M.V. & BRUCE, R.G. (1976b). Migration of lymphoblasts to the small intestine. II. Divergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse. Cellular Immunology 27, 36-46.

ROSENTHAL, M. (1977). Enhanced phagocytosis of immune complexes in pregnancy. Clinical and Experimental Immunology 28, 189-191.

RUGH, R. (1968). The Mouse: its Reproduction and Development. Burgess Publishing Co., Minneapolis.

SABLOVIC, N. & DeROMEMONT, D. (1978). T and B lymphocytes during pregnancy. Biomedicine 29, 212-217.

SAOWAKONTHA, S. (1975). The effect of trichinosis on weight gain and food intake of rats fed low and high protein diet. South East Asian Journal of Tropical Medicine and Public Health 6, 247-250.

SCHIFF, R.I., MERCIER, D. & BUCKLEY, R.H. (1975). Inability of gestational hormones to account for the inhibitory effects of pregnancy plasmas on lymphocyte responses in vitro. Cellular Immunology 20, 69-80.

SCHILLER, E.L. (1959a). Experimental studies on morphological variation in the cestode genus Hymenolepis. I. Morphology and development of the cysticercoid of Hymenolepis nana in Tribolium confusum. Experimental Parasitology 8, 91-118.

SCHILLER, E.L. (1959b). Experimental studies on morphological variation in the cestode genus Hymenolepis. III. X-irradiation as a mechanism for facilitating analyses in H. nana. Experimental Parasitology 8, 427-470.

SELBY, G.R. & WAKELIN, D. (1973). Transfer of immunity against Trichuris muris in the mouse by serum and cells. International Journal for Parasitology 3, 717-722.

SELBY, G.R. & WAKELIN, D. (1975). Suppression of the immune response to Trichuris muris in lactating mice. Parasitology 71, 77-85.

SKOWRON-CENDRZAK, A., PTAK, W., BUBAK, M.,& CZARNIK, Z. (1975). Non-specific decrease of CMI in female mice during syngeneic and allogeneic pregnancy. Folia biologica (Praha) 21, 70-74.

SMITHERS, S.R. & TERRY, R. (1969). The immunology of schistosomiasis. Advances in Parasitology 7, 41-95.

SMYTH, J.D. (1962). An Introduction to Animal Parasitology. English Universities Press.

SMYTH, J.D. (1963). Secretory activity by the scolex of Echinococcus granulosus in vitro. Nature 199, 402.

SMYTH, J.D. (1964). Observations on the scolex of Echinococcus granulosus, with special reference to the occurrence and cytochemistry of secretory cells in the rostellum. Parasitology 54, 515-526.

SMYTH, J.D. (1969). Parasites as biological models. Parasitology 59, 73-91.

SNEDECOR, G.W. & COCHRAN, W.G.(1967). Statistical methods. 6th Edition, Iowa State University Press.

SPRENT, J. (1973) Circulating T and B lymphocytes of the mouse. I. migratory properties. Cellular Immunology 7, 10-39.

STIMSON, W.H., HUNTER, I.C. & MANOS, C. (1977). Comparison of the effects of human pregnancy serum and anti-inflammatory compounds on the release of lysosomal enzymes from macrophages. British Journal of Experimental Pathology 58, 434-437.

TAKAYANAGI, T., KAMBARA, H. & ENRIQUEZ, G.L. (1973). Trypanosoma gambiense: immunity with spleen cell and antiserum transfer in mice. Experimental Parasitology 33, 429-432.

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.

TAN, B.D. & JONES, A.W. (1966). X-ray induced abnormalities and recovery in Hymenolepis microstoma. Experimental Parasitology 18, 355-373.

TAN, B.D. & JONES, A.W. (1967). Autoelimination by means of X-rays: distinguishing the crowding factor from others in premunition caused by the mouse bile duct cestode Hymenolepis microstoma. Experimental Parasitology 20, 147-155.

TAN, B.D. & JONES, A.W. (1968). Resistance of mice to infection with the bile duct cestode Hymenolepis microstoma. Experimental Parasitology 22, 250-255.

TAYLOR, M.G., JAMES, E.R., BICKLE, Q.D., HUSSEIN, M.F., ANDREWS, B.J., DOBINSON, A.R. & NELSON, G.S. (1979). Immunisation of sheep against Schistosoma bovis using an irradiated schistosomular vaccine. Journal of Helminthology 53, 1-5.

TAYLOR, M.G., JAMES, E.R., NELSON, G.S., BICKLE, Q.D., DUNNE, D.W. & WEBBE, G. (1976). Immunisation of sheep against Schistosoma mattheei using either irradiated cercariae or irradiated schistosomula. Journal of Helminthology 50, 1-9.

TEWARI, H.C. & SINGH, K.S. (1977). Acquired immunity in dogs against Schistosoma incognitum. Journal of Parasitology 63, 945-956

THONG, Y.H. & FERRANTE, A. (1979). Inhibition of mitogen-induced human lymphocyte proliferative responses by tetracycline analogues. Clinical and Experimental Immunology 35, 443-446.

THREADGOLD, L.T. & BEFUS, L.T. (1977). Hymenolepis diminuta: ultrastructural localisation of immunoglobulin-binding sites on the tegument. Experimental Parasitology 43, 169-179.

TILNEY, N.L. (1971). Patterns of lymphatic drainage in the adult laboratory rat. Journal of Anatomy 109, 369-383.

TURTON, J.A. (1968). The growth and development of Hymenolepis diminuta and H. nana in vitro and in vivo. Ph.D. Thesis, University of Glasgow.

TURTON, J.A. (1971). Distribution and growth of Hymenolepis diminuta in the rat, hamster and mouse. Zeitschrift fur Parasitenkunde 37, 315-329.

UHLENHUTH, P.T. (1900). Neuer Beitrag zum spezifischen Nachweis von Eireiweiss auf biologischem Wege. Deutsche Medicinische Wochenschrift 26, 734-735.

VIJAY, H.M. & BUTTAR, H.S. (1977). Evaluation of humoral response with ragweed antigen in pregnant rats. Experientia 33, 674-675.

VILELLA, J.B., GOULD, S.E. & GOMBERG, H.J. (1960). Effect of Cobalt 60 and X-ray on infectivity of cysticercoids of Hymenolepis diminuta. Journal of Parasitology 46, 165-169.

VOGE, M. & TURNER, J.A. (1956). Effect of temperature on larval development of the cestode, Hymenolepis diminuta. Experimental Parasitology 5, 580-586.

Von SHOULTZ, B., STIGBRAND, T. & TARNVIK, A. (1973). Inhibition of PHA-induced stimulation by the pregnancy zone protein. FEBS Letters 38, 23-26.

WAGLAND, B.M. & DINEEN, J.K. (1965). The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of guinea-pig. Australian Journal of Experimental Biology and Medical Science 43, 429-43.

WAKELIN, D. (1970). The stimulation of immunity and the induction of unresponsiveness to Trichuris muris in various strains of laboratory mice. Zeitschrift fur Parasitenkunde 35, 162-168.

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 273, 617-620.

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. (1973). Functional antigens of Trichuris muris. The stimulation of immunity by vaccination of mice with somatic antigen preparations. International Journal for Parasitology 3, 711-715.

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 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. (1979a). T and B cells in the transfer of immunity against Trichinella spiralis in mice. Immunology 37, 103-109.

WAKELIN, D. & WILSON, M.M. (1979b). Immunity to Trichinella spiralis in irradiated mice.

WALLIS, V.J., LEUCHARS, E., CHAUDHURI, M. & DAVIES, A.J.S. (1979). Studies on hyperlymphoid mice. Immunology 38, 163-171.

WEINMANN, C.J. (1966). Immunity mechanisms in cestode infections. In Biology of Parasites (ed. E.J.L. Soulsby), 301-320. Academic Press, New York.

WEINMANN, C.J. (1970). Cestodes and Acanthocephala. In Immunity to Parasitic Animals (ed. G.J. Jackson, R. Herman & I. Singer) Vol. 2, 1021-1059. Appleton-Century-Crofts, New York.

WELLS, P.C.U., BURRELS, C. & MARTIN, W.B. (1977). Reduced mitogenic responses in cultures of lymphocytes from newly calved cows. Clinical and Experimental Immunology 29, 159-161.

WERTHAMER, S., GOVINDARAJ, S. & AMARAL, L. (1976). Placenta, transcortin and localised immune response. Journal of Clinical Investigation 57, 1000-1008.

WISEMAN, G. (1964). Absorption from the Intestine. Academic Press, London & New York.

WORTIS, H.H. (1971). Immunological responses of 'nude' mice. Clinical and Experimental Immunology 8, 305-317.

YACHNIN, S. (1975). The immunoregulatory properties of purified human α-fetoprotein (HAFP). Blood 46, 1035.

YOUSEF, M.K. & LUICK, J.R. (1969). Selenomethionine-^{75}Se turnover as an index of protein metabolism during cold exposure of white mice. Canadian Journal of Physiological Pharmacology 47, 273-276.

ZAWADOWESKY, M.M. & ZVJAGUINTZER, S.N. (1933). The seasonal fluctuation in the number of eggs of Nematodirus spp. in faeces. Journal of Parasitology 19, 270-279.

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